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Aims and Scope

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Editor in Chief: Prof. Dr. Nuray ERKAN

Address: Istanbul University, Faculty of Aquatic Sciences, Department of Food Safety, Kalenderhane Mah. 16 Mart Şehitleri Cad. No: 2, 34134 Fatih/Istanbul, Türkiye

E-mail: **nurerkan@istanbul.edu.tr**

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Importance and applicability analysis of the health and safety measures taken against the coronavirus disease on merchant vessels

Tuba KEÇECİ

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Istanbul Technical University, Faculty of Maritime, Department of Maritime Transportation Management Engineering, Istanbul, Türkiye

ORCID IDs of the author(s):

T.K. 0000-0001-6259-3858

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Correspondence:
Tuba KEÇECİ
E-mail: kececit@itu.edu.tr

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ABSTRACT

The coronavirus (COVID-19) outbreak has affected seafarers worldwide. This paper demonstrates the measures taken to prevent the spread of the coronavirus on merchant ships and evaluates them in terms of importance and applicability. Contamination reducing measures were determined through expert opinion and literature review. While their importance values were calculated using the fuzzy analytic hierarchy process method in which the total integral value with optimism index was applied, the applicability levels were revealed using a five-point Likert scale. The imbalance in rest and working hours was clearly seen in the results. Immune system protective measures were the most critical measures; however, two of them have the lowest applicability value among all criteria. They were followed by the measures taken through training, the measures to be applied in case of personnel showing disease symptoms, and the temperature measurement. In terms of the ship locations and ship operations, maintaining physical distance on deck at the port was found more critical. Its applicability level was slightly below average. This paper is the first study in the literature in which the measures taken to prevent the spread of the coronavirus pandemic on merchant ships were demonstrated in detail and evaluated with scientific methods in terms of importance and applicability. The research findings will help companies in the risk assessment process and contribute to the enhancement of the preparedness of the maritime industry for such situations by helping to protect the seafarers' health and safety.

Keywords: COVID-19, Fuzzy MCDM, Health and Safety, Maritime transportation, Seafarer, Virus disease outbreak

Introduction

The COVID-19 (coronavirus disease 2019) Pandemic, which has become a major global health threat, has infected more than 400 million people and caused the death of 5,798,628 people as of February 10, 2022 (Worldometer, n.d.). Within the pandemic period, to prevent the spread of the coronavirus, alternative forms of work such as rotational work and remote work have been adopted in some workplaces. Employees who have to continue working have faced many risks and problems. Seafarers, who have an essential role in the transportation of goods, energy, food, medicine, and many other products vital for daily needs, to all parts of the world, are also one of the groups of workers who have to continue to work. Crew changeovers and repatriation, restrictions on border crossings with border closures, abandonment, resupply and ship surveys, renewals of certificates and licensing of seafarers, and quarantine requirements were serious challenges seafarers faced during the pandemic (Doumbia-Henry, 2020). Besides governments, the International Maritime Organization (IMO), World Health Organization (WHO), International Labor Organization (ILO), the International Transport Workers Federation (ITF), the International Chamber of Shipping (ICS), the International Seafarer's Welfare Association (ISWAN), the International Maritime Health Association (IMHA), and many more organizations have worked to minimize the risk for global trade and human health (Doumbia-Henry, 2020; Stannard, 2020).

No study has been encountered yet in the literature that clearly provides information on the number of ships with COVID-19 cases and the number of seafarers infected with the coronavirus. The British Registered Diamond Princess was the first cruise ship to have coronavirus outbreak on board (Dahl, 2020). On March 8, 2020, it was found that 696 of the 3,711 people on the Diamond Princess cruise ship tested positive for SARS-CoV-2, and seven patients died, while less than twenty days later, it was confirmed that there were COVID-19 cases on 25 more cruise ships (Xu et al., 2020). However, apart from cruise ships, there is no clear information on the number of infected seafarers in merchant ships and fishing vessels. It was known that five crew members caught the coronavirus on the Danish container ship Gjertrud Maersk, which anchored in Zhoushan Port on March 17, 2020 (Dai et al., 2020). Additionally, it was known that there were cases on board ships in Brazil, Antwerp, and Mozambique, and some of these have unfortunately led to the death of seafarers (Stannard, 2020; The Hindu, n.d.).

Under the International Safety Management Code (ISM Code), shipping companies are required to define risks and assess them to ensure the safety of their ships, the environment, and personnel (IMO, 2010). In the light of the risk assessment carried out, the procedure for seafarers' health and safety should be developed and added to the company's Safety Management System (SMS). In the pandemic period, safety procedures including precautions specific to conditions were developed to ensure the safety of seafarers and ship operations' safety and minimize risks. However, due to the unique nature of the working environment on ships, it is not possible to apply some of these measures onboard. In addition, the efficiency level of all measures should be taken into account. There is no study in the literature in which the measures taken to prevent the spread of the coronavirus pandemic on merchant ships were demonstrated in detail and evaluated with scientific methods in terms of importance and applicability. In order to fill this gap, the measures taken on board ships were specified, and the importance values were determined by consulting expert opinion. Calculations were performed by using the Fuzzy Analytic Hierarchy Method (FAHP) in which the total integral value with optimism index was applied. In addition, the extent to which the determined measures are applicable on the ship were found using the five-point Likert scale. This research, which is the first study to investigate the importance and applicability of measures taken against the spread of coronavirus disease on merchant ships based on a scientific method, is an important source that can be used in the risk assessment and procedure development process for maritime companies. The study results, which will also be a reference for safety training, will contribute to the protection of seafarers' health, to ensure job safety, for the maritime industry to become aware of its current state, and to increase its preparedness for such situations.

Protection Measures and Management of Covid-19 Onboard

Under Article IV, paragraphs 1 and 4 of the MLC, 2006, every seafarer has the right to a safe and secure working environment and protection of health, medical care, and well-being. Some recommendations on measures to be taken to protect seafarers, who were recognized as key workers by 45 IMO member states and one associate member country in 2020, have been published during the coronavirus outbreak. ILO has published an information note on maritime labor issues and coronavirus on April 7, 2020 (ILO, 2020). The document emphasizes that flag states should take the necessary measures to protect the seafarers' health on ships flying their flags and ensure that they have access to adequate medical care, including the provision of personal protective equipment and disinfectants, especially during the COVID-19 pandemic (ILO, 2020). In the interim guidance titled 'Operational considerations for managing COVID-19 cases and outbreaks onboard ships' (WHO, 2020a) published by WHO on February 24, 2020, recommendations for seafarers, ship owners, and maritime authorities were presented. In this guide, which was encouraged to be used with the guide titled 'Handbook for management of public health events onboard ships' (WHO, 2016), information about the outbreak management plan, precautions to be taken before boarding the ship, and measures for managing suspicious cases were given. The points to be considered during the quarantine period in case of COVID-19 case detection, the adequacy of the amount of personal protective equipment, training, cleaning, disinfection frequency, waste management were the issues that draw attention in the guide. Another document published by WHO is the interim report titled 'Promoting public health measures in response to COVID-19 on cargo ships and fishing vessels' (WHO, 2020b). Topics mentioned in this report were minimizing the number of non-crew members boarding, hand hygiene, and respiratory etiquette, physical distancing, use of masks, managing COVID-19 cases and their contacts, access to medical facilities, digital tools, and mobile applications, training, mental health and psychosocial support, and public health measures for shore-side visits. ICS has published Coronavirus (COVID-19) Guidance for Ship Operators for the Protection of the Health of Seafarers (ICS, 2020) to help seafarers and ship operators follow health advice from United Nations agencies and other organizations. Measures to be taken to protect from infection were stated as monitoring and screening, using personal protective equipment (PPE), testing and assessment, shipboard self-distancing (SSD), and cleaning and disinfection. It has been emphasized that the training recommended for hand and respiratory hygiene is of vital importance. Minimizing interaction with shore personnel during port operations, giving health-self declaration, regular temperature measurement, frequent disinfection of equipment, using stairs outside the accommodation whenever possible, eating meals in the cabin, trying to spend rest hours in the cabin, getting enough sleep, paying attention to healthy nutrition, using masks, hanging informative posters, giving importance to mental health and adhering to promoting cough etiquette were among the measures mentioned in this document.

Besides the organizations mentioned above, country-specific organizations also provide preventive measures. Minimizing shore leave, avoiding touching face with unwashed hands, and monitoring of crew for signs and symptoms of coronavirus were some of the recommendations presented in the interim guidance (CDC, 2020) prepared by the Centres for Disease Control and Prevention, one of these organizations, on the management of suspected coronavirus cases. The other measures stated in this document were avoiding sharing personal items such as laptops and other hand-held devices and blankets, encouraging the use of non-contact methods of greeting, wearing a facemask, assigning crew to single-occupancy cabins with private bathrooms, placing hand sanitizer in multiple locations and ensuring handwashing facilities are well stocked with paper towels, soap, and a waste receptacle. The CDC also makes recommendations for maritime pilots to protect themselves and slow the spread of coronavirus. Using external stairs to access the vessel bridge, reminding the master to limit the crew involved in vessel navigation while the pilot is on board, using personal hand sanitizer, cleaning and disinfecting portable pilot units, radios etc. after each pilot job, wearing a face shield and avoiding contact with frequently touched bridge surfaces unless it is necessary were noteworthy measures in the document.

Shipping companies determine their own safety measures in addition to the measures stated in the guidelines and resources mentioned in this section for the implementation of protection measures related to coronavirus on their ships. All the measures certainly have an essential role in reducing the risk of coronavirus transmission. However, due to the unique nature of ship operations, not all preventive health and safety measures taken on the ship will be equally applicable, and their effectiveness in preventing the spread of the virus will also be different. Measuring the importance and applicability of the precautions taken in this critical period will be beneficial to ship operators and seafarers in risk assessment studies and in taking additional measures. For this purpose, taking into account ship locations and ship operations, the measures discussed in this section were examined. The measures that were compiled by removing repetitive ones were evaluated after being combined with the safety measures obtained from expert opinions.

Material and Methods

The criteria were determined by reviewing the resources mentioned in the previous section. All measures were presented in a hierarchical structure in the Results and Discussion section. Six maritime experts scored the importance of each measure in preventing the risk of coronavirus transmission. Among those experts, five keep the license of Ocean-going Ship Master, and one keeps the Ocean-going Chief Officer license. While four experts served on the ship, one worked as Executive Director (fleet operations) during this study. The average service period of experts was 11 years. The types of ships that they worked on were container ships, chemical tankers, bulk carriers, dry cargo vessels, and Ro-Ro ships. The fact that the experts who worked on the ship during the coronavirus outbreak worked on different ship types was valuable in that the results of this study would cover different ship types. Scoring aimed to reveal the rankings of measures by comparing them. Analytic Hierarchy Process (AHP) technique (Saaty, 1980) was chosen as the multi-criteria decision-making technique to emergein this order. Fuzzy set theory (Zadeh, 1965) was used to add blurriness in the mentality of experts to this technique in which crisp values were used. The total integral value with optimism index (Liou and Wang, 1992) was used with FAHP to get more reliable results. During the analysis process, an MS excel file in which the application steps of the method were formulated was used for calculations. The 9-point evaluation scale was used to collect expert judgments, and the scale which is given in Table 1 was used in fuzzy AHP calculations. The mean of expert opinions was calculated by the geometric mean method.

Table 1. Fuzzy Evaluation Scale (Yuen and Lau, 2011)

Fuzzy value	Fuzzy Reciprocal value
(8,9,9)	(1/9,1/9,1/8)
(7,8,9)	(1/9,1/8,1/7)
(6,7,8)	(1/8,1/7,1/6)
(5,6,7)	(1/7,1/6,1/5)
(4,5,6)	(1/6,1/5,1/4)
(3,4,5)	(1/5,1/4,1/3)
(2,3,4)	(1/4,1/3,1/2)
(1,2,3)	(1/3,1/2,1)
(1,1,1)	(1,1,1)

In addition, experts were asked to rate the applicability of the protection measures on the ship. A five-point Likert scale was used in scoring. The expert opinion was obtained by the questionnaire technique.

Fuzzy AHP

Introduced to the literature by Saaty (1980), the AHP evaluation process relies on the decision maker's subjective judgment which is uncertain. The uncertainty in the mind set of the decision-maker was tried to be removed by integrating the Fuzzy logic presented in the literature by Zadeh (1965) into comparison matrices. The first study in which Fuzzy AHP was used was presented to the literature by van Laarhoven and Pedrycz (1983). Later, Buckley (1985) showed that the trapezoidal-shaped fuzzy numbers and the geometric mean method could be used in the analysis (Kwong and Bai, 2002). Then, Chang (1996) proposed a new approach to Fuzzy AHP using the extent analysis method for the synthetic extent value of the pairwise comparison. The steps of finding the synthetic extent value with the extent analysis method is given below.

A triangular fuzzy number (TFN) expresses the relative strength of each pair of elements in the same hierarchy and can be indicated as $\widetilde{M} = (l, m, u)$, where $l \leq m \leq u$, the parameters l stands for the lower value, m for the middle value, and u for the upper value (Chang, 1996; Onut et al., 2008). A triangular membership function of $\widetilde{M}$ can be calculated as in Equation (1) (Onut et.al., 2008).

$$\mu_{\tilde{M}}(x) = \begin{cases} 0, & x < l \\ \dfrac{x-l}{m-l}, & l \leq x \leq m \\ \dfrac{u-x}{u-m}, & m \leq x \leq u \\ 0, & x > u \end{cases}$$

(1)

A triangular membership function is demonstrated in Figure 1.

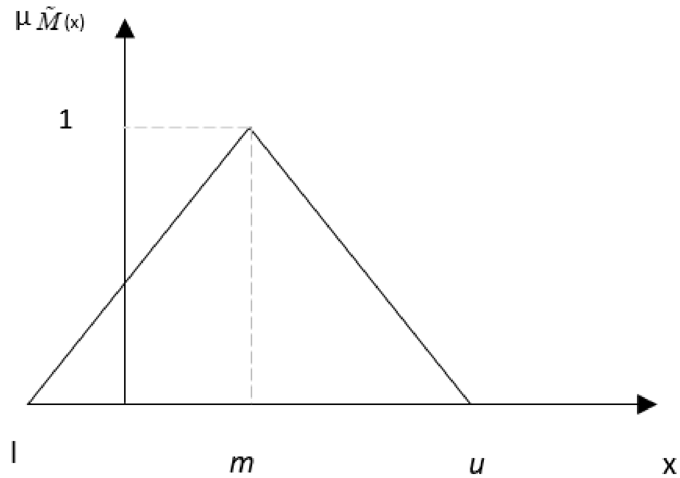

Figure 1. Triangular membership function

The application steps of the extent analysis method were detailed as follows (Chang, 1996).

Let $X = \{x_1, x_2, ..., x_n\}$ represent an object set, and $U = \{u_1, u_2, \ldots, u_m\}$ be a goal set. We take each object and perform the extent analysis for each goal, respectively. We get m extent analysis values for each object, with the following signs;

$$M_{g_i}^1, M_{g_i}^2, M_{g_i}^3 ..., M_{g_i}^m, \quad i=1, 2, 3..., n,$$

(2) where all the $M_{g_i}^j$ (j= 1,2,3 ..., m) are TFNs.

The value of fuzzy synthetic extent with respect to the *i*-th object is found as (Chang, 1992),

$$S_i = \sum_j^m M_{g_i}^j \otimes \left[\sum_i^n \sum_j^m M_{g_i}^j \right]^{-1}$$

(3)

The extent analysis method sometimes causes some of the weight values to appear as zero. The total integral value method with optimism index was applied in this study in order to obtain a more reliable result since if more than one value is zero, the ranking of the criteria cannot be determined clearly.

The Total Integral Value with Optimism Index

Wang et al. (2008) express that the degree of possibility calculated by Chang's (1996) extent analysis method is an index for comparing two TFNs to show to what degree a TFN is larger than the others rather than presenting their relative importance. The total integral value with optimism index was developed by Liou and Wang (1992) to solve this problem and used in many studies (Akyildiz and Mentes, 2017; Alipour et al., 2017; Baysal and Çetin, 2018; De Felice et al., 2019; Duman et al., 2017; Flores-Carrillo et al., 2017; Şen and Çınar, 2010) in the literature. Equation 4 can derive the synthetic extent values of A.

$$I_T^\alpha(\widetilde{S_i}) = \frac{1}{2}\alpha(m_i + u_i) + \frac{1}{2}(1-\alpha)(l_i + m_i)$$
$$= \frac{1}{2}[\alpha u_i + m_i + (1-\alpha) l_i]$$

(4)

where ∝ is the index of optimism indicates the degree of optimism for decision-makers (Liou and Wang, 1992). If ∝ approaches 0 in the [0; 1] interval, it shows the decision-makers are more pessimistic (Şen and Çınar, 2010). For a neutral or moderately objective decision-maker, ∝ value equals 0.5 (Akyildiz and Mentes, 2017).

The normalized importance weight vector $W = (w_1, w_2, ..., w_n)^T$ of fuzzy matrix A can be calculated using Equation 5 (Şen and Çınar, 2010).

$$W_i = \frac{I_T^\alpha(\widetilde{S_i})}{\sum_i^n I_T^\alpha(\widetilde{S_i})}$$

(5)

In this study, the synthetic extent values were determined by using Equation (3) and integrated into the Fuzzy AHP method by applying the total integral value with the optimism index.

Results and Discussion

The measures taken on board to reduce the risk of coronavirus transmission were categorized and presented as criteria in a hierarchical structure which was given in Table 2.

The main criteria that make up the first level were determined as Physical Distancing (C_1), Hygiene Precautions (C_2), Body Temperature Screening (C_3), Immune System Protective Measures (C_4), Training (C_5), and Management of a Suspected Case of COVID-19 (C_6).

At the second level, there were sub-criteria. While determining the sub-criteria of the Physical distancing (C_1), the operational processes of the ship were taken into consideration. These processes were arrival/departure maneuvers, open sea navigation, and staying at the port during cargo handling.

The main locations of the ship were taken into consideration while determining the sub-sub criteria under Physical distancing at port (C_{11}). The areas where people interact at the port were specified as the deck, the accommodation, and the engine room. While defining the sub-sub criteria under Physical distancing during open sea navigation (C_{12}) and Physical distancing during arrival/departure maneuvers (C_{13}), the bridge area was also included among these ship locations.

Table 2. The hierarchical structure of the measures taken on board against COVID-19

Main criteria		Sub- criteria			Sub-sub criteria
C1	Physical Distancing	C11	Physical distancing at port	C111	Physical distancing on deck
				C112	Physical distancing in the accommodation
				C113	Physical distancing in the engine room
		C12	Physical distancing during open sea navigation	C121	Physical distancingthe on bridge
				C122	Physical distancing on deck
				C123	Physical distancing in the accommodation
				C124	Physical distancing in the engine room
		C13	Physical distancing during departure-arrival manouvre	C131	Physical distancing on the bridge
				C132	Physical distancing on deck
				C133	Physical distancing in the accommodation
				C134	Physical distancing in the engine room
C2	Hygiene Precautions	C21	Personal hygiene	C211	Cleaning hands frequently
				C212	Wearing medical masks/changing as often as necessary
				C213	Using personal disinfectants
				C214	Avoiding personal protective equipment sharing
				C215	Using protective googles in case of absence of/not using a face shield
				C216	Using gloves
				C217	Using separate overalls and shoes at the port on deck and in accommodation
				C218	Using disposable coveralls, masks, gloves, and face shield while working on the deck at a risky port
		C22	Ventilation	C221	Ventilation of cabins
				C222	Ventilation of accommodation
				C223	Ventilation at port
		C23	Common area disinfection	C231	Providing disinfectants in corridors and common areas
				C232	Placing anti-bacterial soap in common toilets
				C233	Disinfection of shared devices/equipment (computer, bridge devices etc.)
		C24	Hygiene measures taken regarding visitors	C241	Cleaning the accommodation with bleach at departure from risky port
				C242	Washing the accommodation with sea water from outside at departure from risky port
				C243	Placing pans with bleach to wipe the shoes of visitors at the gangway and accommodation entrances
				C244	Daily cleaning of the areas where visitors are hosted at port
				C245	Making sure visitors (pilot, authority, dock worker etc.) are wearing masks/gloves
				C246	Making sure visitors are wearing overshoes
				C247	Providing the food service to pilots in long maneuvers with disposable materials
				C248	Entering the accommodation from a single point
		C25	Waste management	C251	Treating masks, overalls etc. as medical waste
		C26	Food safety precautions	C261	Prevention of taking food at risky ports
				C262	Proper washing of vegetables/fruits that are eaten raw
C3	Body Temperature Screening	C31	Temperature screening of ship crew twice a day		
		C32	Temperature screening of visitors		
C4	Immune System Protective Measures	C41	Getting enough sleep		
		C42	Exercise		
		C43	Eating adequate/nutritious foods		
C5	Training	C51	Providing training for ship crew on COVID-19 measures with sufficient time prior to port arrival		
		C52	Hanging informative posters on visible parts of the ship		
		C53	Training the new crew members joining the ship on COVID-19		
C6	Management of a Suspected Case of COVID-19	C61	Isolation in single-occupancy cabin		
		C62	Using dedicated or disposable dish and food service utensils		
		C63	Treating the wastes as medical waste		
		C64	Ventilation of the cabin from the light port		
		C65	Checking on the suspected person 3 times a day		
		C66	Making sure the laundry is washed by soap and water		
		C67	Regular support from medical services		

Fuzzy Integrated Total Integral Value Method Results

In this section, by integrating Liou and Wang's (1992) total integral value with optimism index into Chang's (1996)] extent analysis method, the weight vectors of the comparison matrices of the criteria in all levels of the hierarchical structure were calculated.

In the first step, fuzzy synthetic values of the main criteria were obtained using Chang's (1996) extended analysis method. For this purpose, six decision matrices belonging to experts were transformed into a single decision matrix by using the geometric mean method. The aggregated matrix is given in Table 3.

As seen in the matrix given in Table 3, the fuzzy sums of each row were calculated. Using the Equation (3), fuzzy synthetic values of the criteria were determined as S_{C1}= (0.12, 0.19, 0.29), S_{C2}= (0.09, 0.15, 0.26), S_{C3}= (0.04, 0.07, 0.11), S_{C4}= (0.12, 0.19, 0.29), S_{C5}= (0.09, 0.15, 0.26), and S_{C6}= (0.16, 0.24, 0.35).

In the second step, by following the steps of the total integral value method, the fuzzy number sequencing process was carried out with the help of the synthetic values. Considering that experts made an impartial assessment, ∝ the degree of optimism value was determined as 0.5. According to Liou and Wang's (1992) total integral value with optimism index method, using Equation 4,

(SC_1)= 0.202

(SC_2)= 0.165

(SC_3)= 0.071

(SC_4)= 0.202

(SC_5)= 0.165

(SC_6)= 0.248

were obtained. Accordingly, the weight vector of the criteria was expressed as W= (0.202, 0.165, 0.071, 0.202, 0.165, 0.248). Using the Equation 5, the normalized weight vector calculated was W= (0.192, 0.157, 0.067, 0.192, 0.157, 0.236)^T.

All calculations made for main criteria were also performed for the sub-criteria and the sub-sub-criteria. The aggregated FAHP Pairwise comparison matrix and the synthetic values obtained for the sub-criteria were given in Table 4, for the sub-sub-criteria of C_1 were given in Table 5 and for the sub-sub-criteria of C_2 were given in Table 6.

Table 3. Aggregated FAHP Pairwise Comparison Matrix for Criteria

	C_1			C_2			C_3			C_4			C_5			C_6			Total		
C_1	1.00	1.00	1.00	0.76	1.26	1.73	2.26	3.03	3.71	1.00	1.00	1.00	0.76	1.26	1.73	0.76	0.79	0.83	6.56	8.34	10.01
C_2	0.58	0.79	1.31	1.00	1.00	1.00	1.42	2.29	3.31	0.58	0.79	1.31	1.00	1.00	1.00	0.48	0.63	1.00	5.06	6.51	8.93
C_3	0.27	0.33	0.44	0.30	0.44	0.70	1.00	1.00	1.00	0.27	0.33	0.44	0.30	0.44	0.70	0.23	0.28	0.34	2.38	2.81	3.63
C_4	1.00	1.00	1.00	0.76	1.26	1.73	2.26	3.03	3.71	1.00	1.00	1.00	0.76	1.26	1.73	0.76	0.79	0.83	6.56	8.34	10.01
C_5	0.58	0.79	1.31	1.00	1.00	1.00	1.42	2.29	3.31	0.58	0.79	1.31	1.00	1.00	1.00	0.48	0.63	1.00	5.06	6.51	8.93
C_6	1.20	1.26	1.31	1.00	1.59	2.08	2.96	3.63	4.28	1.20	1.26	1.31	1.00	1.59	2.08	1.00	1.00	1.00	8.36	10.33	12.05

Table 4. The aggregated FAHP pairwise comparison matrix and the synthetic values obtained for the sub-criteria

	C_{11}			C_{12}			C_{13}			Total		
C_{11}	1.00	1,00	1,00	1,32	1,59	1,86	1,00	1,41	1,73	3,32	4,00	4,59
C_{12}	0.54	0.63	0.75	1.00	1.00	1.00	0.69	0.89	1.31	2.23	2.52	3.06
C_{13}	0.58	0.71	1.00	0.76	1.12	1.44	1.00	1.00	1.00	2.34	2.83	3.44

SC_{11}=(0.30, 0.43, 0.58), SC_{12}=(0.20, 0.27, 0.39), SC_{13}=(0.21, 0.30, 0.44).

	C_{21}			C_{22}			C_{23}			C_{24}			C_{25}			C_{26}			Total		
C_{21}	1.00	1.00	1.00	1.00	1.12	1.20	1.00	1.26	1.44	1.00	1.12	1.20	1.73	2.52	3.22	1.66	2.00	2.26	7.39	9.02	10.33
C_{22}	0.83	0.89	1.00	1.00	1.00	1.00	1.00	1.12	1.20	1.00	1.00	1.00	1.44	2.24	2.96	1.38	1.78	2.08	6.66	8.04	9.24
C_{23}	0.69	0.79	1.00	0.83	0.89	1.00	1.00	1.00	1.00	0.83	0.89	1.00	1.20	2.00	2.72	1.15	1.59	2.08	5.71	7.16	8.80
C_{24}	0.83	0.89	1.00	1.00	1.00	1.00	1.00	1.12	1.20	1.00	1.00	1.00	1.44	2.24	2.96	1.38	1.78	2.08	6.66	8.04	9.24
C_{25}	0.31	0.40	0.58	0.34	0.45	0.69	0.37	0.50	0.83	0.34	0.45	0.69	1.00	1.00	1.00	0.61	0.79	1.20	2.96	3.58	5.00
C_{26}	0.44	0.50	0.60	0.48	0.56	0.72	0.48	0.63	0.87	0.48	0.56	0.72	0.83	1.26	1.64	1.00	1.00	1.00	3.72	4.51	5.55

SC_{21}= (0.15, 0.22, 0.31), SC_{22}= (0.14, 0.20, 0.28), SC_{23}= (0.12, 0.18, 0.27), SC_{24}= (0.14, 0.20, 0.28), SC_{25}= (0.06, 0.09, 0.15), SC_{26}= (0.08, 0.11, 0.17).

	C_{31}			C_{32}			Total		
C_{31}	1.00	1.00	1.00	0.83	0.89	1.00	1.83	1.89	2.00
C_{32}	1.00	1.12	1.20	1.00	1.00	1.00	2.00	2.12	2.20

SC_{31}= (0.44, 0.47, 0.52), SC_{32}=(0.48, 0.53, 0.57).

	C_{41}			C_{42}			C_{43}			Total		
C_{41}	1.00	1.00	1.00	1.00	1.12	1.20	1.00	1.00	1.00	3.00	3.12	3.20
C_{42}	0.83	0.89	1.00	1.00	1.00	1.00	0.83	0.89	1.00	2.67	2.78	3.00
C_{43}	1.00	1.00	1.00	1.00	1.12	1.20	1.00	1.00	1.00	3.00	3.12	3.20

SC_{41}=(0.32, 0.35, 0.37), SC_{42}=(0.28, 0.31, 0.35), SC_{43}=(0.32, 0.35, 0.37).

	C_{51}			C_{52}			C_{53}			Total		
C_{51}	1.00	1.00	1.00	0.83	1.00	1.20	1.00	1.00	1.00	2.83	3.00	3.20
C_{52}	0.83	1.00	1.20	1.00	1.00	1.00	0.83	1.00	1.20	2.67	3.00	3.40
C_{53}	1.00	1.00	1.00	0.83	1.00	1.20	1.00	1.00	1.00	2.83	3.00	3.20

SC_{51}=(0.29, 0.33, 0.38), SC_{52}=(0.27, 0.33, 0.41), SC_{53}=(0.29, 0.33, 0.38).

	C_{61}			C_{62}			C_{63}			C_{64}			C_{65}			C_{66}			C_{67}			Total		
C_{61}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.12	1.20	1.38	1.78	2.08	2.17	2.40	2.61	1.20	1.26	1.31	8.76	9.57	10.20
C_{62}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.12	1.20	1.38	1.78	2.08	2.17	2.40	2.61	1.20	1.26	1.31	8.76	9.57	10.20
C_{63}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.12	1.20	1.38	1.78	2.08	2.17	2.40	2.61	1.20	1.26	1.31	8.76	9.57	10.20
C_{64}	0.83	0.89	1.00	0.83	0.89	1.00	0.83	0.89	1.00	1.00	1.00	1.00	1.31	1.70	1.99	2.05	2.29	2.50	1.00	1.12	1.31	7.86	8.78	9.80
C_{65}	0.48	0.56	0.72	0.48	0.56	0.72	0.48	0.56	0.72	0.50	0.59	0.76	1.00	1.00	1.00	1.09	1.35	1.66	0.58	0.71	0.87	4.61	5.33	6.46
C_{66}	0.38	0.42	0.46	0.38	0.42	0.46	0.38	0.42	0.46	0.40	0.44	0.49	0.60	0.74	0.92	1.00	1.00	1.00	0.50	0.52	0.55	3.65	3.95	4.34
C_{67}	0.76	0.79	0.83	0.76	0.79	0.83	0.76	0.79	0.83	0.76	0.89	1.00	1.15	1.41	1.73	1.81	1.91	1.99	1.00	1.00	1.00	7.02	7.59	8.22

SC61=(0.15, 0.18, 0.21), SC62=(0.15, 0.18, 0.21), SC63= (0.15, 0.18, 0.21), SC64=(0.13, 0.16, 0.20), SC65=(0.08, 0.10, 0.13), SC66=(0.06, 0.07, 0.09), SC67=(0.12, 0.14, 0.17).

Table 5. The aggregated FAHP pairwise comparison matrix and the synthetic values obtained for the sub-sub-criteria of C_1

	C_{111}			C_{112}			C_{113}			Total		
C_{111}	1.00	1.00	1.00	0.83	1.12	1.44	0.83	1.12	1.44	2.67	3.24	3.88
C_{112}	0.69	0.89	1.20	1.00	1.00	1.00	1.00	1.00	1.00	2.69	2.89	3.20
C_{113}	0.69	0.89	1.20	1.00	1.00	1.00	1.00	1.00	1.00	2.69	2.89	3.20

SC_{111}=(0.26, 0.36, 0.48), SC_{112}=(0.26, 0.32, 0.40), SC_{113}=(0.26, 0.32, 0.40).

	C_{121}			C_{122}			C_{123}			C_{124}			Total		
C_{121}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	4.00	4.00	4.00
C_{122}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	4.00	4.00	4.00
C_{123}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	4.00	4.00	4.00
C_{124}	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	4.00	4.00	4.00

SC_{121}= (0.25, 0.25, 0.25), SC_{122}=(0.25, 0.25, 0.25), SC_{123}=(0.25, 0.25, 0.25), SC_{124}=(0.25, 0.25, 0.25).

	C_{131}			C_{132}			C_{133}			C_{134}			Total		
C_{131}	1.00	1.00	1.00	1.00	1.78	2.50	1.78	2.50	3.17	1.00	1.78	2.50	4.78	7.06	9.17
C_{132}	0.40	0.56	1.00	1.00	1.00	1.00	1.00	1.78	2.50	1.00	1.00	1.00	3.40	4.34	5.50
C_{133}	0.31	0.40	0.56	0.40	0.56	1.00	1.00	1.00	1.00	0.40	0.56	1.00	2.12	2.52	3.56
C_{134}	0.40	0.56	1.00	1.00	1.00	1.00	1.00	1.78	2.50	1.00	1.00	1.00	3.40	4.34	5.50

SC_{131}= (0.20, 0.39, 0.67), SC_{132}=(0.14, 0.24, 0.40), SC_{133}=(0.09, 0.14, 0.26), SC_{134}=(0.14, 0.24, 0.40).

Table 6. The aggregated FAHP pairwise comparison matrix and the synthetic values obtained for the sub-sub-criteria of C_2

	C_{211}			C_{212}			C_{213}			C_{214}			C_{215}			C_{216}			C_{217}			C_{218}			Total		
C_{211}	1.00	1.00	1.00	1.38	1.59	1.73	1.31	1.51	1.66	1.20	1.26	1.31	1.31	1.70	1.99	2.50	3.03	3.46	2.30	2.52	2.72	2.17	2.40	2.61	13.17	15.01	16.48
C_{212}	0.58	0.63	0.72	1.00	1.00	1.00	0.83	0.89	1.00	0.69	0.79	0.87	0.83	1.00	1.20	1.71	2.04	2.30	1.57	1.70	1.81	1.44	1.59	1.71	8.66	9.64	10.61
C_{213}	0.60	0.66	0.76	1.00	1.12	1.20	1.00	1.00	1.00	0.72	0.83	0.92	1.00	1.12	1.20	1.71	2.29	2.76	1.57	1.91	2.17	1.44	1.78	2.05	9.05	10.72	12.07
C_{214}	0.76	0.79	0.83	1.15	1.26	1.44	1.09	1.20	1.38	1.00	1.00	1.00	1.09	1.35	1.66	2.17	2.70	3.13	1.99	2.24	2.47	1.66	2.00	2.26	10.92	12.54	14.18
C_{215}	0.50	0.59	0.76	0.83	1.00	1.20	0.83	0.89	1.00	0.60	0.74	0.92	1.00	1.00	1.00	1.71	2.04	2.30	1.31	1.70	1.99	1.20	1.59	1.89	7.99	9.55	11.06
C_{216}	0.29	0.33	0.40	0.44	0.49	0.58	0.36	0.44	0.58	0.32	0.37	0.46	0.44	0.49	0.58	1.00	1.00	1.00	0.72	0.83	0.92	0.60	0.74	0.92	4.17	4.69	5.45
C_{217}	0.37	0.40	0.44	0.55	0.59	0.64	0.46	0.52	0.64	0.41	0.45	0.50	0.50	0.59	0.76	1.09	1.20	1.38	1.00	1.00	1.00	0.83	0.89	1.00	5.21	5.64	6.36
C_{218}	0.38	0.42	0.46	0.58	0.63	0.69	0.49	0.56	0.69	0.44	0.50	0.60	0.53	0.63	0.83	1.09	1.35	1.66	1.00	1.12	1.20	1.00	1.00	1.00	5.52	6.21	7.14

SC_{211}=(0.16, 0.20, 0.25), SC_{212}=(0.10, 0.13, 0.16), SC_{213}=(0.11, 0.14, 0.19), SC_{214}=(0.13, 0.17, 0.22), SC_{215}=(0.10, 0.13, 0.17), SC_{216}=(0.05, 0.06, 0.08), SC_{217}=(0.06, 0.08, 0.10), SC_{218}=(0.07, 0.08, 0.11).

	C_{221}			C_{222}			C_{223}			Total		
C_{221}	1.00	1.00	1.00	1.00	1.00	1.00	2.37	2.88	3.31	4.37	4.88	5.31
C_{222}	1.00	1.00	1.00	1.00	1.00	1.00	2.37	2.88	3.31	4.37	4.88	5.31
C_{223}	0.30	0.35	0.42	0.30	0.35	0.42	1.00	1.00	1.00	1.60	1.69	1.85

SC_{221}=(0.35, 0.43, 0.51), SC_{222}=(0.35, 0.43, 0.51), SC_{223}=(0.13, 0.15, 0.18).

	C_{231}			C_{232}			C_{233}			Total		
C_{231}	1.00	1.00	1.00	1.00	1.12	1.20	1.31	1.35	1.38	3.31	3.47	3.58
C_{232}	0.83	0.89	1.00	1.00	1.00	1.00	1.20	1.26	1.31	3.03	3.15	3.31
C_{233}	0.72	0.74	0.76	0.76	0.79	0.83	1.00	1.00	1.00	2.49	2.54	2.60

SC_{231}=(0.35, 0.38, 0.41), SC_{232}=(0.32, 0.34, 0.37), SC_{233}=(0.26, 0.28, 0.29).

	C_{241}			C_{242}			C_{243}			C_{244}			C_{245}			C_{246}			C_{247}			C_{248}			Total		
C_{241}	1.00	1.00	1.00	2.50	3.17	3.82	1.20	1.59	1.89	0.83	1.00	1.20	0.83	1.00	1.31	1.44	2.24	2.96	1.00	1.26	1.57	0.83	1.26	1.89	9.64	12.53	15.64
C_{242}	0.26	0.31	0.40	1.00	1.00	1.00	0.41	0.50	0.69	0.24	0.31	0.48	0.27	0.33	0.44	0.58	0.71	1.00	0.31	0.40	0.58	0.36	0.44	0.58	3.42	4.00	5.18
C_{243}	0.53	0.63	0.83	1.44	2.00	2.47	1.00	1.00	1.00	0.48	0.63	1.00	0.53	0.63	0.83	1.00	1.41	1.73	0.69	0.79	1.00	0.55	0.83	1.10	6.23	7.93	9.97
C_{244}	0.83	1.00	1.20	2.08	3.17	4.22	1.00	1.59	2.08	1.00	1.00	1.00	0.83	1.00	1.20	1.20	2.24	3.27	1.00	1.26	1.44	0.76	1.26	1.89	8.71	12.53	16.29
C_{245}	0.76	1.00	1.20	2.26	3.03	3.71	1.20	1.59	1.89	0.83	1.00	1.20	1.00	1.00	1.00	1.31	2.14	2.88	1.00	1.26	1.44	0.87	1.32	1.73	9.24	12.34	15.05
C_{246}	0.34	0.45	0.69	1.00	1.41	1.73	0.58	0.71	1.00	0.31	0.45	0.83	0.35	0.47	0.76	1.00	1.00	1.00	0.44	0.56	0.83	0.52	0.62	0.70	4.53	5.66	7.56
C_{247}	0.64	0.79	1.00	1.73	2.52	3.22	1.00	1.26	1.44	0.69	0.79	1.00	0.69	0.79	1.00	1.20	1.78	2.26	1.00	1.00	1.00	0.60	1.05	1.59	7.56	9.99	12.52
C_{248}	0.53	0.79	1.20	1.71	2.29	2.76	0.91	1.20	1.81	0.53	0.79	1.31	0.58	0.76	1.15	1.42	1.62	1.91	0.63	0.95	1.66	1.00	1.00	1.00	7.31	9.41	12.80

SC_{241}=(0.10, 0.17, 0.28), SC_{242}=(0.04, 0.05, 0.09); SC_{243}=(0.07, 0.11, 0.18), SC_{244}=(0.09, 0.17, 0.29), SC_{245}=(0.10, 0.17, 0.27), SC_{246}=(0.05, 0.08, 0.13), SC_{247}=(0.08, 0.13, 0.22), SC_{248}=(0.08, 0.13, 0.23).

	C_{261}			C_{262}			Total		
C_{261}	1.00	1.00	1.00	0.60	0.66	0.76	1.60	1.66	1.76
C_{262}	1.31	1.51	1.66	1.00	1.00	1.00	2.31	2.51	2.66

SC_{261}=(0.36, 0.40, 0.45), SC_{262}=(0.52, 0.60, 0.68).

The I_T^{α} values and weights calculated for the sub-criteria and sub-sub criteria were given in Table 7 and Table 8, respectively.

Table 7. I_T^α Values and weights of sub-criteria

Criteria	I_T^α	W^T
C_{11}	0.434	0.422
C_{12}	0.282	0.274
C_{13}	0.313	0.304
C_{21}	0.228	0.220
C_{22}	0.204	0.197
C_{23}	0.185	0.178
C_{24}	0.204	0.197
C_{25}	0.097	0.094
C_{26}	0.117	0.113
C_{31}	0.475	0.474
C_{32}	0.527	0,526
C_{41}	0.345	0.345
C_{42}	0.312	0.311
C_{43}	0.345	0.345
C_{51}	0.335	0.333
C_{52}	0.337	0.335
C_{53}	0.335	0.333
C_{61}	0.176	0.175
C_{62}	0.176	0.175
C_{63}	0.176	0.175
C_{64}	0.163	0.162
C_{65}	0.101	0.100
C_{66}	0.074	0.073
C_{67}	0.141	0.140

Table 8. I_T^α Values and weights of sub-sub-criteria

Sub-sub criteria	I_T^α	W^T
C_{111}	0.365	0.360
C_{112}	0.325	0.320
C_{113}	0.325	0.320
C_{121}	0.250	0.250
C_{122}	0.250	0.250
C_{123}	0.250	0.250
C_{124}	0.250	0.250
C_{131}	0.411	0.381
C_{132}	0.255	0.237
C_{133}	0.156	0.145
C_{134}	0.255	0.237
C_{211}	0.205	0.201
C_{212}	0.132	0.130
C_{213}	0.146	0.144
C_{214}	0.172	0.170
C_{215}	0.131	0.129
C_{216}	0.065	0.064
C_{217}	0.078	0.077
C_{218}	0.086	0.085
C_{221}	0.429	0.425
C_{222}	0.429	0.425
C_{223}	0.151	0.149
C_{231}	0.378	0.378
C_{232}	0.346	0.345
C_{233}	0.278	0.277
C_{241}	0.179	0.167
C_{242}	0.059	0.055
C_{243}	0.114	0.106
C_{244}	0.179	0.168
C_{245}	0.174	0.163
C_{246}	0.083	0.078
C_{247}	0.142	0.133
C_{248}	0.139	0.130
C_{261}	0.402	0.401
C_{262}	0.602	0.599

After this step, the global weights were found by multiplying the local weights of all criteria with the local weights of their sub-criteria and sub-sub criteria. The obtained results are presented in Table 9.

Table 9. Global weight results of fuzzy total integral value method

Criteria	w	Sub-criteria	w	Sub-sub criteria	w	Global weight
C_1	0.192	C_{11}	0.422	C_{111}	0.360	0.029
				C_{112}	0.320	0.026
				C_{113}	0.320	0.026
		C_{12}	0.274	C_{121}	0.250	0.013
				C_{122}	0.250	0.013
				C_{123}	0.250	0.013
				C_{124}	0.250	0.013
		C_{13}	0.304	C_{131}	0.381	0.022
				C_{132}	0.237	0.014
				C_{133}	0.145	0.008
				C_{134}	0.237	0.014
C_2	0.157	C_{21}	0.220	C_{211}	0.201	0.007
				C_{212}	0.130	0.004
				C_{213}	0.144	0.005
				C_{214}	0.170	0.006
				C_{215}	0.129	0.004
				C_{216}	0.064	0.002
				C_{217}	0.077	0.003
				C_{218}	0.085	0.003
		C_{22}	0.197	C_{221}	0.425	0.013
				C_{222}	0.425	0.013
				C_{223}	0.149	0.005
		C_{23}	0.178	C_{231}	0.378	0.011
				C_{232}	0.345	0.010
				C_{233}	0,277	0.008
		C_{24}	0.197	C_{241}	0,167	0.005
				C_{242}	0.055	0.002
				C_{243}	0.106	0.003
				C_{244}	0.168	0.005
				C_{245}	0.163	0.005
				C_{246}	0.078	0.002
				C_{247}	0.133	0.004
				C_{248}	0.130	0.004
		C_{25}	0.094	C_{251}	1.000	0.015
		C_{26}	0.113	C_{261}	0.401	0.007
				C_{262}	0.599	0.011
C_3	0.067	C_{31}	0.474			0.032
		C_{32}	0.526			0.035
C_4	0.192	C_{41}	0.345			0.066
		C_{42}	0.311			0.060
		C_{43}	0.345			0.066
C_5	0.157	C_{51}	0.333			0.052
		C_{52}	0.335			0.053
		C_{53}	0.333			0.052
C_6	0.236	C_{61}	0.175			0.041
		C_{62}	0.175			0.041
		C_{63}	0.175			0.041
		C_{64}	0.162			0.038
		C_{65}	0.100			0.024
		C_{66}	0.073			0.017
		C_{67}	0.140			0.033

The Applicability Analysis Results

The applicability of the measures taken on board ships were found by calculating the average of the scores given by the experts to the measures in the hierarchical structure. The geometric mean method was used to determine the mean of expert opinions. The applicability analysis results are presented in Table 10.

Table 10. The applicability of the measures taken on board ships

Criteria	Applicability	Sub-criteria	Applicablity	Sub-Sub criteria	Applicability
C_1	4.1	C_{11}	2.9	C_{111}	2.9
				C_{112}	3.1
				C_{113}	3.1
		C_{12}	4.5	C_{121}	4.1
				C_{122}	4.1
				C_{123}	3.5
				C_{124}	3.0
		C_{13}	2.6	C_{131}	3.2
				C_{132}	3.1
				C_{133}	3.6
				C_{134}	2.8
C_2	3.6	C_{21}	4.3	C_{211}	4.6
				C_{212}	3.5
				C_{213}	4.1
				C_{214}	4.6
				C_{215}	3.1
				C_{216}	3.5
				C_{217}	1.5
				C_{218}	2.4
		C_{22}	3.8	C_{221}	4.6
				C_{222}	4.6
				C_{223}	3.9
		C_{23}	3.5	C_{231}	5.0
				C_{232}	4.8
				C_{233}	3.2
		C_{24}	3.3	C_{241}	3.6
				C_{242}	3.1
				C_{243}	3.4
				C_{244}	4.3
				C_{245}	4.2
				C_{246}	2.5
				C_{247}	3.8
				C_{248}	4.6
		C_{25}	4.8	C_{251}	3.3
		C_{26}	1.9	C_{261}	3.4
				C_{262}	3.7
C_3	5.0	C_{31}	5.0		
		C_{32}	4.8		
C_4	2.1	C_{41}	1.6		
		C_{42}	1.9		
		C_{43}	2.8		
C_5	5.0	C_{51}	4.6		
		C_{52}	5.0		
		C_{53}	4.8		
C_6	3.9	C_{61}	4.8		
		C_{62}	4.6		
		C_{63}	4.8		
		C_{64}	3.8		
		C_{65}	4.3		
		C_{66}	2.6		
		C_{67}	3.8		

According to the analysis results, the order of the main criteria was $C_6>C_1=C_4> C_2=C_5>C_3$. In the ranking made according to the importance of the measures to prevent the risk of coronavirus transmission, 'Getting Enough Sleep', ‘Eating adequate/nutritious foods’ and 'Exercise' measures were on the top two places. One of the most striking results of this study was that 'Getting Enough Sleep' and ‘Exercise’ measures were two of the three criteria with the lowest applicability value among all criteria. The imbalance between the work and rest hours of seafarers becomes apparent here as well. Seafarers' work and rest hours do not reflect reality and it is a real problem known to everyone in the maritime industry. This problem needs to be solved immediately. Baumler et.al. (2020) conducted a study on seafarers' rest hours. It was revealed that the working/rest hour records of all seafarers participating in the study were adjusted. The results of this paper also support the conclusions that Baumler et al. (2020) referred to regarding the imbalance in the working hours of seafarers. Violations during the rest hours due to commercial pressure lead to a departure from the culture of "safety first" and in this environment, which suggests that human health is not valued enough, "work first" culture takes its place. In order to solve this problem, it is thought that the regulations regarding the minimum number of seafarers employed on the ships should be reviewed and a new regulation should be made by investigating the balance of workload and rest hours.

Measures taken through training, measures to be applied in case of personnel showing disease symptoms, and temperature measurement were also at the top of the importance ranking. The applicability values of these measures were also high. In the *10th* and *11th* row of the efficiency ranking, there were measures related to maintaining physical distance at port. When these results were analyzed in terms of ship locations, it was revealed that it was more important to maintain physical distance on deck at the port. The applicability level of maintaining physical distance onboard during port operations was slightly below average. It is believed that observing the recommended working zones on the ship will reduce the risk during port operations. When the measures between the *12th* and *20th* places of the importance ranking were examined, it was seen that the measures with below-average applicability level were ‘Washing the laundry of the personnel with disease symptoms by hand’ and ‘Maintaining physical distance in the engine room during the arrival/departure maneuver’. It is thought that the reason for the low applicability of hand washing the clothes of the person with symptoms of illness in the ship environment was that the cabin environment was not suitable, and the person may not have the body strength to do this. In the engine control room, which is a closed environment, it was deduced that because of the possibility of violations in maintaining the physical distancing due to the need to work punctually as a team, the applicability of ‘Maintaining physical distancing in the engine room during arrival/departure’ was considered to be low. When the measures between the *27th* and the last place of the ranking were examined, the measures that had the lowest level of applicability were ‘Use of disposable overalls-masks-gloves-visors by the personnel working on the deck at the risky port’, ‘Using separate overalls-shoes at the port and in the accommodation’, and ‘Making sure visitors from the port are wearing overshoes’. It was concluded that the applicability levels were low because it would not be easy to change overalls and shoes in such a busy working environment. It is thought that the reason for the low applicability of the measure of making sure visitors arriving at the accommodation are wearing overshoes was that it was not known whether the overshoe could reduce the safety in a dangerous working environment where safety shoes are worn and whether visitors would pay attention to the use of overshoes.

Conclusion

In the face of the global pandemic of the COVID-19, shipping has continued at the cost of seafarers’ health and safety. There are some studies (Baygi et al., 2021; Lucas et al., 2021; Luchenko and Georgiievskyi, 2021; Pauksztat et al., 2022) on the impact of the ongoing pandemic on seafarers’ health and well-being. Unlike these studies, this paper focused on the importance and applicability of the safety measures taken against COVID-19 to reduce the risk of contamination on board the ships. Fuzzy AHP with total integral value with optimism index and a five-point Likert scale was used for calculation. The quantitative analysis showed that immune system protective measures were the most important measures; however, two of them have the lowest applicability value among all criteria. The present work has explored the imbalance in rest and working hours of seafarers. When analyzing the ship locations and operations, maintaining physical distance on deck at port was found more important. Its applicability level was slightly below average.

The findings of this study will be of great interest to maritime policymakers, all kinds of shipping companies, seafarers, and researchers. This research is a resource that shipping companies can use in the risk assessment process and during the procedure development phase. The study will create awareness in the maritime sector and contribute to the protection of seafarers' health and safety, thus increasing the overall quality of the maritime industry.

In future studies, the results of this study can be expanded by using different multi-criteria decision-making methods and by conducting a study to reveal the safety climate during the COVID-19 pandemic with the participation of crew members at all levels.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: The current study had been approved by the Social Sciences and Humanities Ethics Committee of Istanbul Technical University.

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Ordu İli kentsel kıyı yerleşim yerlerinden denize verilen evsel atık su deşarjlarının araştırılması

Sezai ATAKLI[1], **İsmet BALIK**[2]

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[1] Ordu Büyükşehir Belediyesi, Su ve Kanalizasyon İdaresi Genel Müdürlüğü, Ordu, Türkiye

[2] Akdeniz Üniversitesi, Kemer Denizcilik Fakültesi, Kampüs, Pınarbaşı, Konyaaltı, Antalya, Türkiye

ORCID IDs of the author(s):
S.A. 0000-0001-8335-6683
İ.B. 0000-0003-2168-8572

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Correspondence:
İsmet BALIK
E-mail: ibalik@akdeniz.edu.tr

ÖZ

Bu çalışmada, Ordu İli kentsel kıyı yerleşim yerlerinden denize yapılan evsel atık su deşarjlarının yönetmeliklerde belirtilen kriterlere uygunluk durumları araştırılmıştır. Bu amaçla, kentsel kıyı yerleşim yerlerinde bulunan yedi evsel atık su arıtma istasyonunun atık su girişi ve atık su çıkışından ve bir adet doğrudan alıcı ortama deşarj noktasından 2018 yılında her ay, ayda iki kez olmak üzere örnek alınmıştır. Atık su örneklerinde $BOİ_5$ (Biyolojik Oksijen İhtiyacı), KOİ (Kimyasal Oksijen İhtiyacı), TP (Toplam Fosfor), TN (Toplam Azot), AKM (Askıda Katı Madde) ve pH analizi yapılmıştır. Elde edilen verilerin değerlendirilmesinde Kentsel Atık Su Arıtımı Yönetmeliği ve Su Kirliliği Kontrol Yönetmeliği'nde yer alan parametreler ve üst limit değerleri esas alınmıştır. Araştırmada, Altınordu-Durugöl, Altınordu-Kumbaşı, Gülyalı, Ünye-Doğu ve Ünye-Batı arıtma istasyonlarının işlevlerini sorunsuz bir şekilde sürdürmekte oldukları saptanmıştır. Fatsa İlçesi'nde bulunan evsel atık su arıtma istasyonlarında, fiziksel ön arıtma sonrası atık sular denize deşarj edilmektedir. Bu ilçedeki arıtma istasyonlarında atık su girişi ve atık su çıkışı parametre değerleri arasında önemli bir fark olmadığı saptanmıştır. Buda, Fatsa İlçesi'ndeki arıtma istasyonlarında evsel atık suların yeterince arıtılmadan denize deşarj edildiğini göstermektedir. Perşembe İlçesi'nde ise evsel atık sular doğrudan alıcı ortama deşarj edilmektedir. Araştırma sonuçlarına göre, Perşembe ve Fatsa ilçelerinde ihtiyacı karşılayacak kapasite ve özelliklerde atık su arıtma tesisleri kurulmalı ve faaliyete geçirilmelidir. Ayrıca, Ordu İli genelinde faaliyet gösteren küçük, büyük sanayi siteleri ile Organize Sanayi bölgelerindeki atık suların evsel atık sulara karışmaması için sanayi bölgelerinde ön arıtma tesislerinin kurulmasında yarar vardır. Ordu İli genelinde bitişik sistemde çalışan evsel atık su ve yağmur suyu şebekesi, ayrı ayrı şebeke hatlarına dönüştürülmelidir.

Anahtar Kelimeler: Evsel atık su, Deniz deşarjı, Atık su analizi, $BOİ_5$, KOİ, TP, TN, AKM, pH

ABSTRACT

Investigation of domestic wastewater discharged to the sea from urban coastal settlements in Ordu Province

In this study, the compliance of domestic wastewater discharges to the sea from the urban coastal settlements of Ordu Province with the criteria specified in the regulations was investigated. For this purpose, in 2018, samples were taken from the wastewater inlet and wastewater outlet at 8 different domestic wastewater treatment stations located in urban coastal settlements, twice a month, every month 2018. BOD_5 (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TP (Total Phosphorus), TN (Total Nitrogen), TSS (Suspended Solids), and pH analysis were performed in the wastewater samples. In the evaluation of the data obtained, the parameters and upper limit values are given in the Urban Wastewater Treatment Regulation and in the Water Pollution Control Regulation were taken as a basis. In the research, it was determined that they were able to function smoothly in Altınordu-Durugöl, Altınordu-Kumbaşı, Gülyalı, Ünye-Doğu and Ünye-Batı treatment stations. At the domestic wastewater treatment stations in Fatsa District, wastewater is discharged into the sea after physical pre-treatment. It has been determined that there is no significant difference between the wastewater inlet and wastewater outlet parameter values at the treatment stations in this district. This shows that domestic wastewater is discharged into the sea without being treated sufficiently at the treatment stations in Fatsa District. In Persembe District, domestic wastewater is directly discharged into the receiving environment. As a result, wastewater treatment plants with the capacity and characteristics to meet the needs should be established and put into operation in the districts of Perşembe and Fatsa. In addition, it is beneficial to establish pre-treatment facilities in the industrial zones in order to prevent the wastewater from small and large industrial sites operating in the province of Ordu and Organized Industrial Zones from mixing with domestic wastewater. Domestic wastewater and stormwater networks operating in the adjacent system throughout the province of Ordu should be converted into separate network lines.

Keywords: Domestic wastewater, Marine discharge, Wastewater analysis, BOD_5, COD, TP, TN, TSS, pH

Giriş

Atık su, Su Kirliliği Kontrol Yönetmeliğinde "evsel, endüstriyel, tarımsal ve diğer kullanımlar sonucunda kirlenmiş veya özellikleri kısmen veya tamamen değişmiş sular ile maden ocakları ve cevher hazırlama tesislerinden kaynaklanan sular ve yapılaşmış kaplamalı ve kaplamasız şehir bölgelerinden cadde, otopark ve benzeri alanlardan yağışların yüzey veya yüzey altı akışa dönüşmesi sonucunda gelen sular" olarak tanımlanmaktadır (SKKY, 2004). En yaygın ortaya çıkan atık su ise, evsel atık sulardır. Özellikle kentsel yerleşim yerlerinde önemli çevresel sorunlara neden olan evsel atık sular, yaygın olarak yerleşim bölgelerinden ve çoğunlukla evsel faaliyetler ile insanların günlük yaşam faaliyetlerinin yer aldığı okul, hastane, otel gibi hizmet sektörlerinden kaynaklanmaktadır (KAAY, 2006).

Evsel atık suyun bileşimi ve miktarı, yerleşim yerleri arasında önemli farklılıklar gösterebilir. Bunda, toplumlar arasındaki sosyo-ekonomik farklılıklar, yerleşim yerlerinin coğrafi ve iklim özellikleri arasındaki farklar, büyük sanayi kuruluşları veya tatil bölgelerine yakınlık durumları gibi faktörler önemli ölçüde etkilidir (Operation of Municipal Wastewater Treatment Plants, 2008). Bunların yanı sıra özellikle ülkemizde kanalizasyon şebeke hatlarının birleşik sistemde olduğu yerleşim yerlerinde yer alan küçük ve büyük organize sanayi bölgelerinin hemen hemen tamamı atık sularını belediye kanalizasyonuna vermektedir. Bu da evsel atık suların bileşim ve miktarını etkileyen bir başka faktördür.

Kıyı yerleşim yerlerinden arıtım sonrası denize deşarj edilecek evsel atık sularda analizi ya da ölçümü yapılacak parametrelerin üst sınır değerleri SKKY (2004) ve KAAY (2006)'de belirtilen değerlerin üzerinde olmamalıdır. Bu da ancak yerleşim yerlerinde uygun kapasite ve özelliklerde arıtma tesislerinin kurulmasıyla mümkündür.

Atık su arıtımı genellikle ön arıtım, birincil arıtma, ikincil arıtma ve ileri arıtım başlıkları altında incelenir. Bu arıtma aşamalarından ön arıtma ve birincil arıtma, fiziksel arıtma yöntemleridir. Ön arıtmada, atık suda bulunan yüzebilen kaba katıların, diğer büyük materyallerin, kumun uzaklaştırılması amaçlanmaktadır. Bu materyallerin atık sudan uzaklaştırılması her şeyden önce, müteakip arıtma ünitelerinin korunması ve etkinliğinin artırılması için gereklidir. Birincil arıtmanın amacı ise çökebilen katıların ve organik maddelerin bir kısmının giderilmesidir (Libhaber ve Jaramillo, 2012; Sperling, 2007; Balcıgil, 2013). İkincil arıtma ise, kalan çözünmüş organik ve askıda katı maddelerin uzaklaştırılması için birincil arıtmadan çıkan atık suyun genellikle biyolojik arıtma prosesleri ile arıtılmasıdır. İleri arıtma, ikincil arıtımla giderilemeyen bazı atık su bileşenlerinin, nütrientlerin (azot, fosfor) giderilmesi veya ikincil arıtımla elde edilenden daha büyük arıtma verimi sağlamak için kullanılan fiziksel, kimyasal veya biyolojik proseslerdir (FAO, 2019; Biological Nutrient Removal (BNR) Operation in Wastewater Treatment Plants, 2005; Spellman, 2009). Dört ana grupta toplanan, atık sulardan uzaklaştırılması istenen organik ve inorganik kolloidal ve askıda katılar, çözünmüş organik bileşikler, çözünmüş inorganik bileşikler ve biyolojik bileşenler derin filtrasyon, yüzey filtrasyonu, mikrofiltrasyon ve ultrafiltrasyon, ters osmoz, elektrodiyaliz, adsorbsiyon, hava sıyırma, iyon değişimi, ileri oksidasyon, distilasyon, kimyasal çöktürme ve kimyasal oksidasyon prosesleri v.b. prosesler kullanılarak uzaklaştırılabilirler (Tchobanoglous ve ark., 2003). İleri arıtım prosesleri ile atık sudaki kirleticilerin %99'undan fazlası uzaklaştırabilir ve neredeyse içme suyu kalitesinde çıkış suyu elde edilebilir (Spellman, 2009).

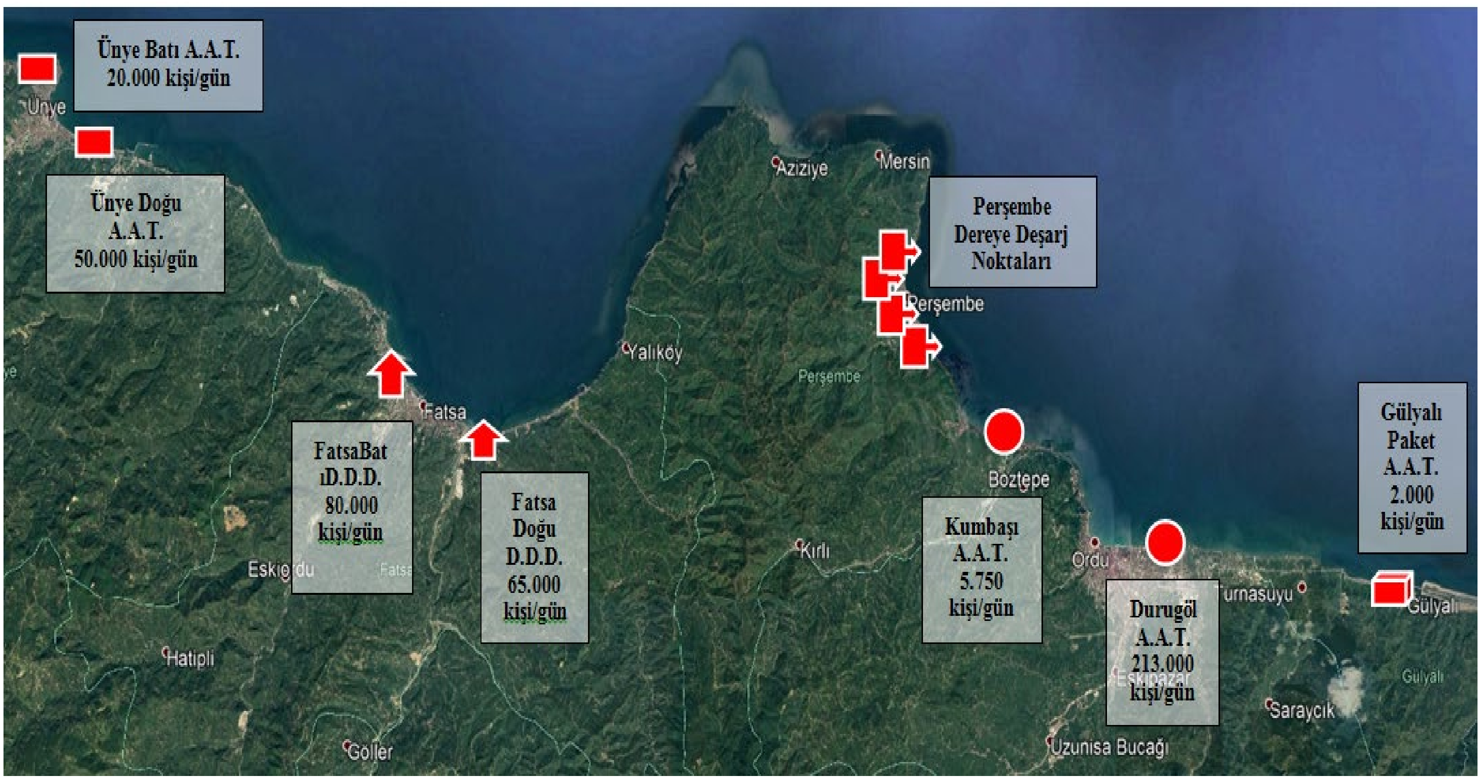
Evsel atık su sorunu, hayvansal canlıların yaşamsal faaliyetlerinin sonucunda ortaya çıkan ve özellikle de toplu yerleşim yerlerinde bertaraf edilmesi bir takım başka sorunların ortaya çıkmasına da neden olan bir durumdur. Bilhassa denize kıyısı olan yerleşim yerlerinde genellikle arıtılmadan ya da farklı arıtma yöntemleri uygulandıktan sonra denize deşarj edilmektedir. Ancak, bu uygulamada denizlerde bir takım organik ya da biyolojik kirlenmeye yol açabilmektedir. Ordu İli, Karadeniz'e 100 km uzunluğunda kıyısı olan, 2020 yılı verilerine göre yaklaşık 761.400 insanın yaşadığı bir büyükşehirdir. Kıyı şeridinde kentsel yerleşim yeri olarak 224.100 nüfusa sahip merkez ilçe Altınordu'nun yanı sıra, doğusunda 8.244 nüfuslu Gülyalı, batısında 30.997 nüfuslu Perşembe, 123008 nüfuslu Fatsa ve 130464 nüfuslu Ünye ilçeleri yer almaktadır (TUİK, 2021). Bu yerleşim yerlerinden Altınordu, Gülyalı ve Ünye ilçelerinde evsel atık sular farklı özelliklere sahip arıtma tesislerinde arıtıldıktan sonra denize deşarj edilmektedir. Fatsa İlçesi'nde ise atık sular sadece fiziksel ön arıtma sonrası derin deniz deşarjı yapılırken, Perşembe İlçesi'nde doğrudan alıcı ortama (dere, deniz) deşarj yapılmaktadır.

Bu araştırmada, söz konusu yerleşim yerlerinde yer alan arıtma istasyonlarında atık su giriş ve çıkışında analizi yapılan parametrelerin değerleri ile söz konusu parametrelerce arıtma tesislerinin özelliklerine göre SKKY (2004) ve KAAY (2006)'da yer alan değerler karşılaştırılmıştır. Ayrıca, hiç bir arıtıma tabi tutulmaksızın doğrudan alıcı ortama deşarj edilen Perşembe İlçesi evsel atık sularında da aynı işlemler yapılarak elde edilen değerler diğer arıtma istasyonlarından denize deşarj edilen değerler ile karşılaştırılmıştır.

Materyal ve Metot

Bu araştırma, Ordu İli kıyı şeridinde yer alan Altınordu, Gülyalı, Perşembe, Fatsa ve Ünye ilçelerinde yapılmıştır. Araştırmanın ana materyalini, söz konusu ilçelerde bulunan birincil, ikincil (biyolojik karbon giderim) ve üçüncül arıtma (biyolojik ileri arıtma, azot ve fosfor giderimli) istasyonlarına giren ve arıtma tesislerinden çıkan evsel atık sulardan alınan atık su örnekleri oluşturmuştur (Şekil 1). Alınan atık su örneklerinde $BOİ_5$ (Biyolojik Oksijen İhtiyacı), KOİ (Kimyasal Oksijen İhtiyacı), TP (Toplam Fosfat), TN (Toplam Nitrat), AKM (Askıda Katı Madde) ve pH analizleri yapılmıştır. Elde edilen değerler, SKKY (2004)'de ve KAAY (2006)'de belirtilen üst sınır değerler ile karşılaştırılmıştır.

Su Kirliliği Kontrol Yönetmeliği'nin Geçici 5. Maddesi'nde "Bu Yönetmeliğin 32 nci maddesinin ikinci fıkrasının (b), (c) ve (d) bentleri ile bu Yönetmeliğin ekinde yer alan Tablo 21.2, Tablo 21.3 ve Tablo 21.4, 31/12/2017 tarihine kadar uygulanır. Bu tarihten sonra söz konusu hükümler yerine, Kentsel Atık Su Arıtımı Yönetmeliği'nin ilgili hükümleri uygulanır." denilmektedir (SKKY, 2004). Yönetmeliklerde belirtilen düzenlemeler esas alınarak Ordu İli'ndeki atık su örneklerinin analiz sonuçlarının değerlendirilmesinde kapasitesi 100.000 kişi/gün'den fazla olan Altınordu-Durugöl ileri biyolojik atık su arıtma tesisi için KAAY (2006)'da yer alan Tablo/Tablo-2' de verilen $BOİ_5$, KOİ, TP, TN ve AKM; kapasiteleri 2000-10000 kişi/gün olan Altınordu-Kumbaşı ileri biyolojik atık su arıtma tesisi ve Gülyalı paket arıtma tesisi ile kapasitesi 10000-100000 kişi/gün olan Ünye-Doğu ve Ünye-Batı biyolojik (Konvansiyonel) atık su arıtma tesisleri için de Tablo 1/Tablo 2'de yer alan $BOİ_5$, KOİ, TP, TN ve AKM; evsel atık suların ön arıtma sorası derin denize deşarj edilerek bertraf edildiği Fatsa-Doğu ve Fatsa-Batı istasyonları için SKKY (2004)'de yer alan Tablo 22'de verilen $BOİ_5$, KOİ, TP, TN, AKM ve pH değerleri esas alınmıştır. Atık suların doğrudan alıcı ortama (dere, deniz, vs.) deşarj edildiği Perşembe İlçesi'nden alınan örneklerin analiz sonuçları ise diğer arıtma tesisleri için elde edilen değerler ile karşılaştırılarak, bu ilçeden denize deşarj edilen atık suların kirleticilik düzeyleri belirlenmeye çalışılmıştır.



Şekil 1. Ordu İli kıyı şeridi kentsel yerleşim yerlerinde atık su arıtma tesisleri, derin deniz deşarj ve direk alıcı ortama (dere, deniz vb.) deşarj noktaları

Figure 1. Wastewater treatment facilities, deep sea discharge, and direct discharge point to the receiving environment (stream, sea, etc.) in the urban settlements of Ordu Province.

Atık Su Örneklerinin Alınması

Atık su örnekleri, arıtma istasyonlarında atık su girişi ve atık su çıkışından, Perşembe İlçesi'nde ise atık suların doğrudan alıcı ortama deşarj edildiği noktalardan, 2018 yılında her ay, ayda iki kez olmak üzere iki litrelik plastik şişelere alınmıştır. Atık su örneklerinin analizi, Ordu Büyükşehir Belediyesi Su ve Kanalizasyon İdaresi Kimya Laboratuvarında yapılmıştır.

Analiz Yöntemleri

Alınan evsel atık su örneklerinde $BOİ_5$ analizleri Bok Track II marka cihaz, Hach Lange/SM 5210 D metodu ile; KOİ analizleri Merck Pharo 300 marka cihaz, SM 5220 D Kapalı Reflux Kolrometrik ve SM 5220 B Açık Reflux Titirimetrik metodu ile; TP ve TN analizleri Merck Pharo 300 marka cihaz, KİT metodu ile; AKM deneyleri Etüv Hassas Terazi marka cihaz, SM 2540 D Gravimetrik metodu ile; pH deneyleri Hach Lange HQ40D marka cihaz, Elektrometrik yöntemi ile yapılmıştır (Rice ve ark., 2012). Atık su analiz sonuçları KAAY (2006)'da yer alan Tablo 1/Tablo 2 ve SKKY (2004)'da yer alan Tablo 22'de verilen yer alan referans değerler ile karşılaştırılmıştır.

İstatistiksel Değerlendirmeler

Verilerin istatistiksel olarak değerlendirilmesinde IBM SPSS Statistics 21 programı kullanılmıştır. Arıtma istasyonlarında atık su girişi ve atık su çıkışından alınan örneklerin analiz sonuçları arasındaki farklar, parametrik olanlar Paired t-testi, parametrik olmayanlar Wilcoxon testi ile karşılaştırılmıştır. Ayrıca, parametre değerlerinin mevsimsel değişim gösterip göstermediklerini belirlemek için parametrik değerlere sahip bağımsız gruplar için Tek Yönlü Varyans Analizi (ANOVA), parametrik olmayan bağımsız gruplar için Kruskal Wallis testi uygulanmıştır.

Bulgular ve Tartışma

SKKY (2004) ve KAAY (2006)'ya göre, Ordu İli kentsel kıyı yerleşim yerlerinde bulunan arıtma istasyonlarında aranan parametreler ve üst sınır değerleri Tablo 1'de verilmiştir.

Tablo 1'de görüldüğü üzere, arıtma istasyonlarının özelliklerine göre aranan parametreler değişiklik arz etmekte ise de, bizim çalışmamızda arıtma istasyonları ve deşarj noktalarının tamamında tüm parametrelerin ($BOİ_5$, KOİ, TP, TN, AKM ve pH) de analizleri yapılmış ve analiz sonuçları Tablo 2'de sunulmuştur.

Arıtma istasyonlarında giren atık sularda en yüksek $BOİ_5$ değeri Fatsa-Doğu; KOİ, TP, TN, AKM ve pH değerleri ise Ünye-Batı arıtma istasyonunda tespit edilmiştir. Tablo 2'de verilen değerlerden de anlaşılacağı üzere, Fatsa ve Ünye ilçeleri arıtma istasyonlarında giren evsel atık sularda tespit edilen parametre değerleri, Altınordu ve Gülyalı ilçeleri arıtma istasyonlarında tespit edilen parametre değerlerine göre daha düşüktür. Bunun, başta kanalizasyon sistemine dahil olan diğer sular, içeriği ve miktarları gibi bir çok farklı nedeni olabilir. Ordu İli kıyı şeridi yerleşim yerlerinde atık sular ayrık alt yapı sistemi ile yerleşim yerlerinden uzaklaştırılacak şekilde planlanmış ise de, eski yapılaşma olan yerler ile imara açık olmayan bölgelerde yüzey yağmur suları, bina çatı suları ve bahçe suları usulsüz bir şekilde kanalizasyon sistemine verilmektedir. Bu farklı alt yapı şebekelerinden gelen evsel atık su ve yağmur suyunun arıtma istasyonlarında toplanmasının da analiz sonuçları üzerinde etkili olduğu düşünülmektedir.

Tablo 1. Arıtma istasyonlarında aranan parametreler ve üst limit değerleri

Table 1. Parameters sought in treatment stations and their upper limit values

İstasyon	$BOİ_5$ (mg/L)	KOİ (mg/L)	TP (mg/L)	TN (mg/L)	AKM (mg/L)	pH
Durugöl	<25	<125	<1	<10	<35	-
Kumbaşı	<25	<125	-	-	<60	-
Gülyalı	<25	<125	-	-	<60	-
Perşembe	-	-	-	-	-	-
Fatsa-Doğu	<250	<400	<10	<40	<350	6 - 9
Fatsa-Batı	<250	<400	<10	<40	<350	6 - 9
Ünye-Doğu	<25	<125	-	-	<35	-
Ünye-Batı	<25	<125	-	-	<35	-

Tablo 2. Arıtma istasyonlarında atık su girişi ve atık su çıkışı ile deşarj noktalarından alınan örneklerde yapılan analiz sonuçları [$BOİ_5$, KOİ, TP, TN, AKM (mg/L) ve pH]

Table 2. Analysis results of samples taken from wastewater inlet and wastewater outlet and discharge points at treatment stations [BOD_5, COD, TP, TN, TSS (mg/L), and pH]

İstasyonlar	Giriş Değerleri						Çıkış Değerleri					
	$BOİ_5$	KOİ	TP	TN	AKM	pH	$BOİ_5$	KOİ	TP	TN	AKM	pH
Durugöl	118.57	295.21	4.09	30.78	186.33	7.19	16.15	39.38	0.32	7.70	8.83	7.26
Kumbaşı	86.43	222.38	3.75	24.37	169.29	7.20	16.00	40.38	1.32	10.83	13.58	7.32
Gülyalı	67.27	183.21	2.86	25.12	149.38	7.06	21.19	75.96	1.54	12.87	44.21	7.17
Perşembe	-	-	-	-	-	-	73.16	181.63	2.30	15.36	118.01	7.37
Fatsa-Doğu	155.54	359.25	3.95	30.20	228.88	7.21	147.21	300.67	3.86	27.97	215.75	7.20
Fatsa-Batı	144.94	363.33	4.34	33.32	207.54	7.24	127.82	296.54	4.21	31.41	203.58	7.26
Ünye-Doğu	130.17	315.83	4.73	36.64	207.67	7.17	16.85	43.38	1.40	11.95	16.92	7.20
Ünye-Batı	144.40	365.88	5.30	40.51	239.92	7.28	14.66	35.96	1.35	12.43	9.13	7.46

Arıtma istasyonlarından çıkan atık sularda ise en yüksek $BOİ_5$, KOİ, TP ve AKM değerleri Fatsa-Doğu, TN değeri Fatsa-Batı, pH değeri ise Ünye-Batı arıtma istasyonunda saptanmıştır. Fatsa İlçesi'nden denize deşarj edilen atık sularda, pH dışında, diğer parametrelerin değerleri özellikle Altınordu ve Gülyalı ilçelerine göre oldukça yüksektir. Çünkü, Fatsa İlçesi'ndeki arıtma istasyonlarına giren evsel atık sularda sadece fiziksel ön arıtma yapılarak katı kirleticilerin denize deşarjı önlenmektedir. Dolayısıyla, Fatsa İlçesi'nden denize deşarj edilen evsel atık suların arıtma tesisi bulunan diğer yerleşim yerlerinden denize deşarj edilen atık sulara göre denizi çok daha fazla kirlettiği açıktır. Atık suların doğrudan denize deşarj edildiği Perşembe İlçesi'nde ise, atık sulardaki kirletici parametre değerleri Altınordu, Gülyalı ve Ünye ilçelerine göre oldukça yüksek bulunurken, Fatsa İlçesine göre düşük bulunmuştur. Bunda, Perşembe İlçesinin daha küçük bir yerleşim yeri olması, sanayi tesisinin bulunmaması ve halkın daha doğal bir yaşam sürdürüyor olması gibi faktörlerin etkili olduğu söylenebilir. Bu konuda Tokat Belediyesi Atık Su Tesisi (Çiğdem, 2019), Taşköprü (Kastamonu) Atık Su Arıtma Tesisi (Şama, 2017), Kayseri İncesu İlçesi Subaşı ve Şeyhşaban Köyü Doğal Artıma Sistemleri (Duygulu, 2016), Ankara Merkezi Atık Su Arıtma Tesisi (Azman, 2005) ve Tunceli Evsel Atık Su Arıtma Tesisinde (Tanyol ve Uslu, 2013) yapılan çalışmalarda farklı sonuçların bildirildiği anlaşılmaktadır (Tablo 3).

Tablo 3. Türkiye'deki bazı arıtma istasyonlarında atık su girişi ve atık su çıkışı için bildirilen [BOD_5, COD, TP, TN, TSS (mg L^{-1}) ve pH] değerleri

Table 3. Reported values for wastewater inlet and wastewater output at some treatment stations in Turkey [BOD_5, COD, TP, TN, TSS (mg/L), and pH]

	Giriş Değerleri						Çıkış Değerleri					
	$BOİ_5$	KOİ	TP	TN	AKM	pH	$BOİ_5$	KOİ	TP	TN	AKM	pH
Çiğdem (2019)	208.8	449.07	5.59	55.29	200	-	19.28	63.93	3.47	29.33	20.87	-
Şama (2017)	118.5	378.8	4.8	40.7	156.2	-	5.7	19	1.2	9	13.7	-
Duygulu (2016)	298	174.1	8.2	25.3	85.8	7.6	249	90.9	6.3	14.1	73.6	7.8
Azman (2005)	128.1	235.8	-	-	126	-	10.7	39.1	-	-	14.6	-
Tanyol ve Uslu (2013)	-	-	-	-	-	7.4	10-25	25-76-	-	-	9-22	7.7

Çiğdem (2019)'in Tokat İli'nde yapmış olduğu araştırmada, Tokat Belediyesi atık su arıtma tesisinde üç yılın ortalama $BOİ_5$ giriş değeri 208.8 mg/L ve çıkış değeri 19.28 mg/L; KOİ giriş değeri 449.07 mg/L, çıkış değeri 63.93 mg/L; TP giriş değeri 5.59 mg/L, çıkış değeri 3.47 mg/L; TN giriş değeri 55.29 mg/L, çıkış değeri 29,33 mg/L; AKM giriş değeri 200 mg/L, çıkış değeri 20.87 mg/L olarak tespit edilmiştir. Bizim araştırmamızda tespit edilen, AKM dışındaki, parametrelerin atık su girişi değerleri bu değerlerden daha düşüktür. AKM değeri ise, Altınordu ve Gülyalı ilçelerinde daha düşük iken, Fatsa ve Ünye ilçelerinde daha yüksektir. Atık su çıkışında ise $BOİ_5$, KOİ ve AKM değerleri Altınordu ve Ünye ilçelerinde daha düşük iken, diğer arıtma istasyonlarında daha yüksektir. TP değeri, sadece Fatsa'da daha yüksek iken, arıtma istasyonlarının tamamında TN değeri Tokat İli'ne göre daha düşük ya da benzerdir.

Şama (2017) tarafından yapılan çalışmada da Taşköprü (Kastamonu) Atık Su Arıtma Tesisinde atık suların ortalama $BOİ_5$ giriş değeri 118.5 mg/L, çıkış değeri 5.7 mg/L; KOİ giriş değeri 378.8 mg/L, çıkış değeri 19 mg/L; TP giriş değeri 4.8 mg/L, çıkış değeri 1.2 mg/L; TN giriş değeri 40.7 mg/L, çıkış değeri 9.0 mg/L; AKM giriş değeri 156.2 mg/L, çıkış değeri 13.7 mg/L ve pH giriş değeri 7.6, çıkış değeri 7.8 bulunmuştur. Bizim araştırmamızda tespit edilen KOİ, TP ve TN değerleri söz konusu araştırmada tespit edilen değerlerden daha düşüktür. $BOİ_5$ değeri ise Fatsa ve Ünye ilçelerinde daha yüksek, Altınordu-Durugöl'de benzer, Altınordu-Kumbaşı ve Gülyalı'da daha düşüktür. AKM değeri ise, Gülyalı ilçesi dışındaki diğer tesislerde daha yüksektir. Bu sonuçlardan, Ordu İli kıyı şeridi yerleşim yerleri ile Taşköprü İlçesi evsel atık sularının önemli farklılıklar gösterdiği anlaşılmaktadır. Arıtma istasyonlarından çıkan atık sular arasında yapılan karşılaştırmalarda ise bizim araştırmamızda tespit edilen $BOİ_5$ ve KOİ değerleri, Taşköprü İlçesi'nde tespit edilen değerlere göre çok daha yüksektir. TP ve TN değerleri Altınordu-Durugöl'de, AKM değeri Altınordu-Durugöl, Altınordu-Kumbaşı ve Ünye-Batı arıtma istasyonlarında, Taşköprü İlçesi için bildirilen değerlerden daha düşüktür.

Duygulu (2016) tarafından Kayseri İlinde yapılan araştırmada, İncesu İlçesi Subaşı ve Şeyhşaban Köyü doğal artıma sistemlerinde atık suların ortalama $BOİ_5$ giriş değeri 298 mg/L, çıkış değeri 249 mg/L; KOİ giriş değeri 174.1 mg/L, çıkış değeri 90.9 mg/L; TP giriş değeri 8.2 mg/L, çıkış değeri 6.3 mg/L; TN giriş değeri 25.3 mg/L, çıkış değeri 14.1 mg/L; AKM giriş değeri 85.8 mg/L, çıkış değeri 73.6 mg/L; pH giriş değeri 7.4 ve çıkış değeri 7.7 olarak bildirilmektedir. Ordu İli kıyı şeridi yerleşim yerlerinde tespit etmiş olduğumuz atık su girişi $BOİ_5$, KOİ ve TP değerleri, Kayseri İncesu İlçesi Subaşı ve Şeyhşaban köyleri atık su arıtma tesisleri için bildirilen değerlerden daha düşük iken, AKM değeri daha yüksektir. Ordu İli'ndeki arıtma istasyonlarında atık su çıkışında tespit edilen $BOİ_5$ ve TP değerleri, Kayseri İncesu İlçesi Subaşı ve Şeyhşaban köyleri arıtma tesislerinde atık su çıkışında tespit edilen $BOİ_5$ ve TP değerlerine göre oldukça düşüktür. KOİ, TN ve AKM değerlerinin de, Fatsa İlçesi arıtma istasyonları dışında, Ordu İli'ndeki arıtma istasyonlarında daha düşük olduğu anlaşılmaktadır. Altınordu-Kumbaşı ve Gülyalı arıtma istasyonları TN değerleri ise söz konusu araştırmada bildirilen değerler ile benzerdir.

Azman (2005)'ın Ankara Merkezi Atık Su Arıtma Tesisi'nde yaptığı çalışmada atık su $BOİ_5$ giriş değeri 128.1 mg/L, çıkış değeri 10.7 mg/L; KOİ giriş değeri 235.8 mg/L, çıkış değeri 38.1 mg/L; AKM giriş değeri 126.1 mg/L, çıkış değeri 14.6 mg/L olarak bildirilmektedir. Altınordu-Durugöl, Altınordu-Kumbaşı ve Gülyalı arıtma istasyonları atık su girişi $BOİ_5$ değeri ile Altınordu-Kumbaşı ve Gülyalı atık su girişi KOİ değerleri Ankara Merkezi Atık Su Arıtma Tesisi için bildirilen değerlerden daha düşük, AKM değeri ise daha yüksektir.

Tunceli Evsel Atık Su Arıtma Tesisinde atık su çıkışı BOI_5, KOİ, AKM ve pH değerleri sırasıyla 10-25 mg/L, 25-76 mg/L, 9-22 mg/L ve 7.97-8.14 arasında değişmiştir (Tanyol ve Uslu, 2013). Fatsa ve Gülyalı ilçeleri atık su çıkışı AKM değerleri, Tunceli İli için bildirilen değerden daha yüksektir. Ayrıca, Ordu İli'ndeki kıyı yerleşim yerlerinde atık su çıkışı için tespit edilen pH değerlerinin de Tunceli için bildirilen pH değerinden çok daha yüksek oldukları saptanmıştır.

Yapılan karşılaştırmalardan, arıtma istasyonlarında atık su girişi parametre değerlerinin, yukarıda da bahsedildiği gibi, kanalizasyon ve yağmur suyu alt yapısı, sanayi atık suyu karışımı, halkın yaşam tarzı ve tüketim alışkanlıkları gibi bir çok farklı faktöre bağlı önemli farklılar gösterdiği anlaşılmaktadır. Atık su çıkış değerleri arasındaki fark ise giren atık suyun kirlilik seviyesi ve bileşimin yanı sıra arıtma tesisinin kapasitesi ve arıtma özelliğiyle ilgili bir durumdur.

Altınordu-Durugöl, Altınordu-Kumbaşı, Gülyalı ve Ünye-Batı istasyonlarında tüm parametrelerin atık su girişi ve atık su çıkışı değerleri arasındaki farkların önemli olduğu ($P<0.05$) tespit edilmiştir. Ünye-Doğu istasyonunda da, pH dışında, diğer parametrelerin atık su girişi ve atık su çıkışı parametre değerleri arasındaki farkların önemli ($P<0.05$) olduğu anlaşılmıştır. Yapılan t-testi, Fatsa-Doğu istasyonunda $BOİ_5$ ve pH değerleri bakımından atık su giriş ve atık su çıkış değerleri arasında önemli fark olmadığını ($P>0.05$), TP bakımından ise atık su giriş ve atık su çıkış değerleri arasındaki farkın önemli olduğunu ($P<0.05$) göstermiştir. Fatsa-Doğu istasyonunda yapılan Wilcoxon testi, atık su giriş ve çıkış değerlerinin KOİ ve TN değerleri bakımından farklı ($P<0.05$), AKM değerleri bakımından ise aralarında fark olmadığını ($P>0.05$) ortaya koymuştur. Bu sonuçlardan Altınordu, Gülyalı ve Ünye ilçelerinde bulunan arıtma istasyonlarının arıtım işlevlerini yerine getirdiklerini gösterirken, Fatsa İlçesi'ndeki arıtma istasyonlarının ise kayda değer bir arıtım işlevi olmadığını göstermektedir.

Arıtma istasyonlarından alınan atık su giriş ve atık su çıkış örneklerinin analizleri sonucunda elde edilen parametrelerin mevsimsel değerleri Tablo 4'de verilmiştir. Mevsimlere göre oluşturulan grupların varyansları arasında yapılan Levene Testi, tüm parametrelerce varyanslar arası fark olmadığını ($P>0.05$) göstermiştir. Ayrıca, analizi yapılan parametrelerin mevsimlere göre sınıflandırılması sonucu elde edilen değerlerin normal dağılıma sahip oldukları ($P>0.05$) belirlenmiştir.

Parametrelerin atık su giriş ve atık su çıkış değerlerinin mevsimlere göre farklılaşıp farklılaşmadığını belirlemek amacıyla yapılan bağımsız gruplar arası tek yönlü varyans analizi, grupların ortalamaları arasındaki farkların anlamlı olmadığını ortaya koymuştur ($P>0,05$).

Henze ve ark. (2002), $BOİ_5$ değerinin KOİ değerine oranı ile atık suyun karakteristiğinin belirlenebileceğini ifade etmektedir. Kimyasal olarak oksitlenebilecek bileşikler, biyolojik olarak oksitlenebileceklerden daha fazla olduğundan, kimyasal oksijen ihtiyacı, biyolojik oksijen ihtiyacından daha büyüktür. Arıtılmamış atıksular için BOİ5 /KOİ = 0.4-0.8 (ortalama 0.65) alınabilir. Ordu İli kıyı şeridinde yer alan yerleşim yerlerinde bulunan atık su arıtma istasyonları için hesaplanan parametreler arası oransal değerler Tablo 5'de verilmiştir.

Tablo 4. Arıtma istasyonlarında atık su girişi ve atık su çıkışı mevsimsel analiz sonuçları (mg/L)

Table 4. Seasonal analysis results of wastewater inlet and wastewater outlet at treatment stations (mg/L)

	$BOİ_5$		KOİ		TP		TN		AKM	
	Giriş	Çıkış	Giriş	Çıkış	Giriş	Çıkış	Giriş	Çıkış	Giriş	Çıkış
İlkbahar	110.720	53.740	277.293	128.209	3.720	2.085	28.014	17.171	163.291	76.751
Yaz	112.776	59.256	272.749	138.791	3.393	1.856	27.074	15.843	157.541	76.918
Sonbahar	102.079	50.113	251.376	112.360	3.611	2.000	27.341	2.000	187.229	76.208
Kış	98.089	53.414	251.128	130.579	3.785	2.215	28.031	15.983	186.436	85.128

Tablo 5. Arıtma istasyonları ve deşarj noktaları için hesaplanan parametreler arası oransal değerler

Table 5. Proportional values between parameters calculated for treatment stations and discharge points

İstasyonlar	Giriş Değerleri						Çıkış Değerleri	
	$BOİ_5$ / KOİ	KOİ / $BOİ_5$	$BOİ_5$ / AKM	AKM / $BOİ_5$	KOİ / AKM	AKM / KOİ	$BOİ_5$ / KOİ	KOİ / $BOİ_5$
Durugöl	0.40	2.49	0.64	1.57	1.58	0.63	0.41	2.44
Kumbaşı	0.39	2.57	0.51	1.96	1.31	0.76	0.40	2.52
Gülyalı	0.37	2.72	0.45	2.22	1.23	0.82	0.29	3.58
Perşembe	-	-	-	-	-	-	0.40	2.48
Fatsa-Doğu	0.43	2.31	0.68	1.47	1.57	0.64	0.48	2.04
Fatsa-Batı	0.40	2.51	0.70	1.43	1.75	0.57	0.43	2.32
Ünye-Doğu	0.41	2.43	0.63	1.60	1.52	0.66	0.39	2.57
Ünye-Batı	0.40	2.53	0.60	1.66	1.53	0.66	0.41	2.45

Bizim araştırmamızda hesaplanan $BOİ_5$/KOİ oranları atık su girişinde 0.37-0.41, atık su çıkışında 0.29-0.43 arasında değişmiştir. Bu oranlar, Tchobanoglous ve ark. (1991)'nda 0.30 – 0.80 olarak verilen oranlar ile uyumludur. KOİ/BOİ oranı, tesiste uygulanacak biyolojik arıtma yöntemleri için belirleyici olmaktadır. Evsel bir atıksu için KOİ/BOİ oranı 1.5-3 arasında değişmektedir. Bu oranın düşmesi durumunda kimyasal arıtma sisteminin de devreye girmesi gerekecektir (Azman, 2005). Henze ve ark. (2008)'nda KOİ/$BOİ_5$ oranı 1.50-2.00 arası düşük; 2.00-2.50 arası orta; 2.50-3.50 arası yüksek olarak yorumlanmaktadır. Bu yoruma göre, Ordu İli için elde etmiş olduğumuz atık su giriş ve atık su çıkış değerlerinin orta seviyede karakteristik özelliklere sahip olduğu anlaşılmaktadır. Tchobanoglous ve ark. (1991) ve Henze ve ark. (2008)'nın çalışmalarında, AKM/KOİ oranının 0.22-0.80 arasında değiştiği belirtilmektedir. Bizim araştırmamızda elde edilen 0.57-0.82 arasında değişen AKM/KOİ oranı söz konusu çalışmalarda bildirilen oranlardan azda olsa daha yüksektir.

$BOİ_5$/KOİ oranı, biyolojik olarak arıtılabilirliğin bir göstergesidir. Daha doğrusu kolayca ve yavaşça biyolojik olarak parçalanabilen organik maddenin kaba bir oranı olarak düşünülebilir (Orhon ve ark., 1997). Eğer $BOİ_5$/KOİ oranı 0.5 ve 0.5'den büyükse, biyolojik olarak kolayca arıtılabileceği düşünülür. Bu oran yaklaşık 0.3 ise bakteriler tarafından zor parçalanan ya da parçalanamayan organiklerin biyolojik arıtma tesisinde varlığını göstermektedir (Tchobanoglous ve ark., 1991). Ordu İli'nde gerek atık su girişi gerekse atık su çıkışı için hesaplanan $BOİ_5$/KOİ oranının 0,5'den daha küçük olması biyolojik arıtmanın yapılamayacağını, kimyasal arıtma yapılması gerektiğini göstermektedir. Adana Büyükşehir Belediyesi'ne ait arıtma istasyonunda $BOİ_5$/KOİ oranı Ersü (2000) tarafından 0.77; Yılmaz (2018) tarafından 0.73 olarak bildirilmektedir. Bu $BOİ_5$/KOİ oranları, Adana'daki evsel atık suların biyolojik olarak kolaylıkla arıtılabildiğini göstermektedir. Bu veriler ışığında Ordu İli evsel atık suyundaki organik maddelerin parçalanmaya dayanıklı olması ya da atık sudaki bazı maddelerin organik madde kullanan bakterilerin inhibasyonuna yol açması gibi durumların söz konusu olabileceği anlaşılmaktadır.

Sonuç

Bu araştırmada elde edilen sonuçları aşağıdaki şekilde özetlemek mümkündür:

- Ordu İli kıyı şeridinde yer alan kentsel yerleşim yerlerinde faaliyette olan evsel atık su arıtma istasyonlarından Altınordu-Durugöl ve Altınordu-Kumbaşı, Gülyalı, Ünye-Doğu ve Ünye-Batı arıtma tesisleri işlevlerini sorunsuz bir şeklide yerine getirmektedir.
- Perşembe İlçesi'nde doğrudan alıcı ortama deşarj edilen evsel atık sularda tespit edilen $BOİ_5$, KOİ, TN, TP ve AKM değerleri, araştırmada incelenen diğer arıtma istasyonlarında çıkan atık sularda tespit edilen söz konusu parametrelerin değerlerinden çok daha yüksektir.
- Su Kirliliği Kontrol Yönetmeliği'nde belirtilen parametrelere ve değerlerine göre, Fatsa İlçesi'nde bulunan arıtma istasyonlarından, fiziksel arıtmaya tabi tutulan atık suların derin deniz deşarjı yapılmasında herhangi bir sorun olmadığı anlaşılmaktadır. Ancak, Fatsa İlçesi'nde yer alan arıtma istasyonları için SKKY (2004)'de belirtilen parametrelerin üst sınır değerleri, araştırmada incelenen diğer arıtma istasyonları için KAAY (2006)'da belirtilen üst sınır değerlerine göre oldukça yüksektir. Bu nedenle, Fatsa İlçesi'nden deşarj edilen atık suların denizi kirletmediği sonucunu çıkarmak mümkün değildir.

Öneriler;

- Atık su arıtma tesisi bulunmayan Perşembe İlçesi'nde coğrafik konumu da dikkate alınarak atık suların arıtımı için yeterli kapasite ve özelliklerde arıtma tesisi/tesisleri kurulmalı ve faaliyete geçirilmelidir.
- Evsel atık suların sadece ön arıtma sonrası derin denize deşarj edildiği yaklaşık 120 bin nüfusun yaşadığı Fatsa İlçesinde de, vakit geçirilmeksizin yeterli kapasiteye sahip atık su arıtma tesisi/tesisleri kurulmalıdır.
- Ordu İli genelinde faaliyet gösteren küçük, büyük sanayi siteleri ile Organize Sanayi bölgelerindeki atık suların evsel atık sulara karışmaması için sanayi bölgelerinde ön arıtma tesislerinin kurulmasında yarar vardır.
- Ordu İlinin genelinde evsel atık su ve yağmur su yu şebekesi birleşiktir. Birleşik sistemde çalışan hatlar ayrı şebeke hatlarına dönüştürülmelidir.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışmasının olmadığını beyan eder.

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Research Article

Population dynamics of deep-water pink shrimp (*Parapenaeus longirostris* Lucas, 1846) (Decapoda, Penaeidae) in the coastal waters of Tuzla (Eastern part of the Sea of Marmara)

Erhan ÇİLOĞLU[1], Celal ATEŞ[2]

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[1] Recep Tayyip Erdogan University, Turgut Kıran Maritime Faculty, 53900 Derepazarı/Rize, Türkiye

[2] Muğla Sıtkı Koçman University, Fisheries Faculty, 48170 Kötekli/Muğla, Türkiye

ORCID IDs of the author(s):

E.Ç. 0000-0002-9995-0377

C.A. 0000-0002-7336-0387

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Correspondence:

Erhan ÇİLOĞLU

E-mail: erhan.ciloglu@erdogan.edu.tr

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ABSTRACT

In this study, the population parameters, recruitment period, exploitation rate, and stock status of deepwater pink shrimp (*Parapenaeus longirostris* Lucas, 1846) in the eastern Sea of Marmara were investigated. Growth showed negative allometry, and, b-values were significantly lower than 3 for females′ and males′ ($p < 0.05$). Females and males' growth performance indexes (φ) of *P. longirostris* were determined as 2.91 and 2.89, respectively. Asymptotic carapace lengths (CL_∞), growth coefficients (K), and theoretical birth ages (t_0) of females and males were calculated as 32.55, 28.67 mm, and 0.760, 0.950, and –0.233, –0769, respectively. The maximum exploitation rates (E_{max}) were calculated as 0.82 for females and as 0.90 for males. The first capture lengths (CL_{50}) for females and males were estimated to be 18.78 and 18.07 mm, respectively. Two recruitment periods were determined for *P. longirostris* for females and males. The recruitment period was estimated to be between February and April, July and November. The yield per-recruit (YPR) analysis for females and males revealed that $E_{0.1}$ = 0.72 and 0.80 approached the maximum exploitation rate (E_{max} = 0.82 and 0.90), respectively, indicating that exploitation for *P. longirostris* was in quadrant category "C" in the Eastern Marmara Sea. This status demonstrated active fishing, according to the quadrant rule.

Keywords: Growth, Exploitation Rate, Mortality, *Parapenaeus longirostris*, Recruitment, Stock, Eastern Sea of Marmara

Introduction

Parapenaeus longirostris (Lucas, 1846) spreads from the State of Massachusetts, the USA to French Guiana in the West Atlantic, and from Portugal to the entire Mediterranean Sea, including Namibia and the Sea of Marmara (Holthuis, 1980; Kocataş, 1981; Morri *et al.,* 1999). According to a study conducted by Sobrino et al., (2005), *P. longirostris* is located between 20 and 400 m in Guinea gulf, and from 50 to 500 m off Congo (Crosnier et al., 1970), as well as other marine areas of tropical Africa (Crosnier and Forest, 1973).

P. longirostris is a species of economic value in Europe and Turkey (Anonymous, 2021; FAO, 2008). It can be said to be the most important invertebrate species in the East Atlantic and the entire Mediterranean in countries such as France, Spain, Tunisia, Italy, and Greece (Deval *et al*, 2006).

The Marmara and Aegean coasts are well known for their high economic sea product potential (Bayhan *et al*, 2006). Among the ten commercially evaluated shrimp species (*Penaeus semisulcatus*, *Melicertus kerathurus*, *Marsupenaeus japonicus*, *Parapenaeus longirostris*, *Metapenaeus monoceros*, *Metapenaeus stebbingi*, *Trachypenaeus curvirostris*, and *Melicertus hathostris*), *P. longirostris* is the most caught species. According to data obtained, a total of 125.126,9 tonnes of pink shrimp have been captured from the Marmara Sea in the last ten years (from 2010 to 2020) (Anonymous, 2021). The amount of shrimp captured in the Sea of Marmara constitutes approximately 54.9% of the total amount of shrimp product in all Turkish seas. A total of 537 fishing boats with a length of 7–30 m, 9–670 HP engine power, and trawlers and two-way trawlers are widely used in shrimp fishing in the Sea of Marmara (Anonymous, 2020).

The Sea of Marmara is an inland sea connecting the Mediterranean and to the Black Sea. Therefore, the Sea of Marmara is a part of the Mediterranean water system. Various aquatic species live in the Sea of Marmara. One of the most important species is the deep-water rose shrimp (*P. longirostris* Lucas, 1846) (Kocataş, 1981). The number of studies on the stock structure of *P. longirostris* living in the East Marmara Sea is not enough, and such a study will be crucial for the best management of this resource. On the Marmara and Aegean Sea coasts, *P. longirostris* has significant economic potential (Bayhan *et al*., 2006; Zengin *et al*., 2007). In a study comparing the sizes of *P. longirostris* individuals living in the Sea of Marmara and in the Aegean Sea, it was reported that the difference between the ages and lengths of *P. longirostris* individuals in these two seas was insignificant (Tosunoğlu *et al*, 2009). In a study on the biodiversity of the Marmara Sea, İhsanoğlu and İşmen (2020) reported that the areas where *P. longirostris* is most abundant are the southern Marmara region and the waters of the Kapıdağ peninsula. It has been determined that *P. longirostris*, which is the target species in South eastern Marmara waters, constitutes 64.5% of the total product (Bayhan *et al*, 2006). Öztürk (2009) conducted a study in the Sea of Marmara, stating that in 1987, the pink shrimp shell length varied between 85 mm and 306 mm, but the maximum shell length measured in his study was 126 mm. He emphasised that according to this result, shrimp stocks were in danger due to fishing pressure. In another study conducted in the Marmara Sea, the effect of trawl net material and different net cod-ends on the amount of *P. longirostris* was investigated, and it was stated that 32 mm PE and 32 mm PA net cod-ends were not efficient for shrimp fishing (Deval *et al*, 2006). The product quantities obtained from the bottom trawl and beam trawl operations of *P. longirostris* in the Marmara Sea were calculated by the "area scanning method" as 229.8 ±57.3 kg/m^2 and 409.3 ±152 kg/m^2, respectively (Zengin *et al*, 2007). The bathymetric distribution of *P. longirostris*, as well as seasonal growth and mortality rates according to Elefan, Projmat, and SLCA methods, was investigated in a study conducted in Saros Bay, in the Aegean Sea. It was reported that the shrimp stocks exploited in Saros Bay were compatible with the shrimp stocks in other regions, and ELEFAN was the most suitable method for the shrimp population among the mentioned seasonal growth patterns (Bilgin *et al*, 2009). In a study conducted in the Mediterranean (Babadillimanı bay) on the bio-ecology and population dynamics of *P. longirostris*, it was reported that the most intensive breeding occurred between December and March, and April and June. In the same study, it was reported that the total mortality rate (Z), natural mortality rate (M), fishing mortality rate (F), and exploitation rate (E) were 4.0, 1.29, 2.71, and 0.67, respectively (Manaşırlı *at al*. 2011).

In this study, we evaluated the length at first capture, the recruitment model, the relative yield per-recruit, and the relative biomass per-recruit, as well as the growth and mortality parameters that are needed for stock management of *P. longirostris* in the eastern Sea of Marmara.

Material and Methods

Sampling Area and Sample Collection

This research was conducted in the coastal waters of Tuzla, Eastern Marmara Sea (Figure 1). The sampling area's endpoint coordinates are lower right, 40^0 43′ 52″ N; 29^0 21′ 04″ E, and upper left, 40^0 50′ 00″ N; 29^0 08′ 08″ E. *P. longirostris* specimens were sampled from 11 fishing areas (Figure 1). Samples were caught using a beam trawl in eastern the

Marmara Sea at depths ranging from 90 to 120 m. The width of the beam trawls used by the fishermen was 3 m, and the height was 40 cm. Shrimp were randomly sampled from commercial fishing vessels between February 2008 and May 2009.

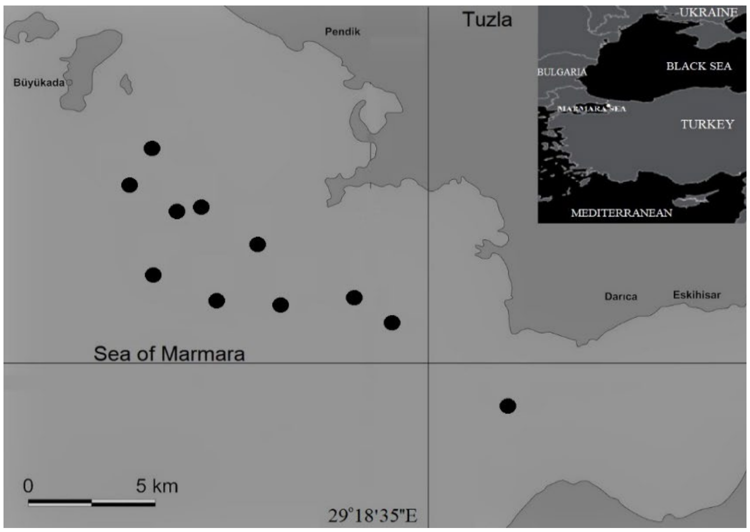

Figure 1. Study area (Sampling points ●)

Data Analysis

P. longirostris samples collected monthly during the study were directly brought to the laboratory. A total of 3826 shrimp were sampled in the study. The carapace length (CL) was measured to the nearest 0.1 mm as the shortest distance from the orbital edge to the mid-dorsal posterior margin of the carapace using vernier callipers. The total length (TL) was measured to the nearest 1 mm, from the tip of the rostrum to the end of the telson. The body weight (W) was measured to the nearest 0.01 g using a digital balance. Differences in the size distribution of shrimp between males and females were evaluated using the Kolmogorov-Smirnov two-sample test in the "Statistica" program. The sex-ratio was calculated and compared to the 1:1 proportion using the chi-square ($\chi 2$) goodness fit test.

Growth Parameters

The relationship between size and weight for females and males was determined by the following non-linear equation: Length-weight relationships were calculated considering the allometric equation. $W = a * CL^b$ (Sparre *et al*, 1989), where W is the shrimp weight, CL is the shrimp carapace length, "a" is the regression constant, and "b" is the coefficient of allometry. The growth type was defined by using Student's t-test by the equation according to Sokal and Rohlf (1987). Length-frequency distribution analysis was used to estimate growth in *P. longirostris* following the Von Bertalanffy technique. The von Bertalanffy Growth Function (VBGF) adapted to FISAT II was used to attain the growth indexes, where the growth rate (K), the asymptotic carapace length (CL_∞) and the growth performance index(φ). The Powell-Wetherall Plot adapted to FISAT II was used to obtain the rate of Z/K for the fish species evaluated. Growth of individual fishes on average towards L_∞ at K with length at the time (t) was calculated using the VBGF (Pauly, 1979):

$L_t = L_\infty\ (1 - e^{-k(t-t_0)})$. The theoretical age at birth was estimated separately, employing the mathematical equality: $log_{10}(-t_0) = -0.3922 - 0.275 * log_{10}L_\infty - 1.038 * log_{10}K$

(Maximum longevity (Tmax) was calculated by the following equation: $T_{max} = {}^{3}/_{K} + t_0$ (Pauly, 1983). The φ was calculated by the formula: $(\varphi) = 2\ \mathrm{Log}L_\infty + Log\ K$ (Munro and Pauly, 1983).

Mortality

The total mortality rate (Z) was estimated using the length-converted catc h curve Pauly (1984) approach. The natural mortality rate (M) was estimated using Pauly's (1980) empirical relationship.

$$Log(M) = -0.0066 - 0.279\ Log(CL\infty) + 0.6543\ Log(K) + 0.4634\ Log(T)$$

where: CL_∞ is the asymptotic carapace length, M is the natural mortality, K is the VBGF growth coefficient, and T is the average annual seawater water temperature (°C). The input requirement in the procedure was the value of T = 15.5 ° C (Anonymous, 2010). After M and Z were estimated, fishing mortality was determined employing the following relationship:

$F = Z – M$

where Z= is the total mortality, F= is the fishing mortality, and M= is the natural mortality. The exploitation rate (E) was calculated by the Gulland (1971) formula E=F/Z.

Length at First Capture (Lc50)

The probability of capturing each length class equipped with FISAT II was estimated using the increasing left arm of the length-converted catch curve. The CL at first capturing (Lc_{50}) was determined by plotting the cumulative probability of capturing against the mid-length of the resulting curve. In addition, the lengths of the 25% and 75% catches were calculated to represent the cumulative possibilities of 25% and 75%, respectively.

Recruitment Pattern

The recruitment pattern was inferred using a backwards analysis of the length axis of the length-frequency data set defined in FISAT. This method produced the recruitment pulse from a time series of length-frequency data to determine the number of pulses each year and the pulse's relative strength (Amin et al, 2008). Input parameters contained both L_∞ and K. The Normal Distribution of the recruitment pattern was estimated employing the NORMSEP FISAT (Pauly and Caddy, 1985).

Relative Yield per-Recruit (Y'/R) and Relative Biomass Per−Recruit (B'/R)

The relative biomass per recruit (B'/R) was estimated as B'/R = (Y'/R)/F. The E_{max}, which refers to an exploitation rate producing maximum yield, $E_{0.1}$ suggesting an exploitation rate at which the marginal increase of Y'/R is 10% of its virgin stock with $E_{0.5}$ indicating an exploitation rate under which the stock is reduced to half of its virgin biomass, was computed using the procedure incorporated in the FiSAT II Tool, using the Knife-edge option.

Results and Discussion

Length-Frequency Distribution

CL frequency distributions of samples ranged from 8.00 mm to 31.00 mm for females and from 11.00 mm to 28.00 mm for males (Figure 2). The sex ratio proportion was found to be 1:1.7 (F:M). The females represented 63.0% and the males' represend 37.0% of the analyzed population. The sex ratio proportion presented a statistically difference from the 1:1 proportion ($p < 0.05$, $\chi 2$). The mean W of females and males were 7.06 g (± 2.72 SE) and 5.17 g (± 1.043 SE), respectively. Female and male CL distributions were significantly different (Kolmogorov-Smirnov two-sample test: nf = 2410, nm = 1416, D = 0.1290, $p < 0.05$).

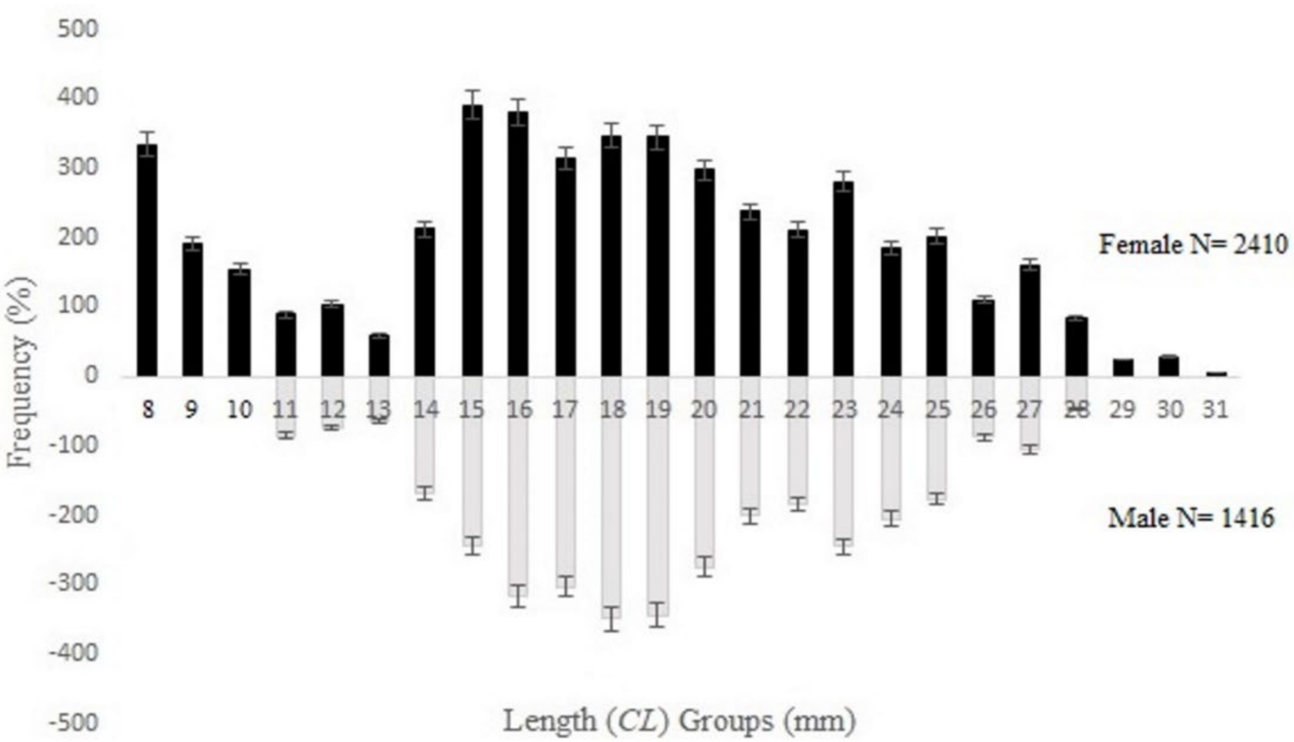

Figure 2. Length-frequency of *P. longirostris* in coastal waters of Tuzla, eastern Sea of Marmara

Growth Parameters

The relationship between carapace length and weights was determined as $W= 0.0105CL^{2.6866}$ and $W= 0.0126CL^{2.6103}$ for females and males, respectively. Both the CL and the weight values were determined seasonally and annually. The values did not show a statistically significant different considering seasons and years ($p > 0.05$). The CL-weight relationship for both sexes indicated negative allometric growth ($b<3$; t-test, $P<0.05$).

The estimated VBGF parameters for the Sea of Marmara *P. longirostris* females and males were: $CL_\infty = 32.55$, 28.67 mm, $K = 0.760$, 0.950 yr^{-1}, respectively (Figure 3, Table 1)). The estimated growth performance indexes (φ) for females and males were 2.91 and 2.90, respectively. Figure 3. A and B show reconstructed length-frequency data superimposed on the estimated growth curve, demostrating approximately three cohorts for females and almost two cohorts for males. The calculated t_0 and T_{max} for females and males were estimated to be –0.233, 3.71 and –0.769, 2.39, respectively (Table 1).

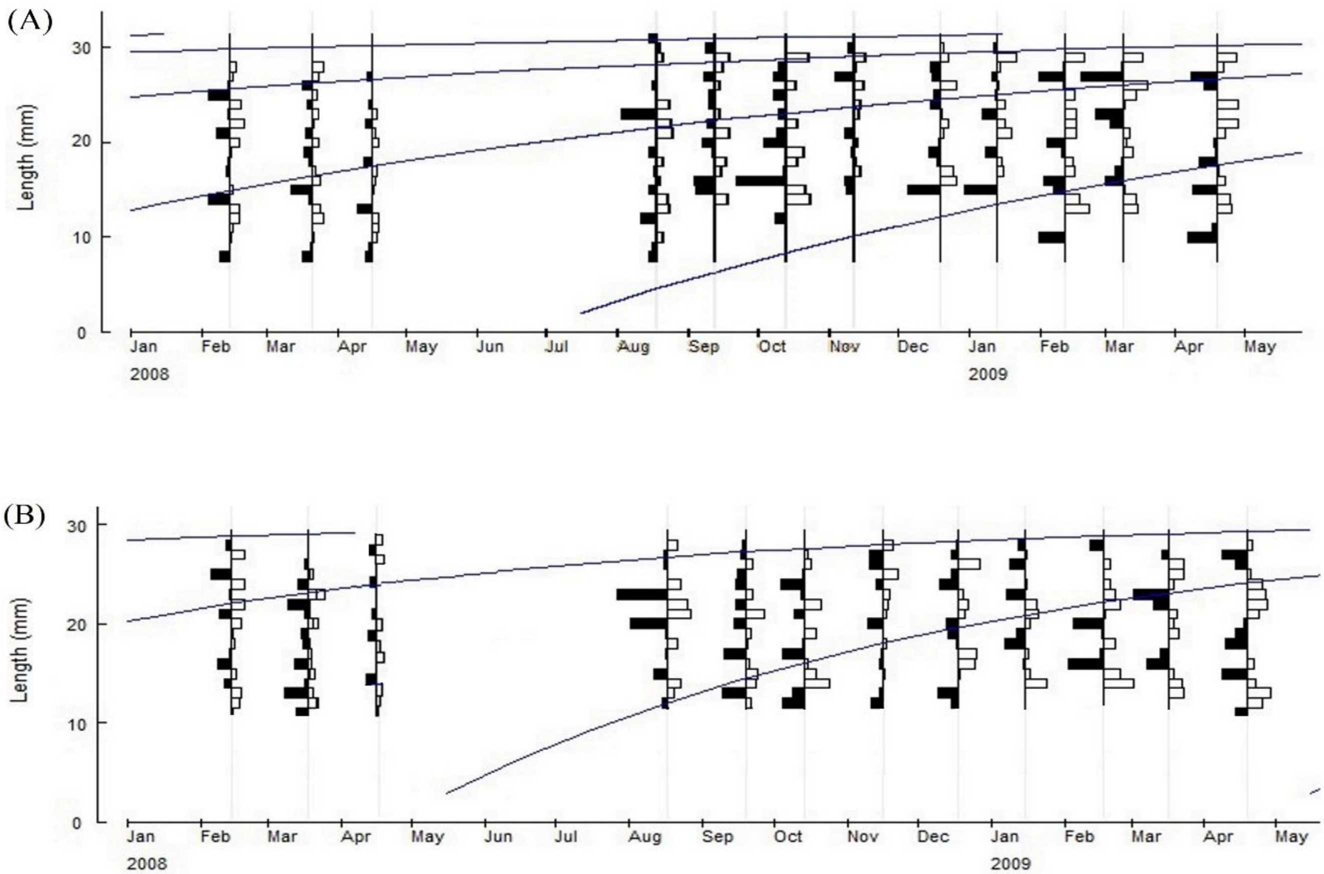

Figure 3. Von Bertalanffy growth curves for females and males. *P. longirostris*, for females (A) and males (B), is superimposed on the re-structured carapace length-frequency histograms. The black and white bars of three length groups represent pseudo-cohorts.

Table 1. Population parameters of *P. longirostris* by sex in the coastal waters of Tuzla, eastern Sea of Marmara

Population parameters	**Female**	**Male**
Asymptotic Length (CL∞) in mm	32.55	28.67
Growth coefficient (K, yr^{-1})	0.760	0.950
Theoretical age at birth (t_0)	−0.233	−0.769
Lifespan (t_{max})	3.71	2.39
Growth performance index (φ)	2.91	2.89
Natural mortality (M, yr^{-1})	1.09	1.33
Fishing Mortality (F, yr^{-1})	1.20	0.71
Total mortality (Z, yr^{-1})	2.29	2.04
Exploitation rate (E)	0.52	0.35
M/K	1.43	1.40
Lc_{50}/L_∞	0.58	0.63
Allowable limit of exploitation (E_{max})	0.82	0.90
$E_{0.5}$	0.39	0.40
$E_{0.1}$	0.72	0.80
Mean surface temperature (ºC)	15.50	15.50
Number of samples (N)	2410	1416

For each monthly sampling, mode lengths were estimated by cohorts. The modal length groups of the *P. longirostris* population were estimated to consist of maximum three age groups in females and two age groups in males, ranging from 17.61 mm to 30.44 mm and from 14.22 mm to 24.34 mm, respectively (Figure 4, Table 2).

Mortality and Exploitation Rate

Using the length-converted capture curve, the total mortality rates (Z) for female and male shrimp were estimated to be 2.29 and 2.04 yr^{-1}, respectively (Figure 5). Based on the total mortality rate (Z) obtained from the length-converted catch curve, the fishing mortality rates (F) of males and females were determined as 1.20 and 0.71 yr^{-1}, respectively (Table 1).

Based on these data, the exploitation rates (E) for female and male *P. longirostris* in the eastern sea of Marmara were calculated as 0.52 and 0.35, respectively (Table 1).

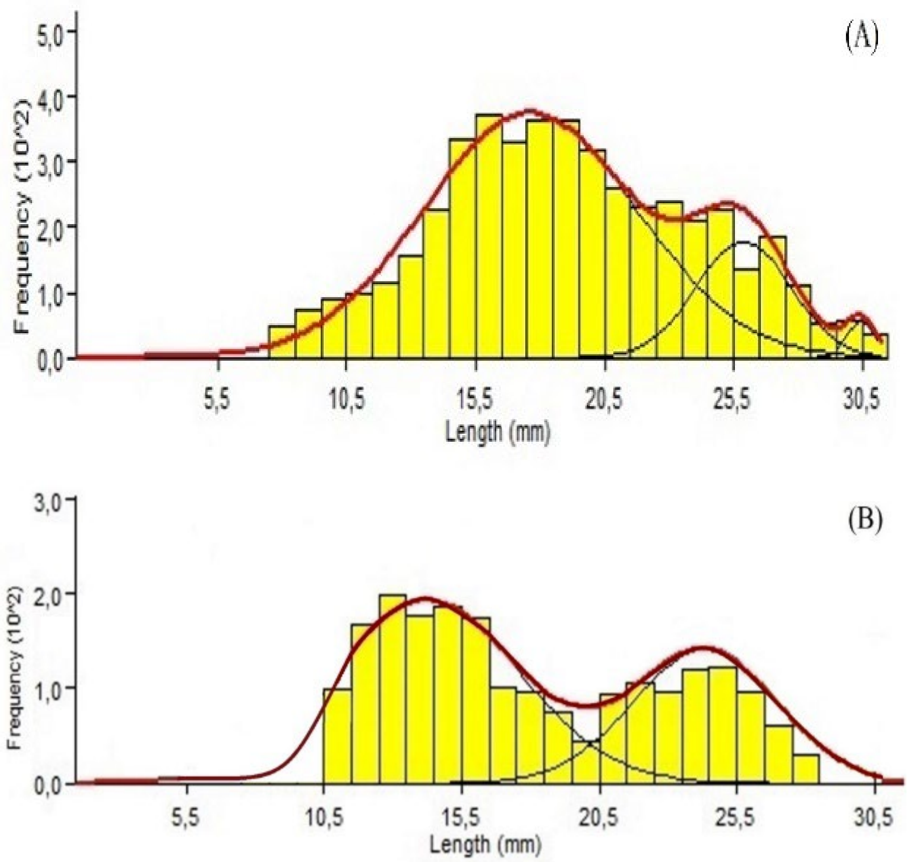

Figure 4. Age estimation of female (A) and male (B) individuals of *P. longirostris* according to Bhattacharya′s method

Table 2. Result of Bhattacharya method to identify *Parapeneus longirostris* modal components (annual cohort) by sex in the coastal waters of Tuzla, the eastern sea of Marmara.

Sex	Cohort	Computed mean	SD	SI
Female	1	17.61	3.430	n.a
	2	25.88	2.840	2.210
	3	30.44	0.520	2.160
Male	1	14.22	2.370	n.a
	2	24.34	2.620	2.470
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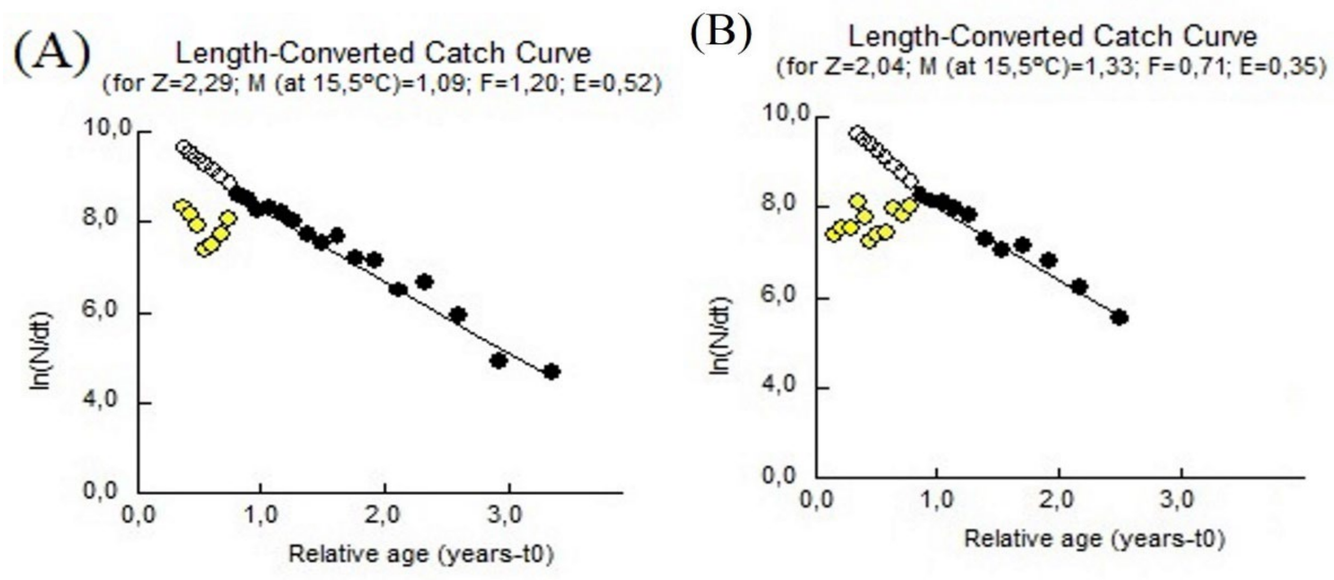

Figure 5. In the length-converted capture curve for females (A) and males (B), the darkened full points represent the points used in the calculation via least-square linear regression, and the open points represent either the not fully recruited point or the estimate of total mortality near t_0.

Length at First Capture (CL_{50})

As part of the length-converted catch curve analysis, the CL_{50} (at which 50% of the population of *P. longirostris* is vulnerable to fishing gear) was estimated. The estimated values for females and males were CL_{50} = 18.78 and 18.07 mm, respectively. The lengths at which 25% and 75% of the females and males caught with beam trawls were determined by the analysis were CL_{25}% = 14.56, 15.46 mm, and CL_{75}% = 22.99, 20.69 mm, respectively (Figure 6).

Recruitment Pattern

The recruitment pattern of *P. longirostris* had two peaks in both sexes during the year. 1^{st} and 2^{nd} recruit rates of females and males were 26.28%, 15.32% and 15.54%, 13.11%, respectively. Significant recruitment took place in October for females and in March for males. The highest and lowest recruitments for females and males occurred in October, November and March, August, respectively (Figure 7).

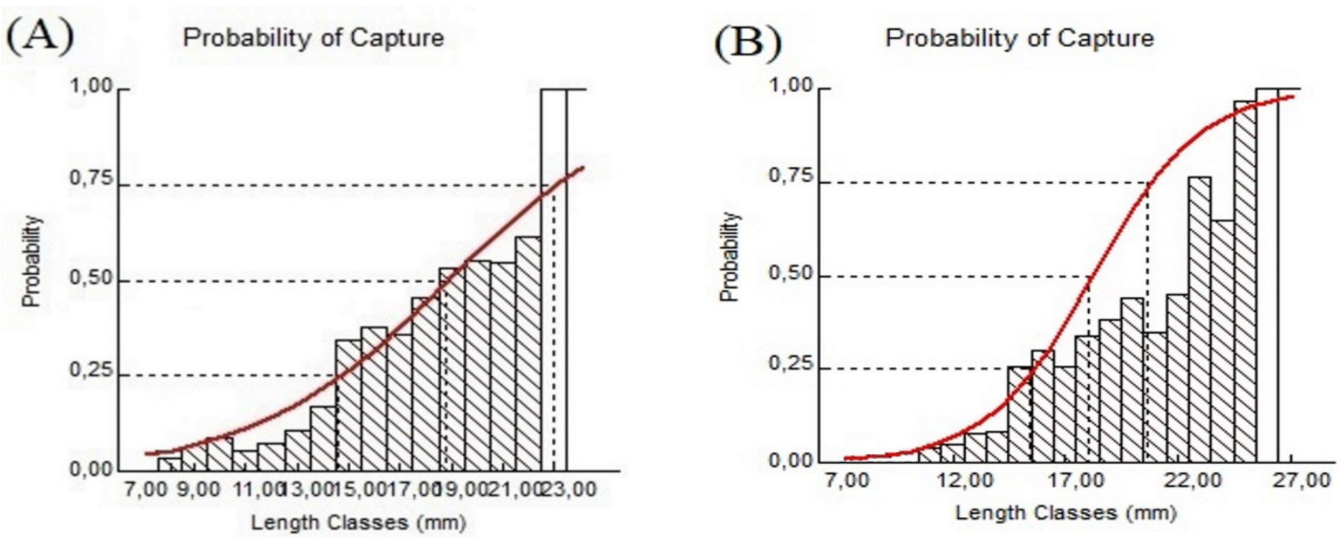

Figure 6. Probability of capture of each Carapace length (CL_{50}) class of females (A) and males (B)

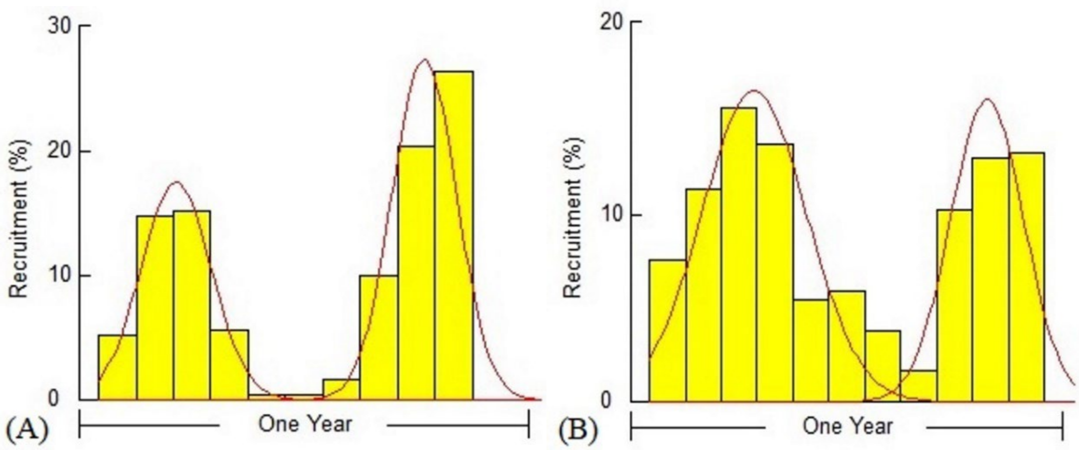

Figure 7. Recruitment pattern of (A) female and (B) male *P. longirostris* in the coastal waters of Tuzla, eastern Sea of Marmara showing two main peaks.

***Relative Yield Per-Recruit* (*Y′/R*) *and Relative Biomass Per-Recruit* (*B′/R*)**

The simplest assumption is that selectivity is knife-edge at some standardized length Lc_{50}, i.e. all fish smaller than Lc_{50} is not vulnerable to fishing mortality and only experience natural mortality, while all fish larger than L_{c50} is fully vulnerable to fishing mortality. The relative Y′/R and B′/R analyses of females and males *P. longirostris* were estimated using the knife-edge method assumptions. The input parameters for females and males for the model were the values of Lc_{50}/L_∞= 0.58 and 0.63, and M/K = 1.43 and 1.40, respectively. The maximum exploitation rate (E_{max}) providing maximum relative yield per recruit, was calculated from the analysis.

The maximum exploitation level (E_{max}) giving the maximum relative yield per stock was calculated as 0.82 and 0.90 for females and males, respectively (Figure 8). The economic exploitation rates ($E_{0.1}$) of females and males were 0.72 and 0.80, where the marginal increment in relative yield per recruit was 10% of the marginal increase estimated at the slight rate in exploitation (Table 1). The rate of exploitation ($E_{0.5}$) of the non-exploited stock was 0.40, corresponding to 50% of relative biomass per-recruit. Yield per recruit for male and female *P. longirostris* populations in Tuzla coastal waters of the Eastern Marmara Sea was estimated using a wide E range and fishing pressure in both isopleths and CL_{50} (Figure 8).

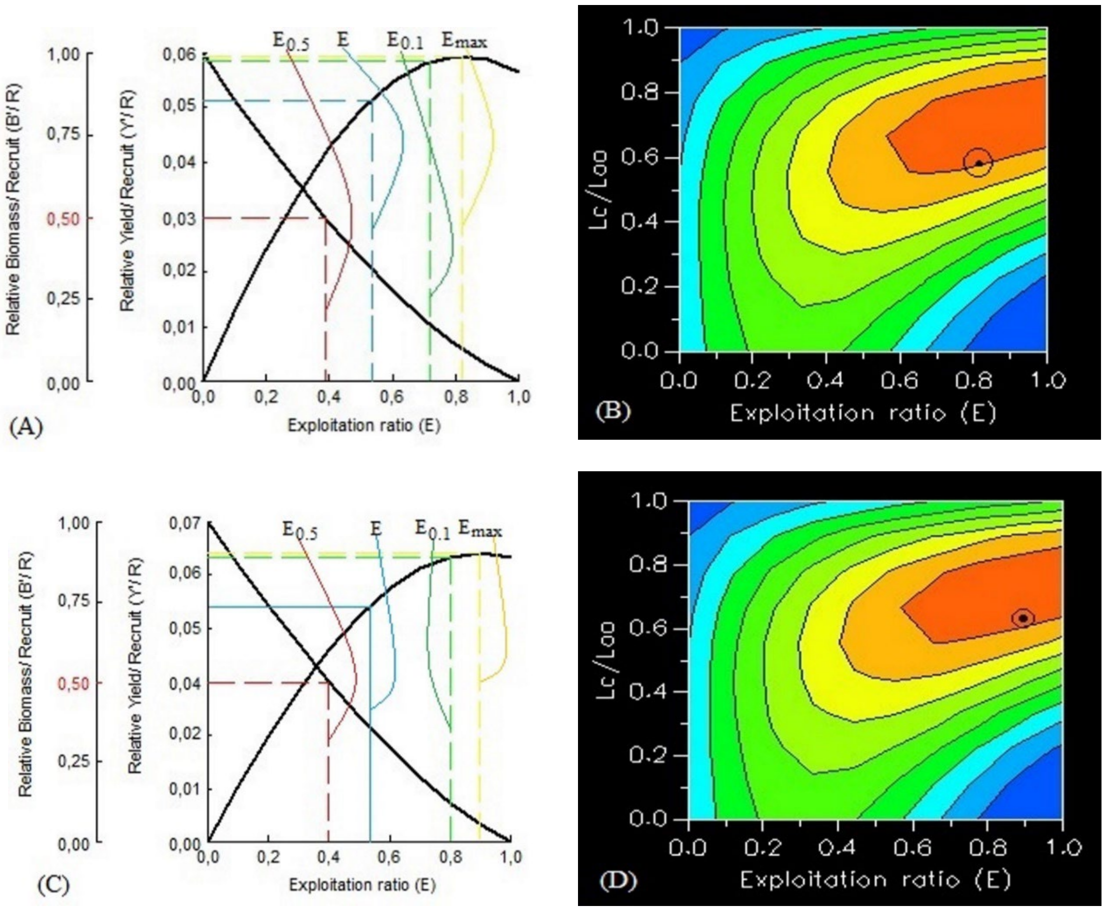

Figure 8. Relative yield per recruit Y′/R) and relative biomass per recruit (B′/R) for females (A) and males (C) and using knife-edge procedure for yield isopleths diagrams for females (B) and males (D) in Tuzla coastal waters, eastern Sea of Marmara

In the former studies in the Marmara Sea, the male and female percentages of *Parapenaeus longirostris* were 44.2% and 55.8% (İhsanoğlu and İşmen, 2020) and 35.4% and 64.6% (Tosunoğlu *et al*., 2009) and 44.2% and 55.8% (Zengin *et al*., 2007), 37.7 and 62.3% (Bayhan *et al*., 2006). In a previous study in the Mediterranean, the percentages of males and females were found to be 72.75% and 23.75 (Manaşırlı, *et al*., 2011). In our study, a significant skewness was observed in the sex ratio in the Marmara Sea and in the Mediterranean, but no bias in the sex ratio was observed in the Aegean Sea.

In our study, 3 age groups for females and 2 age groups for males were determined. The 3^+ age group for *P. longirostris* females and males was determined in a study conducted in the Eastern Mediterranean Babadil Bay (Manaşırlı *et al*., 2011). In another study conducted with samples obtained from the north, south, and west of the Marmara Sea, 4 age groups for females and 3 age groups for males were determined (İhsanoğlu and İşmen, 2020). Other studies reported a maximum of 6 years in the Aegean Sea (Tosunoğlu *et al*., 2009). A study conducted previously in the sea of Marmara indicated 3 years of age of the *P. longirostris* (Zengin *et al*., 2007). It can be thought that this difference is due to environmental conditions, nutrient abundance, sampling season, and sampling methods.

In this study, CL_∞ and K of female and male *P. longirostris* were determined as 32.55, 28.67 mm and 0.760, 0.950 yr^{-1}, respectively. In the Eastern Mediterranean coastal waters of Turkey, Manaşırlı et al. (2011) estimated L_∞ and K values to be 32.30, 31.20 mm and 0.76, 0.77 for female and male individuals, respectively. These results are very close to the results of our study. Deval *et al*., (2006) used three different cod ends to investigate the effect of beam code end size and net materials on fishing activity and mortality in the Sea of Marmara. These are results, total mortality (Z), natural mortality (M), fishing mortality (F), and exploitation rate (E) were calculated between 1.16 and 2.83, 0.97 and 1.16, 0.60 and 1.86, 0.45 and 0.65, respectively. In a study conducted in

Sığacık Gulf, the Aegean Sea, the CL_∞ and K values for females and males were calculated as 41, 257 mm, 0.314 and 34.991 mm, 0.41, respectively. In the same study, the total mortality rate (Z) for females and males was 2.48 and 1.21, the fishing mortality rate (F) was 1.71 and 0.54, the natural mortality rate (M) was 0.77 and 0.67, and the exploitation rate (E) was 0.69 and 0.45, respectively (Dereli, 2010). İhsanoğlu and İşmen (2020), in a study conducted in the north, south and west of the Marmara Sea, calculated the CL_∞ and K values for females and males as 41 mm, 0.27 and 36.8 mm, 0.37, respectively. In the same study, the total mortality rate (Z) for females and males was 1.09 and 2.22, the fishing mortality rate (F) was 0.57 and 1.56, and the natural mortality rate (M) was 0.52 and 0.66, respectively. The exploitation rate (E) for females and males was found to be 0.52 and 0.70, respectively. Accordingly, when the results of these studies are compared, it can be said that the total mortality rates (Z) and fisheries mortality rates are close to each other, while the natural mortality rates (M) and exploitation rates are slightly higher in our study results. This difference can be associated with habitat conditions and fishing pressure.

In their research in the Gulf of Saros (Aegean Sea), Bilgin et al. (2009) estimated CL_∞ to be 34.7 mm and 27.0 mm, K to be 1.05 and 1.49, and Z to be 1.19 and 4.73 for *P. longirostris*. These results are quite close to the findings of our study, but the K value is higher. This situation can be associated with environmental conditions and nutrient abundance. In another study conducted in the Mediterranean waters of Morocco, the L_∞ (52.87 mm) value was found to be high, while the K value was determined as 0.39 Awadh and Aksissou (2020). In the same study, Z was calculated as 3.49. M and F were calculated by two different methods and estimated to be 1.98, 1.36 and 1.51, 2.13, respectively. These differences can be attributed to environmental factors (temperature, nutrients, predators, etc.). Although the total mortality rate was higher, M and F estimates were somewhat closer to each other in our findings. In two different locations in the South Tyrrhenian Sea, Arculeo *et al*., (2014) determined the shortest CL and largest CL_{max} for male and female *P. longirostris* at Terrasini location was estimated to be 9.0 mm and 32.0 mm, respectively. The VBGF was CL_∞ = 38.5 mm, F = 0.65 $years^{-1}$ for females, and CL_∞ = 32.5 mm, F = 0.8 $years^{-1}$ for males. The smallest and largest carapace lengths (CL) for females and males at the Porticelli location were 8 mm, 31 mm, 13 mm and, 26 mm, respectively. VBG parameters for female and male individuals were CL_∞ = 40 mm, F = 0.60 yr^{-1} and CL_∞ = 30 mm, F = 0.76 yr^{-1}, respectively (Arculeo *et al*., 2014). These results are close to the findings estimated for male and female individuals in our study. The smallest carapace length measured in our study was 8.00 mm for females and males, and the CL_{max} was 31.28 mm. The largest and smallest measured CL were very close to each other. *P. longirostris* was estimated to have a CL_∞ of 45 mm and a K of 0.34 yr^{-1} in a study conducted in the Gulf of Alicante (Spain) (Garca-Rodriguez *et al*., 2009). Study findings in the Bay of Alicante showed a longer asymptotic length but a lower K value than our findings. An increase in CL_∞ was striking as it approached from the east of the Mediterranean Sea to the west of the Atlantic Ocean. These differences may be due to the abundance of nutrients in the environment and seawater temperatures (Ren *et al*., 2021). In another study conducted in the Central Mediterranean, VBGF results were close to our findings, but K = 0.63 was lower (Levi *et al.,* 1995). This may be due to environmental conditions (Ren *et al*., 2021). The mortality rates (Z = 1.4, M = 0.17, and F = 0.79) were considerably lower than the findings of this study. On the other hand, the exploitation rate (E = 0.67) was higher. In another study conducted in West Africa, the CL_∞ and K value were estimated as 17.61 mm and K = 0.78, respectively (Yacouba *et al*., 2014). While the K value (0.78) was close to our findings, the CL_∞ = 17.61 was lower than our study result. These differences can be thought to be due to environmental conditions. The mortality rates were close to our findings. However, (F = 3.74) and (E = 0.77) were higher. These differences are due to the high rate of exploitation due to fishing pressure (Colloco *et al*., 2017).

In a study conducted in the Strait of Sicily and Tunisian waters, the length CL_∞ for females and males was calculated as 43.0, 42.41 mm, 34.3, and 32.82 mm, respectively. The K and M values for females and males estimated were estimated as 0.68, 0.66, 1.07, 1.03 and 0.73, 0.73, 1.53, 0.86, respectively (Knittweis *et al*., 2013). Estimated CL_∞ for males and females was lower in our study, but these values were also higher in the studies of İhsanoğlu and İşmen (2020), and Dereli (2010). K values were found to be higher for both sexes. The M were estimated higher in our results for both sexes. All these differences can be attributed to the ecological conditions of the Mediterranean waters and the bio-ecological conditions of the East Marmara Sea waters (Ceraulo *et al*., 2018).

In our study, two peaks were determined for the recruitment of *P. longirostris*, occurring in February and July and August for females and males. In their study conducted in the Eastern Mediterranean Turkish waters, Manaşırlı *et al*., (2011) stated that the recruitment period was in March, and the spawning period was in the 2^{nd} period. Yacouba *et al*., (2014) found two peaks for the recruitment of *P. longirostris*, corresponding to February and September, in his research in West African waters. All these results support the findings of our study. These results might be influenced by the number of predators and competitors as well as the fishing pressure and mesh size of fishing gear (Sparre and Venema, 1992). The length of the first capture is a crucial input in the estimation of the relative yield-per-recruit and relative biomass-per-recruit. In this study, the highest Y′/R and E_{max} was calculated as 0.82 and 0.90, respectively. Pauly and Soriano (1986) classified the relative yield isopleths into four groups (or quadrants) based on the critical size level (Lc_{50}/L_∞) (related to mesh size).

In this study, isopleths with Lc_{50}/L_∞, $E_{0.1}$, and E_{max} are seen in Figure 8 (A, B, C, D). It indicated an exploitation ratio where the marginal increase in Y'/R which is 10% of the virgin stock ($E_{0.1}$), and the E_{max} is close to each other. The fishing regime for *P. longirostris* in the eastern Marmara Sea was estimated in the "C" quadrant, indicating advanced fishing (Pauly and Soriano, 1986). Results from the analysis of the E based on the mortality estimate and the Y′/R indicate that the fishing is above the level of optimum based on the $E_{0.1}$ principle. The state of *P. longirostris* populations is of concern. If these rates of exploitations and fishing pressures are sustained, stocks of *P. longirostris* could be damaged in a short time. *P. longirostris* stocks will suffer if current fishing pressure continues.

Conclusion

The population and stock status of *P. longirostris*, which is an economically valuable resource, should be routinely evaluated and stock management should be planned well. In long-term stock management for *P. longirostris*, we think that the findings of this study will contribute to stock management together with the results of other studies.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

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Distribution of soft-bottom benthic molluscs in the Gulf of Oran (Western Algeria)

Ahmed KERFOUF[1], Patricio R. De los RÍOS-ESCALANTE[2,3], Kamel Belhadj TAHAR[1], Françoise DENIS[4]

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[1] Laboratory of Eco-Development of Space, University Djillali Liabes, Sidi Bel Abbès, 22000, Algeria

[2] Departamento de Ciencias Biológicas y Químicas, Facultad de Recursos Naturales, Universidad Católica de Temuco, Casilla 15-D, Temuco, Chile

[3] Núcleo de Estudios Ambientales UC Temuco

[4] Marine Biology Station (MNHN), UMR 7208 'BOREA', 29182, Concarneau, France

ORCID IDs of the author(s):
A.K. 0000-0002-4466-1201
P.R.R.E. 0000-0001-5056-7003
K.B.T. 0000-0002-0711-9296
F.D. 0000-0002-0006-8238

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Correspondence:
Ahmed KERFOUF
E-mail: kerfoufahmed@yahoo.fr / ahmed.kerfouf@univ-sba.dz

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http://aquatres.scientificwebjournals.com

ABSTRACT

A total of 46 samples were taken in the softbottom of the Gulf of Oran, in order to study the nature of the sediments and the spatial distribution of the mollusc communities. As a result of sampling in the study area, at depth range (30 m -106 m), a total of 29 species were identified, included in two classes: Gastropoda and Bivalvia. The use of multivariate statistical analyzes, as well as particle size analysis, made it possible to identify the main mollusc communities. Six mollusc communities were defined from faunistic and sedimentologic affinities. The malacological fauna of the Gulf of Oran is dominated by *Saccella commutata* (33.33%), *Limatula subauriculata* (20.83%) and *Nucula sulcata* (20.83%). The main ecological stocks are mixicole and coastal detritus species. The malacofauna of the Gulf of Oran is scarce and less diversified due to the nature of the soft bottoms (sand and gravel) and the absence of terrigenous inputs.

Keywords: Molluscs, Gastropoda, Bivalvia, softbottom, Gulf of Oran, Western Algeria

Introduction

As the majority of the other emerged coasts all around the Mediterranean Sea, the northern extremity of the African continent is prolonged under the sea by a very tight submarine bordure (Leclaire, 1972). The gulf is subject to strong human pressure: harbours activities, urban and industrial wastewater discharges (Remili and Kerfouf, 2013; Benali et al., 2017; Rouabhi, 2020). The gulf of Oran is supplied by waters originated from the Atlantic Ocean and the circulation seems to be very turbulent along the African continent. These turbulences favour the dispersion of eventual pollution sources and permit a so relative important food chain development (Millot, 1999).

The objective of the study was related to the spatial distribution of mollusc communities in the Gulf of Oran. The majority of research on marine mollusc diversity and spatial distribution are incomprehensive and focuses only on a small geographical area. Nevertheless, this region was considered poor in terms of species (Pallary, 1900; Llabadore, 1935; Vaissière et al., 1963; Amar, 1998; Kerfouf et al., 2007). On the Algerian coast, few synthesis works has been carried out regarding the marine macrobenthic fauna (Dautzenberg, 1895; Meziane and Kerfouf, 2018; Bakalem, 1979). Molluscs communities structure analysis is a good method in the study of environmental modifications caused both by natural and anthropic perturbations (Palaz, 2005). In fact, some results show the response of the molluscs communities to different disturbance sources in a simple way.

Material and Methods

Sampling

The Gulf of Oran, on the Algerian Mediterranean coast, is located between the industrial Gulf of Arzew in the east (Cap Carbon), and cap Falcon in the west, mainly represented by two important harbours Oran and Mers El Kebir. According to the bathymetry of this zone, 23 stations have been prospected. The nearest coast station was at 30 m depth and the most far was at 106 m depth (Figure 1). Aberdeen benne (Smith McIntyre) has been used for the sediments and benthos sampling operations. All fauna and sediment were fixed in 10% formalin.

Data analysis

The first segregation of the samplings was made according to the great zoological groups. Then the molluscs species were identified by using several references (Hinton, 1972; Springsteen and Leobrera, 1986; Poppe, 2008; Wong, 2011). The frequency of each inventoried species was noted, in order to establish species abundance and dominance calculations.

The sediments were analyzed in order to determine the nature and the texture of the substrate (Caulet, 1972). The method used consists of passing the dried sediment (100 g) through a column of 16 superimposed sieves (AFNOR, 1996). The granulometric analysis allows the size and the distribution evaluations of the particles constituting the sediment (Table 1).

Several classical and synthetic methods were used in order to evaluate the distribution and faunal structure such as abundance, and species richness.

As a first step, the results were processed using the multi-variable analysis method HCA (hierarchical ascendant classification), and also the sedimentary analysis permitted, to highlight the structure of the main malacofauna communities of the Gulf of Oran.

In a second step, a matrix correlation analysis was applied, in order to calculate a checkerboard score ("C-score"), which is a quantitative index of occurrence that measures the extent to which species coexist less frequently than predicted by luck (Gotelli, 2000). A community is structured by competition when the C-score is significantly larger than expected by chance (Tondoh, 2006; Tiho and Josens, 2007). It compared the co-occurrence patterns with null expectations. Gotelli and Ellison (2013) suggest using the statistical null model Fixed-Fixed, as in this model, when the row and column sums of the matrix are preserved. The null model analyses were likewise performed using the R software (R Development Core Team, 2009) and the EcosimR package (Gotelli and Graves, 1996; Gotelli and Ellison, 2013; De los Ríos-Escalante et al., 2020).

Table 1. Geographical coordinates and physical characteristics of the studied sites

Site	Coordinates N	Coordinates E	Depth (m)	Median (mm)	Gravel (%)	Sands (%)	Muddy (%)
1.7	35°45’45’’	00°42’65’’	70	0.386	58.86	35.71	5.43
1.9	35°47’23’’	00°41’55’’	90	0.286	48.86	49.71	1.43
2.4	35°46’70’’	00°40’60’’	82	0.346	12.43	74.03	13.54
3.1	35°44’38’’	00°40’25’’	61	0.372	59.71	30.14	10.14
4.2	35°43’05’’	00°39’00’’	66	0.215	31.57	51.71	16.71
4.3	35°44’05’’	00°39’00’’	74	0.343	15.57	75.00	9.43
4.4	35°44’09’’	00°38’09’’	77	0.209	54.14	42.71	3.14
4.6	35°47’03’’	00°78’01’’	82	0.328	48.86	46.70	4.44
5.6	35°43’08’’	00°37’05’’	60	-	-	-	-
5.7	35°45’00’’	00°37’00’’	70	-	-	-	-
5.9	35°48’06’’	00°36’05’’	94	0.316	10.43	76.03	13.54
5.10	35°48’04’’	00°36’08’’	106	0.364	12.13	77.13	10.54
6.4	35°44’68’’	00°35’67’’	39	0.305	6.00	93.57	0.43
6.5	35°45’42’’	00°35’70’’	55	0.186	48.86	45.71	5.43
7.4	35°47’10’’	00°34’60’’	60	0.339	73.14	26.43	0.43
7.5	35°46’77’’	00°34’45’’	70	0.624	48.29	49.00	2.71
7.7	35°47’10’’	00°34’60’’	60	0.496	77.29	22.70	0.01
7.8	35°48’15’’	00°35’20’’	70	rocky	rocky	rocky	rocky
7.9	35°48’50’’	00°35’50’’	80	0.365	4.00	95.57	2.43
8.3	35°47’10’’	00°33’30’’	32	0.514	19.43	80.29	0.29
8.4	35°47’40’’	00°33’50’’	41	0.186	48.86	45.71	5.43
8.5	35°47’60’’	00°33’65’’	49	0.551	80.00	19.14	0.86
8.10	35°49’78’’	00°34’95’’	95	0. 462	72.86	25.86	1.29

Results and Discussion

Data Collection and Analysis

A total of 46 samples of all macrobenthic fauna were collected at 23 sites of the continental shelf of the Gulf of Oran at a depth of between 30 m and 106 m. A total of 116 individuals and 29 species belonging to two classes were identified in this area (WORMS, 2022). Each species has an ecological status from a bibliographic synthesis based on the following works (Picard, 1965; Falconetti, 1980; Stora, 1982; Glemarec and Grall, 2000; Costa et al., 2010). This status corresponds to its affinity concerning the substrate (the nature of the sediment fraction) and the quality of the environment (Table 2).

Population’s Characterization

Using Ascending Hierarchical Classification (CHA) for the stations made it possible to regroup stations according to the faunal composition and the substrate nature. The communities identified in the Oran's Gulf (and in most studies concerning the Mediterranean Sea), organized following the classification of biocoenoses proposed by Pérès and Picard (1964). The grain size analysis made it possible to associate each station a sedimentary type. The ascending hierarchical classification (AHC/species/stations), showing six great groups G1, G2, G3, G4, G5 and G6 (Figure 2).

Table 2. Faunistic list and ecological significance

Class	Order	Families	Genus	Species
	Mytiloida	Mytilidae	Lioberus	*Lioberus agglutinans* (Cantraine, 1835) Sspr
			Modiolula	*Modiolula phaseolina* (Philippi, 1844) Sspr
	Pectinoida	Pectinidae	Pseudamussium	*Pseudamussium clavatum* (Poli, 1795) Sspr
	Limoida	Limidae	Limatula	*Limatula subauriculata (*Montagu, 1808) *Sspr*
	Carditoida	Carditidae	Centrocardita	*Centrocardita aculeata* (Poli, 1795) Mix
			Cardites	*Cardites antiquatus* (Linnaeus, 1758) Sspr
		Astartidae	Gonilia	Gonilia calliglypta (Dall, 1903) Sspr
	Myoida,	Corbulidae	Varicorbula	*Varicorbula gibba* (Olivi, 1792) LRE/IMO
	Euheterodonta	Hiatellidae	Hiatella	*Hiatella arctica* (Linnaeus, 1767) LRE
	Lucinoida	Lucinidae	Loripes	*Loripes orbiculatus* (Poli, 1795) Sspr
Bivalvia	Anomalodesma-	Lyonsiidae	Lyonsia	*Lyonsia norwegica* (Gmelin, 1791) Mix
	taVeneroida,	Cardiidae	Papillicardium	*Papillicardium papillosum* (Poli, 1791) G
				Parvicardium scabrum (Philippi, 1844) Sspr
		Veneridae	Hysteroconcha	*Hysteroconcha dione* (Linnaeus, 1758) LRE/ DC
				Timoclea ovata (Pennant, 1777) Mix
			Timoclea	*Moerella donacina* (Linnaeus, 1758) DC
		Tellinidae	Moerella	*Moerella distorta* (Poli, 1791) DC
			Moerella	*Gari costulata* (Turton, 1822) Sspr
	Nuculanoida	Psammobiidae	Gari	*Saccella commutata* (Philippi, 1848) Mix
	Nuculida	Nuculanidae	Saccella	*Nucula turgida* (Gould, 1846) IP
		Nuculidae	Nucula	*Nucula sulcata* (Bronn, 1831*)* V
				Nucula nucleus (Linnaeus, 1758) Mix
	Cardiida	*Tellinidae*	Gastrana	*Gastrana fragilis* (Linnaeus, 1758) Sspr
Gasteropoda	Neogastropoda	Nassariidae	Tritia	*Tritia lima* (Dillwyn,1817) Sspr
				Tritia reticulata (Linnaeus, 1758) Sspr
		Fasciolariidae	Gracilipurpura	*Gracilipurpura rostrata* (Olivi, 1792) Mix
		Buccinidae	Euthria	*Euthria cornea* (Linnaeus, 1758) Sspr
		Naticidae	Notocochlis	*Notocochlis dillwynii (*Payraudeau, 1826) Sspr
Total	13	20	24	29

G*: gravel,* ***DC****: coastal detritic,* ***IP****: indicator of pollution,* ***IMO****: organic matter indicator,* ***LRE****: species with wide ecological distribution,* ***Mix****: mixicole,* ***S****: sandy species,* ***Sspr****: species with no specified ecological significance,* ***V****: muddy species.*

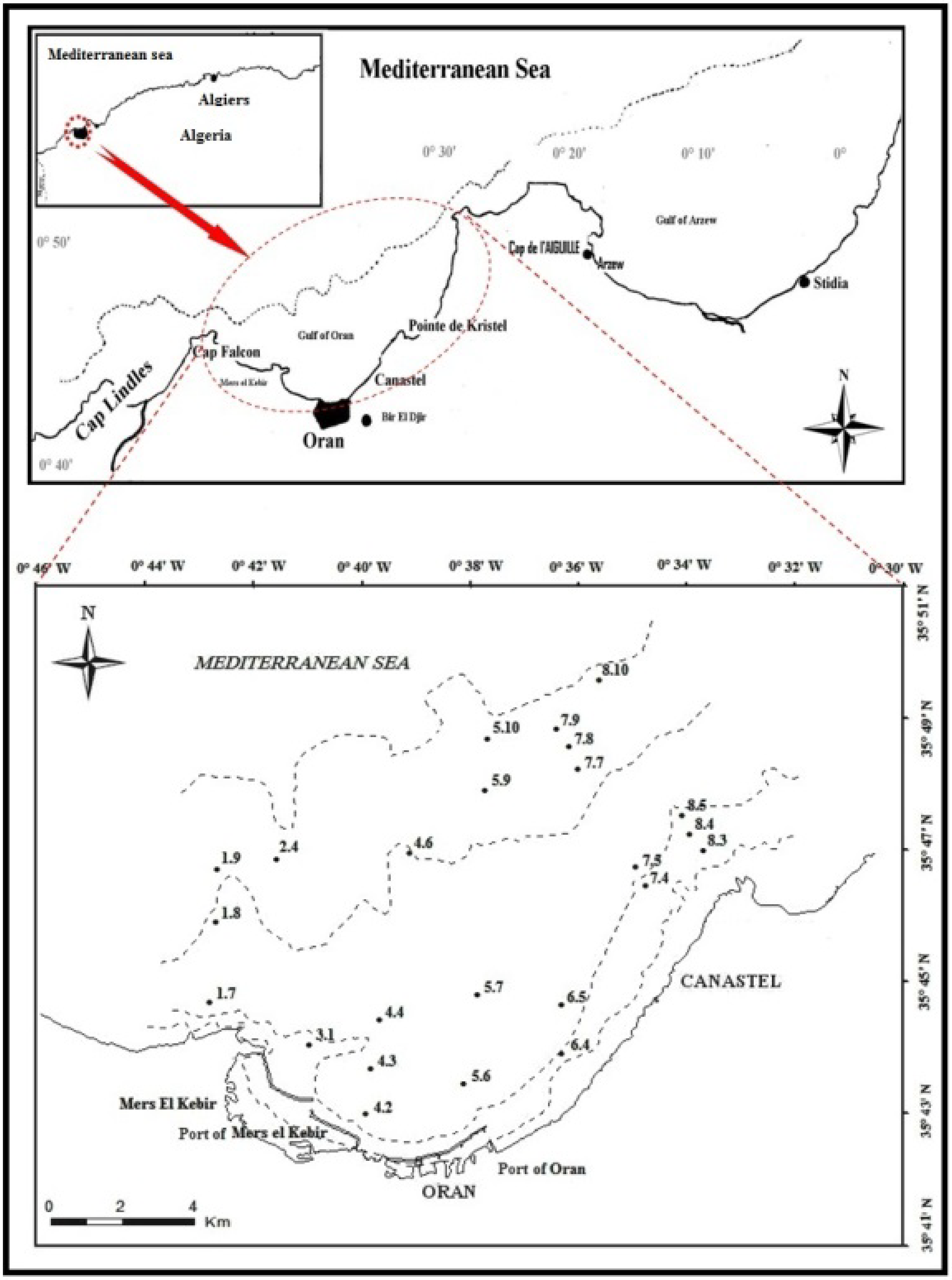

Figure 1. The locations of the sampling sites.

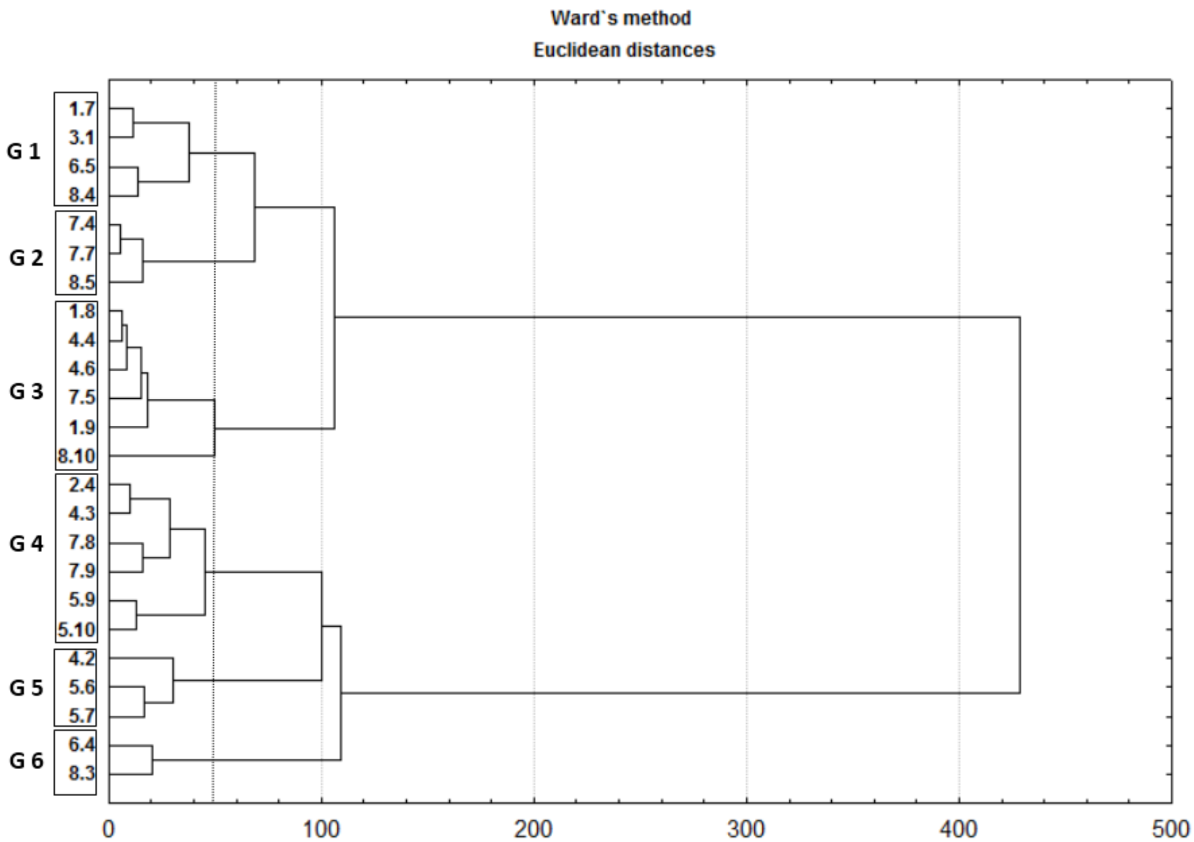

Figure 2. Ascending hierarchical analysis (AHC)

Six mollusc communities were defined from faunistic and sedimentologic affinities (figure 3).

The G1 group is made of 4 stations (1.7, 3.1, 6.5, and 8.4). The G2 group is composed of 3 stations (7.4, 7.7, and 8.5). G3 group and G4 are comprised of 6 stations (1.8, 1.9, 4.4, 4.6, 7.5, 8.10), and (2.4, 4.3, 5.9, 5.10, 7.8, and 7.9), respectively. The G5 and G6 groups are formed of 3 stations (4.2, 5.6, and 5.7) and 2 stations (6.4, 8.3).

The main communities are represented by the groups of stations: G3 and G4. They are each represented by 6 stations. G3 represents the community of *Nuculana commutata* (27.27%) and *Papillicardium papillosum* (18.18%) with mixed sediments (gravel 50%, sands 47%, muddy 3%), and G4 represents the community of *Nucula turgida* (23.80%) and *Nuculana commutata* (23.80) located on a sandy substrate (sands 80%, muddy 10%, gravel 10%). Both communities are far from the coast at a depth of 80m. The diversity of sedimentary habitats provides this community with good conditions for development. The characteristic species of Group G3 are *Nuculana commutata, Papillicardium papillosum, Amygdalum phaseolinum, Limatula subauriculata, Nassa limata, Nucula nucleus, Nucula turgida* and *Timoclea ovata.* For group G4, the pool of characteristic species is made up of *Nucula turgida, Nuculana commutate, Hiatella arctica, Cardita aculeta, Varicorbula gibba, Gracilipurpura rostrata, Limatula subauriculata, Nassa limata, Nucula nucleus, Nucula sulcata, Parvicardium scabrum,* and *Hysteroconcha dione.*

The G1 group is formed of 4 stations representing the community of *Nuculana commutata* (63.15%). This community is located at a depth between – 40m and -70 m, and on a mixed soft-bottom (sands 40%, gravel 55%, muddy 5%). The malacological composition of this community is represented by *Nuculana commutata, Collochiton larvis, Euthria cornea, Limatula subauriculata, Lyonsia norwegica, Nucula sulcate, Tellina donacina, and Timoclea ovata.*

Groups G2 and G5 are each made up of 3 stations, and represent respectively the community of *Nucula sulcata* (53.84%) and the community of *Parvicardium scabrum* (22.22%) and *Notocochlis dillwynii* (22.22%). These two communities are located in the central area of the gulf, at a depth between - 55m and - 65m. The two species *Nucula sulcata* and *Tellina donacina* are present in both communities.

Finally, the community of *Nucula turgida* (27.27%) and *Venericardia antiquate* (18.18%), constitutes the G6 group. This group is located almost along the coast of the gulf (depth < -40m), and sand is the dominant sediment (>80%). The specific composition of this malacological community is represented by *Nucula turgida, Cardites antiquatus, Varicorbula gibba, Gari costulata, Tritia reticulata*, *Limatula subauriculata, Loripes orbiculatus, Peplum clavatum.*

In general, the malacological fauna of the Gulf of Oran is dominated by *Nuculana commutata* (33.33%), *Limatula subauriculata* (20.83%) and *Nucula sulcata* (20.83%). The large ecological stocks are mixicole species and coastal detritus species. The malacofauna of the Gulf of Oran is scarce and less diversified (29 species) due to the nature of the soft bottoms (sand and gravel) and the absence of terrigenous inputs.

The results of the null model analysis revealed that species associations are structured; this means that these are not random (observed index > mean index, and "p" value lower than 0.05). Whereas the niche sharing revealed that species share ecological niche, and in consequence, there are interspecific competition (see observed index > mean index; and "p" value lower than 0.05) (Table 3).

The distribution of macrobenthic species depends on the nature of the substrate and the quality of the water due to hydrodynamics and anthropic activities.

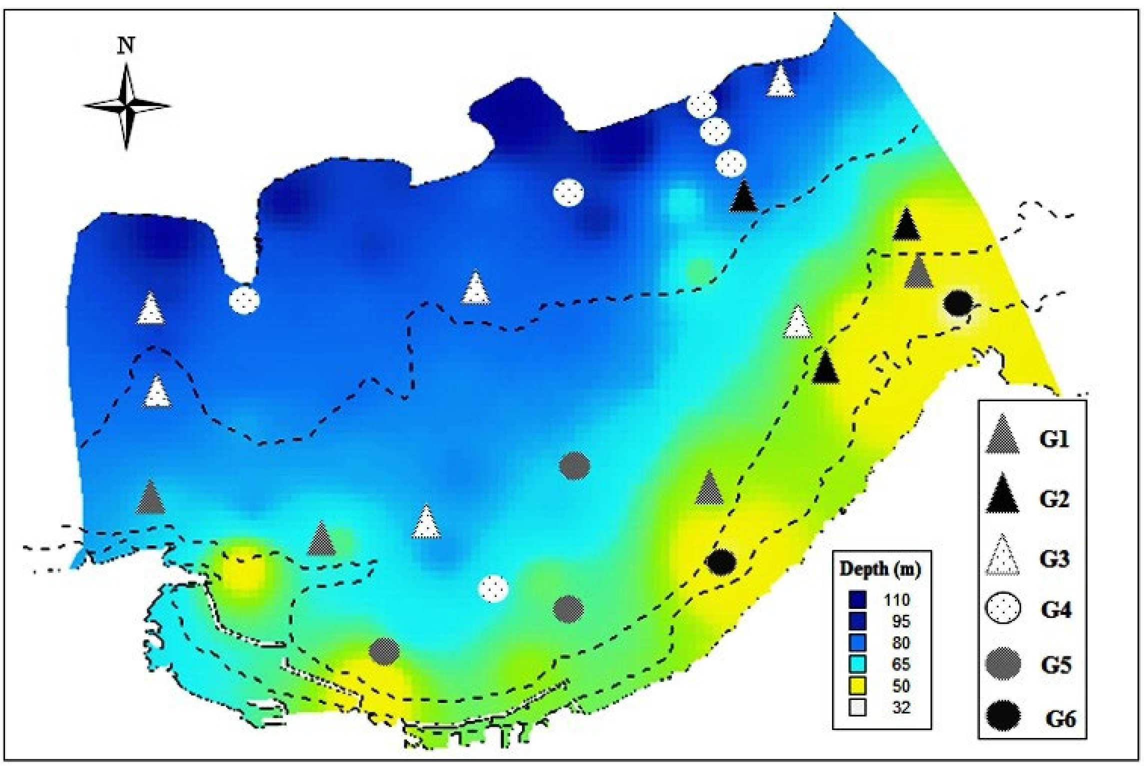

Figure 3. Mollusc communities in the Gulf of Oran

Table 3. Results of null model analysis in the present study

	Mean index	Observed index	Variance	Standard effect size	P
Species co-occurence	3.0924	3.2037	0.0017	2.6982	< 0.001
Niche sharing	0.1076	0.1419	0.0001	10.8170	<0.001

The present study indicates that the malacological fauna is less diverse than previously reported in other studied areas of the Algerian coast (Hassam, 1991; Oulmi, 1991). Data analysis such as zoological composition, nature of the sediments and the depth of the Molluscs of the Gulf of Oran has shown the six main communities. The results of the null model analysis revealed that species associations are structured. The results confirm that the malacological fauna of the Gulf of Oran is relatively poor from a specific point of view, both qualitatively and quantitatively, compared to what is known along the Algerian coast (Rebzani-Zahaf, 1992; Amar, 1998; Meziane et al., 2020; Bakalem et al., 2020) because only 29 species were observed. The conclusions also provide insight into the impact of the sedimentary compartment and the quality of the environment on the biogeography of the mollusc group. The knowledge of the mollusc assemblage in an area is a key requirement for the design of conservation measures (Lodeiros, 2011). Thus, surveys of benthic invertebrate assemblages in marine habitats, such as the anthropogenic region studied, are of fundamental importance to biomonitoring and the development of conservation strategies (Lima, 2017).

Conclusion

As a conclusion, a total of 29 species of benthic molluscs were identified. This study on the benthic malacofauna sampled on the Gulf of Oran constitutes an inventory of the coastal ecosystem and complements the database because bionomic research is rarely studied and fragmented. This study also provides a clear description of the main characteristics of six mollusc communities.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: Ethics committee approval is not required.

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Research Article

Pathological aspects of experimental infection of *Lactococcus garvieae* in European Sea Bass (*Dicentrarchus labrax* L.): Clinical, hematological, and histopathological parameters

Tülay AKAYLI[1], Çiğdem ÜRKÜ[1], Sena Zeynep GÖKEN[2]

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[1] Istanbul University, Faculty of Aquatic Science, 34134 Istanbul, Türkiye

[2] Istanbul University, Institute of Science, 34470 Istanbul, Türkiye

ORCID IDs of the author(s):
T.A. 0000-0003-2375-2224
Ç.Ü. 0000-0003-0381-9321
S.Z.G. 0000-0002-4102-8574

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Correspondence:
Tülay AKAYLI
E-mail: takayli@istanbul.edu.tr

ABSTRACT

This study, it was aimed to examine the clinical, hematological, and histopathological aspects of lactococcosis induced in European sea bass (*Dicentrarchus labrax*), which was experimentally infected with *Lactococcus garvieae*. For this purpose, the infection was induced intraperitoneally with *L. garvieae* strain (10^8 CFU/mL), and blood samples were collected from the infected fish on different days (6, 18, 26, 31, 36, 44, and 48th) of infection. The morphological structures, erythrocyte and leukocyte count, hematocrit value, sedimentation rate, and coagulation time of the blood cells in the collected samples were calculated. As a result of the infection, while there was a decrease in erythrocyte count, hematocrit value, and coagulation rate, in addition to changes in the morphological structure of blood cells, it was determined that there was a significant increase in the leukocyte count and sedimentation rate. Furthermore, histopathological examination was also performed in the organs of infected fish such as the brain, liver, spleen, kidney, heart, gill, and intestine. Histopathologically, in the infected sea bass, while hemorrhage, diffuse necrosis, and hyaline droplets were detected in the granular brain tissue, hyperemia and hemorrhage were noted in the liver and spleen, and widespread necrosis in the hematopoietic tissue of the kidney, in the gills intensive hyperplasia, an increase in the goblet cell were detected. Although there are studies on lactococcosis in various marine fish species around the world, the infection of *L. garvieae* in farmed sea bass and various parameters and pathological aspects were investigated in detail for the first time in this study. *L. garvieae* was determined to have clinical significance for European sea bass with a high economic value.

Keywords: Lactococcosis, European sea bass, Experimental infection, Blood, Histopathology

Introduction

Lactococcosis is a bacterial infection caused by *Lactococcus garvieae* and characterized by hemorrhagic septicemia (Vendrell et al., 2006; Austin and Austin, 2012). The infectious agent is zoonotic (Lee et al., 2020), and it has spread to many countries of the world and causes significant economic losses in marine and freshwater farmed fish (Vendrell et al., 2006; Ortega et al., 2020; Duman et al., 2020). The wide range of strains reported in many countries suggests that *L. garvieae* can be an opportunist bacteria cohabitant in different fish farming systems (Austin and Austin, 2012; Shahi et al., 2018). Previous studies indicate that it has been reported that the causative agent of lactococcosis causes disease in marine fish such as yellowtail (*Seriola quinqueradiata*) (Kawanishi et al., 2005), mullet (*Mugil cephalus*) (Chen et al., 2002), black rockfish (*Sebastes schlegeli*) (Kang et al., 2004), olive flounder (*Paralichthys olivaceous*) (Lee et al., 2001) and wild red sea wrasse (*Coris aygula*) (Colorni et al., 2003) and sorubim (*Pseudoplatystoma* sp.) (Rodrigues et al., 2020). However, there is no report stating that it causes disease in European sea bass (*Dicentrarchus labrax*).

It has been reported that lactococcosis develops depending on fish species in experimental infection studies performed on different fish species (Muzquiz et al. 1999; Chen et al., 2002; Urku and Timur, 2014), and trout has been reported to be the most susceptible species to the disease (Vendrell et al., 2006). Regardless of the infected fish species, exophthalmos, hemorrhage in the periorbital and intraocular area, base of the fins, perianal region, operculum, and swollen abdomen are among the typical external clinical findings of the disease. Internally, different researchers (Muzquiz et al., 1999; Chen et al., 2002; Urku and Timur, 2014; Didinen et al., 2014) have reported fluid accumulation in the peritoneal space, diffuse hemorrhage in the internal organs, splenomegaly, focal necrosis in the spleen and liver.

In recent studies, it has been reported that hematological analyses provide important data on the physiological status and health of cultured fish (Fazio, 2019). It has been stated that changes in hematological values may develop due to stress or environmental factors or in the presence of an infection (Gbore et al., 2006; Fazio et al., 2012). Therefore, hematological examinations should be performed to detect changes in blood parameters that occur in fish blood depending on the source of infection (Chen et al., 2005; Alsaid et al., 2014).

Lactococcosis is an acute systemic disease histopathologically characterized by congestion and hemorrhage (Vendrell et al., 2006; Roberts, 2012). It causes necrosis and diffuses hemorrhage and hyperemia in hemapoietic tissues such as the spleen, kidneys, and liver. It has been reported to cause inflammatory cell infiltration and melanomacrophage centers in the kidney tissue (Chen et al. 2002; Urku and Timur, 2014) as well as pericarditis (Chang et al., 2002; Didinen et al., 2014), panophthalmitis (Chen et al. 2002; Avcı et al. 2010; Didinen et al. 2014) in the fish infected with *L. garvieae.*

European sea bass is extensive and very significant commercial marine fish species in the Mediterranean Sea. According to FAO data (2014), Turkey ranks second among the countries producing sea bass such as Italy (67.00 tons), Greece (42.500 tons), Spain (14.455 tons), and Egypt (13,798 tons) with a production of 65.512 tons. (Di Trapani, et al., 2014). According to TUIK 2020 data, it has been reported that sea bass is the most common fish with a production of 148.907 tons. Due to its high economic value, the sea bass was selected as a model for inducing experimental lactococcosis. The aim of this study is to evaluate the clinical, hematological, and histopathological changes of lactococcosis induced in sea bass experimentally infected with *L. garvieae*.

Material and Methods

Fish Material and Experimental Groups

The experimental study plan was designed according to previous studies (Chang et al., 2002; Urku and Timur, 2014; Rodrigues et al., 2020). 130 European sea bass weighing 50-70 g on average were obtained from a commercial enterprise in the Aegean Sea. The fish were adapted to laboratory conditions for two weeks. TSA and NB media were used in this study. The number of bacteria was calculated according to Plumb and Bowser (1983). For determining the LD_{50} dose, bacterial concentrations were identified to be 10^6, 10^7, and 10^8 CFU/mL. For every dose, five fish were infected intraperitoneally. The LD_{50} dose was found to be 10^8 CFU/mL. After determining the LD_{50} dose, 100 sea bass were divided into two groups. For this purpose, two fiberglass tanks with a diameter of 1 m and a depth of 70 cm were used in the experiment. The *L. garvieae* strain isolated from diseased fish in an enterprise in Fethiye/Turkey was used in the experimental infection study. Bacteria incubated in Nutrient Broth (NB) medium for 24 hours were washed with PBS at 2500 rpm, then the optical density was adjusted to 1 (approximately 10^9 CFU/mL) at 540 nm in the spectrophotometer (Barnes et al., 2002) and then it was diluted and the bacterial count was adjusted to 10^8 CFU/mL. The experimental infection was induced by the intraperitoneal injection of 0.1 mL of bacterial culture (10^8 CFU/mL) per fish in the experimental group. 0.1 mL sterile PBS was administered intraperitoneally to 50 fish in the control group. During the experimental period, fish were fed twice a day. The water temperature was maintained at 22-23ºC, salinity at 35‰, and oxygen at 5-6, and the fish were monitored for clinical signs and mortality for 50 days.

Hematological Examination

Hematological sampling was performed considering the clinical signs of the disease. For this purpose, blood samples were taken from five fish showing clinical signs on the post-infection (dpi) days (6, 18, 26, 31, 36, 44, and 48th) when mortality were detected in the experimental group. Approximately 0.5 mL of blood sample was taken from each fish. There was a decrease in blood volume due to anemia developing towards the end of the experiment (0.1-0.2 mL/ each fish).

The blood was withdrawn from the caudal vessel into a sterile syringe containing lithium heparin anticoagulant (Svobodova and Vykusova, 1991), and leucocyte (WBC) and erythrocyte counts (RBC) were determined manually by utilizing a Neubauer hemocyter using Natt-Herrick's stain (Natt and Herrick, 1952). Haematocrits (Hct) were estimated using a microhematocrit machine (Goldenfarb et al., 1971). The micro-ESR method, described by Murachi (1959) was used to determine the erythrocyte sedimentation rate, and the slide coagulation method described by Wolf (1959) was used to determine the coagulation time of the blood taken from infected fish. The results were compared with the control group and the average of all the data obtained was taken according to Office Excel. To determine morphological changes in blood cells and confirm the presence of inoculated bacteria in the blood, thin blood smears were routinely prepared and stained by Giemsa/May-Grunwald (Blaxhall and Daisley, 1973).

Histopathological Examination

Experimental fish were anesthetized with 2-phenoxyethanol. For this purpose, fish samples were exposed to concentrations of 0.15 mL/L 2-Phenoxyethanol dissolved in aerated and dechlorinated tap water. Tissue samples taken from experimentally infected sea bass (heart, liver, kidney, gills, intestines, spleen, eyes, and brain) were fixed in 10% neutral-buffer formalin solution, embedded in paraffin, sectioned in 4-5 µm and stained routinely with hematoxylin and eosin (H&E) (Roberts, 2012).

Results and Discussion

The detection of hematological and tissue changes caused by pathogens in fish organs by experimental infection studies plays a significant role in controlling the outbreaks of bacterial fish diseases (Alsaid et al., 2014; Fazio et al., 2019). In particular, the evaluation of these data gives detailed information about the pathogenesis, diagnosis, control, and treatment of the disease. Although few studies have been carried out to assess the blood parameters, tissue changes and clinical findings of lactococcosis infected freshwater fish species (Avsever et al., 2014; Khosravi et al., 2018), the present study describes the details of changes in clinical, hematological, and histopathological parameters induced by lactococcosis in European sea bass for the first time.

After the 6th day of the experimental infection, it was observed that deaths also started with the decrease in feed intake and slowing of movements in infected fish. At the end of the experiment, it was observed that four fish remained in the experimental group. Mortality caused by the *L. garvieae* strain is given in Figure 1 in detail. It was observed that an administration dose of 10^8 CFU/mL caused mortality in experimental fish.

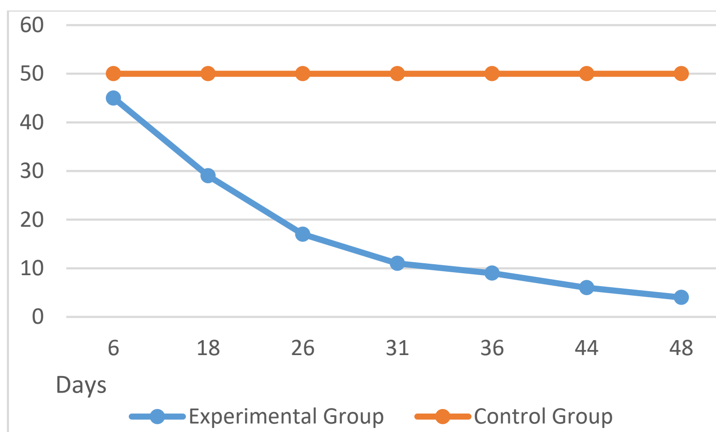

Figure 1. Mortality range of European sea bass infected with *L. garvieae*

Experimental infection studies have reported that the disease-related symptoms develop at different times depending on the fish species and cause mortality. It has been stated that this time is 2-3 days in yellowtail (Itami et al., 1996), 2 days in mullet (Chen et al., 2002), and 3-4 days in trout (Muzquiz et al., 1999). In a previous study, *L. garvieae* (2×10^6 CFU/mL) did not cause death and any clinical signs in sea bass (Türe et al., 2014). However, in the present study, administration dose of 10^8 CFU fish^{-1} caused deaths in experimental fish. In this study, it was determined that deaths occurred with a decrease in feed intake and slowing of movements on the 6th day of inoculation in the infected sea bass, as reported by Vendrell et al. (2006). Considering all the studies on experimental infection, the fact that *L. garvieae* has a short incubation period and causes death by the virulence mechanism is also clinically important for other fish species that are economically valuable and farmed.

External clinical findings such as hemorrhage in the abdomen, operculum (Figure 2a) and eyes, darkening of the skin in the back region, and scoliosis were detected in sea bass experimentally infected with *L. garvieae*. Furthermore, internal clinical findings such as fatty degeneration in the liver, hyperemia and hemorrhage in the visceral organs, splenomegaly, and necrosis in the anterior kidney were observed (Figure 2b). No clinical findings were noted in the control group fish.

External clinical findings, such as hemorrhage in the intraocular area, the base of the fins, and operculum, and darkening, reported in the fish infected with *L. garvieae* were also detected in the infected sea bass in this study (Prieta, 1993; Eldar and Ghittino, 1999; Mu´zquiz et al., 1999). Exophthalmos, defined as the typical clinical manifestation of the disease, was not observed in the sea bass infected in this study. Scoliosis, which has not been reported until the present day, was detected in the infected sea bass in this study for the first time. Furthermore, as reported by (Eldar and Ghittino, 1999; Mu´zquiz et al., 1999; Urku and Timur, 2014) depending on the course of the disease, diffuse hemorrhage and hyperemia, splenomegaly in the internal organs, focal necrosis in the spleen and liver were also observed in the infected sea bass.

As a result of the 50-day experimental infection study, it was determined that the hematocrit value (Hct) of the fish in the control group remained constant at 30%, while it was observed to decrease to 4% in the blood samples taken from the infected fish (Table 1). While the sedimentation rate of the blood samples taken from the control group fish was determined to be 3 mm during the experiment, this value increased to 34 mm in the experimental group (Table 1). It was noted that the coagulation time in the control group fish was 13 minutes throughout the whole experiment, while it decreased to 1.5 minutes in the infected fish (Table 1). While the erythrocyte count (RBC) in the control group fish was between 3.2×10^6-5.1×10^6 /mL, it was found that this value was 4.2×10^6/mL in the fish infected with *L. garvieae* at the beginning and decreased to an average of 0.8 $\times10^6$/mL at the end of the experiment. While the leukocyte count (WBC) was between 0.8×10^3-1.6×10^3 /mL in the control fish, the average leukocyte count increased to 8.3 $\times10^3$/mL in the infected fish (48th day) (Table 1).

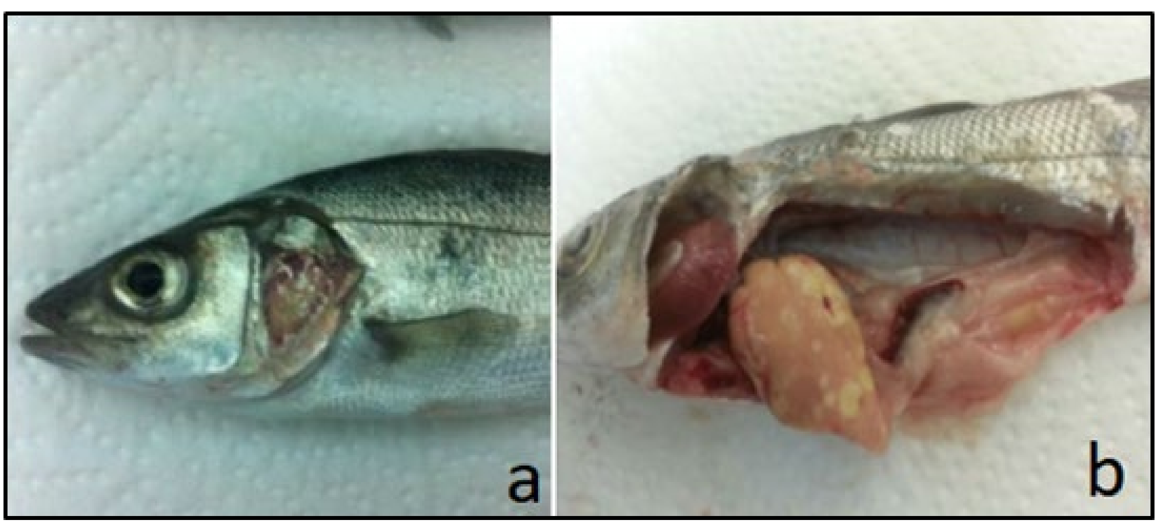

Figure 2. European sea bass infected with *L. garvieae:* (a) Large hemorrhage in the operculum; (b) Fatty degeneration, hyperemia in the liver, necrosis in the anterior kidney

Table 1. Hematological findings in infected and non-infected European sea bass

Experimental groups	Hct (%)	Sedimentation rate (mm)	Coagulation time (min)	RBC ($\times10^6$ µL^{-1})	WBC ($\times10^3$ µL^{-1})
Post-infection days					
6	21	4	12	3.01	0.9
18	18	8	10	2.66	1.5
26	13	15	9	2.56	2.8
31	10	19	7	2.05	3.6
36	8	24	4	1.45	5.8
44	5	29	2	1.23	7.0
48	4	34	1.5	0.8	8.3
Control	30	3	13	3.2- 5.1	0.8x-1.6

When the blood smears of the diseased fish in the experimental group were compared with the control group (Figure 3a), defects were detected in the morphological structure of erythrocytes from the first week of the inoculation (Figure 3b, c). In the later days of the infection, in addition to lymphocytes, monocyte cells that phagocytized bacterial cells were detected (Figure 3d).

Hematological analysis such as sedimentation quantity, total leukocyte count, and type provides information about the existence of the bacterial fish disease (Fazio, 2019; Alsaid et al., 2014). The erythrocyte count is an important blood parameter used to determine the functions of hemopoietic tissues (Witeska, 2005). Different researchers have reported that bacterial infections in fish usually result in a decrease in the erythrocyte count (Pathiratne and Rajapakshe, 1998; Rehulka, 2002; Chen et al., 2004; Alsaid et al., 2014). However, although it has been stated that there is no change in this value in the Nile tilapia infected with *Enterococcus* sp., which is closely related to the genus of *Lactococcus* (Martins et al., 2008), a decrease in RBC and hematocrit values has been reported in rainbow trout (Barham et al., 1980) and tilapia (Alsaid et al., 2014) infected with *Streptococcus* sp., a closely related species and sorubim infected with *L. garvieae* (Rodrigues et al., 2020). The decrease in the erythrocyte count detected in this study may have developed as a result of the destruction in the hemopoietic tissues of organs such as the spleen and kidney, which we detected histopathologically. Furthermore, it has been reported that the decrease in hematocrit percentage, as well as the erythrocyte count, is associated with anemia (Rajendiran et al., 2008). In this study, it is thought that anemia occurred at the end of the experiment in relation to hemorrhage and hyperemia that we detected in the spleen and liver tissues of the infected sea bass.

Leukocytes are one of the cellular components associated with the immune system for bacteria and foreign material in the blood (Magnadóttir, 2006; Castro and Tafalla, 2015). An increase in the WBC count may indicate the presence of bacterial pathogens, especially as a response of the non-specific immune system. As reported in trout infected with *L. garvieae* (Avsever et al., 2014; Khosravi et al., 2018) increase in the WBC value was also detected in the infected sea bass in this study.

It has been reported that the hematocrit value of healthy fish living in seawater varies between 15.3-52.5% according to different species (Satheeshkumar et al., 2012), while this value is 51.18% in sea bass (Fazio, 2019). As reported in the farmed Korean rockfish (*Sebastas schlegeli*) and rainbow trout (*O. mykiss*) infected with *L. garvieae* (Kobayashi et al., 2004; Khosravi et al., 2018), the decrease in the hematocrit value due to the disease was also detected in the infected sea bass in this study, and this value was found to be 4%.

Cells located in the inner wall of the vessel activate many cells and systems involved in the coagulation mechanism. Fish need very effective hemostatic mechanisms to respond to vascular damage and other general injuries in the case of infection (Tavares-Dias and Oliveira, 2009). However, the knowledge of the coagulation system in fish is rather limited. It has been reported that *L. garvieae* causes lesions in the vascular endothelium and damages these cells (Prieta, 1993; Vendrell et al., 2006). In this study, we predict that the gradual decrease in the coagulation level originates from the pathomorphological changes caused by the agent in the infected fish's vascular systems, as reported by Prieta (1993).

The sedimentation rate is a parameter related to erythrocytes and can change in situations such as disease, stress, and exposure to pollutants (Kori-Siakpere and Ubogu, 2008). In these cases, it is reported that the platelet counts and coagulation time, which are coagulation-related parameters, may change (Tavares-Dias and Oliveira, 2009). In the presence of an infection, protein structures such as fibrinogen and globulin in the plasma combine with red blood cells, causing erythrocytes to precipitate faster, and the sedimentation rate of the blood with anemia due to infection increases (Fazio, 2019). Likewise, in this study, a significant increase in sedimentation rate was detected due to the decrease in the erythrocyte count in infected fish, in addition to the picture of anemia formed as a result of severe hyperemia and hemorrhage in the last days of the infection.

Histopathologically diffuse necrotic areas (Figure 4a), hemorrhage, hyperemia, and melanomacrophage foci in the spleen (Figure 4b); vacuolar degeneration, hyperemia, and hemorrhage in the liver (Figure 4c); necrosis, hyperemia, and tubular edema in the inter-renal hematopoietic tissue of the kidney (Figure 4d); hyperemia, large necrotic areas, and hyaline droplets in the brain tissue (Figure 4e) were detected in the infected sea bass. Furthermore, diffuse necrosis and hemorrhages were observed in the cardiac muscle cells of heart, and as a result of necrosis in the enterocyte cells covering the intestinal lumen, spillage into the lumen of the necrotic enterocyte cells (Figure 4g) and severe hyperplasia in the gills and an increase in the goblet cell count (Figure 4ı) were observed.

Pathomorphological changes in the fish infected with *L. garvieae* indicate acute systemic disease characterized by congestion and hemorrhages in internal organs (Prieta, 1993; Aizpurua et al., 1999). Likewise, diffuse hemorrhage and hyperemia were detected in the spleen and liver tissue samples in this study, when sections obtained from spleen and liver compared with control fish group. It was determined that this picture transformed into anemia with the change in blood values in the last days of the infection. Furthermore, other researchers (Eldar and Ghittino, 1999; Vendrell et al., 2006; Avci et al., 2010; Urku and Timur, 2014) have already described hemorrhages among the myocardial muscles of the heart and necrosis, hemorrhage and hyperemia in the liver, spleen, and kidney, observed in this study. It has been reported that some histopathological findings we detected in the infected sea bass are also similar to the pathological findings observed in diseases with hemorrhagic septicemia (Vendrell et al., 2006; Roberts, 2012). Furthermore, although hyaline droplets have been reported in the tubular lumens of the kidney tissue of infected trout (Chang et al., 2002; Didinen et al., 2014), the presence of hyaline droplets in the brain tissue of infected sea bass was detected for the first time in this study.

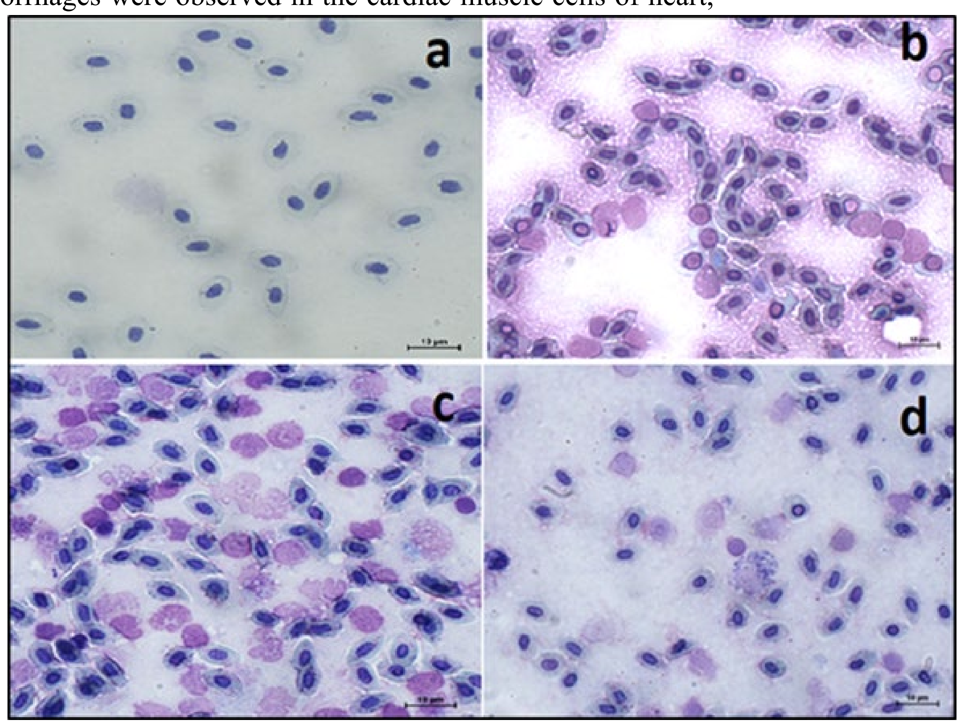

Figure 3. Morphological changes seen in blood cells of infected sea bass: (a) Control group; (b) Erythrocyte cells with altered morphological structures adhered to each other (18^{th} day); (c) Lymphocyte cells on day 26^{th} of infection; (d) Monocyte cells phagocytosed bacterial cells.

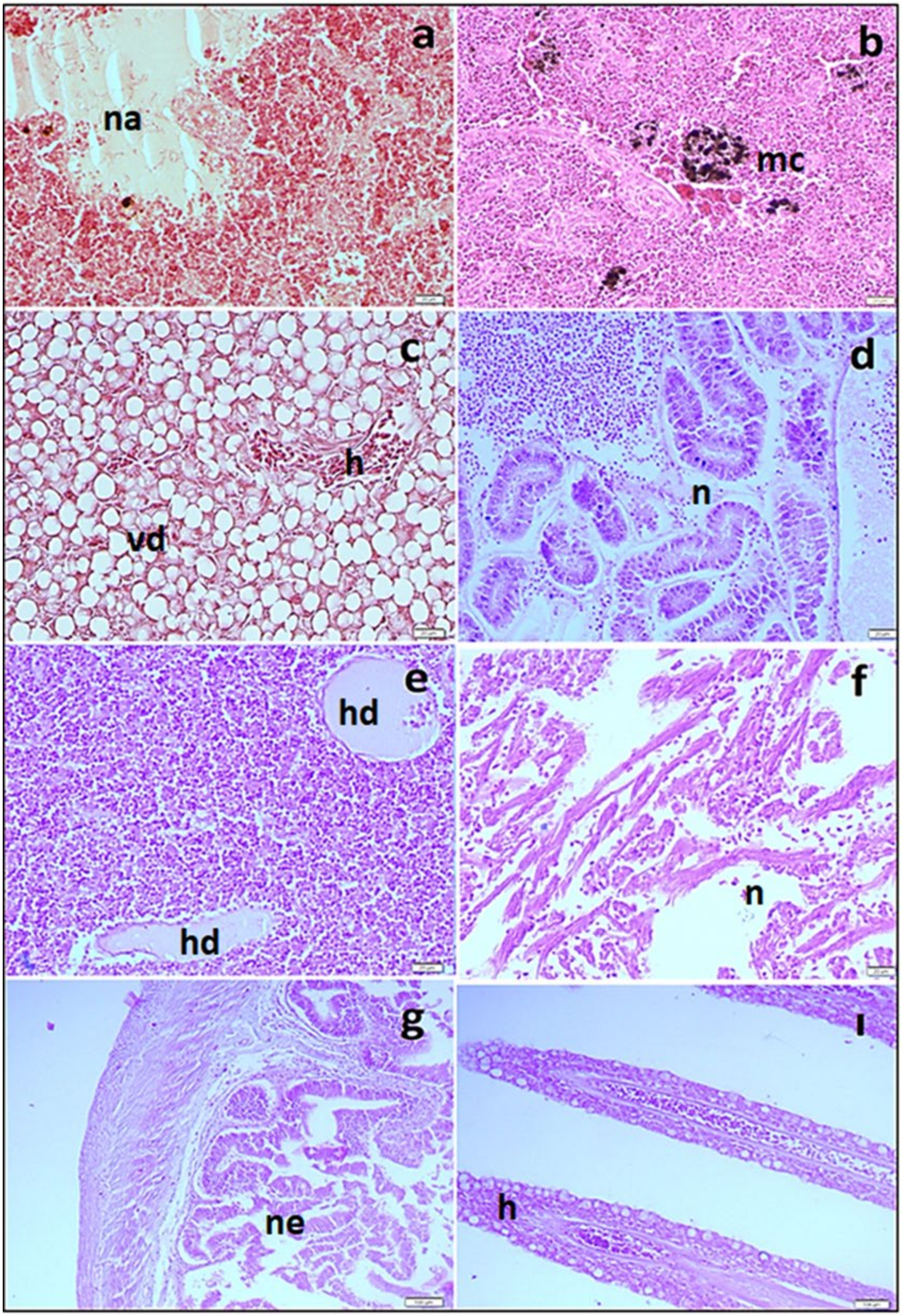

Figure 4. European sea bass infected with *L. garvieae*: (a) Diffuse necrotic areas (na); (b) hemorrhage, hyperemia, and melanomacrophage centers (mc) in the spleen; (c) vacuolar degeneration (vd), hemorrhage and hyperemia (h) in the liver; (d) necrosis (n) in the inter-renal hematopoietic tissue, tubular edema in the kidney; (e) hyaline droplets (hd) in the brain tissue; (f) necrosis (n) in the heart muscles and hemorrhage; (g) spillage into the lumen of the necrotic enterocyte (ne) cells; (ı) intensive hyperplasia (h) in the gills and an increase in the goblet cell

Conclusion

In this study, clinical, hematological, and histopathological changes caused by the infection were revealed in sea bass experimentally infected with *L. garvieae.* It was determined that this pathogen bacterium caused mortality in sea bass by creating a pathomorphological structure. Furthermore, it was revealed that *L. garvieae* caused acute systemic disease characterized by congestion and hemorrhage in sea bass histopathologically, and this clinical finding was confirmed by hematological data. Clinically, it was observed that the structure of anemia took shape in infected fish in the later stages of the disease. As a result, *L. garvieae* was found to have clinical significance, especially for sea bass with high economic value.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: Experimental protocol was approved by the Ethics Committee for Animal Experiments of the University of Istanbul (2014/47).

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Disclosure: -

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Additional record of *Anatolichthys marassantensis* from Simenlik-Akgöl Lagoon in lower Yeşilırmak Drainage (Türkiye)

Melek ÖZPİÇAK, Semra SAYGIN, Savaş YILMAZ, Nazmi POLAT

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Ondokuz Mayıs University, Sciences and Arts Faculty, Biology Department, Samsun, Türkiye

ORCID IDs of the author(s):
M.Ö. 0000-0003-3506-4242
S.S. 0000-0002-3249-5074
S.Y. 0000-0003-2859-4886
N.P. 0000-0001-9785-9927

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Correspondence:
Melek ÖZBIÇAK
E-mail: melek.zengin@omu.edu.tr

ABSTRACT

Anatolichthys marassantensis widely distributed in Kızılırmak drainage, and few records also available from Yeşilırmak drainage. However, the existing literature has not provided a morphological comparison between Kızılırmak and Yeşilırmak populations in a systematic approach. The present study examines, for the first time, the morphology/morphometry of *A. marassantensis* from both of the drainages based on additional material from Simenlik-Akgöl Lagoon Lake in Yeşilırmak drainage and published data (type materials) from Kızılırmak River. A total of 40 specimens from Simenlik-Akgöl Lagoon were compared for morphometric and morphological characters with the type measurements. According to the results of this study, morphological characters largely overlapped between selected populations of Yeşilırmak and Kızılırmak Rivers. The results obtained from this study clearly demonstrate the presence of *A. marassantensis* in a new location in the Yeşilırmak drainage with consistent morphological data.

Keywords: *Anatolichthys marassantensis,* Simenlik-Akgöl Lagoon Lake, Aphaniidae, New record

Introduction

Freshwater fish diversity and endemism are high in important biodiversity hotspots such as the Caucasus, Iran-Anatolia, and the Mediterranean basin (Perea et al., 2010). Türkiye has a rich diversity and endemism in terms of freshwater fish, because of having both European- and Asian origin species (Tarkan et al., 2015). Killifishes of the family Aphaniidae is one of the largest groups of fishes in the Western Palaearctic and represented by the highest number of species in Central Anatolia (Hrbek & Meyer, 2003; Freyhof &Yoğurtcuoglu, 2020). The killifishes in Anatolia are also unique such that represented by four genera, two of which are endemic, i.e. the monospecific *Kosswigichthys* and the most species-rich genus *Anatolichthys*. While many of the species of *Anatolichthys* are restricted to a single lake basin with a definite distribution, others are widely distributed over a wide range in larger river drainages with poorer data on their ranges. *Anatolichthys marassantensis* is one of such wider species distributed in Kızılırmak and Yeşilırmak drainages.

Freyhof & Yoğurtçuoğlu (2020) reported that 21 species of killifishes are distributed in Anatolia and many of which are threatened and poorly explored. The populations from Kızılırmak and Sultan marshes were collectively treated as *Aphanius danfordii* until the description of populations from Kızılırmak River drainage as *A. marassantensis* by Pfleiderer et al. (2014). Since that time, *Anatolichthys marassantensis* was demonstrated particularly from Hirfanlı Reservoir and Kızılırmak River Delta (Yoğurtçuoğlu & Ekmekçi, 2013). Gül and Atasağun (2022) have reported from Delice River (Kızılırmak). Hrbek et al. (2002) reported population from lower Yeşilırmak River. Yoğurtçuoğlu & Ekmekçi (2017) have reported new records of the *Aphanius marassantensis* from Central Yeşilırmak, and Benzer (2018) have reported from Süreyyabey Dam Lake from Yeşilırmak. After a recent taxonomic revision the species of *A. anatoliae* group was re-transferred to *Anatolichthys* (Freyhof & Yoğurtçuoğlu, 2020).

Although these publications has tried to reveal the exact distribution of *Anatolichthys marassantensis,* none of them provided a morphological comparison between Kızılırmak and Yeşilırmak River populations. Therefore, the aim of this study is to test if populations from Kızılırmak and Yeşilırmak River differed. Moreover, improving the knowledge of the distribution of *A. marassantensis* is crucial in order to adequately inform conservation planning and thus guarantee its long-term survival. In this study, we have proved the occurrence of *A. marassantensis* in the Simenlik-Akgöl Lagoon Lake from Samsun. Also, *A. marassantensis* is a new record for this area and fish fauna of Samsun.

Material and Methods

Sampling Site

Simenlik-Akgöl Lagoon Lake is located in 25 km to the center of Terme in the province of Samsun. This area is located within the borders of the Yeşilırmak Delta. Simenlik Lake, with an area of 80 hectares and a delta depth of 0.5-3 m, connects to the Black Sea by a channel from the northwest; it creates a lagoon complex by linking to Akgöl, which has an area of 50 hectares and an average depth of 3 m, via a channel from the southeast (Anonymous, 1990). Simenlik Lake and its surrounding lakes and wetlands (16043.0 he) were first declared as a conservation area in 1975 (Anonymous, 2013; Karaer et al., 2017). After this decision, Simenlik-Akgöl Lagoon Lake and other aquatic ecosystems around it were evaluated together and taken under protection. The fact that Simenlik Lake is connected to the Black Sea as a result of rising water levels during rainy seasons affects the lake's salinity. As a result, numerous freshwater animals are impacted by this situation (Karaer et al., 2017). *Anatolichthys marassantensis* samples were collected from the following coordinates, 41°16' 41.952" N - 36°56'29.868" E. *Mugil cephalus*, *Mugil saliens*, *Esox lucius*, *Carassius gibelio*, *Tinca tinca* and *Abramis brama* were recorded from Simenlik by Uğurlu (Helli) & Polat, 2003. In addition, Uğurlu et al. (2008) and Polat & Uğurlu (2011) were added *Syngnathus abaster*, *Platichthys flesus*, *Atherina boyeri*, *Gasterosteus aculeatus*, *Neogobius melanostomus* and *Proterorhinus marmoratus* to fish fauna of Simenlik-Akgöl Lagoon Lake. We followed Eschmeyer's Catalog of Fishes (Fricke et al., 2022) for the most recent and valid taxonomy.

Table 1. Morphometric data of *Anatolichthys marassantensis* from Terme Stream (Black Sea-Türkiye)

Morphological variables	Holotype	This study		Pfleiderer et al. (2014)	
		Female N=15	Male N=24	Female N=15	Male N=14
Standard length (mm)	32.61	31.27-37.31	25.70-30.30	31.3-42.6	28.3-37.5
In percent of standard length					
Head length	35.87	26.59-38.32 (29.80 ±1.8)	27.41-40.73 (33.89 ±0.6)	26.5-30.8 (29.1 ±1.1)	27.9-32.2 (30.5 ±1.2)
Predorsal length	70.77	64.95-76.06 (69.03 ±2.8)	62.18-75.40 (66.68 ±0.7)	61.1-65.3 (63.4 ±1.4)	57.0-63.0 (60.5 ±1.8)
Preanal length	72.79	68.91-79.36 (74.48 ±1.5)	56.40-78.22 (69.94 ±1.3)	68.4-74.6 (70.8 ±1.7)	63.6-69.9 (66.3 ±2.2)
Prepelvic length	56.67	54.98-63.39 (58.04 ±2.3)	50.48-70.17 (58.27 ±0.9)	52.8-59.2 (55.9 ±2.1)	51.1-55.6 (53.5 ±1.3)
Height of dorsal fin	16.56	12.82-24.18 (18.58 ±3.1)	18.61-29.33 (24.21 ±2.7)	*	*
Length of dorsal fin base length	21.92	13.98-23.47 (16.77 ±2.6)	17.18-25.39 (21.40 ±2.3)	11.0-16.5 (13.6 ±1.3)	14.6-20.1 (17.4 ±1.5)
Length of pectoral fin	13.95	12.03-18.04 (14.09 ±1.6)	13.56-21.00 (16.84 ±2.0)	12.6-17.1 (15.4 ±1.0)	16.1-20.2 (17.8 ±1.0)
Length of anal fin base	14.54	9.47-15.98 (12.40 ±1.9)	11.75-18.58 (14.87 ±1.7)	8.9-11.3 (10.0 ±0.7)	10.9-16.5 (13.8 ±1.6)
Length of pelvic fin	12.57	6.40-13.77 (10.70 ±2.0)	9.70-17.26 (12.29 ±2.1)	8.1-12.1 (10.4 ±1.0)	12.0-14.2 (12.7 ±0.6)
Length of upper caudal fin lobe	18.89	17.85-25.70 (20.94 ±1.8)	19.14-27.71 (22.94 ±2.3)	*	*
Distance between dorsal and caudal fin orgin	38.91	30.68-43.91 (36.15 ±3.1)	26.79-65.06 (41.33 ±2.9)	*	*
Distance between pectoral and ventral fin	26.04	23.81-29.51 (27.28 ±1.7)	19.07-30.31 (23.91 ±2.8)	20.6-26.9 (24.7 ±1.7)	20.3-26.7 (20.3 ±1.8)
Distance between ventral and anal fin	18.12	16.21-24.92 (20.02 ±2.6)	14.97-28.17 (19.14 ±2.9)	14.9-20.0 (16.5 ±1.3)	12.4-17.4 (14.4 ±1.5)
Distance between anal and caudal fin	30.21	21.89-30.86 (26.36 ±1.3)	25.73-34.58 (30.40 ±2.7)	*	*
Caudal peduncle length	17.54	15.47-21.49 (17.90 ±1.6)	17.11-24.11 (20.41 ±1.4)	16.3-18.7 (17.3 ±0.7)	18.9-23.9 (20.9 ±1.2)
Minimum body depth	15.88	13.64-17.26 (15.87 ±1.0)	16.54-22.40 (19.27 ±1.4)	*	*
Maximum body depth	29.90	29.48-37.08 (31.79 ±2.1)	28.15-37.77 (33.68 ±2.0)	19.2-24.5 (21.6 ±1.6)	16.8-23.2 (19.9 ±1.8)
In percent of head length					
Head depth at eye	65.56	60.16-79.83 (70.12 ±4.1)	54.52-82.69 (68.90 ±3.8)	59.0-67.0 (62.7 ±2.8)	61.0-72.0 (64.0 ±2.9)
Eye diameter	27.27	23.90-34.33 (30.00 ±2.1)	22.77-43.41 (30.70 ±1.2)	29.0-33.0 (30.1 ±1.4)	30.0-35.0 (32.2 ±1.6)
Interorbital distance	32.81	29.97-46.53 (38.08 ±2.7)	25.81-38.27 (32.16 ±2.1)	38.0-43.0 (40.4 ±1.5)	38.0-44.0 (40.7 ±2.1)
Preorbital distance	16.15	14.76-29.56 (21.50 ±3.1)	11.41-32.15 (22.67 ±2.8)	*	*
Postorbital distance	49.91	41.71-65.58 (50.95 ±2.9)	41.26-63.57 (50.16 ±3.8)	41.0-48.0 (45.3 ±1.9)	40.0-45.0 (42.7 ±1.5)
Snout length	7.89	7.19-16.34 (10.83 ±2.4)	6.07-15.50 (9.97 ±2.0)	17.0-23.0 (19.9 ±2.0)	16.0-21.0 (18.5 ±1.4)

*There is no data in Pfleiderer et al. (2014)

Morphological Analyses

A total of 40 *A. marassantensis* individuals were collected from Simenlik-Akgöl Lagoon (Samsun) on 20 May 2021 with an electro-fishing device. Fish were immediately fixed in 4% formaldehyde in the field and stored permanently in 70% ethanol. Identification of the specimens was based on the morphological characters following Pfleiderer et al. (2014), Yoğurtçuoğlu & Ekmekçi (2017) and Freyhof & Yoğurtcuoğlu (2020). Sex determination was based on external coloration of individuals. Weight was measured to the nearest 0.01 g. A total of 25 morphometric characters were measured from samples (Table 1). All measurements were made point to point taken on the left side of each specimen with a digital caliper by the same person according to Pfleiderer et al. (2014) and Kottelat & Freyhof (2007) (±0.01 mm). Also, morphological data in Pfleiderer et al. (2014) were used for comprasions between specimens from Kızılırmak and Yeşilırmak drainages.

Results and Discussion

Türkiye is a very important fauna detection center due to its geological location. Periodic monitoring of ichthyofauna and updating fish fauna are very important due to global climate change. Our findings provide light on distribution of *A. marassantensis*. This study is very important as it is the first record of the species for the Yeşilırmak Lagoon Region (Simenit-Akgöl Lagoon).

The general body shape of *A. marassantensis* from Simenlik-Akgöl Lagoon Lake is displayed in Figure 1. The minimum and maximum total lengths and weights of the samples are 32.63-45.26 mm and 0.60-1.89 g, respectively. And also, morphological data were offered in Table 1. Samples were sexed as 24 male and 16 female. The formulations of the fins are 9-10 branched rays in dorsal, 8-10 branched rays in anal, 14-16 rays in pectoral. Caudal fin is truncate or rounded. There is sexual dimorphism between males and females. Colouration varies between males and females (Figure 1). Males have 12–13 dark-brown lateral bars in flank. Pfleiderer et al. (2014) indicated 8-13 dark-brown lateral bars in flank of males. These differences observed in morphology and morphometric measurements may be due to phenotypic plasticity and environmental variables. Also, in terms of fisheries management and biology, it is important to determine the phenotypic variations caused by environmental factors (Chen et al., 2015; Freire et al., 2017; Chavarie et al., 2021; Schroeder et al., 2022). Dorsal and ventral profiles convex between tip of snout and dorsal- and anal-fin origins, rarely straight; straight or slightly concave along caudal peduncle. There are no lateral bars in females. All characteristics of the captured samples were determined by comparing them with Pfleiderer et al. (2014). According to Pfleiderer et al. (2014), minimum and maximum total lengths are 28.3-42.6 mm, samples in present study are bigger. However, the percentage values of morphometric data at standard length and head length are similar with Pfleiderer et al. (2014).

Although records were given from Kızılırmak and Yeşilırmak Basins in previous studies on *A. marassantensis* (Hrbek et al., 2002; Pfleiderer et al., 2014; Yoğurtçuoğlu & Ekmekçi, 2017; Benzer, 2018; Benzer, 2021a; Benzer, 2021b; Gül & Atasağun, 2022), there is no record from Simenlik-Akgöl Lagoon, which is located in Yeşilırmak Basin, too (Figure 2-Table 2). The fact that the species was not found in previous studies carried out in the Simenlik-Akgöl lagoon may be related to the sampling gears. When the literature is examined, there are different studies about morphometry and phylogenetic of Aphaniid species (Wildekamp, 1993; Parker & Kornfield, 1995; Hrbek et al., 2002; Esmaeili et al., 2014; Teimori et al., 2014; Benzer, 2018; Esmaeili et al., 2020; Freyhof & Yoğurtçuoğlu, 2020; Kuyumcu, 2021). Molecular and morphometric investigations have corroborated taxonomic classification of *A. marassantensis* (Pfleiderer et al., 2014; Freyhof & Yoğurtçuoğlu, 2020).

Documenting biodiversity data is of crucial importance for a first step in conservation studies. Here the exact and most recent distribution data of endemic *A. marassantensis* is provided.

Table 2. Old and new records of *Aphanius marassantensis* from literature

Drainage	Province	Coordinates	References
Kızılırmak	Kayseri	38° 40' 0" N 35° 17' 59" E	Fredie
Kızılırmak	Kayseri	38° 41' 52" N 35° 19' 62" E	Hrbek et al. (2002)
Kızılırmak	Kayseri	38°23'25" N 35°21'56" E	Freyhof et al. (2017)
Kızılırmak	Kırşehir	38°59′15" N 34°06′58" E	Bardakci et al. (2004)
Kızılırmak	Ankara	39° 09' 32.4" N 33° 36' 42.5" E	Yoğurtçuoğlu (2010)
Yeşilırmak	Çorum	40° 22' N 35° 13' E	Yoğurtçuoğlu and Ekmekçi (2017)
Yeşilırmak	Çorum	40° 23'N 35° 15'E	Yoğurtçuoğlu and Ekmekçi (2017)
Yeşilırmak	Çorum	40° 26' N 35° 16' E	Yoğurtçuoğlu and Ekmekçi (2017)
Kızılırmak	Sinop	42° 21' 24" N 35° 1' 5" E	Karsli and Aral (2010)
Kızılırmak	Ankara	39° 0' 0" N 33° 01' 00" E	Yoğurtçuoğlu (2009)
Kızılırmak	Kırşehir	39° 00' 00" N 33° 00' 00" E	Yoğurtçuoğlu (2009)
Yeşilırmak	Süreyyabey Dam-Yozgat	35°28' N 39°55' E	Benzer (2018)
Kızılırmak	Karasu Brook	39° 19' 55.63" N 34° 48' 12.77" E	Gül and Atasagun (2022)
Kızılırmak	Kanak Brook	39° 30' 22.40" N 34° 48' 12.77" E	Gül and Atasagun (2022)
Yeşilırmak	Samsun-Simenlik-Akgöl Lagoon	41°16' 41.952" N 36° 56' 29.868" E	This study

(a)

(b)

Figure 1. General body shape of *Anatolichthys marassantensis* (a) Female (35.81 mm SL), (b) Male (30.30 mm SL), black bar represents 10 mm

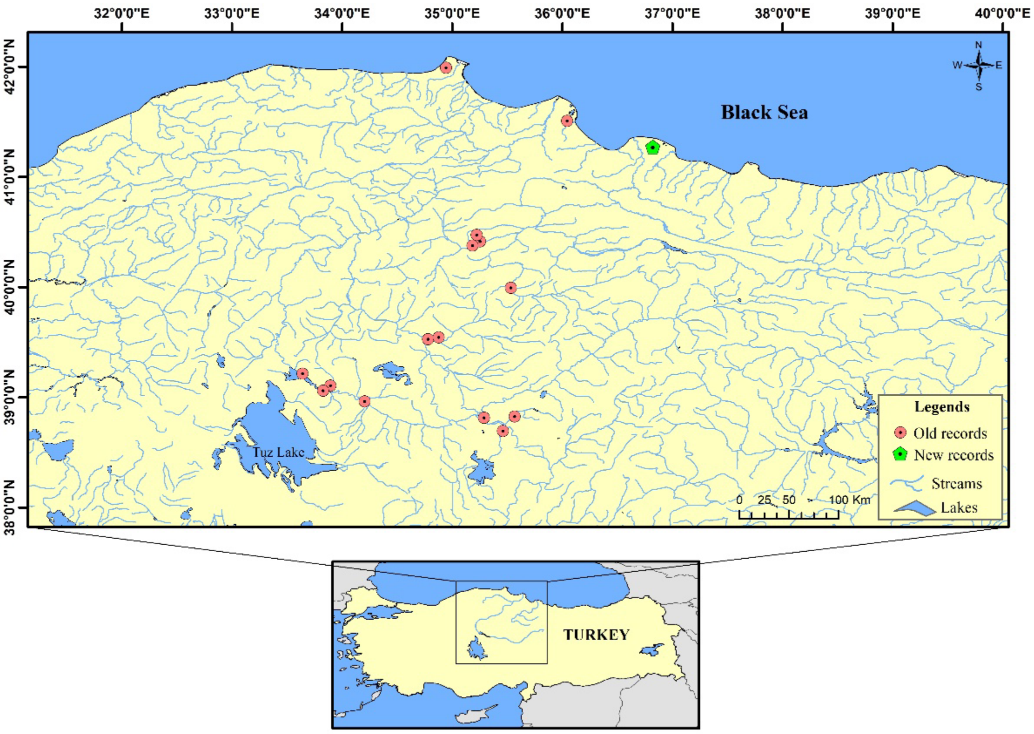

Figure 2. Distribution of *Anatolichthys marassantensis* in Kızılırmak and Yeşilırmak drainages

Conclusion

In this study, we report a new record of *A. marassantensis* from Simenlik-Akgöl Lagoon. The results of this study reveal that the existence of a new fish species has been recorded for the fish fauna of Simenlik-Akgöl Lagoon, and the distribution area of *A. marassantensis* has reached a different location in Yeşilırmak drainage.

Studies on fish populations is important from various viewpoints including evolution, ecology, behavior, conservation, water resource management, and stock assessment (AnvariFar et al., 2011). Unfortunately, changes in climatic conditions which are affecting the water regime, and anthropological activities are the main threats for the basin and lakes. It is obvious that all of these negative factors will have a detrimental impact on the species' range. The study of genetic stocks of endemic species and the identification of populations will lead to the appropriate and successful management of fish stocks. For all these reasons, a conservation strategy should be created for *A. marassantensis*.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

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Black soldier fly (*Hermetia illucens*) larvae meal improves growth performance, feed utilization, amino acids profile, and economic benefits of Nile tilapia (*Oreochromis niloticus,* L.)

Simon M. SHATI[1*], **Mary A. OPIYO**[2], **Rita N. NAIRUTI**[2], **Amon P. SHOKO**[3], **Fridah MUNYI**[4], **Erick O. OGELLO**[1]

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1. Maseno University, Department of Animal and Fisheries Sciences, Private Bag, Maseno, Kenya
2. Kenya Marine and Fisheries Research Institute, National Aquaculture Research Development and Training Center, P.O. Box 451-10230, Sagana, Kenya
3. Tanzania Fisheries Research Institute, Institute Headquarters, P.O. Box 9750, Dar es Salaam, Tanzania
4. Kenya Marine and Fisheries Research Institute, P.O Box 81651-80100, Mombasa, Kenya

ORCID IDs of the author(s):
S.M.S. 0000-0003-0552-7728
M.A.O. 0000-0001-9660-5888
R.N.N. 0000-0001-7188-7516
A.P.S. 0000-0002-2174-8857
F.M. 0000-0003-2513-3558
E.O.O. 0000-0001-9250-7869

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Correspondence:
Simon M. SHATI
E-mail: shatisimon2015@gmail.com

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ABSTRACT

This study investigated the effect of replacing soybean meal (SBM) with black soldier fly larvae meal (BSFLM) on the growth performance, feed utilization, carcass body composition, and amino acids profile of Nile tilapia (*Oreochromis niloticus)*. Three isonitrogenous (30% crude protein) diets containing BSFLM in varying proportions of 0% ($BSFLM_0$), 50% ($BSFLM_{50}$), and 100% ($BSFLM_{100}$), were formulated to replace SBM. A commercial diet ($COMM_0$) sourced from the local market was used as a positive control. Male sex-reversed *O. niloticus* juveniles of a mean weight 20.88 ±0.16 g were stocked in 12 cages each at a density of 12.5 fish m^{-3}. Fish were hand-fed at 5% (28 days), 3% (54 days), and 2.5% (84 days) of the body weight twice a day (1000 hrs and 1600 hrs). Significant differences ($P < 0.05$) were found in the final body weight, body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR), survival rate, and condition factor (K). The best growth performance and feed utilization was recorded in fish fed on $BSFLM_{100}$. The different diets had significant effects on the body composition and amino acid profiles of the experimental fish ($P<0.05$). Fish fed on $BSFLM_{100}$ exhibited highest values for phenylalanine, threonine, Isoleucine, lysine, proline, and glutamic acid amino acids. The partial enterprise budget analysis indicated that replacing SBM with BSFLM at 50% and 100% reduced the cost of production compared to the control diet ($BSFLM_0$) and commercial diet ($COMM_0$). The study demonstrated that BSFLM is a cost-effective alternative to SBM in the diets of *Oreochromis niloticus* hence can replace soybean meal up to 100% without negative effect on growth and carcass body composition.

Keywords: Black soldier fly, *Hermetia illucens*, Nile tilapia, Soybean meal

Introduction

Aquaculture plays a crucial role in global food security and has further demonstrated potential to meet future food demand, particularly the need for protein (FAO, 2020). However, aquaculture production has been constrained by the over dependence on fish meal and fish oil as the main ingredients in fish feeds (Chang et al., 2016). Fish meal has been the major protein source in fish feeds due to its high-quality protein, well-balanced amino acid profile, high essential fatty acids, and phospholipids near to requirement levels of most cultivated aquatic organisms (Bruni et al., 2021; Luthada-Raswiswi et al., 2021). A steady decline in capture fisheries and the competition with humans and animal feed industry have resulted to rapid decrease in the global supplies of fish meal and fish oil, leading to high costs (Dee Roos et al., 2017). This in turn has prompted the search for cheaper alternative protein sources for fish feed formulation.

Soybean meal has become the key ingredient used in tilapia feeds following technological innovation in feed production and high incentives for seeking high quality and affordable alternatives for fish meal and fish oil (Chang et al., 2016). The extensive utilization of soybean meal (SBM) is attributed to its well-balanced amino acid profile and relatively high crude protein contents (Arriaga-Hernández et al., 2021) with the latter being identified as a key and most expensive dietary component in the production of fish feeds. Despite soybean's pivotal role in animal production, its use in fish feeds is constrained by the competition from the livestock feed industry, human food consumption, and industrial production of ethanol and bio-diesel (Lu et al., 2020). This has contributed to the increasing scarcity of soya beans coupled with the effects of climate change and high land-use competition.

Moreover, soybean meal is characterized by nutritional deficiencies with lysine, methionine and threonine being limited in soy-based fish diets (Malcorps et al., 2019). Great amounts of anti-nutritional components in soya have been associated with reduced efficiencies in protein and lipid utilization and digestion, mineral utilization, and anti-vitamins consequently, leading to depressed growth of fish (He et al., 2020). Locally, limited soybean processing has rendered SBM susceptible to contamination, and growth of mycotoxin producing molds mainly aflatoxins that compromises fish health and growth when used in feeds (Niyibituronsa et al., 2016).

Nonetheless, studies have demonstrated that insects represent a valid substitute for the global problems associated with the expensive, unsustainable protein sources for fish feed (Huis et al., 2013). Among insect species, black soldier fly (*Hermetia illucens)* is considered as one of the most promising protein source substitute due to its wide distribution in both tropical and subtropical climate zones (Zurbrügg et al., 2018). It is also easy to culture due to the capability to convert various forms of organic wastes into biomass of high quality proteins, essential amino acids (EAA), fat, vitamins, and minerals (Gasco et al., 2019). The Black soldier fly (BSF) also reproduces easily, has a high growth rate, can control bacteria favoring low risk of zoonotic disease transmission and in addition has a prebiotic effect on the fish (Gariglio et al., 2019).

The nutritive value of *H. illucens* larvae in aquafeeds has been tested with success on several fish species including Nile tilapia (Devic et al., 2018; Agbohessou et al., 2021; Taufek, et al., 2021; Tippayadara et al., 2021), Pacific white shrimp (*Litopenaeus vannamei)*, Cummins et al., 2017), Jian carp (*Cyprinus carpio*) (Zhou et al., 2018), Atlantic salmon (*Salmo salar)* (Li et al.,2020) and Juvenile grass carp (*Ctenopharyngodon idella*) (Lu et al., 2020). However, most of the studies have focused on fish meal protein replacement with black soldier fly larvae meal (BSFLM). Thus scarcity of information on the effects of replacing SBM with BSFLM in the diets of Nile tilapia. In addition, most of the studies are focused on a specific life stage of the fish in different culture systems, especially at the larval rearing and juvenile stage hence discrepancies on the best BSFLM inclusion levels for grow out fish. This study, therefore, evaluated the utilization of defatted black soldier fly larvae (*H. illucens*) meal as SBM replacement in the diet of grow-out Nile tilapia in terms of growth performance and economic aspects during the entire production cycle.

Material and Methods

Study Area and Diet Preparation

The experiment was conducted at Kenya Marine and Fisheries Research Institute, (KMFRI) Sagana Aquaculture Centre (0º19´S and 37º12´E), Kirinyaga County, Kenya. The cultured BSFL was processed by Biobuu Limited, Kilifi, Kenya. Other feed ingredients (Table 1) were purchased from the local agrovet shop and ground separately into finer particles using hammer mill (Thomas-Wiley intermediate mill, 3348-L10 series, USA). Proximate analysis of test ingredients was conducted to guide formulation of three isonitrogenous (30 % crude protein) experimental diets. The diets were prepared by replacing SBM with BSFLM at 0% ($BSFLM_0$) (Control), 50% ($BSFLM_{50}$) and 100% ($BSFLM_{100}$). A commercial diet ($COMM_0$), sourced from local feed manufacturer in Nairobi was used as a positive control. In the production of diets: $BSFLM_0$, $BSFLM_{50}$ and $BSFLM_{100}$, the ingredients were mixed thoroughly with water to make homogenous dough and pelletized using 2-4 mm diet commercial pelletizing machine into floating pellets. The pellets were sundried, packed and stored in clean, dry and cool environment.

Proximate Analysis of the Experimental Diets and Fish Body Composition

The analysis of experimental diets, the initial and final fish body composition was carried out in triplicates following standard methods by the Association of Official Analytical Chemists (AOAC, 1995). Dry matter (DM) was determined gravimetrically by reweighing 2 grams of the sample that had been oven-dried for six hours to constant weight at 105°C. Crude protein (CP) was analyzed by Copper Catalyst Kjeldahl Method. Analysis was done by taking 1g of each sample and 2 tablets of catalyst (Kjeltabs) which were digested in 15 ml concentrated sulfuric acid at 420°C. The samples were cooled and automatically distilled in Kjeldahl equipment with 40% NaOH and ammonia gas trapped in 4% Boric acid was reverse titrated using 0.2N HCl. The nitrogen content was determined and converted to crude protein content using a nitrogen factor for the crude protein calculation of 6.25. Ash was determined by expressing the weight of 2 g of the ground sample burnt at 600°C for 3 hours in a muffle furnace as a percentage of the un-burnt sample weight. Crude fat was extracted by heating 3g of the sample in diethyl ether under reflux at 105°C for 30 min in a VELP Solvent Extraction unit. Ether extract was calculated as the difference between the original sample and the ether extract residue. Crude fibre (CF) was determined gravimetrically by chemical digestion and solubilization, and quantified by: CF (%) = dried sample (g) – ashed sample (g)/initial sample weight × 100). Nitrogen free extract (NFE) was determined by the difference between the original weight of the sample and sum of the weights of its moisture, crude protein (CP), crude fat (CF), ash and crude fibre as: 100 – (crude protein + crude fat + crude fibre + moisture + ash). Amino acids were analyzed using ion exchange liquid chromatography via continuous flow chromatography. The compounds were identified and quantified using an authentic sample mixture (amino acid standard solution (AAS 18) from Sigma-Aldrich (Chemie GmbH, Munich, Germany).

Experimental Design

A total of six hundred sex reversed male Nile tilapia (*O. niloticus)* juveniles of 20.88 ±0.16 g mean weight were sourced from Kenya Marine and Fisheries Research Institute and acclimatized for seven days prior to the experiment. The experimental fish were randomly stocked in 12 cages each at a density of 12.5 fish/m^3 with four cages suspended in 150 m^2 earthen pond. The fish were randomly assigned to the four experimental diets containing BSFLM in varying proportions of 0% ($BSFLM_0$), 50% ($BSFLM_{50}$), 100 % $BSFLM_{100}$) and a commercial diet ($COMM_0$) in triplicates. The experimental fish were hand fed twice a day (1000 hrs and 1600 hrs) at 5 % of body weight for 28 days, 3 % of body weight for 54 days and 2.5 % of body weight for 84 days.

Table 1. Ingredients and proximate composition of the experimental diets

Ingredient inclusion (g kg^{-1})	$BSFLM_0$	$BSFLM_{50}$	$BSFLM_{100}$	
Wheat bran	28	28	28	
Soybean meal	30	15	0	
Black soldier fly larvae meal	0	15	30	
Maize germ	18	18	18	
Freshwater shrimp	12	12	12	
Sunflower seed cake	9	9	9	
Mono-calcium phosphate	1	1	1	
Vitamin premix	1	1	1	
Soybean oil	1	1	1	
Proximate composition (%)	**$BSFLM_0$**	**BSFLM50**	**$BSFLM_{100}$**	**$COMM_0$**
Crude protein	30.2 ±0.32	30.28 ±0.61	30.34 ±0.15	30.5 ±0.22
Crude fat	6.35 ±0.03	6.31 ±0.02	6.46 ±0.00	6.8 ±0.00
Ash (dry)	7.75 ±0.26	7.99 ±0.12	8.23 ±0.42	7.3 ±0.16
Nitrogen free extract (NFE)	42.00	45.40	45.90	48.10

*Black soldier fly treatments - $BSFLM_0$ (0%); $BSFLM_{50}$ (50%); $BSFLM_{100}$ (100%); $COMM_0$ (Commercial feed)
*Soybean treament- $BSFLM_0$ (100%); $BSFLM_{50}$ (50%); $BSFLM_{100}$ (0%); $COMM_0$ (Commercial feed)

Sampling

Fish sampling was done every 28 days. The individual length (cm) and weight (g) of the fish were determined using a measuring board and an electronic weighing scale (model EHB-3000, China) respectively. To evaluate the growth and feed efficiency the following standard formulas were used (Hopkins, 1992):

Specific growth rate (SGR, %) = 100 × [(Ln BW final (g) – Ln BW initial (g)) / days of experiment] … (1)

Body weight gain (BWG, g) = Final weight (g) - Initial weight (g)……………………..………..(2)

Feed conversion ratio (FCR) = feed provided/live weight gain (g) … (3)

Fulton's condition factor (K) = $100(W/L^3)$……………..(4)

Length- weight relationship was determined by the formula=$W = aL^b$, where W = weight (g) and L = total length (cm), a is the regression slope and b is the y intercept……….(5)

Percentage Survival (%) = 100 × (final number of fish)/ (initial number of fish)………........ (6)

Water quality parameters were measured weekly at 1000 hrs using a multi-parameter water quality meter (YSI industries, Yellow Springs, OH, USA). Water samples were collected using 250 ml plastic bottles after every two weeks for ammonia, nitrites, nitrates and phosphorus analysis following the procedures by APHA (1995). The mean values of water quality parameters of the pond water were stable with minimal variations during the experiment period. The mean values were as follows: Temperature (25.31 ±0.22°C), dissolved oxygen (DO) (5.47 ±0.08 mg L^{-1}), conductivity (104.60 ±1.68 µS cm^{-1}), total dissolved solids (67.05 ±1.08 mg L^{-1}), and pH (8.35 ±0.04). Relatively low values for nutrients were recorded as follows: Phosphates (0.003 ±0.0001 mg L^{-1}), nitrites (0.001 ±0.0001 mg L^{-1}), Nitrates (0.004 ±0.0003 mg L^{-1}) and Ammonium (0.002 ±0.0001 mg L^{-1}). All the parameters were within the recommended levels for Nile tilapia.

Enterprise Budget Analysis

Partial enterprise budget analysis was used to evaluate the economic implications of substituting SBM with BSFLM in the diets of Nile tilapia. Variable costs included the cost of labor, feeds, and fingerlings (Table 2). The cost of formulated diets ($BSFLM_0$, $BSFLM_{50}$, and $BSFLM_{100}$) was calculated based on the cost of the ingredients in each diet and the cost of feed production while the price of the commercial diet was based on feed company retail prices. Labor costs were based on the prevailing market prices within the experiment site. The US dollar exchange rate against Kenya shillings was pegged at KES 107.15. The study assumed that all other costs of production were constant for all dietary treatments and thus not considered. Fish harvested per treatment was sold at USD 3.73 kg^{-1}, which is the market price of Nile tilapia. Further, to gauge the profitability of the alternative the break-even price was calculated by dividing total costs per unit price of fish (Musa et al.,2021).

Data Analysis

The data was cleaned and subjected to the Shapiro–Wilk test of normality. Statistical analyses were performed using MS excel and Statistical Package for the Social Sciences (SPSS version 23). Mean comparisons were done by the analysis of variance (one-way ANOVA) followed by Tukey HSD post hoc test to determine the pairwise differences among the diets. Significant differences were considered at $P < 0.05$. Percentage data were arcsine transformed prior to analysis to normalize data.

Table 2. Partial enterprise budget input expenditures

Item	Units	Quantity	Unit cost (USD)	Total(USD)
Nile tilapia fingerlings	Pcs/cage	50	0.09	4.5
$BSFLM_0$	Kg/cage	12.57	1.06	13.32
$BSFLM_{50}$	Kg/cage	12.68	0.95	12.05
$BSFLM_{100}$	Kg/cage	13.74	0.86	11.82
$COMM_0$	Kg/cage	13.31	0.94	12.51
Labor costs	USD/cage/Month	6	0.4	2.4
Cost of packaging fish	USD/Kg	-	0.009	-
Cost of transporting fish	USD/Kg	-	0.027	-
Tax on sale of fish	USd/Diet	-	0.47	-
Total yield $BSFLM_0$	Kg	17.77		
Total yield $BSFLM_{50}$	Kg	16.08		
Total yield $BSFLM_{100}$	Kg	20.54		
Total yield $COMM_0$	Kg	20.81		

Results and Discussion

Growth Performance and Nutrient Utilization

The BSFLM replacement level had a significant effect on the growth of the fish ($P < 0.05$). $BSFLM_{100}$ recorded the highest weight gain followed by $COMM_0$, $BSFLM_{50}$ and finally $BSFLM_0$ (Table 3). Fish body weight increased by 89 % in fish fed on $BSFLM_{100}$ and $COMM_0$, while an increase of 87% was observed in fish fed on $BSFLM_0$ and $BSFLM_{50}$. Fish fed on $BSFLM_{100}$ had significantly higher BWG and SGR ($P<0.05$) and the lowest FCR compared to those fed on $BSFLM_0$ and $BSFLM_{50}$. Fish fed on $BSFLM_0$ presented significantly lower BWG, SGR and the highest FCR. All the diets recorded a positive correlation co-efficient (R^2) that was not significantly different among the diets ($P > 0.05$). The values of condition factor recorded for all the fish ranged between 1.86 and 1.89. There were significant differences ($P<0.05$) in Fulton's condition factor (K) between the $BSFLM_0$, $BSFLM_{50}$, $BSFLM_{100}$, and $COMM_0$ with control diet ($COMM_0$) having the highest condition factor. Survival rates were relatively higher in all the treatments with significant variations ($P<0.05$) among the diets. The highest survival rates were recorded in diet $BSFLM_0$ (95.33%) while the lowest in $BSFLM_{100}$ (92.67 %).

Body Composition Analysis

Significant difference ($P<0.05$) was recorded for crude protein, crude fat, and Nitrogen free extracts (NFE) in the body composition of the fish (Table 4). However, no significant differences ($P>0.05$) was recorded in ash content in fish in all the treatments. Fish fed on $BSFLM_{100}$ exhibited significantly ($P<0.05$) higher values for phenylalanine, threonine, isoleucine, lysine, histidine and leucine, while for non-essential amino acids: glutamic acid, cysteine, proline were higher in fish fed in $BSFLM_{100}$ as compared to control diets ($COMM_0$ and $BSFLM_0$) (Table 5). Fish fed on $COMM_0$ registered highest mean values for arginine, glycine, methionine, tyrosine, tryptophan and tyrosine although, they were statistically similar to fish fed on $BSFLM_{100}$. The lowest values for glycine, alanine, lysine, cysteine, glutamic acid, methionine, and isoleucine were registered in fish fed on $BSFLM_{50}$ while arginine, histidine, tryptophan, leucine threonine, proline were lowest in fish fed on $BSFLM_0$ (control).

Table 3. Growth performance parameters of *O. niloticus* fed on diets with different levels of defatted black soldier fly larvae meal and commercial diet

Parameter	$BSFLM_0$	$BSFLM_{50}$	$BSFLM_{100}$	$COMM_0$
Initial length (cm)	10.57 ±0.07	10.54 ±0.06	10.57 ± 0.06	10.56 ± 0.05
Initial weight (g)	20.83 ±0.33	20.86 ±0.33	20.85 ±0.33	20.88 ±0.30
Final length (cm)	21.04 ±0.13[a]	21.35 ±0.13[a]	21.67 ±0.12[b]	21.53 ±0.17[b]
Final weight (g)	166.19 ±3.18[a]	169.03 ±2.91[a]	193.35 ±3.69[b]	188.56 ±5.46[b]
SGR (% day $^{-1}$)	1.23 ±0.02[a]	1.24 ±0.01[a]	1.32 ±0.013[b]	1.29 ±0.02[b]
BWG (g)	145.36 ±3.22[a]	148.17 ±2.94[a]	172.40 ±3.66[b]	167.68 ±5.46[b]
FCR	1.87 ±0.04[a]	1.86 ±0.046[b]	1.79 ±0.03[a]	1.82 ±0.05[a]
Condition factor (K)	1.86 ±0.01[a]	1.86 ±0.01[a]	1.88 ±0.01[ab]	1.89 ±0.01[b]
R^2	0.98[a]	0.98[a]	0.98[b]	0.98[b]
Survival (%)	95.33 ±0.10[a]	94.67 ±0.10[b]	92.67 ±0.20[c]	93.33 ±0.26[d]

* Means within the same row with different superscript letters are significantly different at $P < 0.05$, n=30

Table 4. Body carcass composition of Nile tilapia fed on diets with different levels of defatted black soldier fly larvae meal and commercial diet

Parameter (%)	**$BSFLM_0$**	**$BSFLM_{50}$**	**$BSFLM_{100}$**	**$COMM_0$**
Ash	7.0 ±0.59	7.2 ±0.39	6.8 ±0.15	6.9 ±0.49
Crude protein	72.4 ±0.81[a]	73.8 ±0.46[a]	75.3 ±0.96[b]	75.7 ±1.03[b]
Crude fat	3.3 ±0.01[a]	2.9 ±0.29[b]	5.0 ±0.31[c]	8.3 ±0.26[d]
NFE	5.5[a]	6.5[b]	2.3[c]	1.13[d]

** Means within the same row with different superscript letters are significantly different at $P < 0.05$; n=3; NFE - Nitrogen free extract

Table 5. Amino acids profile of *O. niloticus* fed on diets with different levels of defatted black soldier fly larvae meal and commercial diet

Amino acids (%)	$BSFLM_0$	$BSFLM_{50}$	$BSFLM_{100}$	$COMM_0$
Essential amino acids				
Phenylalanine	18.17 ±2.30[a]	17.70 ±2.90[b]	19.79 ±5.40[c]	19.44 ±4.00[d]
Threonine	3.87 ±0.10[a]	5.37±0.70[b]	5.78 ±1.60[c]	5.25 ±1.10[d]
Arginine	0.31 ±0.10[a]	0.45 ±0.30[a]	0.43 ±0.50[b]	0.51 ±0.60[b]
Valine	6.13 ±0.60[a]	4.33 ±1.10[b]	4.64 ±1.00[c]	4.2 ±1.00[d]
Isoleucine	2.72 ±0.40[a]	2.43 ±0.50[b]	2.94 ±0.80[c]	2.82 ±0.60[d]
Methionine	2.25 ±0.20[a]	1.29 ±0.10[b]	1.46 ±0.70[c]	1.98 ±1.10[d]
Tryptophan	12.98 ±0.40[a]	20.71 ±1.90[b]	13.02 ±2.70[c]	15.06 ±5.40[d]
Lysine	11.88 ±1.20[a]	14.52 ±1.30[b]	15.73 ±3.60[c]	15.3 ±3.70[d]
Histidine	1.36 ±0.10[a]	1.24 ±0.50[a]	1.43 ±0.30[b]	1.47 ±0.30[b]
Leucine	0.93 ±0.10[a]	0.91 ±0.40[a]	0.99 ±0.40[b]	0.75 ±0.30[b]
Non-essential amino acids				
Alanine	2.87 ±0.20[a]	2.73 ±1.10[a]	2.59 ±0.70[b]	2.58 ±0.80[b]
Tyrosine	1.16 ±0.10[a]	1.21 ±0.60[a]	0.84 ±0.20[a]	0.93 ±0.20[a]
Glycine	23.60 ±.60[a]	17.47 ±1.00[b]	20.32 ±8.70[c]	21.9 ±4.10[d]
Glutamic acid	3.41 ±0.30[a]	2.88 ±1.10[b]	3.65 ±0.60[c]	2.25 ±0.70[d]
Cysteine	2.72 ±0.30[a]	2.12 ±0.70[a]	2.33 ±0.90[b]	1.92 ±0.40[c]
Proline	5.59 ±0.60[a]	3.57 ±0.60[b]	4.29 ±0.90[c]	3.72 ±1.20[d]

* Means within the same row with different superscript letters are significantly different at $P < 0.05$; n=3

Partial Enterprise Budget Analysis

The mean yield was higher in the fish fed on $COMM_0$ and $BSFL_{100}$ followed $BSFLM_0$ and $BSFLM_{50}$ (Table 6). All the experimental diets registered significantly positive net return above total costs ($P<0.05$). The total cost and variable cost decreased gradually as SBM was replaced with BSFLM with lowest values exhibited by $BSFLM_{100}$. Break-even price were significantly different with lowest values (USD 2.36 ±0.01) being recorded in $BSFLM_{100}$ ($P<0.05$).

The present study was conducted to evaluate the effect of replacing soybean meal (SBM) with black soldier fly larvae meal (BSFLM) on the growth performance, feed utilization, carcass body composition and amino acid profile of Nile tilapia (*Oreochromis niloticus*). All the diets registered gradual increase in the mean weight. This can be attributed to the similarity in the initial crude protein and fat content of the extruded experimental diets coupled with the well-balanced and formulated diets. Fish fed on $BSFLM_{100}$ registered statistically similar final weight to control diet $COMM_0$ though significantly higher than diets $BSFLM_0$ and $BSFLM_{50}$. Similar findings have been reported in juvenile grass carp (*Cyprinus carpio*) by Lu et al., (2020) but contrary to (Dietz & Liebert, 2018). in Nile tilapia that registered lowest weight with highest soybean concentrate replacement with BSFLM.

Fish body weight increased by 89% in fish fed on $BSFLM_{100}$ and $COMM_0$ while 87% in fish fed on $BSFLM_0$ and $BSFLM_{50}$. Previous studies on Nile tilapia, have reported contrasting results to this study, for instance, a lower body weight increase of 30% (Tippayadara et al., 2021), 64% (Devic et al., 2018), 73% (Muin et al., 2017) and higher body weight gain of 96% (Rana, 2009) have been reported in diets containing 100% BSFLM. The differences in the increase of the body weight between the present and previous trials may have been mainly associated with varying fish development stages and study periods of between 32 and 96 days compared to the present study that lasted for a period of 168 days.

Table 6. Partial enterprise budget for the dietary treatments with different levels of defatted black soldier fly larvae meal and commercial diet

Parameters	Units	Treatments			
		$BSFLM_0$	$BSFLM_{50}$	$BSFLM_{100}$	$COMM_0$
Total yield	Kg	17.77 ± 0.01^a	16.08 ± 0.01^b	20.54 ± 0.01^c	20.81 ± 0.02^d
Gross revenue	USD	66.28 ± 0.01^a	59.98 ± 0.01^b	76.61 ± 0.01^c	77.6 ± 0.35^d
Variable costs	USD	59.5 ± 0.12^a	57.42 ± 0.01^b	47.93 ± 0.00^c	58.97 ± 0.02^d
Fixed costs	USD	0.47 ± 0.02	0.47 ± 0.00	0.47 ± 0.00	0.47 ± 0.00
Total costs	USD	59.97 ± 0.01^a	57.88 ± 0.01^b	48.4 ± 0.23^c	59.44 ± 0.01^d
Net return above total costs	USD	6.32 ± 0.01^a	2.09 ± 0.00^b	28.21 ± 0.01^c	18.16 ± 0.02^d
Unit selling price	USD	3.73 ± 0.01	3.73 ± 0.02	3.73 ± 0.00	3.73 ± 0.01
Breakeven price (total costs)	USD	3.37 ± 0.01^a	3.6 ± 0.01^b	2.36 ± 0.01^c	2.86 ± 0.01^d
Breakeven yield (total costs)	Kg	16.08 ± 0.02^a	15.52 ± 0.01^b	12.98 ± 0.01^c	15.96 ± 0.01^b

* Values are means ± S.E. Values with the same superscripts in a row are not significantly different

The lowest BWG and SGR were recorded in fish fed on $BSFLM_0$ and $BSFLM_{50}$ with 100 and 50% soybean inclusion levels respectively. Similar results were reported by Lu et al. (2020) in juvenile grass carp but contrary to Dietz & Liebert (2018) in Nile tilapia fed on BSFL meal based diets. Previous studies have also reported reduced feeding and growth as a result of higher levels of dietary soybean proteins and recommended different optimal inclusion levels of 25% (Sharda et al., 2017 & Abdel-Warith et al., 2020), 4% (Amesa et al., 2018), 4.5% (Godoy et al., 2019) in Nile tilapia. Soybean meal has been reported to have growth-inhibitory effect which has been linked to anti-nutritional factors (Lu et al., 2020) that lower nutrient digestibility, nutrient bio-availability and palatability due to excessive degrees of non-soluble fibre and starch (Daniel, 2018). Similarly, the depressed growth in fish fed on diets with high inclusions (50 and 100 %) of SBM could be attributed to lack of phytase enzyme in tilapia to hydrolyze phytates in soybean that causes unavailability of amino acids.

Better BWG and FCR exhibited in fish fed on $BSFLM_{100}$ could be due to better amino acid profile with higher levels of glycine, alanine, isoleucine, leucine and phenylalanine compared to control $BSFLM_0$ (El-Hack et al., 2020). Contrary to the findings of this study, lower SGR and BWG in diets containing high inclusion levels of BSFLM have been reported by Devic et al., (2018) in Nile tilapia (*Oreochromis niloticus*) and Lu et al., (2020) in grass carp (*Ctenopharyngodon idellus*). The diminishing performance was linked to imbalances in amino acids and inadequate chitinase activity in diets with higher BSFLM. Similar growth patterns of the fish fed on diets with 100% BSFLM and the commercial diet indicated that the diets had similar nutritional values to support the growth of fish.

Fish fed on $BSFLM_{100}$ registered lowest FCR compared with other diets. The FCR values in the present study were lower than those reported by Muin et al., (2017) and Tippayadara et al., (2021) when BSFLM was included in the diets of tilapia. A lower FCR serves as an indicator of better feed utilization (Devic et al., 2018). The low FCR in fish fed on $BSFLM_{100}$ is in line with Xiao et al., (2018) findings in yellow catfish (*Pelteobagrus fulvidraco)* when conventional protein sources were replaced at 100% with BSFLM. Low FCR in fish fed $BSFLM_{100}$ may have been a factor of utilization of defatted BSFLM. Observations by Basto et al., (2020) indicated that defatting insect meals improved digestibility and utilization of nutrients though the chitin content remained similar between defatted and non-defatted insect meals.

The high values of FCR recorded in fish fed on $BSFLM_0$ and $BSFLM_{50}$ indicates that high content of SBM negatively influenced feed utilization. This could be due to the unavailability of macro and micronutrients in SBM that compromises feed utilization. High levels of soybean in fish diets could have resulted to anti-nutritional factors specifically, tannin which is ascribed to inhibition of digestive enzymes, phytic acid chelation which diminishes bioavailability of calcium, phosphorus, magnesium, manganese, zinc and iron (He et al., 2020). Gossypol in soya bean meal have also been reported to diminish digestibility and feed consumption by lowering the bioavailability of lysine. Soybean processing by thermal heating is a contributing factor in loss of some amino acids that influence protein, carbohydrate and moisture on milliard reactions (Ghosh & Ray, 2017). Length - weight relationship (r≥0.98, b=3) of the fish of the current study indicated isometric growths in all the fish fed on the experimental diets that entailed an increase in weight in every unit increase in length (Daliri, 2012). This suggests good physiological conditions of fish in relation to its welfare associated with optimal physico-chemical and biological qualities of water in fish ponds.

The survival rates from the present study were above 92% in all the dietary treatments. The survival rates agree with results in Nile tilapia (Tippayadara et al., 2021), yellow catfish (*Pelteobagrus fulvidraco*) (Xiao et al., 2018) and juvenile grass carp (*Ctenopharyngodon idellus*) (Lu et al., 2020) fed on black soldier fly larvae meal. As reflected in the condition factor, acclimatization of fish prior to stocking, consistent and appropriate feeding regimes in an optimal environment in favor of fish physiological processes played a major role in high survival rates.

The body carcass composition of fish was significantly influenced by different dietary treatments. There were significant differences in crude protein, crude fibre and total lipids content of the carcass. This may have been associated with the changes in the synthesis, deposition rate in muscle and/or different growth rate (Abdel-Warith et al., 2020). Fish fed on different diets differed significantly in crude protein content (Dietz & Liebert, 2018). Fish fed on control diet and $BSFLM_0$ had lower crude protein levels compared to $BSFLM_{100}$ and $COMM_0$. This could be due to difference in absorption kinetics of free and protein-bound amino acids that create asynchrony in post absorptive availability of individual amino acids. Cummins et al., (2017) found reduced protein and lipid levels in Pacific white shrimp (*Litopenaeus vannamei*) associated with deficiencies in essential amino acid (EAA) as well as increasing imbalances of essential and non-essential amino acids. This is contrary to the findings by Devic et al., (2018) in Nile tilapia where there were no significant differences in protein levels with increasing BSFLM supplementation. Low lipid content in fish fed on $BSFLM_0$ and $BSFLM_{50}$ is similar to the findings in rainbow trout (Sealey et al., 2011).

Protein quality is linked to amino acids content. High quality proteins are readily digestible and contain the dietary essential amino acids (EAA) in quantities that correspond to human requirements. In the current study amino acids concentration in the fish fillet increased with increasing dietary BSFLM. Fish fed on $BSFLM_{100}$ and $COMM_0$ had enhanced and satisfactory amino acid profile compared to fish fed on $BSFLM_0$ and $BSFLM_{50}$. Fish fed on $BSFLM_{100}$ had significantly higher values for six out of the ten identified essential amino acids associated with amino acid balance in the feed coupled with better physiological utilization after digestion, absorption and oxidation (Wu et al., 2014). This is also reflected in low FCR. The amino acids profile of the fish fed on $BSFLM_{100}$ and the fish fed on $COMM_0$ were comparable which means the quality of the feed with 100% BSFM led to the same effect on the amino acid profile of the fish fed on commercial diet ($COMM_0$).

Feed constitutes a major expense in aquaculture species hence profitability is a factor of cost minimization. In the current study feed costs were 46.33% ($BSFLM_{100}$), 48.37% ($BSFLM_{50}$,) 50.79% ($BSFLM_0$) and 53.04% ($COMM_0$) of the total variable costs. Replacing SBM with BSFLM at 50% and 100 % reduced the cost by 8.8% and 12 % respectively compared to control diet $COMM_0$. This was contributed by lower BSFLM price of 0.80 USD/kg against SBM of 0.90 USD/kg at the time of the experiment. Although $COMM_0$ had the highest total yield and gross revenue attributed to good growth performance, the diet registered lower net return above total costs in comparison to $BSFLM_{100}$. Similar results of cost of feed reduction and increased net returns have been reported by Abdel-Tawwab et al., (2020) in European sea bass (*Dicentrarchus labrax)*, Wachira et al., (2021) in Nile tilapia and Rawski et al., (2021) in Siberian sturgeon, when conventional sources of proteins were replaced with BSFLM. In addition, non-utilization of basal ingredients, mainly SBM and oil reduced the costs of formulating $BSFLM_{100}$ hence contributing to its cost effectiveness. Lower net return above total costs values were recorded in fish fed diet $BSFLM_0$ and $BSFLM_{50}$ mainly contributed by high amounts of soybean and fish meal included in these diets to supplement on limiting macro and micro elements. This is also compounded by low utilization of the feeds that contributed to low yields. As evidenced by low values for break-even price (total costs) and break even yield, replacing SBM with 100% BSFLM is economically viable.

Conclusion

This study demonstrated that fish fed on $BSFLM_{100}$ exhibited better growth performance in terms of body weight gain, specific growth rate and feed conversion ratio compared to the commercial diet. Similarly, amino acids deposition in fish fed on $BSFLM_{100}$ was significantly higher than $COMM_0$ confirming enhanced and comparative nutritive effect of $BSFLM_{100}$ and $COMM_0$. The study also confirms BSFLM as a cost effective alternative source of protein to the SBM in the diets of Nile tilapia.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: The experiment was conducted following the standard operating procedures (SOPs) of the Kenya Marine and Fisheries Research Institute (KMFRI) guidelines for handling animals registered with the National Commission for Science, Technology and Innovation (NACOSTI) registration number NACOSTI/2016/05/001. The SOPs comply with the Prevention of cruelty to animals Act 1962, CAP 360 (Revised 2012) of the laws of Kenya and the EU regulation (EC Directive 86/609/EEC).

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Short Comunication

First record of *Sphyrna gilberti* Quattro et al., 2013, on the coast of Campeche, México

Jorge I. ROSALES-VÁSQUEZ[1a], Vicente ANISLADO-TOLENTINO[1b], Andrés IRIGOYEN-SOLIS[1c], Armando T. WAKIDA-KUSUNOKI[2], Luis Fernando DEL MORAL-FLORES[3]

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[1] Grupo de Investigadores Libres Sphyrna; [a]Allende 755-3. Bustamante. 22840, Ensenada, Baja California, Mexico; [b]Boulevard del Cimatario 439. Constelación, 76087, Queretaro, Queretaro. Mexico; [c] Calle 20 SN, entre 27 y 29. Centro. 24460. Seybaya. Campeche

[2] Centro Regional de Investigación Acuicola y Pesquera de Yucalpetén. Instituto Nacional de Pesca y Acuacultura. Boulevard del Pescador s/n esquina Antigua Carretera a Chelem 97320. Yucalpetén, Yucatán, México

[3] Laboratorio de zoología, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Av. de los Barrios No. 1, Los Reyes Iztacala, C.P. 54090 Tlalnepantla, Estado de México, Mexico

ORCID IDs of the author(s):

J.I.R.V. 0000-0001-5245-3376
V.A.T. 0000-0002-2184-0047
A.I.S. 0000-0002-3528-3245
A.T.W.K. 0000-0002-7917-2651
L.F.D.M.F. 0000-0002-7804-2716

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Correspondence:
Jorge I. ROSALES-VÁSQUEZ
E-mail: gilsmexico@gmail.com

ABSTRACT

Sphyrna gilberti is a large hammerhead shark described off South Carolina and data distribution on other regions of the Western Atlantic are unknown. An adult male with a total length of 240 cm was collected in 2021 on Campeche, Southwestern Gulf of México. The precaudal vertebral count of the examined individual was 89, and a large head and a robust caudal peduncle were observed. This study documents the first record of *S. gilberti* in Mexican waters.

Keywords: *Sphyrna gilberti*, Cephalofoil, distribution, First-record, Campeche

Introduction

The hammerhead sharks (Carcharhiniformes: Sphyrnidae) are characterized with their distinctive feature being the presence of a modified head with two folds or blades expanded and compressed dorso-ventrally, known as cephalofoil (Compagno, 1984). In general, species identification of hammerhead sharks is based mainly on the morphological differences that occur in the cephalofoil. Within the family are currently accepted two valid genera *Eusphyra* and *Sphyrna* (Compagno, 1984), and ten recognized species (*Eusphyra*= 1, *Sphyrna*= 9) (Ebert et al., 2021). Hammerhead sharks are inhabitants of coastal and insular waters, distributing in tropical and subtropical zones of all the oceans (Compagno, 1984). Some of the sphyrnid sharks, such as the scalloped hammerhead (*Sphyrna lewini*) and the great hammerhead (*Sphyrna mokarran*), are considered as nomadic and migratory (Commission SSGotISS, 2007). Hammerhead sharks can be divided into two large groups by the shape of their cephalofoil: the large cephalofoil group *(E. blochii, S. mokarran, S. zygaena* and the *S. lewini-gilberti,* group) and the small cephalofoil group (*S. tudes, S. corona, S. tiburo* and *S. media*) (Cavalcanti, 2007). Within these groups, the *"lewini-gilberti* group" is of great interest due to the complexity of species identification.

The presence of seven species of hammerhead shark have been reported in Mexican waters being these for the Pacific Ocean: *S. corona, S. lewini*, *S. media*, *S. mokarran*, *S. zygaena* (Pérez-Jimenez, 2014) and *S. vespertina* (Del Moral-Flores et al., 2016), and for the Gulf of México and Caribbean Sea: *S. lewini*, *S. mokarran*, *S. tiburo* (Salomón-Aguilar, 2012). To date, there is no record of the presence of *S. gilberti* for Mexican waters.

The South Carolina hammerhead shark *Sphyrna gilberti* is the most recently described species of the family Sphyrnidae, considered a cryptic species of *S. lewini*, presents as diagnostic characteristic 81 to 90 precaudal vertebrae (Quattro et al., 2013). To date, the distribution range of *S. gilberti* in Western Atlantic Ocean is recorded only off the coast of South Carolina, United States (Quattro et al., 2013). Aspects about its biology are unknown mainly due to incorrect identification and confusion with *S. lewini*, but it is believed to share habitat, show similar behavior and have the same reproduction type (Ebert et al., 2021). In the present study, authors report on the first record of *S. gilberti* off the Mexican coasts of the Gulf of México.

Material and Methods

On 10th of March, 2021, a male specimen of a hammerhead shark (Sphyrnidae) with 240 cm of stretched total length (SLT) was incidentally caught by a snapper bottom longline with 250 m of length, with 100 snoods attached to No. 8 fishing hooks and operating at a deep between 20-30 m in the southern coast of the state of Campeche, México, near of the 91°30'36"W 19°49'26"N point (Fig. 1).

Initially, it was supposed that it was a specimen of *Sphyrna lewini* (Griffith & Smith, 1834), however, there were doubts due to notable differences in the length of the head and a some robustness in the caudal peduncle that the fishermen did not recognized for *S. lewini.* Due to its size, the specimen was processed as fillets, so photographs and measurements were taken *in situ*, the vertebrae were counted after processing, and the jaw was examined by the third author and preserved by the fishermen who catch it. The photographs taken in the landing site were used to calculate, as far as possible, the measurements proposed by Compagno (1984, 1988) using Photoshop ® following Reiss's recommendations (Reiss, 2007). Measurement comparisons were made using the proportions published by Quattro et al. (2013) for *S gilberti* and *S. lewini*. Vertebrae counting was performed after traditional commercial processing. Being *S. gilberti* a species only identifiable by the vertebral counts (Quattro et al., 2013) and by the unexpected presence of the examined specimen, the lack of freezing infrastructure, lack of quick access to conservation chemicals (alcohol), and the speed of the filleting and sale of the shark at the commercial processing zone made impossible to preserve a tissue sample for genetic analysis.

Results and Discussion

The specimen presented a 240 cm of stretched TL and 160 of precaudal length. The posterior margins of the pelvic fins are weakly convex with an angle of just over 160° (Figure 2A, 2B & 2C), it was a mature male with well-calcified clasper and mating bruises (Figure 2D) and semen flowing under the pressure of the siphonal sac.

A total of 25 morphometric measurements were taken (Table 1). Of the morphological characteristics was highlighted that the specimen had 89 precaudal vertebrae, a head length (HDL) to total length ratio of 25 % and its dental formulae was 15-1-15/14-1-14/ (Figure 3), a remarkable characteristic was the rounded caudal peduncle (Figure 4A).

Table 1. Morphometric proportions of *Sphyrna lewini, S. gilberti,* and the specimen studied in this work * according to Gilbert (1967)

	Quattro et al., 2013		Present study	
Morfometrics	***Sphyrna lewini******	***Sphyrna gilberti***	**Specimen**	**Measurements in cm**
Stretched total length				240
Precaudal length		66	67	160
Furcal length		77	76	182
Natural total length		97	96	230
Head length	22.34	24	25	60
Prepectoral length	21.36	23	22	53
Prepelvic length	44.67	46	48	115
Preanal length	57.02	58	60	144
Pectoral-pelvic space	18.56	19	21	50
Pelvic-anal space	7.75	7	7	16
Caudal anal space	6.46	7	6	14.5
Caudal pelvic space	18.65	18	18	42
Head width	28.01	30	29	70
Indentation half lateral space	6.74	7	8	19
Posterior margin of the head	7.88	7	8	18
Nose length	6.79	8	7	17
Nacelle length	8.66	10	11	27
Nacelles height	2.65	3	3	7
Eye length	2.16	2	2	5
Eye height	1.93	2	2	5
Internarinal space	13.3	14	15	36
Mouth wide	6.92	7	7	16
Mouth length	3.75	4	4	9
Interbranchial length	5.58	6	6	15
Number of vertebrae	92-99	87	89	
Dental formulae	U 15 to 16 - 0 to 2-15 to 16, L 15 to 16-1 to 2-15 to 16*	11 to U 15-1 to 2-11 to 15, L 11 to 14-0 to 2-11 to 14	U 15-1-15, L14-1-14	

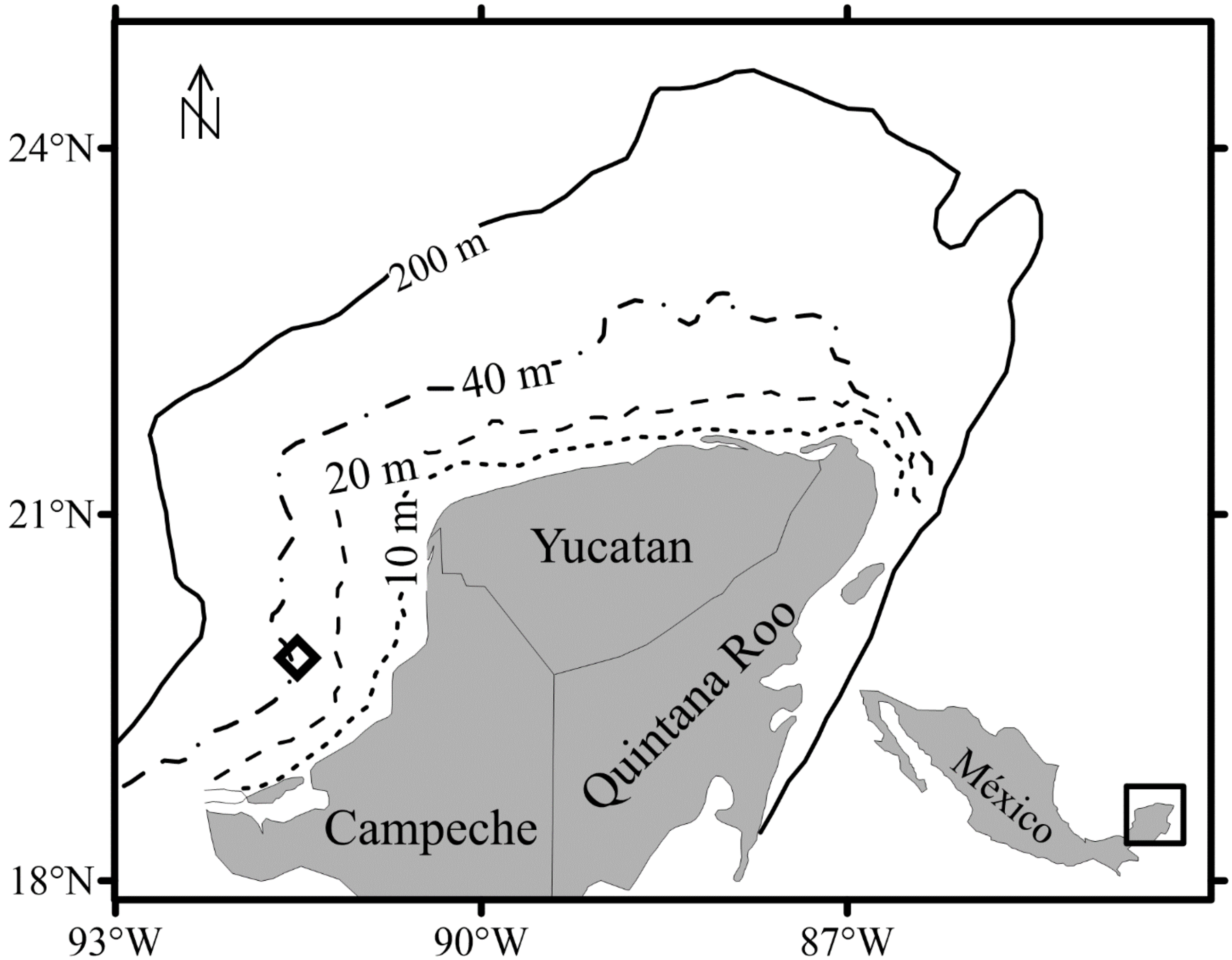

Figure 1.-Point of capture of the specimen (open rhombi) of *Sphyrna gilberti* in Southwestern Gulf of México

The examined hammerhead shark presented the main characteristic that Quattro et al. (2013) mention as diagnoses of *S. gilberti,* being this, that the count of precaudal vertebrae of the specimen (89) is in the range of reported for *S. gilberti,* which is from 81 to 90, while *S. lewini* has 92 to 99 (Quattro et al., 2013). Moreover, the expanded head (HDL to TOT = 25 %) of the examined specimen indicates that it belongs to *S. gilberti*, which Quatro et al. (2013) mention to be greater than 23 % for the species. Dentition of the examined specimen are very similar to *S. lewini* in form, however, the dental formula of palatoquadrate and Meckel's cartilage is more similar to *S. gilberti*. That is, it has fewer teeth than *S. lewini* (Gilbert, 1967; Quattro et al., 2013). On the other hand, in the detailed descriptions made for *S. lewini* and *S. gilberti* (Gilbert, 1967; Quattro et al., 2013; Ebert et al., 2021), the shape of the caudal peduncle has not been considered.

It is interesting to know that in the area of capture of the examined specimen, the fishermen recognize two groups of common hammerhead sharks: those with round peduncle (*S. gilberti*, Figure 4A) and oval peduncles (*S. lewini*, Figure 4B), being a diagnostic characteristic to be explored.

It's also noteworthy that Barker et al. (2019) found that there's a hybridization between *S. gilberti* and *S. lewini* on the distribution range, being *S. gilberti* nearly always the maternal species. Unfortunately, Barker et al. (2019) did not analyze morphological or meristical characteristics of the hybrids so the only way to detect hybrids is by genetic methods. For further encounters of *S. gilberti* at the locality, it is necessary to be prepared to take tissue samples for genetic analysis in order to correlate meristic and genetic data to identify the possible presence of hybrids.

This shows that the analyzed specimen belongs to the species *Sphyrna gilberti,* which confirmed the extension of range to south at Mexican waters.

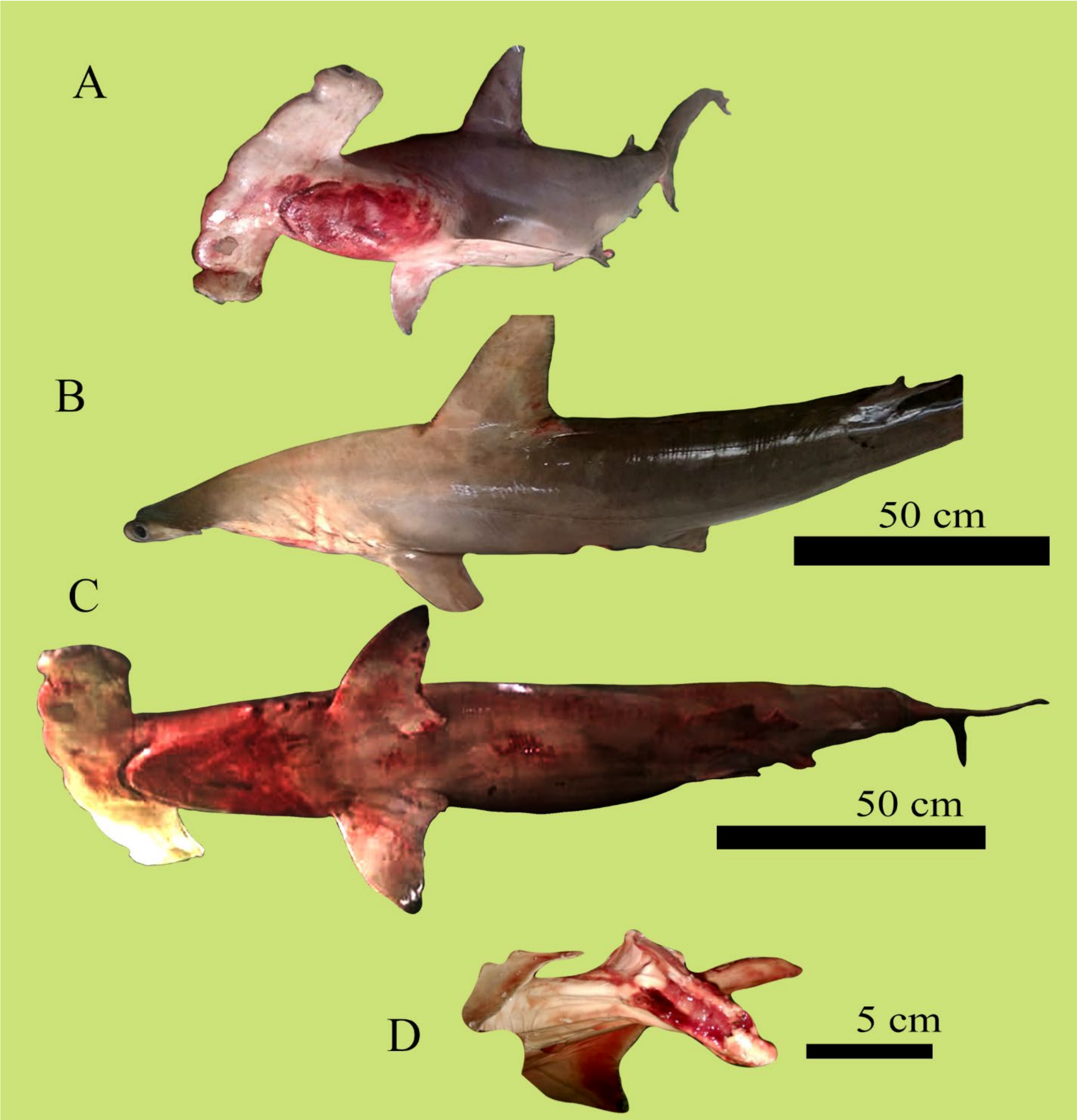

Figure 2. View of the specimen analyzed (A, B and C), clasper in extension and with mating hematomas (D). Photographs by Andrés Irigoyen-Solís

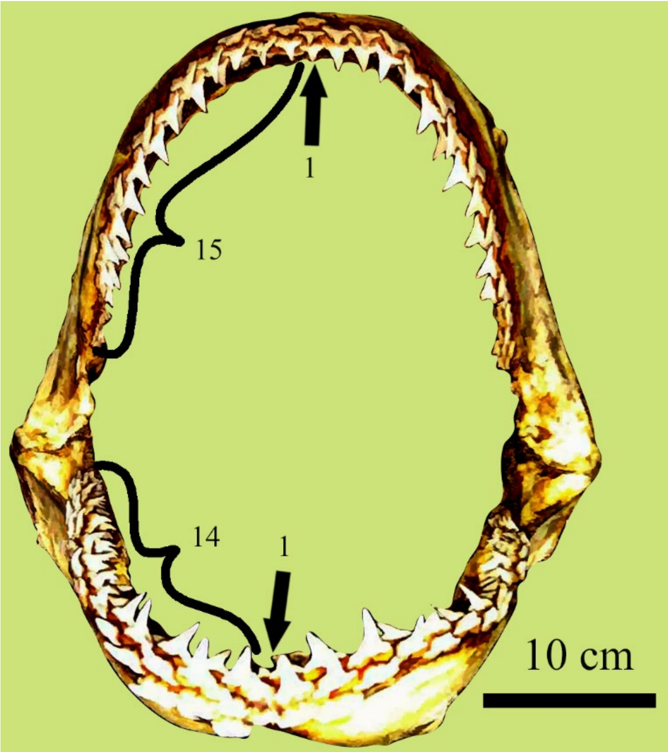

Figure 3. Jaws of analyzed specimen of *Sphyrna gilberti*, number is the countable teeth. Photograph by Andrés Irigoyen-Solís.

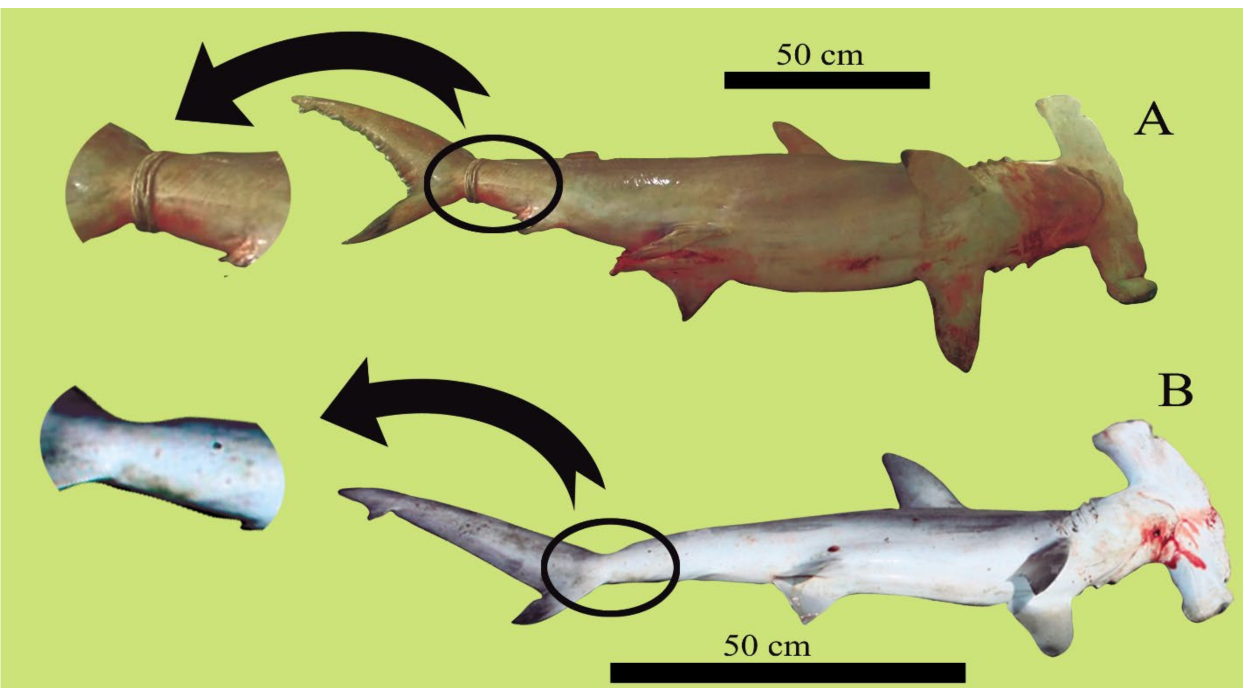

Figure 4. A graphical comparative of caudal peduncle of *Sphyrna gilberti* (A, male 240 cm of stretched total length of this work) and *Sphyrna lewini* (B, female 125 cm of Stretched total length of Oaxaca coast). Photographs A by Andres Irigoyen-Solis, and B by Vicente Anislado Tolentino.

Conclusion

The confirmation of the presence of *S. gilberti* in Mexican waters highlights the need for greater taxonomic control of the identifications of hammerheads made in the studies by the researchers, this due to great similarities with *S. lewini*. It also generates new challenges for the conservation and management of the hammerhead sharks (Sphyrnidae) on the southern coasts of the Gulf of México and Caribbean Sea. Is necessary better-trained staff for the identification of this two species to generate more realistic fisheries data for a better stock assessment, management and inspection and surveillance. On the future, this also can lead to a possible *S. gilberti* inclusion to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) for more control on the international trade of this large hammerhead species.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: The present study is within the legal framework established in México: General Law of Sustainable Fisheries and Aquaculture, Official Mexican Regulations for recreational sport fishing activities in the waters of federal jurisdiction of the United Mexican States (NOM-017-PESC-1994), and Official Mexican Regulations for Environmental Protection-Mexican native species of wild flora and fauna, risk categories and specifications for their inclusion, exclusion or change to the list of species at risk (NOM-059-SEMARNAT-2001).

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Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following forms during the initial submission.

- Copyright Transfer Form,
- Author Contributions Form (one form for copyright and contributions available in **https://dergipark.org.tr/en/download/journal-file/19583**)
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Manuscripts prepared in Microsoft Word must be converted into a single file before submission. Please start with the title page and insert your graphics (schemes, figures, *etc.*), tables in the main text.

Title (should be clear, descriptive, and not too long)

Full Name(s) and Surname (s) of author(s)

ORCID ID for all author (s) (http://orcid.org/)

Address (es) of affiliations and e-mail (s)

Complete correspondence address and e-mail

Abstract

Keywords (indexing terms), normally 3-6 items

Introduction

Material and Methods

Results and Discussion

Conclusion

Compliance with Ethical Standard

Conflict of Interest: When you (or your employer or sponsor) have a financial, commercial, legal, or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

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Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

Funding: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Disclosure: Explanations about your scientific / article work that you consider ethically important.

References

Tables (all tables given in the main text)

Figures (all figures/photos given in the main text)

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. **The main text should contain "Introduction", "Materials and Methods", "Results and Discussion" and "Conclusion" sections.**

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

Review Articles: Reviews prepared by authors who have extensive knowledge of a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in research and should guide future studies. The main text should start with the Introduction and end with the Conclusion sections. Authors may choose to use any subheading in between those sections.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Short Communication" Readers can also present their comments on the published manuscripts in the form of a "Short Communication". **The main text should contain Introduction, "Materials and Methods", "Results and Discussion" and "Conclusion" sections.**

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted in main document WORD files (in JPEG or PNG format) through the submission system. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in the USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

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References

Reference System is APA 6th Edition

In-text Citation with APA

The APA style calls for three kinds of information to be included in in-text citations. The **author's last name** and the work's **date of publication** must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Crockatt, 1995).

Direct quote from the text

"The potentially contradictory nature of Moscow's priorities surfaced first in its policies towards East Germany and Yugoslavia," (Crockatt, 1995, p. 1).

Major Citations for a Reference List in Table 2.

Note: All second and third lines in the APA Bibliography should be indented.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 15 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

Table 2. Major Citations for a Reference List

Material Type	Reference List/Bibliography
A book in print	**Baxter, C. (1997).** *Race equality in health care and education.* Philadelphia: Ballière Tindall, p. 110-115, ISBN 4546465465
A book chapter, print version	**Haybron, D.M. (2008).** Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (p. 17-43). New York, NY: Guilford Press. ISBN 4546469999
An eBook	**Millbower, L. (2003).** *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. p. 92-90. Retrieved from http://www.amacombooks.org/ (accessed 10.10.15)
An article in a print journal	**Carter, S., Dunbar-Odom, D. (2009).** The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy,* 14(1), 38-48.
Preview article in a journal with DOI	**Gaudio, J.L., Snowdon, C.T. (2008).** Spatial cues more salient than color cues in cotton-top tamarins (*Saguinus oedipus*) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
Websites - professional or personal sites	***The World Famous Hot Dog Site.* (1999, July 7).** Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.2015)
Websites - online government publications	**U.S. Department of Justice. (2006, September 10).** Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.2015)
Photograph (from book, magazine or webpage)	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.2015)
Artwork - from library database	**Clark, L. (c.a. 1960's).** *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
Artwork - from website	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.2015)