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Aims and Scope

AQUATIC RESEARCH

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Aquatic Biology, Aquatic Ecology, Aquatic Environment and Pollutants, Aquaculture, Conservation and Management of Aquatic Source, Economics and Management of Fisheries, Fish Diseases and Health, Fisheries Resources and Management, Genetics of Aquatic Organisms, Limnology, Maritime Sciences, Marine Accidents, Marine Navigation, and Safety, Marine and Coastal Ecology, Oceanography, Seafood Processing, and Quality Control, Seafood Safety Systems, Sustainability in Marine and Freshwater Systems The target audience of the journal includes specialists and professionals working and interested in all disciplines of marine and aquatic sciences.

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The journal's target audience includes specialists and professionals working and interested in all disciplines of marine and aquatic Sciences.

The editorial and publication processes of the journal are shaped by the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal conforms to the principles of

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RESEARCH ARTICLES

SHORT COMMUNICATION

AQUATIC RESEARCH

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Aquat Res 6(2), 83-96 (2023) • https://doi.org/10.3153/AR23009 *Research Article*

An investigation of the maritime accident in the Aegean Sea Turkish search and rescue region

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ABSTRACT

The Aegean Sea is risky for marine accidents due to its geography and dense vessel traffic. Revealing the patterns of marine accidents in the region is essential for preventing such accidents in the future. With this motivation, this study analyzes the data on maritime accidents in the Aegean Sea Turkish Search and Rescue Region. For this purpose, the descriptive analysis of the 576 marine accidents in the region between 2001 and 2020 was accomplished. Moreover, by applying hypothesis tests, the relationships between the factors that affect the occurrence of marine accidents were investigated. As a result, the most common contributing factors to the event of accidents were revealed. The relationships between the variables of the data set were determined. Accordingly, the type of ship with the most accident are yacht/recreational boat. Hull/machinery failure is the most common type of accident, and summer is the most frequent season for maritime accidents. It was revealed that there are significant relationships between the variables such as the type of accident, the sub-region where the accident occurred, the time of the accident, and the ship type. Finally, deficiencies were evaluated, and suggestions were made for more effective investigation and prevention of accidents.

Keywords: Aegean sea, Maritime casualty, Accident analysis, Search and rescue

Introduction

Geographically, the Aegean Sea is a semi-closed sea located between 41°-35° north latitudes and 23°-27°/28° east longitudes (Başeren, 2006). Its average depth is approximately 350 meters. Its length in the north-south line is 660 km. The length of the east-west line is 270 km in the north, 150 km in the middle, and 400 km in the south. Together with the Turkish Straits, the Aegean Sea forms a vital waterway connecting the Black Sea and the Mediterranean.

Maritime trading, tourism, and fishing take place in the Aegean Sea intensely. Aegean Sea shipping is 75% of all marine trade to Turkish ports. Furthermore, ships sailing in this area supply 75% of Türkiye's oil demands (Kurumahmut, 1998). Regarding marine tourism, approximately 5.4 million tourists visited Muğla and İzmir cities on the coasts of the Aegean Sea in 2019 (TÜİK, 2020). In addition, the Aegean's coasts and islands are the regions where cruise ships frequently visit and where marine activities are intense (Akay, 2020; DTO, 2019). Geographically, there are narrow passages and straits created by more than 1800 islands. Therefore, maritime accidents frequently occur in the area and pose severe risks to life, economic and environmental issues. Analyzing marine accidents and taking measures to prevent them will contribute to mitigating or eliminating these risks.

Directorate General of Coastal Safety of Türkiye provides vessel traffic services in the Aegean Sea to control maritime traffic effectively and reduce maritime accidents. İzmir Vessel Traffic Center and Turkish Straits Vessel Traffic Center, affiliated with the Directorate General of Coastal Safety, serve in the Aegean Sea Turkish Search and Rescue Region. While the marine area between Babakale and Çeşme and the İzmir Bay is under the responsibility of İzmir vessel traffic services, the marine area being used to approach the Çanakkale Strait is under the obligation of the Turkish Straits Vessel Traffic Services (KGM, 2020).

This study performed a statistical analysis of maritime accidents that occurred over 19 years in the Turkish search and rescue region of the Aegean Sea. In addition, hypothesis tests were performed in the SPSS statistics among the variables that make up the data set. The study aims to present the pattern of marine accidents in the relevant region and to present a way out of measures that can be taken to reduce such accidents in the future. The findings obtained are thought to be useful for safe management for maritime stakeholders. In the ongoing sections, the geographical field of the study, data collection process, methods, application of the method, findings, discussion, and conclusion sections are included, respectively.

Literature Review

A survey of the literature reveals that the majority of research that analyzes marine accidents included statistical analysis, causation investigation, and the determination of hazard or risk maps. Ece (2011), in the paper on marine casualties in the İstanbul Strait, studied statistical analysis of the time of the accidents, accident types, and the types of ships involved (Ece, 2011). Büber and Töz (2017) conducted an accident risk analysis using Geographical Information Systems (GIS) for maritime casualties in the Turkish Port Regions of the Aegean Sea (Büber and Töz, 2017). Kuleyin and Aytekin (2015) statistically analyzed the marine casualties in Çanakkale Strait between 2004-2014 and suggested preventing future accidents (Kuleyin and Aytekin, 2015). In his master's thesis, Kızkapan (2010) performed statistical analysis related to accidents of vessels engaged on international voyages at the coasts of Türkiye between 2004 and 2008 (Kızkapan, 2010). Park and Ahn (2007) analyzed the variance of accident information such as accident time, vessel speed and distance with the SPPS program for a period of 10 years (Park and Ahn, 2007). Aalberg et al. carried out a bivariate t-test and chi-square analysis of marine casualties in Norwegian waters in the light of data on ship information, ship behaviour, accident type, and external factors (Aalberg et. al., 2016). Kılıç and Sanal (2015) conducted a statistical analysis of the grounding accidents that took place between the borders of the Çanakkale Strait between 2000 and 2011 and analyzed the causes of these accidents using the fault tree analysis method (Kılıç and Sanal, 2015). Raiyan et al. (2017), in their study, examined the marine casualties that occurred in Bangladesh waters between 1974 and 2014 and obtained findings of the causes of accidents with the event tree analysis (Raiyan et. al., 2017). Mullai and Paulsson (2011) aimed to design a conceptual model for the analysis of marine casualties with their study using metric and non-metric variables with the marine casualty data they obtained from the Swedish Maritime Administration (Mullai and Paulsson, 2011). Dobbins and Abkowitz (2010) have explored how advanced information technologies can be used to assess US sea routes' hazard risk using GIS (Dobbins and Abkowitz, 2010). Shahrzad et al. (2014),

with their study on marine casualties, proposed an accident simulation model to evaluate the accident risk in maritime transportation using Markov Modeling and Markov Chain Monte Carlo Simulation (Shahrzad et. al., 2014). Kujala et al., with their study, examined the marine casualty statistics of the last 10 years to analyze the safety of the maritime traffic in the Gulf of Finland and then evaluated the collision risk of the ships with the theoretical model they developed (Kujala et. al., 2009). Olgaç and Töz (2020) examined cooperation activities and disputes with coastal states regarding the search and rescue activities of marine casualties in Turkish seas (Olgaç and Töz, 2020). Maya et al. proposed a marine casualty learning approach with fuzzy cognitive maps combined with bayesian networks to make risk assessments by determining significance coefficients for each factor that causes marine accidents and to develop and implement risk control options more effectively (Maya et. al., 2020). Yılmaz and İlhan made a retrospective examination of the marine casualty/incidents that resulted in death, injury, or loss involving Turkish-flagged ships in the Turkish Search and Rescue Region (Yılmaz and İlhan, 2018). Seo and Bae analyzed the cause of the accident and the accident statistics by examining the court reports of the marine casualties that occurred over a period of ten years (Seo and Bae, 2002). Nas analyzed the grounding accidents at Yenikale Pass in İzmir Bay and made a risk assessment (Nas, 2011). Arslan et al. investigated the causes of accidents on board that occur during cargo operations at tanker terminals using a fault tree analysis approach and tested the results in a Monte Carlo simulation (Arslan et. al., 2018). Karabacak and Köseoğlu examined the maritime accidents that took place between 2007 and 2017 in Turkish Territorial Waters and aimed to analyze the maritime casualties by data mining method in their paper (Karabacak and Köseoğlu, 2021). In the study he prepared, Olgaç made a literature review of the maritime accident analysis methods used in the analysis of maritime accidents and introduced these analysis methods and gave general information about their use (Olgaç, 2021). Demirci and Gülmez aimed to determine the types of marine accidents caused by human errors on Ro-Ro cargo ships and analyzed the usefulness of the Human Factors Analysis and Classification System method in the classification of these accidents (Demirci and Gülmez, 2021).

Material and Methods

Following the selection of the study's topic, a thorough literature review was conducted to look at earlier studies on maritime accidents. Then, the data were officially provided by the main search and rescue coordination centre (MSRCC). Findings were obtained through descriptive analyzes and hypothesis tests on the data. The findings of the study were compared with the findings of similar studies in the discussion section. Finally, in the conclusion section, suggestions were made for a more effective evaluation of marine accidents. The diagram showing the process of the study is as follows.

Study Area

The Search and Rescue Region of Türkiye in the Aegean Sea geographically limits the scope of this study. The borders of the Search and Rescue Region of Türkiye in the Aegean Sea are determined by Türkiye through The Search and Rescue Regulation of Marine and Air Vehicle Accidents published on October 17, 2020. The Search and Rescue Region of Türkiye in the Aegean Sea is given below in figure 1.

This study divided Türkiye's Aegean Sea Search and Rescue Region into six subregions based on the geographical structure of the coastline. Each subregion was given codes from 1 to 6. The accidents are also divided and distributed according to these six subregions. These six subregions and their codes are shown in figure 2. The subregions are 1-North Agean, 2-Edremit, 3-İzmir, 4-Kuşadası, 5-Bodrum, and 6-Marmaris.

Data Collection

In the scope of the study, the data set, which includes variables such as date, season, accident type, vessel type, subregion, loss of life, and injury, were obtained from the Main Search and Rescue Coordination Center's (MSRCC) under the Ministry of Transport and Infrastructure of the Republic of Türkiye. The centre gave the data set based on the authors' official letter. The data set includes marine accidents in the Aegean Sea Turkish Search and Rescue Region between 2001-2020 (until the 5th of March).

Literature Review

- **Data Bases:** Google Academic, Scopus, Science Direct, Wiley Online
- **Keywords:** Marine casualty, Marine accident, Accident analysis, Accident investigation, Accident analysis, Search, and rescue

Data collection

All datas were obtained from MSRCC

Analysis process

Statistical analysis methods were used

Finding and Discussion

Conclusion

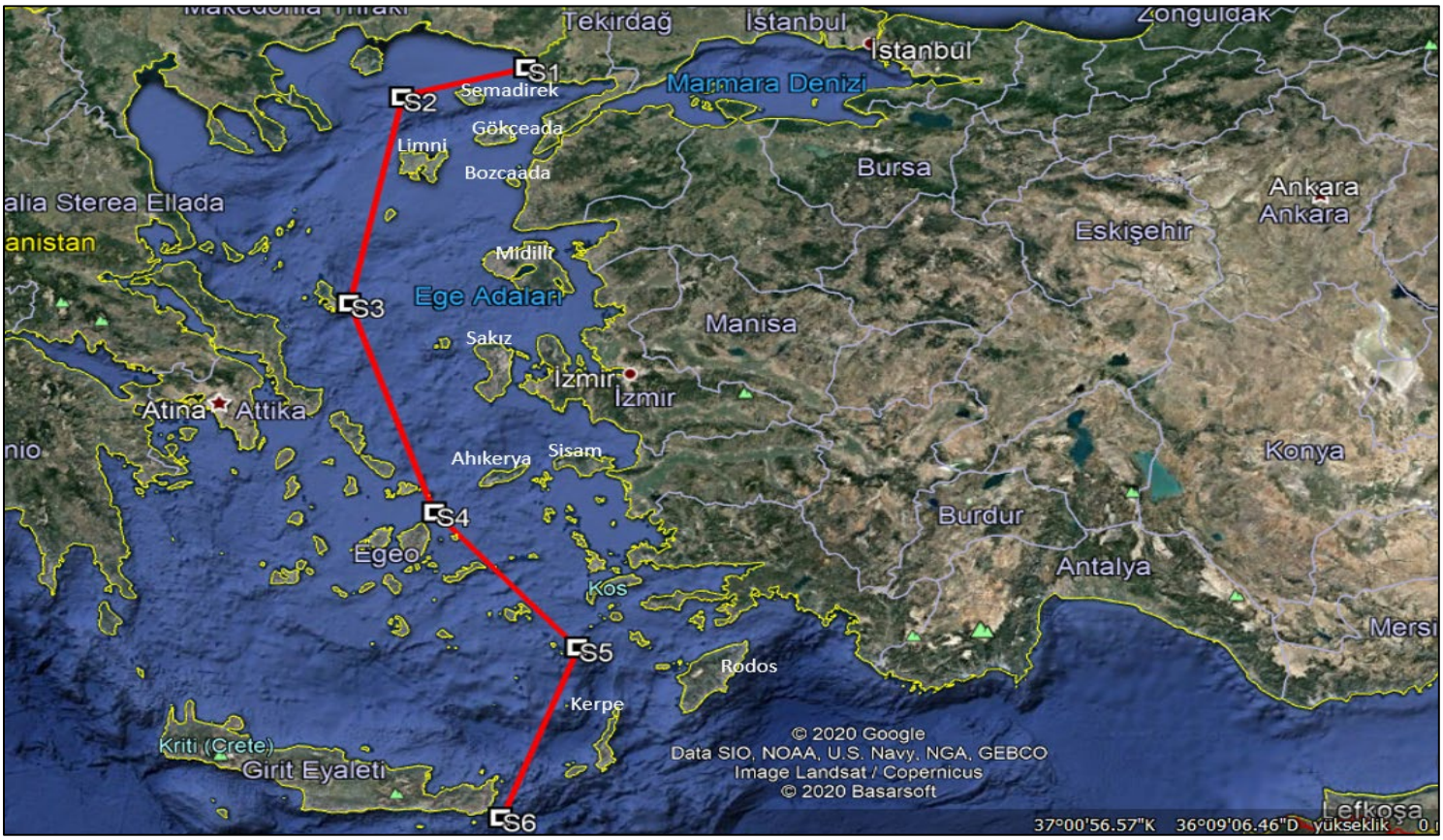


Figure 1. The search and rescue region of Türkiye in the Aegean Sea

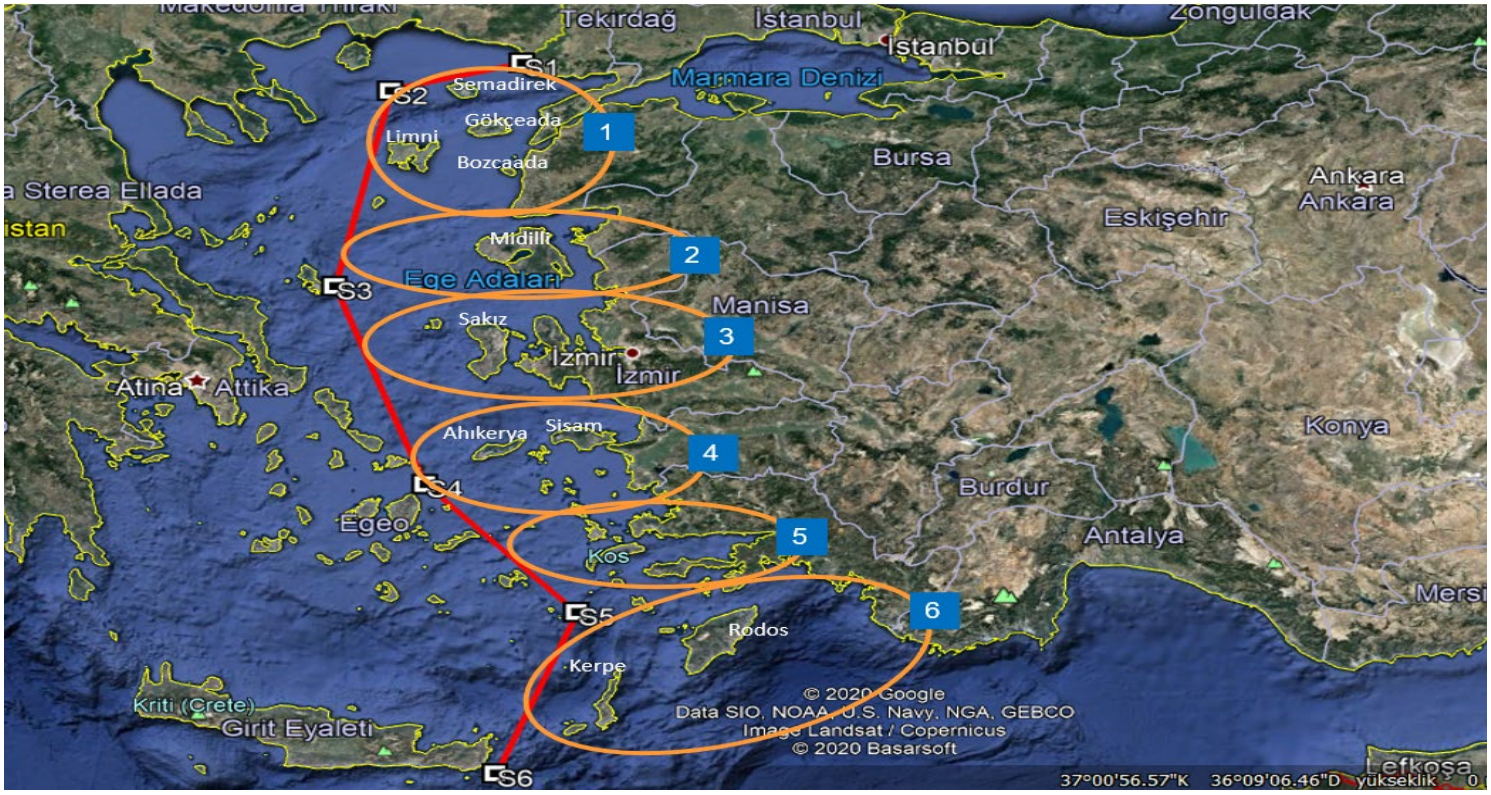


Figure 2. Subregions of the Aegean Sea Turkish search and rescue region

Statistical Analysis Method

Statistical data analysis of the marine accidents that occurred in the Aegean Sea Turkish Search and Rescue Region between 2001-2020 (till 05th March 2020) was performed using SPSS Statistics v.23. Frequency analysis, chi-square tests and Kruskal Wallis tests of the variables that make up the data set were performed. Frequency analysis is a type of content analysis that makes it easy to understand the density and importance of a particular item (Sezginsoy, 2007). The chi-square test was used to investigate significant relationships between variables that made up the data set. A Chi-square test is applied to test the relationship between two nominal variables. The null hypothesis (H0) states that the two nominal variables are independent of each other or, in other words, the absence of a significant relationship between the two nominal variables. The alternative hypothesis (HA or H1) states a significant relationship between the two nominal variables, so these variables are dependent (Güngör and Bulut, 2008). Kruskal Wallis tests were applied to investigate the existence of the relationship between categorical variables and numeric variables. The Kruskal-Wallis test is a nonparametric hypothesis test that explores the relationship between the numerical variable and the categorical variable consisting of more than two groups. The Kruskal-Wallis test is a one-way variance analysis between independent variables of populations (McKight and Najab, 2010).

Frequency Analysis

A total of 576 marine accidents occurred in the Aegean Sea Turkish Search and Rescue Region between 2001 and 2020 were examined. The frequency of these cases based on date (years), seasons, accident types, vessel types, subregions, loss of life, and injury is given below.

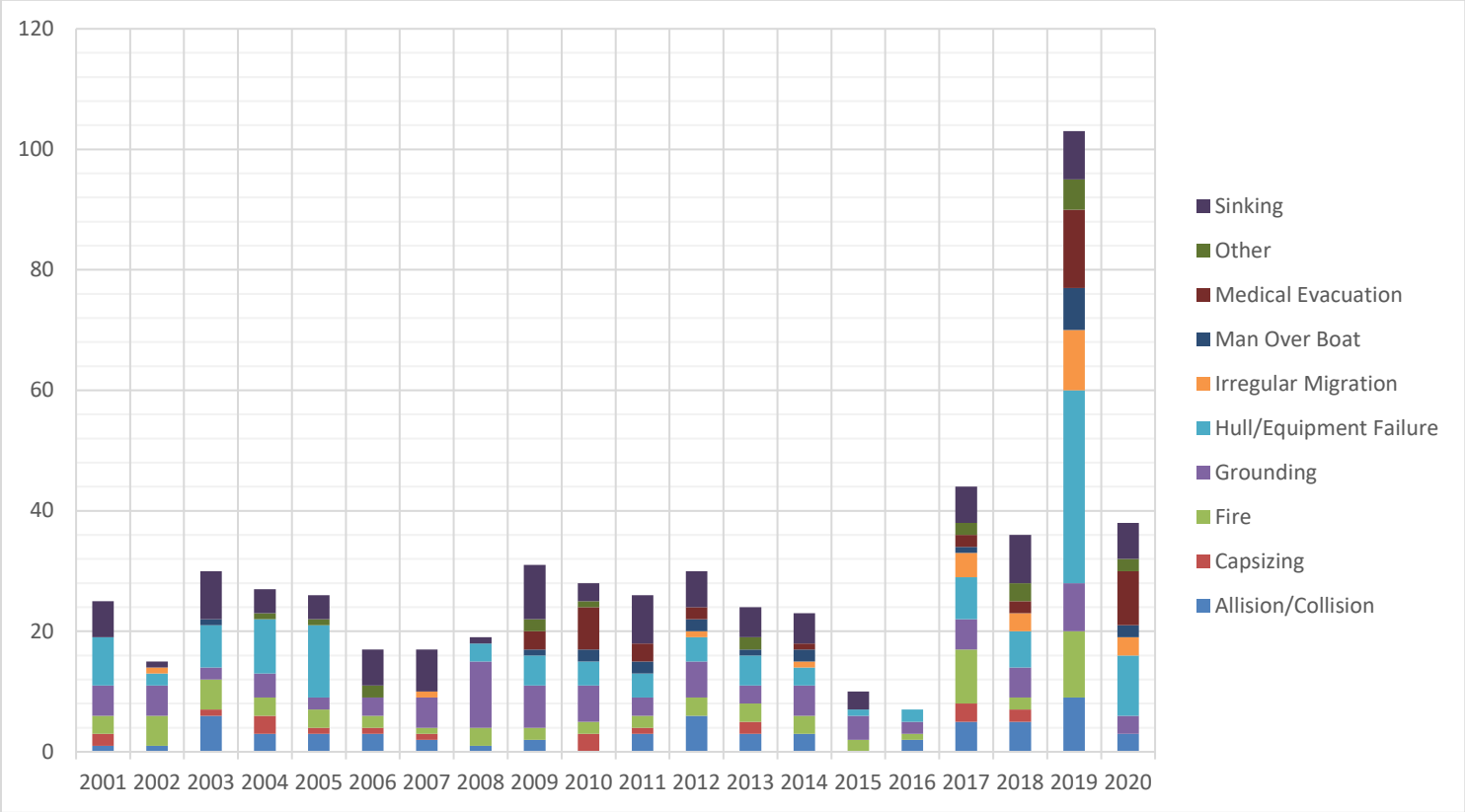


Figure 3. Distribution of accident/incident types by years

As is seen in figure 3, the frequency of accidents are as follows respectively: hull/equipment failure with the number 124, sinking with the number 104, grounding with the number 94, fire with the number 65, allision/collision with the number 61, medical evacuation with the number of 42, irregular migration with the number 24, man overboard with the number 21, other with the number 21 and capsizing with the number 20. When the distribution of marine accidents/incidents in the Aegean Sea by years is examined, it is seen that the highest number of accidents occurred in 2019 with several 103. The year in which the least accidents occurred is 2016 with several 7. Most of the accidents in 2019 took place in the third subregion, on yacht/recreational type ships, and during the summer season.

Spring and summer were the seasons with the least and highest accidents, respectively, according to an analysis of the distribution of accidents by seasons shown in figure 4. It is also observed that the most common type of accident during the summer period is hull/equipment failure, followed by sinking and fire, respectively. It was determined that the most accidents in the summer season occurred in subregion 3 and yacht/recreational vessel type.

When the types of vessels involved in accidents are examined as in figure 5, it is understood that the yacht/recreational boat comes to the fore with the number 274. The most common yacht/recreational boat accident/incident types are hull/equipment failure (77), sinking (64) and fire (42) respectively. Also, It can be seen in the figure that ro-ro vessels have the least accident number during the period under review.

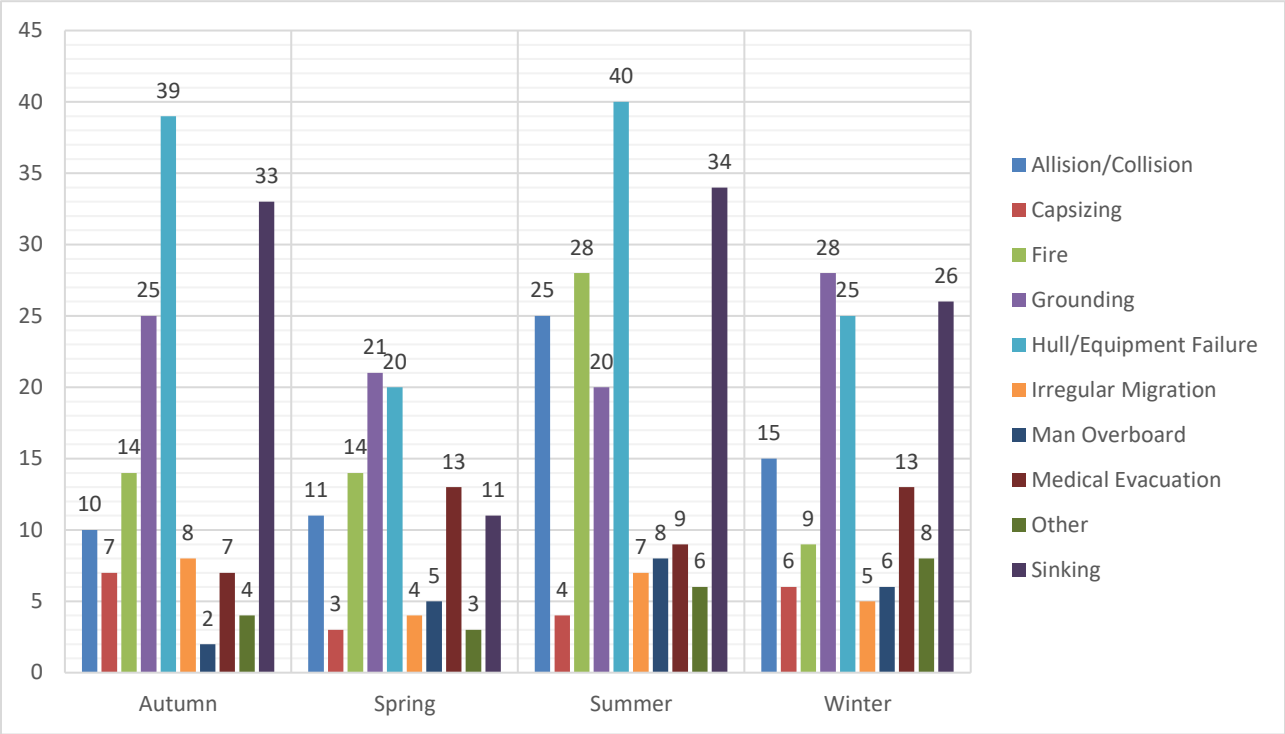
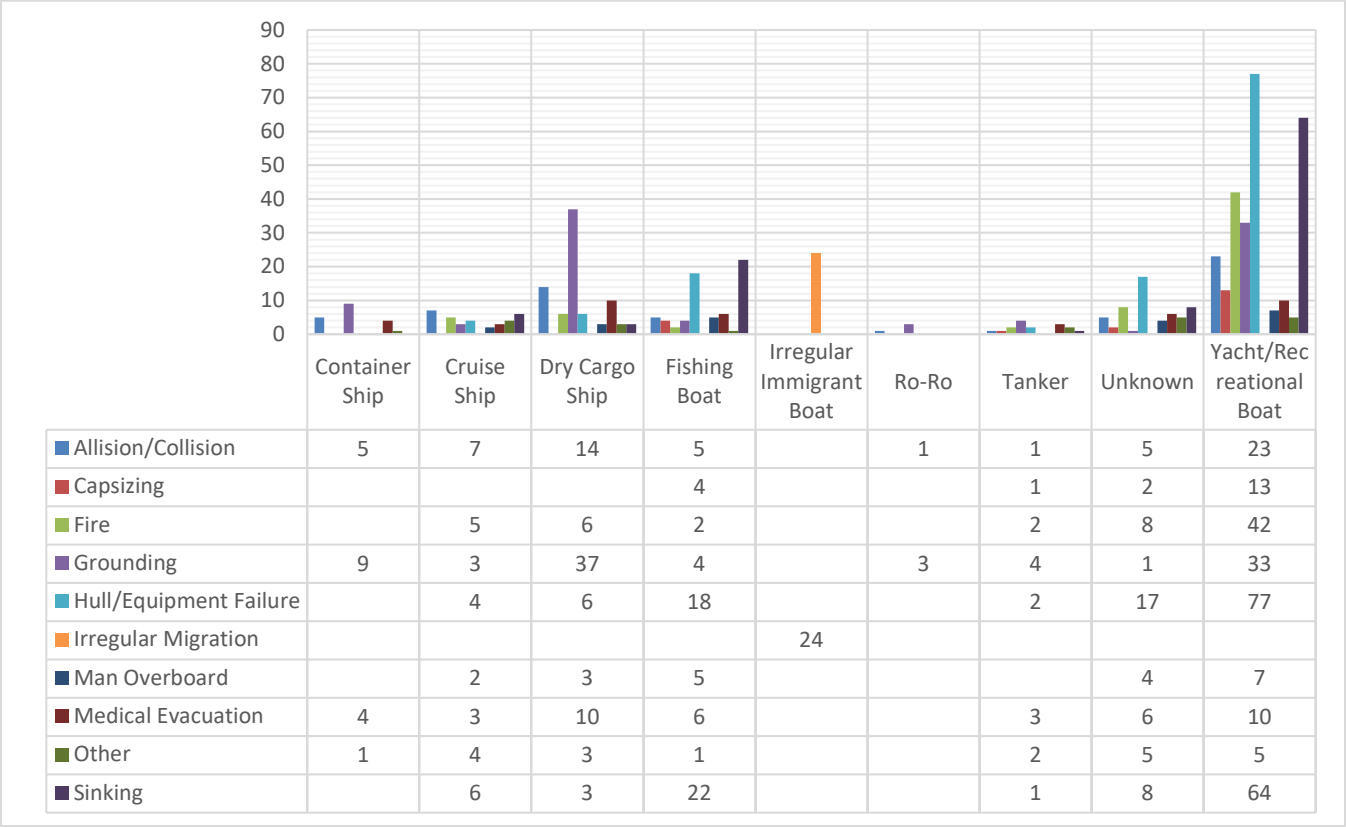


Figure 4. Distribution of accident/incident types by season (from 2001 to 2020)



	Container Ship	Cruise Ship	Dry Cargo Ship	Fishing Boat	Irregular Immigrant Boat	Ro-Ro	Tanker	Unknown	Yacht/Recreational Boat
Allision/Collision	5	7	14	5		1	1	5	23
Capsizing				4			1	2	13
Fire		5	6	2			2	8	42
Grounding	9	3	37	4		3	4	1	33
Hull/Equipment Failure		4	6	18			2	17	77
Irregular Migration					24				
Man Overboard		2	3	5				4	7
Medical Evacuation	4	3	10	6			3	6	10
Other	1	4	3	1			2	5	5
Sinking		6	3	22			1	8	64

Figure 5. Distribution of accident/incident types by vessel types

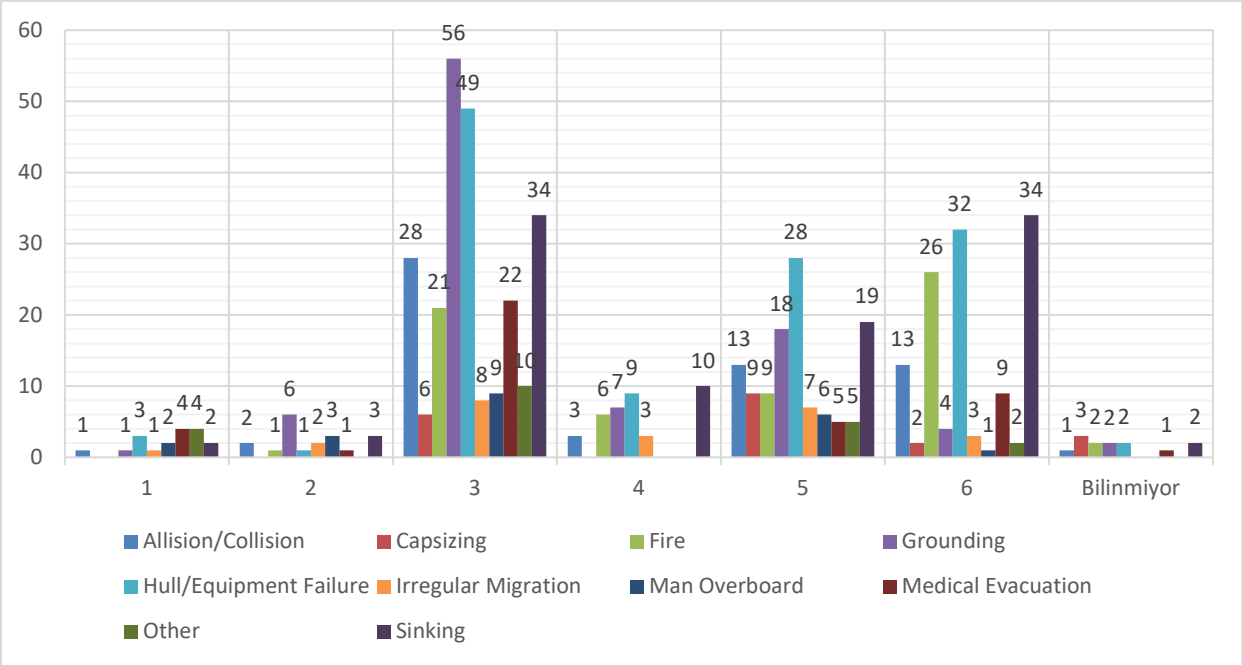


Figure 6. Distribution of accident/incident types by subregions

The subregions where the accidents occurred are examined in figure 6; it is seen that the most accidents occurred in the İzmir subregion (3) with the number of 243, followed by tourism subregions Marmaris (6) with the number of 126, and Bodrum (5) with the number of 119. Grounding is the most common accident type that occurred in the İzmir subregion.

During the period under consideration, 224 people died in 42 accidents/incidents and 114 people were injured in 22 accidents/incidents. Considering the accidents that resulted in the loss of life, it was understood that the groups with the highest number of loss of life cases were the year 2012 with 69 people, the summer season with 105 people, the 4th subregion with 80 people, the irregular migration activity with 145 people, and irregular migrants boats with 145 people. Considering the accidents/incidents that resulted in injuries, it was understood that the groups with the highest number of injury cases were the year 2013 with 34 people, the summer season with 65 people, the type of fire accident with 22 people, yacht/recreational type vessels with 59 people and the 3rd subregion with 54 people.

Binary Hypothesis Tests

Chi-square tests were applied to test the relationships between the 5 categorical variables of the data set, namely date, season, accident type, vessel type, and subregion variables. Since our categorical variables in the data set consist of more than two subgroups, in chi-square tests, a conclusion was reached according to the significance value (p) of the Fischer-Freeman-Helton exact test. To reach the p-value of the Ficher-Freeman-Helton exact test, calculations based on Monte Carlo simulation were performed at a 99% confidence interval and 10,000 sample scales. It is known that the exact test p-value obtained by Monte Carlo simulation (with 10,000 samples and 99% confidence interval) is the same up to the three decimal places with the exact test p-value obtained with the exact option in the SPSS (Mehta and Patel, 2011). In this concept, within the framework of chi-square tests, the hypothesis and Fisher-Freeman-Halton p significance values were formed as follows:

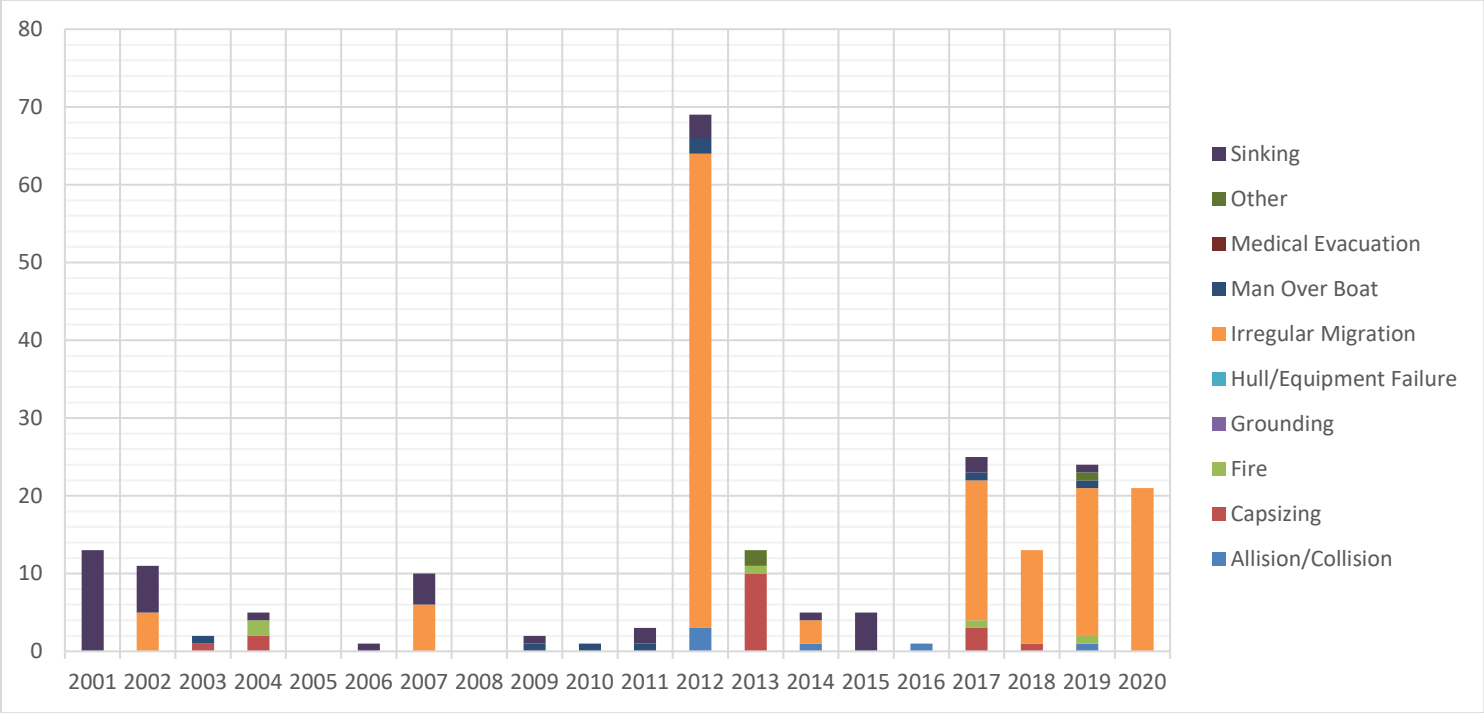


Figure 7. Distribution of loss of life by years

1. Date x Season: $\begin{cases} H_0: No\ significant\ relationship\ between\ date\ and\ season. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

2. Date x Accident Type: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ date\ and\ Acc.Inc.type. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

3. Date x Vessel Type: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ date\ and\ vessel\ type. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

4. Date x Subregion: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ date\ and\ subregion. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

5. Season x Accident Type: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ season\ and\ Acc.Inc.type. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

6. Season x Vessel Type: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ season\ and\ vessel\ type. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

7. Season x Subregion: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ season\ and\ subregion. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

8. Accident Type x Vessel Type: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ Acc.Inc.type\ and\ vessel\ type. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

9. Accident Type x Subregion: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ Acc.Inc.type\ and\ subregion. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

10. Vessel Type x Subregion: $\begin{cases} H_0: No\ significant\ relationship\ betweeen\ vessel\ type\ and\ subregion. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000^b < 0{,}05; so\ H_A\ is\ supported. \end{cases}$

As a result of the chi-square tests carried out within this framework, it has been determined that there are significant relationships between date and season, accident type, vessel type, and subregion; between season and accident type, vessel type and subregion; between accident type and vessel type and subregion; between vessel type and subregion.

According to the normality and homogeneity test results applied to the variables loss of life, and injury, it was understood that these variables don't conform to the normal distribution and their variances were not homogeneous. Therefore, to examine the relationship between categorical variables and these two numerical variables, nonparametric Kruskal Wallis tests were applied instead of the parametric one-way ANOVA test. Hypotheses and P asymptotic values obtained as a result of Kruskal Wallis tests are as follows:

1. Date x Loss of Life: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ date\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}271 > 0{,}05; so\ H_0\ is\ supported. \end{cases}$
2. Season x Loss of Life: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ season\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}642 > 0{,}05; so\ H_0\ is\ supported. \end{cases}$
3. Accident Type x Loss of Life: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ accident\ type\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000 < 0{,}05; so\ H_A\ is\ supported. \end{cases}$
4. Vessel Type x Loss of Life: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ vessel\ type\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000 < 0{,}05; so\ H_A\ is\ supported. \end{cases}$
5. Subregion x Loss of Life: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ subregion\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}553 > 0{,}05; so\ H_0\ is\ supported. \end{cases}$
6. Date x Injury: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ date\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000 < 0{,}05; so\ H_A\ is\ supported. \end{cases}$
7. Season x Injury: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ season\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}043 < 0{,}05; so\ H_A\ is\ supported. \end{cases}$
8. Accident Type x Injury: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ accident\ type\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}000 < 0{,}05; so\ H_A\ is\ supported. \end{cases}$
9. Vessel Type x Injury: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ vessel\ type\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}377 > 0{,}05; so\ H_0\ is\ supported. \end{cases}$
10. Subregion x Injury: $\begin{cases} H_0: The\ medians\ of\ the\ groups\ of\ the\ subregion\ are\ equal. \\ H_A: H_0\ is\ false. \\ p\ value = 0{,}606 > 0{,}05; so\ H_0\ is\ supported. \end{cases}$

According to the results of the Kruskal Wallis tests, it was determined that the medians of the groups in the accident type and vessel type have a significant difference in terms of the number of losses of life. it was also determined that the medians of the groups of the date, season, and subregion do have not a significant difference in terms of the number of losses of life.

In the Kruskal Wallis tests performed for the injury, it was observed that the medians of the groups in the date, season, and accident type have a significant difference in terms of the number of injuries. No significant difference was found between the medians of the groups of subregion and vessel type in terms of the injury.

Results and Discussion

According to the findings obtained within the scope of the study, the region with the highest number of accidents in the Aegean Sea is the İzmir subregion, which includes the İzmir Bay, Çandarlı Bay, and İzmir offshore. When the locations of marine accidents in this region are examined, it is seen that grounding accidents occur frequently in the Yenikale Passage. Büber and Töz (2017) stated that there were many grounding accidents in the Yenikale Passage in İzmir Bay and that this area poses a high risk for ships with large draughts (Büber and Töz, 2017). Nas (2011) reported similar results that the frequent occurrence of grounding accidents at the Yenikale Passage. In the same study, Nas stated that the riskiest action for the Yenikale Passage is collusion during the manoeuvre to avoid grounding, and collusion at this location may stop the marine transport to İzmir Port for a long time (Nas, 2011). Within the Aegean Sea Turkish Search and Rescue Region, it has been observed that summer is the season with the highest number of accidents and the type of ship involved in the most accidents is a yacht/recreational boat. Yılmaz and İlhan (2018), in their study analyzing the marine accidents in the Turkish Search and Rescue Region, in addition to reporting the same results, also stated that more than half of those injured in these accidents were amateur fishermen/amateur sailors (34.6%) and professional seafarers (29.6%) (Yılmaz and İlhan, 2018).

Karabacak and Köseoğlu examined 738 maritime accidents that took place in Turkish territorial waters between 2007-2017 and they reached some statistical findings about these accidents (Karabacak and Köseoğlu, 2021). Karabacak and Köseoğlu found that the most common type of accident was conflict accidents, and the most common type of ship involved in the accident was a dry cargo ship. In the study they prepared, they stated that the majority of the accidents occurred in the Turkish Straits and the Marmara Sea, where maritime traffic is the most intense. In this study, it was found that yacht/recreational boats were the most involved in the accident in the Aegean Sea Turkish Search and Rescue Region, and the accidents resulting in hull/equipment failures were the most common accidents. Due to the geographical structure of the Aegean Sea and the fact that this region is a denser sea area than the Marmara Sea in terms of tourism activities, it is considered that the findings related to the accidents in this sea are different from the study by Karabacak and Köseoğlu.

Aalberg et.al. (2016) analyzed the marine casualties in Norwegian seas by performing t-test and chi-square tests within the framework of data such as ship type, ship's age, ship's flag, route, speed, operation time, accident type, and external factors (traffic density, day or night, etc.) (Aalberg, 2016). It has been understood that studies conducted with data sets containing such detailed information give more healthy and understandable results. Generally, such detailed information is not taking place in the data sets used in the statistical analysis of marine casualties. In this respect, it is important to record data completely after accidents in order to make a more accurate analysis of marine casualties. Also, in order to benefit from previous academic studies on marine casualties, it is crucial to standardize the classification of data such as accident type and ship type, etc. Classification of maritime accidents as specified in the code and directive IMO, 2009/18 / EC published by IMO in 2009 will be useful in achieving this goal (IMO, 2019).

Conclusion

In this study, the marine accidents/incidents that occurred in the years between 2001-2020 in the Aegean Sea Turkish Search and Rescue Area are examined. As a result of the analysis, it was found that the yachts/recreational boats were the most frequent ship type that accidents occurred, and hull/equipment failure was the most common accident type, in the mentioned region and period. Besides, it was determined that in İzmir Bay and Aliağa-Nemrut Port Regions, large ships such as dry cargo ships were involved in accidents such as grounding and allision to the pier.

As a Peninsula country, in Türkiye, the private and commercial activities of yachts are getting increased year by year. Hence, the ascending accident rate of these ships is an expected situation. In order to reduce accidents in yachts/recreational boats, it would be beneficial to handle, analyse and publish such accidents differently from cargo ships. In this manner, the authorities will be assisted in taking effective measures to prevent such accidents. It is vital that ships navigating in the region comply with the guidance of İzmir Vessel Traffic Services and benefit from pilotage and towage services when necessary, in order to prevent accidents.

Due to marine transport density, marine tourism level, and irregular migration activities, the Aegean Sea is a waterway that contains importance and risk together. The effectiveness of marine casualty analysis has of great significance in reducing marine casualties in this region. For an effective accident analysis, it is necessary to keep an accurate and detailed record of the data of each accident. The results obtained from the analysis should be discussed altogether by public institutions, academics, and maritime sector stakeholders and the most effective steps should be taken to prevent accidents.

Compliance with Ethical Standards

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

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Demographic characteristics and exploitation rate of *Dormitator lebretonis* (Pisces: Eleotridae: Steindachner, 1870) from four coastal lagoons of Southern Benin, West Africa

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ABSTRACT

Dormitator lebretonis (Pisces: Eleotridae) is the dominant eleotrid in the Benin coastal waters, where this species constitutes an important fisheries component. The current fisheries survey investigates demographic parameters and the exploitation of D. lebretonis from the Benin coastal waters to contribute to species conservation and sustainable use. Individuals of *D. lebretonis* have been sampled monthly for 18 months at four locations in the Benin coastal waters. Overall, *D. lebretonis* showed moderate asymptotic length ($L\infty$) ranging between 11.55-12.08 cm. The growth rate (K) ranged between 0.74yr^{-1} and 3.1yr^{-1}. The species exhibited moderate longevity ranging between 0.968 and 4.054 years. The growth performance index (φ') varied between 1.994-2.617, indicating that *D. lebretonis* is a relatively slow-growing eleotrid. Annual mortalities dominate population growth ($Z/K>1$), and lengths at first capture ($3.80 \leq L_{50} \leq 5.05$cm) indicated that *D. lebretonis* was exploited at immature stages. Nevertheless, the exploitation rate (E) was under 0.5, showing the stock was underexploited. The species showed two peaks of recruitment, except in Lake Ahémé. Sustainable exploitation of *D. lebretonis* in the coastal waters requires a holistic management scheme that should include fisheries regulation enforcement, spawning ground protection, ecosystem restoration, and follow-up.

Keywords: Coastal waters, *Dormitator lebretonis*, Exploitation rate, Growth parameters, Lengths at first capture, Recruitment/Management

Introduction

In sub-Saharan Africa, fisheries provide low-cost proteins of high nutritional quality accessible to grassroots and urban populations, even those with limited revenues (Joly et al., 2007; Allali, 2017). Primarily, fishermen practice multi-species fisheries that improve their income and, thus, constitute an important component of their annual revenue. Belhabib et al. (2019) reported that African fishery catches increased from 2.1 million metric tons in 1950 to 16.7 million metric tons in 1988. Hence, due to overexploitation and environmental degradation, catches in 2010 were reduced to 12.4 million metric tons, estimated at 1.860.000.000 US dollars. At a country level, the role of fisheries in the economy is quite significant, and its contribution to the total Gross Domestic Product (GDP) of African countries is approximated at 1.12% (De Graaf and Garibaldi, 2014). In Benin, fisheries products remain the most accessible source of animal protein for the majority of the population, with an estimated consumption of 73 965 tons in 2020 (World Bank, 2022). The fisheries sector constitutes an important component of rural development, with about 59114 inland and marine fishermen in Benin (Direction de la Production halieutique du Bénin, 2022). Hence, it substantially contributes to reducing unemployment (JICA, 2022). In addition, fisheries play an important role in the national economy by contributing to about 2.2% of the national PIB (Gross Interior Product) (INSAE, 2019). As reported by the Benin fisheries Department (2019), though specialized inland and marine fishermen approximated 59114, more than 600 000 people (including fishermen, woman sellers, fishmongers, boat makers, boat mechanics, etc.) were directly or indirectly employed in the inland and marine fisheries sector. Hence, because of aquatic ecosystem degradation, habitat fragmentations, and overfishing, almost a quarter of fish populations are threatened worldwide, leading to changes in fish community structures and reduced fish stocks (Adite, 2013; Ifremer, 2019).

At the Benin coastal waters, a recent ichthyological survey indicated five (5) eleotrids, *Dormitator lebretonis*, *Eleotris vittata*, *Eleotris daganensis*, *Eleotris senegalensis,* and *Bostrychus africanus* inhabit the four coastal waters, Lake Nokoué, Porto-Novo Lagoon, Coastal Lagoon, and Lake Ahémé. Among them, *Dormitator lebretonis* is of high fisheries and commercial importance in these brackish waters where it constitutes the most abundant eleotrid making about 57.91% - 87.39% of the squeaker community. Fishbase (2022) states that *D. lebretonis* is only distributed in Africa and endemic to the West African coast from the Senegal River to the Cunene River in Namibia. Specifically, this eleotrid occurred in countries such as Angola, Benin, Cameroon, Côte d'Ivoire, Congo (RDC), Gambia, Ghana, Guinea, Namibia, Nigeria, Senegal, and Sierra Leone.

Benin Fisheries Department (2022) reported that annual catches of Eleotridae species in the Benin coastal waters were estimated at 47.95 metric tons. According to Sonon et al. (2021), *Dormitator lebretonis* was the dominant eleotrid in the Benin coastal waters and accounted for about 67.11% (31.87 metric tons) of the annual catches of Eleotridae. Fishing gear such as traps, cast nets, and seines were used to exploit the species. *Dormitator lebretonis* individuals were caught in aquatic vegetation in the spawning grounds at a depth of less than 50 cm. Also, the species was caught in open water with mean depths ranging between 138.8 ±36.1 5cm and 225.3 ±29.56 cm.

Babatundé (2015) and Sonon et al. (2021) reported that *D. lebretonis* was intensively exploited because when fried, this eleotrid is widely consumed with corn meal and rice. Likewise, for its great organoleptic quality and distinctive taste, *D. lebretonis* is utilized for soup seasoning in replacement with lagoon shrimps whose stock has decreased. In general, there is no prohibition against the fishing *Dormitator lebretonis* as the species is not on the list of endangered species. Nevertheless, there is a fishing regulation from the Benin Department of Fisheries that prohibits the use of cast nets and gillnets of small mesh size (≤10 mm) (Direction de la production halieutiaue, 2018). Adite et al. (2013) and Sonon et al. (2021) reported that the sizes of *D. lebretonis* individuals recorded in Beninese lagoons were moderate due to overexploitation and habitat degradation. These threats to Benin coastal waters, ichthyofauna in general, and *D. lebretonis* in particular, constitute a real obstacle to the renewal of the fish stock and the fishing industry's sustainability. An emergency plan is, therefore, necessary to anticipate the collapse of the natural stock of *D. lebretonis* populations in Benin.

Despite the fisheries and commercial importance of this small-sized eleotrid and the continuing disturbances and degradation of Benin's coastal waters, little is known about the demographic features and exploitation rate of *Dormitator lebretonis*. Efficient conservation of fish, sustainable exploitation, and better decision-making in fisheries industries require knowledge of demographic components such as exploitation rate, fishing and natural mortalities, asymptotic length, growth factors, and species recruitment (Abohwere and Falaye, 2008; Tah et al., 2010; Imorou Sidi et al., 2019).

The current fisheries research aimed to assess the demographic characteristics and exploitation rates of *Dormitator*

lebretonis to contribute to the rational management of its stock in the Benin coastal waters.

Material and Methods

Study Area

The study was carried out in the four (4) coastal waters of Southern Benin. These were Lake Nokoué, Porto-Novo Lagoon, Coastal Lagoon, and Lake Ahémé. Lake Nokoué (140 km^2) is located between 6°20' and 6°30'N, and between 2°20' and 2°35'E. The Salinity of Lake Nokoué was highly variable and ranged between 0 to 40 ‰ because of the permanent connection of this lake with the marine water (Atlantic Ocean) (Adité et al., 2013; Hamil et al., 2018). The average depth varied between 0.25-3.46 m, transparencies were ≤ 98.5 cm, water temperature between 27.5- 31.1ºC, pHs ranged between 5.8 et 7.55, and dissolved oxygen varied from 0.55mg/L to 8.9 mg/L (Sonon et al., 2021). In general, lower dissolved oxygen was recorded in aquatic vegetation and swamp habitats full of organic matter and mud that intensively consume dissolved oxygen. In contrast, open water is a habitat of high transparency where high photosynthesis activities generate a relatively high dissolved oxygen concentration. Porto-Novo Lagoon (35 km^2) is geographically and hydrologically connected to Lake Nokoué and situated between 6°25'and 6°30'N and between 2°30' and 2°38'E, with an average width of 35 m (Gnohossou, 2006) and salinities ≤ 18 ‰. Depth ranged between 88.5-485.3 cm, transparency between 45.3-113.5 cm, water temperature between 26.8-31.5ºC and salinity between 0.11-18 ‰. pH varied between 6.2-8.55, and dissolved oxygen ranged between 0.85-8.2 mg/L (Sonon et al., 2021). The Coastal Lagoon extends 55 km^2 and is located between 6°25' and 6°30'N and between 2°30' and 2°38 East. Depth ranged between 2.2 - 3.85 m, with transparency varying between 2.2 m and 0.92 m. The salinity at the Coastal Lagoon varied between 0.15 and 30‰, and dissolved oxygen fluctuated between 0.82 and 9.8 mg/L. The pH was neutral and ranged between 6.5 and 8.5 (Adite et al., 2013; Sonon et al., 2021). Lake Ahémé (80 km^2**)** lies between 6.20° and 6.40°N and between 1.55° and 2°E. Depth ranged between 0.72 and 4.78 m, and transparencies varied between 0.78 and 1.29 m. The salinity in Lake Ahémé varied between 0.12-22‰, and pH fluctuated from 6.1 to 7.8. The dissolved oxygen concentration ranged from 0.85 to 9.7 mg/L (Sonon et al., 2021).

The four (4) coastal waters' environment shows a sub-equatorial climate type with an annual mean rainfall ranging between 1108.1 and 1307.3 mm (Adite et al., 2013). The evaporation varied from 59.2 to 145 mm (Akouegninou et al., 1993). The soils were sandy, swampy, and red ferric (Gbaguidi and Adite, 2016). The ambient temperature varies from 25°C to 30°C (I.N.S.A.E, 2016). Vegetation species were compounded by two main groups: aquatic floating plants (*Eichornia crassipes, Pistia stratiotes*) and semi-aquatic plants (*Paspalum vaginatum, Cyperus articulenius, Penisetum polystachion, Typha australis, Phragmites australis, Cyclosorus striatus*). Dominant mangrove trees were *Rhizophora* sp and *Aviennia africana* (Chouti, 2011). Regarding aquatic animals, the dominant fish families were Cichlidae, Clariidae, Bagridae, and Claroteidae; the family was the most abundant (Sonon et al., 2021; Vodougnon, 2015). Also, mangrove shellfish such as *Crassostrea tulipa* were intensively exploited and cultured.

Fish Sampling Methods

Twelve (12) sites, five (5) in Lake Nokoué, two (2) in Porto-Novo Lagoon, three (3) in Coastal Lagoon, and two (2) in Lake Ahémé were selected for the fish sampling (Figure 1). At each sampling site, geographic coordinates were determined using a GPS (Garmin Dakota 10). *Dormitator lebretonis* individuals were sampled monthly from April 2017 to September 2018 at the open water and aquatic vegetation habitats. Traps cast net (3m diameter; 10 mm mesh) and seine (3 to 20 mm mesh) was used for *D. lebretonis* samplings. Once collected, a 10% formalin was used to preserve the fish samples transported to the LEMEA laboratory of Abomey-Calavi University (Adite et al., 2013). At the laboratory, the total length (TL) and standard length (SL) of *D. lebretonis* individuals were measured to the nearest 1mm with an ichthyometer and weights (W) were measured to the nearest 1mg with an electronic balance (Adite et al. 2013).

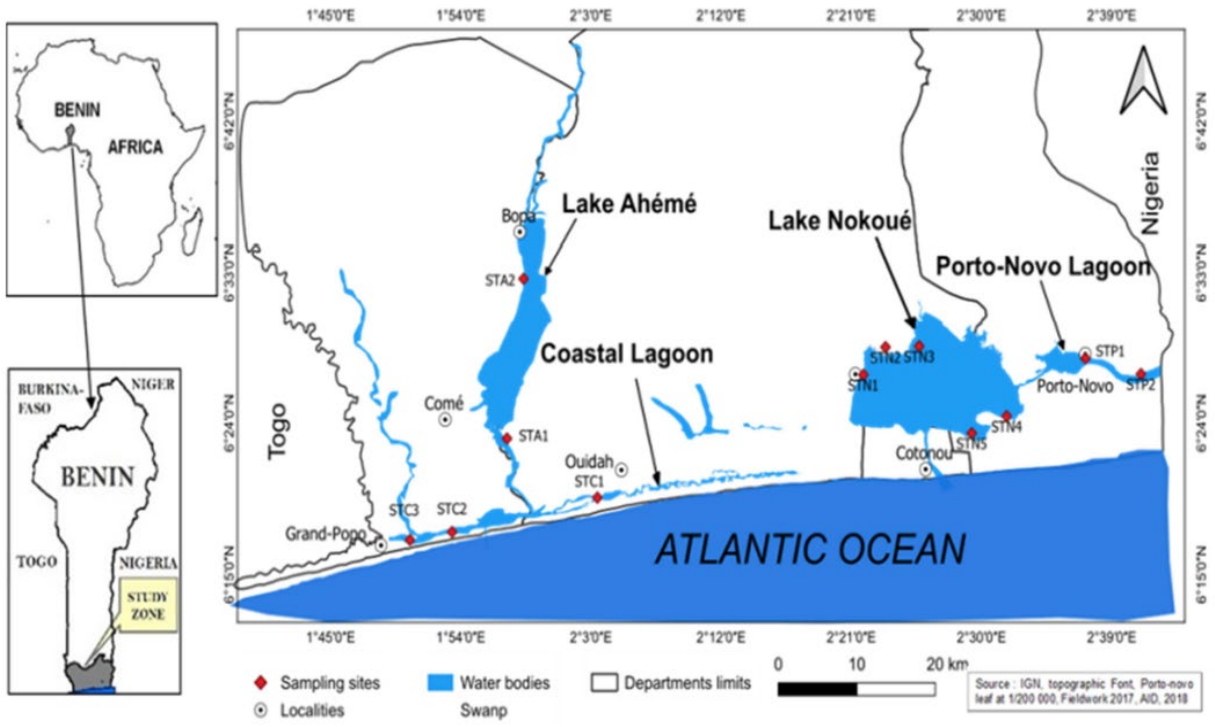
The growth parameters were computed using the VBGF fitted in FISAT II (2005).

Data Analysis

Growth parameters

The growth parameters of *D. lebretonis* individuals were computed using the von Bertalanffy growth function model (VBGF) (1938) of the ELEFAN I program in FiSAT II. The following growth formula was applied to the total length (TL) of *D. lebretonis*:

$$\mathbf{TL = L\infty \{1 - exp [-K(t - t0)]\}}$$



STN= Lake Nokoué sites; STP=Lagoon of Porto-Novo' sites; STC= Coastal Lagoon sites; STA= Lake Ahémé sites.

Figure 1. Map of *D. lebretonis* sampling sites

Where TL (cm) is the fish's total length; L∞ (cm) is the asymptotic length of the fish. L∞ is the maximum size that fish can reach after several years of life; K (yr^{-1}) is the growth coefficient of fish; t_0 (yr) is the theoretical age of fish when its length is zero. t_0 is obtained from the equation of Pauly (1979):

Log 10 ($-t_0$) = -0.392- 0.275 Log10L∞- 1.038 Log10K

Where t_0 (yr) is the inverse of the Bertalanffy growth equation. It represents the age of fish of different sizes. The Growth Performance Index was assessed using Pauly and Munro's (1984) formula:

φ'= Log10K+ 2Log10 L∞

Mortalities and level of exploitation

The total instantaneous mortality rate (Z) was directly estimated in FiSAT II software (FAO, 2005) using a Length-converted Catch Curve that integrates both the asymptotic length (L∞) and the growth coefficient (K) of the species in each of the four (4) coastal waters.

Natural mortality rate (M) was estimated using Pauly's (1980) in FiSAT II software (FAO, 2005) using the following empirical relationship:

$$\log_{10} M = -0.0066 - 0.279 \log_{10} L\infty + 0.6543 \log_{10} K + 0.463 \log_{10} T$$

Where T is the annual temperature of the water body.

The fishing mortality coefficient (F) was estimated by the formula: **F = Z-M**

The longevity was determined by Anato's (1999) formula: **Tmax = 3/K**

The exploitation rate (E) was used to determine whether or not *D. lebretonis* stock is overexploited (Pauly, 1983). was evaluated from the length linearity curve converted into a catch curve: **E = F/Z**

F is the fishing mortality rate, and Z is the annual mortality rate. According to Francis and Sikoki (2007), the maximum level of exploitation of a resource is reached when the exploitation rate is greater than or equal to 0.5 or when fishing mortality (F) is equal to natural mortality (M).

Probability of capture and first capture size

FiSAT software was used to generate the catch curve to assess both the size of the first capture (L_{50}) and the probability of capture.

Recruitment of *Dormitator lebretonis*

Recruitment is the relative abundance of new young fish stages that enter yearly in adult sub-population. The recruitment patterns of *D. lebretonis* in each of the four (4) coastal lagoons were assessed through a recruitment curve generated by FiSAT software based on total length (TL) frequency data (Pauly, 1985).

Results and Discussion

Estimation of the von Bertalanffy Growth Parameters (L∞, K, t) and the Growth Performance Index (φ')

Table 1 presents values of the von Bertalanffy growth parameters generated by FiSAT software. The asymptotic length (L∞) values vary from 11.55 cm (Lake Nokoué, Lake Ahémé, and Coastal Lagoon) to 12.08 cm in the Lagoon of Porto-Novo. Growth rate (K) values ranged between 0.74 yr^{-1} (Coastal Lagoon) to 3.100 yr^{-1} (Lake Ahémé). The highest growth performance index Φ' was recorded in Lake Ahémé (Φ'=2.204), and the lowest performance index was recorded in Coastal Lagoon (Φ'=1.994). Theoretical age at length 0 varied from -0.58 years recorded in the Lagoon of Porto-Novo and the Coastal Lagoon to -0.30 years in Lake Ahémé (Table 1).

Table 1: Growth parameters of *Dormitator lebretonis* from the coastal waters of Benin

Parameter	Lake Nokoué	Lagoon of Porto-Novo	Coastal Lagoon	Lake Ahémé
L∞ (cm)	11.55	12.08	11.55	11.55
K	1.200	0.780	0.740	3.100
t (an)	-0.46	-0.58	-0.58	-0.30
φ’	2.204	2.058	1.994	2.617

Estimation of Mortality Parameters, Exploitation Rate, and Longevity

Table 2 and Figure 2 showed trends of mortality parameters and exploitation rate (E) for *D. lebretonis* from Benin’s four (4) coastal waters investigated. Overall, the total mortality (Z) ranged between 2.21 and 8.29, while the natural mortality (M) recorded varied from 1.93 (Coastal Lagoon) to 4.93 (Lake Ahémé) (Table 2). Also, the exploitation rate (E) varied from 0.13 (Coastal Lagoon) to 0.41 (Lake Ahémé). The longevity of *D. lebretonis* was moderate and ranged between 0.968 years (Lake Ahémé) and 4.054 years (Coastal Lagoon). Values of ratio Z/K were superior to 1 and ranged from 2.67 (Lake Ahémé) to 3.47 (Porto Novo Lagoon). The ratio (L_{50}/L∞) of the size of the first capture and asymptotic length showed low variations and fluctuated between 0.33 (Lake Ahémé) and 0.44 (Lake Nokoué) (table 2).

Probability of Capture and First Capture Size

The size of the first capture (L_{50}) of *D. lebretonis* was moderate in all four (4) lagoons. The lowest value (L_{50} =3.80 cm) was recorded in Lake Ahémé, whereas the highest value (L_{50}=5.05 cm) was recorded in Lake Nokoué (Figures 3-A, B, C, and D).

Recruitment

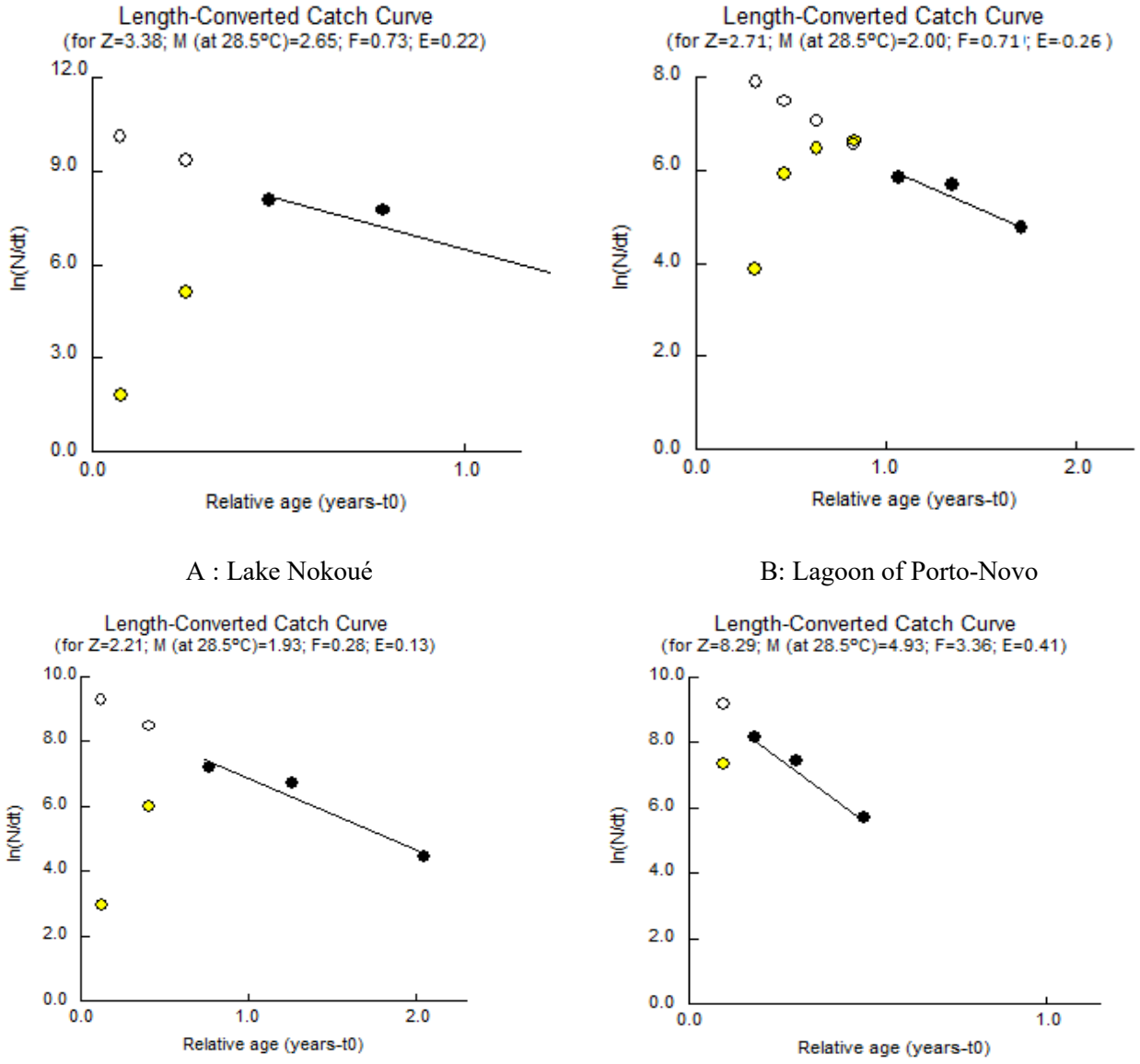
Lake Nokoué, Coastal Lagoon, and Lagoon of Porto-Novo, *D. lebretonis* population exhibited two peaks of recruitment (Figures 4 A, B, and C). Percentages of recruitment significantly varied not only between coastal waters but also between peaks of the same coastal water. In contrast, in Lake Ahémé, *D. lebretonis* showed one recruitment peak during the year, with a higher percentage reaching 20% (Figure 4-D).

Overall, growth parameters and performance indices are efficient tools most fisheries biologists use to assess the status of fish population stocks. In the current fisheries survey of *D. lebretonis*, the asymptotic lengths (11.55< L∞ <12.08) recorded, that is, the maximum total length fish individuals can reach in their population, were moderate and almost similar in the four (4) coastal water studied. These results agreed with that Fishbase (2022) reported in Western Africa (Senegal, Gambia, Guinea, Cote d’Ivoire, Ghana, Nigeria), where the maximum total length recorded for *D. lebretonis* was 12.4 cm. In this study, the relatively lower value (L∞=11.55 cm) recorded in Lake Nokoué, Lake Ahémé, and Coastal Lagoon and even in Lagoon of Porto-Novo (L∞=12.08 cm) may be the result of environmental disturbances such as dumping of domestic wastes, mangrove degradation, the proliferation of floating plants that negatively affect water parameters and water quality (Barro, 1968; Adite, 2013). Also, using sophisticated fishing gear leads to overfishing, with fishery increasingly oriented towards small individuals. Nevertheless, Sonon et al. (2021) records of physicochemical parameters indicated that the water quality of the four (4) coastal waters studied was within the required standards for the survival and growth of *D. lebretonis*. Indeed, the presence of air-breathing accessory organs helps to cope with the harsh conditions (mainly dissolved oxygen: 4.5 ±0.42 mg/L) in swamps and aquatic vegetation habitats. These ecological trends agreed with those reported by Okyere et al. (2011) in the coastal wetland of Ghana, where dissolved oxygen was reduced and averaged 4.2 ±0.9 mg/L.

Table 2: Mortality, longevity, and exploitation rate of *Dormitator lebretonis* from the Benin coastal waters

Ecosystems	M	F	Z	E	Z/K	L_{50}	Tmax	$L_{50}/L\infty$
Lake Nokoué	2.65	0.73	3.38	0.22	2.82	5.05	2.5	0.44
Lagoon of Porto-Novo	2.00	0.71	2.71	0.26	3.47	4.17	3.846	0.34
Coastal Lagoon	1.93	0.28	2.21	0.13	2.98	4.20	4.054	0.36
Lake Ahémé	4.93	3.36	8.29	0.41	2.67	3.80	0.968	0.33

M: Natural mortality; F: Fishing mortality; Z: Total mortality; K: Growth coefficient; E: Exploitation rate; L_{50}: Size of first capture; Tmax: Longevity.



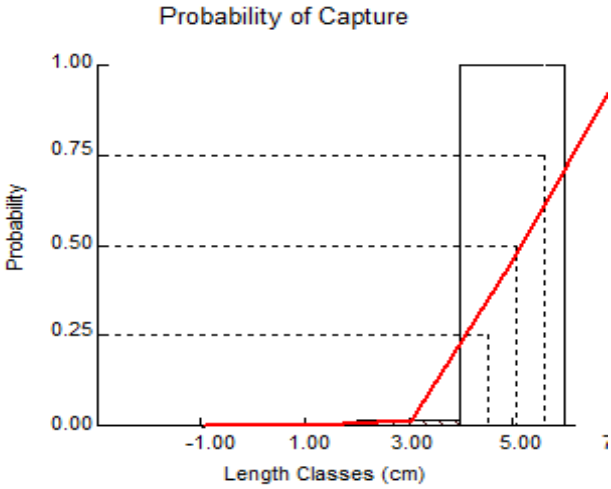
A : Lake Nokoué

B: Lagoon of Porto-Novo

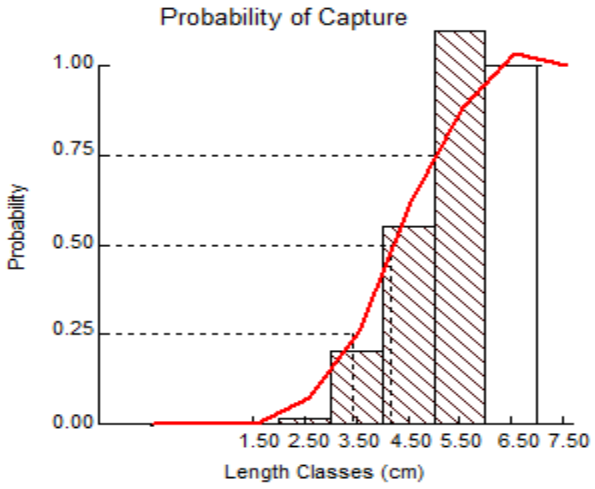
C: Coastal Lagoon

D: Lake Ahémé

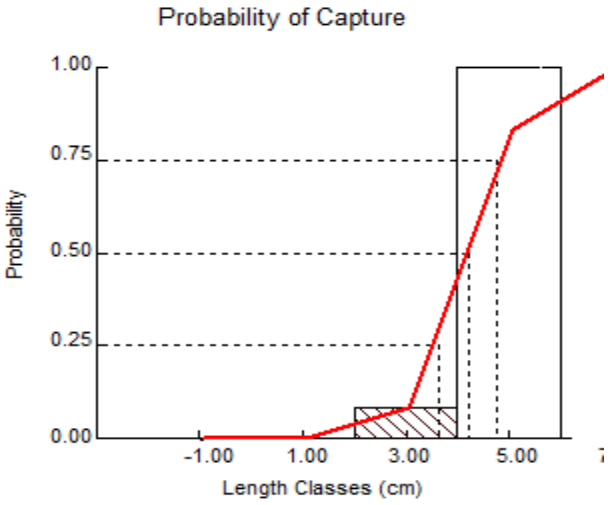
Figure 2 (A, B, C, and D). length converted catch curves of *Dormitaor lebretonis* from the coastal waters of Benin



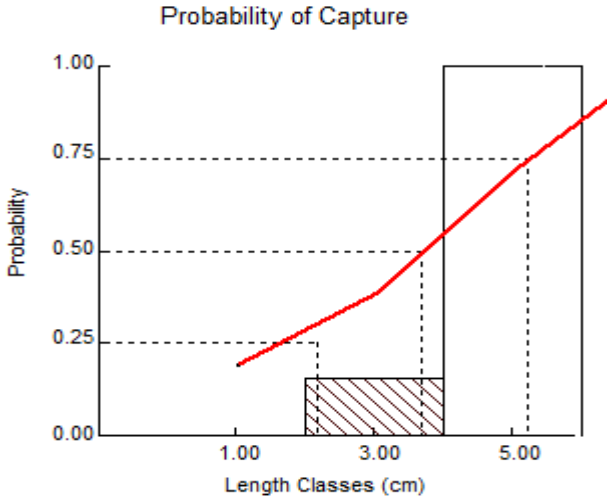
A : Lake Nokoué



B : Lagoon of Porto Novo

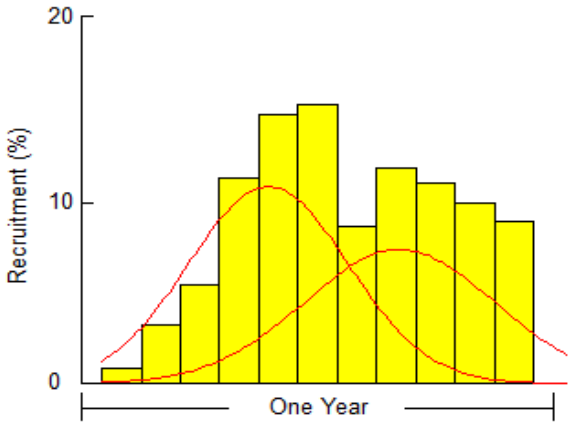


C: Coastal Lagoon

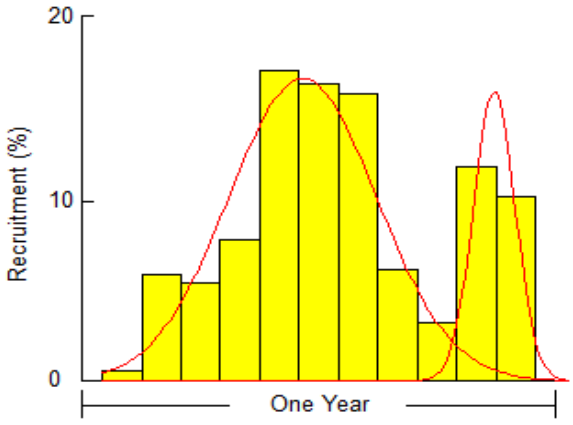


D: Lake Ahémé

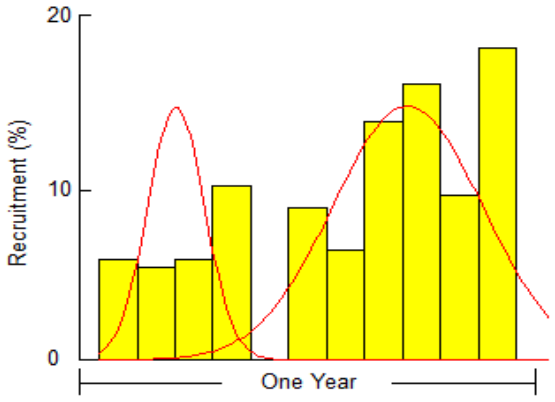
Figure 3 (A, B, C, and D). Probability of capture and length of the first capture of *Dormitator lebretonis* from the Coastal waters of Benin



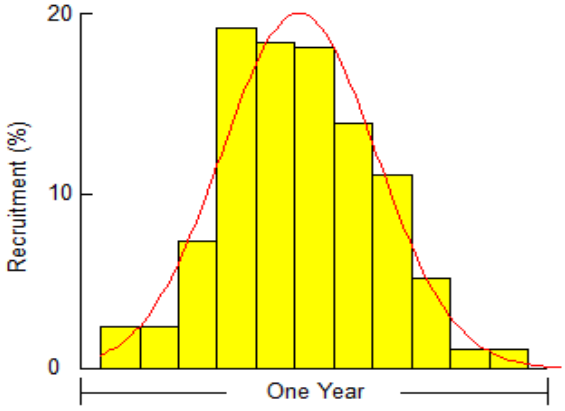
A : Lake Nokoué



B : Lagoon of Porto-Novo



C: Coastal Lagoon



D: Lake Ahémé

Figure 4 (A, B, C, D): Recruitment patterns of *Dormitator lebretonis* from the coastal waters

In the current study, when considering the whole abundance of *D. lebretonis* in the four coastal waters, One-way Analyses of Variance (ANOVA) on the length of this eleotric showed significant ($F_{3,4848} = 21.42$, $P = 0.002$) variations of SL across the four coastal waters. However, physicochemical parameters exhibited insignificant ($p>0.05$) variations across the four coastal waters, indicating that mean physicochemical parameters were nearly identical across the 4 coastal waters (Sonon et al., 2023). These records were probably due to different fishing pressures in each of the four coastal waters and generating different sizes of *D. lebretonis* in each ecosystem.

Among the four (4) coastal waters, Lake Ahémé showed the highest growth rate reaching $K=3.1$ yr^{-1}, whereas those recorded from Lake Nokoué, Lagoon of Porto-Novo, and coastal lagoon were relatively low and ranged between $K=0.74$ yr^{-1} and $K=1.20$ yr^{-1}. As Sidi Imorou et al. (2019) reported, the relatively low abundance of *D. lebretonis* in Lake Ahémé could have reduced intraspecific food competition, enhancing the growth rate of this eleotrid. Also, the presence of suitable habitats for *D. lebretonis* in Lake Ahémé, exempt from floating plants at some sites, could have increased the availability of dissolved oxygen and minerals that made greater the quality of habitats and hence, the growth rate. Likewise, the intensive reinforcement of fishing regulation by the Benin Department of Fisheries in Lake Ahémé compared to Lake Nokoué, Lagoon of Porto-Novo, and Coastal Lagoon could have positively impacted the growth rate of *D. lebretonis.* Also, the value of K recorded in Lake Ahémé was higher than those obtained from some other five (5) teleost fishes *Hemi-*

chromis fasciatus, Marcusenius senegalensis, Shilbe intermedius, Oreochromis niloticus, Brycinus macrolepidotus from the Okpara river in Northern Benin where K ranged between 0.66 yr^{-1} (*Shilbe intermedius*) and 1.2 yr^{-1} (*Marcusenius senegalensis*). However, in the current study, the growth rate range (K=0.74 yr^{-1} - K=1.20 yr^{-1}) recorded for *D. lebretonis* in Lake Nokoué, Lagoon of Porto-Novo and Coastal Lagoon were relatively low and rather agreed with those reported for *H. fasciatus, M. senegalensis, S. intermedius, O. niloticus, B. macrolepidotus* from the Okpara river. Identical trends of low K were recorded in Lake Ayame in Cote d'Ivoire for *Brycinus macrolepidotus* (K= 0.46), *Hemichromis fasciatus* (K=0.57) and *Oreochromis niloticus* (K=0.48). Combined effects of habitat degradations and fragmentations, species tolerance to disturbances, niche breadth, and level of ecosystem productivity could act for spatial and temporal variations of K. (Al-Nadhi et al., 2009).

Longevity depends not only on the species but also on the habitat's ecological status. In the Benin coastal water, *D. lebretonis* exhibited moderate longevity that varied between 0.968 yr (Lake Nokoué) and 4.054 yr (Coastal Lagoon). Though species-dependent, the moderate longevity recorded in Lake Nokoué is probably the result of mangrove destruction, the proliferation of invasive floating vegetation such as water hyacinth, the dumping of domestic and industrial wastes, and overall habitat degradation. In contrast, at the Coastal Lagoon, some mangrove forests are under protection through the reinforcement of Benin fisheries regulation. These findings nearly agreed with those Sidi Imorou et al. (2019) reported for some teleost fishes such as *M. senegalensis*, *S. intermedius B. macrolepidotus,* and *H. fasciatus* from the Okpara river in Northern Benin, where longevity ranged between 1.88 yr and 4.55 yr. Also, these results agreed with those reported for *Chrysicthys nigrodigitatus* in Lake Akata in Benue State in Nigeria, where longevity varied from 1 to 3 yr (Ikongbeh et al., 2015). However, on the Sô River, where ecological disturbances were moderate, Hazoume et al. (2017) reported higher longevity (11.66 yr) for the Claroteidae *Chrysichthys auratus*.

The growth performance index (φ') values recorded on all the water bodies were relatively low and varied from 1.994 (Coastal Lagoon) to 2.617 (Lake Ahémé). This result indicates that *D. lebretonis* is a very slow-growing fish species (Baijot and Moreau, 1997). The various degradations of the aquatic environment due mainly to anthropogenic activities may negatively influence the growth performance of the fish (Sidi Imorou *et al.*, 2019). In this survey, the exploitation rate (E) was under 0.5 in all four (4) coastal waters suggesting that the stocks of *D. lebretonis* were underexploited. This result is similar to those Francis et al. (2007) reported in Andoni River System, Niger Delta in Nigeria, who found many underexploited fish species with an exploitation ratio (E) below 0.50. Some of these underexploited fish species reported were *Galeoides decadactylus* with E =0.20, *Chrysichthys nigrodigitatus* (E= 0.25), *Sarotherodon melanotheron* (E=0.31), *Eucinostomus melanoptenus* (E= 0.45), *Liza grandisquamis* (E= 0.45), *Tilapia guinensis* (E= 0.48).

In the current fisheries survey, the ratio Z/K was greater than 1 and ranged between 2.67 and 3.47. As reported by Barry & Tegner (1989) and Sidi Imorou (2019), these results indicate that annual mortalities (Z) dominate population growth (K). According to Lederoun *et al.* (2015), a ratio Z/K under 2 indicates that mortality predominates overgrowth. Hence, the population of *D. lebretonis* in the Benin coastal waters is threatened. Similar trends of disturbances have been recorded for species such as *Brycinus macrolepidotus, Hemichromis fasciatus, Marcusenius senegalensis, Shilbe intermedius, Oreochromis niloticus* from the Ouémé river in northern Benin where fisheries regulations are underway (Sidi Imorou et al., 2019; Ahouansou Montcho et al., 2011; Welcome and De Merona 1988; Pauly, 1982).

Overall, in this survey, the lengths at first capture (L50) of *D. lebretonis* were relatively small and ranged between 3.80 cm (Lake Ahémé) and 5.05 cm (Lake Nokoué). This indicates that in the four (4) coastal waters, individuals of *D. lebretonis* were practically exploited at immature or early stages. This fishing pressure on juveniles is a threat to the population and could jeopardize the sustainability of the exploitation and the extinction of this eleotrid in the future if nothing is done to restore the fishable stock (Okyere et al. 201).

Except in Lake Ahémé, *D. lebretonis* showed two peaks of recruitment in Lake Nokoué, Lagoon of Porto-Novo, and Coastal Lagoon. Probably, these two peaks originated from two different spawning seasons in the year. Indeed, in general, two rainy seasons occur in Southern Benin. In addition, annual floodplains caused by the Mono River (case of Lake Ahémé, Coastal Lagoon) and by the Ouémé River (case of Lake Nokoue, Porto-Novo Lagoon) could act to affect the breeding periods and hence, the recruitment peaks. As reported by Pauly (1982), Adité et al. (2006), and Ahouansou Montcho et al. (2011), these spawning and recruitment trends are similar for most tropical fishes, and in general, match with rainy and flooding seasons.

In terms of exploitation, fish and shellfish resources from the four coastal waters were intensively and permanently exploited by local professional fishermen for sales and food. The main fishing gear used by fishermen were cast nets, gill-

nets, seines, traps, hooks, longlines, etc. In particular, a fishing/aquaculture method called "Acadja" is mainly utilized in Lake Nokoue. The "Acadja" fishery is a site ranging between 10 m^2 – 10 hectares delimited at the shallower (0.5-1.5 m - depth) parts of the lake where tree or/and palm tree branches are planted in the mud to cover the space. These branches decompose and generate a huge amount of food leading to a high abundance of fish in the "Acadja" that finally serves as growing, spawning, and nursery grounds. Regarding management, a fishery regulation is implemented by the Benin Department of Fisheries. Thus, government fisheries agents remove prohibited fishing gears and mesh sizes. Besides, some traditional regulations, such as fishing prohibited days, are implemented by the grassroots and local population.

Conclusion

The current fisheries survey gives valuable and helpful information on the demographic characteristics of *Dormitator lebretonis* from the four (4) coastal waters of Southern Benin. The coastal waters surveyed were under severe ecological disturbances that negatively impacted demographic traits. This small squeaker generally exhibited moderate asymptotic length ($L\infty$) ranging between 11.55 and 12.08 cm in the four (4) coastal waters studied. In Lake Ahémé, *D. lebretonis* exhibited a higher growth rate (K) that was reduced in Lake Nokoué, Lagoon of Porto-Novo, and Coastal Lagoon. In these coastal ecosystems, the species showed moderate longevity but higher in the Coastal Lagoon. The growth performance index (φ') was relatively low, indicating that *D. lebretonis* is a relatively slow-growing eleotrid. In the four coastal waters, annual mortalities dominate population growth (Z/K >1), and the low lengths at first capture indicated that individuals of *D. lebretonis* were exploited at immature and early stages. Nevertheless, the exploitation rate (E) was under 0.5, indicating that the stock of *D. lebretonis* was underexploited, probably because of the two peaks of recruitment. The current results on the demographic characteristics and exploitation rate of *D. lebretonis* from the Benin coastal waters give valuable information that could serve as reference data for the efficient management of this squeaker. Sustainable exploitation *of D. lebretonis* in the four coastal waters requires a holistic management scheme that should include enforcement of fisheries regulation, spawning ground protection, and ecosystem restoration and follow-up.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required.

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Disclosure: -

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Efficacy of natural and consumer-friend applications to control *Aeromonas hydrophila*, growth in Bluefish

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ABSTRACT

Fish is one of the main transmission routes of *Aeromonas (A.) hydrophila*, an emerging pathogen that threatens public health due to its high antibiotic resistance. This study aimed to control the growth of *A. hydrophila* in cold-stored Bluefish (*Pomatomus saltatrix*) using natural, consumer-friendly practices. Samples were inoculated with *A.hydrophila*, dipped or sprayed with acetic acid, citric acid, ascorbic acid, sodium lactate, or sodium chloride solutions (4%), and stored at 4°C. Dipping was very effective since the growth of *A. hydrophila* was inhibited by all dipping treatments and remained below the inoculation dose after 72 hours. During this time, dipping in acetic acid, ascorbic acid, and citric acid reduced the initial load of *A. hydrophila* (7.03 log cfu/g) to 5.27, 5.51, and 5.64 log cfu/g, respectively. Acetic acid, ascorbic acid, and citric acid dipping treatments reduced the *A. hydrophila* number 1 log/cfu more than other treatments ($P<0.05$). Acetic acid and ascorbic also provided the best results for the sprayed samples. Our results showed that dipping in natural acids such as acetic acid, ascorbic acid, and citric acid yielded successful results in inhibiting *A. hydrophila* growth. Using consumer-friendly, natural substances to ensure food safety by controlling the growth of this emerging pathogen will provide significant benefits for the food industry.

Keywords: *Aeromonas hydrophila*, Bluefish, Organic acid, Dipping, Spraying

Introduction

Aeromonas hydrophila is an important opportunistic foodborne pathogen that causes human gastroenteritis or septicemia. It is widely distributed in the environment and found in the intestinal flora of humans and animals. *Aeromonas hydrophila* and other members of *Aeromonas* spp. have been isolated from many fish species, roe, prawns, shellfish, and ready-to-eat seafood products, and seafood has a crucial role in the transfer of this bacteria to humans. (Vivekanandhan et al., 2005; Di Pinto et al., 2011; Stratev et al., 2015; Praveen et al., 2016). Due to the high resistance of *A. hydrophila* to antibiotics, it is difficult to cure diseases caused by this pathogen, and its importance for public health has become prominent. Therefore, it has been regarded as a foodborne pathogen having an emerging importance; and most (> 85%) of human gastroenteritis cases are associated with *Aeromonas* sp., including *A. hydrophila*. The crucial need for further investigation into the control of this pathogen has been reported due to its role in public health and food safety (Praveen et al., 2016; Pal, 2018; Hoel et al., 2019). Various techniques have been developed for the inhibition of pathogenic bacteria. However, in recent years, consumers have been concerned about using artificial additives or preservation methods even more than the pathogen risk. Therefore, the food industry continuously seeks efficient and natural solutions (Mahmoud, 2014).

A growing significance of *A. hydrophila* as an emerging pathogen and fish has been regarded as one of the main transmission routes. Since *A. hydrophila* can grow at refrigerated temperatures, it may significantly impact cold-stored foods and pose a risk during fish transportation, processing, and marketing (Daskalov, 2006; Praveen et al., 2016). The majority (>85%) of gastroenteritis cases caused by Aeromonas are responsible for three *Aeromonas* species. One is *A. hydrophila* (hybridization group HG1) (Daskalov, 2006). Therefore, this study aimed to control the growth of *A. hydrophila* in cold-stored fish using natural, consumer-friendly practices, and the effects of organic acids or salts were studied.

Material and Methods

Sample Preparation

Fresh Bluefish *Pomatomus saltatrix* (Linnaeus, 1766) were purchased from the Istanbul, Türkiye, wholesale market. Bluefish was chosen as raw material, as it is an economically valuable species (Bal et al., 2018) and a treasure for Turkey (Mol & Varlik, 2019).

The samples were packed in polystyrene boxes with ice and then transferred to the laboratory. The samples' average lengths (cm) were 32 ±1.32, and the average weights (g) were 408.68 ±10.64. The samples were cut into portions approximately 2x5x1 cm (10g), and each sample was decontaminated under a U.V. cabinet (CRYSTE, Korea) at 366 nm for 2 hours. Before inoculation, decontaminated fish samples were analyzed for the presence/absence of *A. hydrophila* (Xanthopoulos et al., 2010).

Preparation of Bacterial Inoculum

Aeromonas hydrophila was obtained from the culture collection of the Department of Aquaculture and Fish Diseases Faculty of Aquatic Sciences, İstanbul University. The bacterial stock culture was kept in Tryptone Soy Broth (TSB) (Merck, 1.05499) with 20% glycerol (v/v) at –80°C before use. *Aeromonas hydrophila* was activated in TSB at 30°C for 24h. After incubation, the culture was centrifuged (EBA 20 Hettich, Germany) at 4000 RPM for 10 min. After centrifugation, the pellet was washed two times in 10 mL TSB. After this process, the supernatant was removed, and the pellet was resuspended in TSB (10mL). Then, serial dilutions were prepared, and the bacteria cell count was determined (Doğruyol et al., 2020).

Sample Inoculation and Treatments

Both sides of the fish samples were inoculated with 0.2 mL of *A. hydrophila* inoculum (10 log cfu/mL) and spread with a sterile Drigalski spatula. The inoculated fish samples were left to stand in a sterile cabinet for 15 min for inoculum attachment. For the natural acid /natural salt treatments, food grade acetic acid (Sigma Aldrich, 4% v/v), citric acid (Sigma Aldrich, 4% w/v), ascorbic acid (Sigma Aldrich, 4% w/v), sodium lactate (Fluka, 4% v/v) and sodium chloride (Merck, 4% w/v) solutions were prepared in sterile distilled water. The inoculated fish samples were divided into thirteen groups. Six batches of these groups were treated by dipping for two minutes in one of 4% acetic acid, 4% citric acid, 4% ascorbic acid, 4% sodium lactate, 4% sodium chloride solution, or sterile distilled water. The ratio of Bluefish samples to treatment solution volume was 1:2 (w/v). After dipping, the fish samples were kept for 15 min at room temperature (20°C) to allow draining. The other six batches were treated by spraying (0.50 mL) one of the formerly mentioned solutions to one side of each fish sample. The fish samples were left to stand for 15 min for the attachment. The same procedure was repeated for the other sides of each sample. The thirteenth group was untreated (UNT). All samples were treated at the same time. Then, samples from different treatment groups were placed in sterile plastic bags, stored at 4 ±1°C for 72 hours, and analyzed every 24 hours.

Enumeration of A. hydrophila

Microbiological analyses were performed in duplicate. Aseptically, 10 g of Bluefish sample was homogenized with 90mL TSB in a stomacher bag using a stomacher (IUL Instruments, Barcelona, Spain) for 60 seconds. Serially diluted samples were spread out (0.1 mL) onto Aeromonas Isolation Medium Base (Himedia, M884), supplemented with Aeromonas selective supplement (HIMEDIA, FD039), for quantitative enumeration. Three agar plates per dilution were incubated at 28°C for 24 hours (Xanthopoulos et al., 2010).

pH Measurement

All pH measurements were performed in triplicate. Fish were homogenized (fish/distilled water; 1:10 w/v), and Hanna pH 211 Micro-processor pH meter was used (Vyncke, 1981).

Statistical Analysis

The experimental study was repeated twice using 12 individuals of Bluefish per treatment (12 fish x 13 treatments x 2 replications). *Aeromonas hydrophila* counts were presented as $\log_{10}$ cfu/g values. The reduction in *A. hydrophila* counts was calculated by subtracting the $\log_{10}$ cfu/g in the treated samples (dipped and sprayed) from the $\log_{10}$ cfu/g in the untreated samples. Analysis of variance (ANOVA) was used to compare the results of mean bacterial levels. Significance was determined at the $P < 0.05$. Data were analyzed using the IBM SPSS 21 software program.

Results and Discussion

Effect of Treatments on Growth of A. hydrophila

Overall results indicated that pre-processing organic acids, sodium lactate, and NaCI salt treatments as dipping solutions might provide more effective inhibition compared to treatments with sprayed solutions and untreated samples. Some similar studies demonstrated that dipping treatment considerably reduced bacterial load (Anderson et al., 1988; Okolocha & Ellerbroek, 2005; Leceta et al., 2015). Organic acids have been reported to be more effective in decreasing microbial load (Xiong et al., 1998; Phillips, 1999; Samelis et al., 2001; Geornaras et al., 2006; Rio et al., 2007; Neetoo et al., 2008; Schelegueda et al., 2012; Gonzales-Fandos & Herrera, 2014; Zaki et al., 2015; Mohan & Pohlman, 2016). Before the treatment, the uninoculated fish samples were found to be free of *A. hydrophila*. After the inoculation, *A. hydrophila* load was found to be 7.03 ±0.06 log cfu/g, then reached 9.51 ±0.19 log cfu/g after 72 hours at 4°C in UNT samples. The populations of *A. hydrophila* in treated Bluefish samples, either by dipping or spraying, are presented in Table 1. Dipping treatment resulted in significantly ($P<0.05$) lower *A. hydrophila* counts. The growth of *A. hydrophila* was suppressed by all dipping treatments, and its amount was reduced below the inoculation dose after 72 hours (Table 1). Spraying also reduced the initial load, but *A. hydrophila* counts were significantly higher ($P<0.05$) than dipped samples and exceeded the initial load after 24 hours of storage. Sterile water treatment also succeeded in reducing the initial load.

Table 1. *A. hydrophila* counts on bluefish samples treated with different organic acids under refrigerated storage

			Storage Hours			
Applications method	**Groups**	**Chemical solutions**	**0 hour**	**24th hour**	**48th hour**	**72th hour**
Spraying	S1	Sterile Water	6.97 ±0.12 ^{aeA}	7.86 ±0.04 ^{adB}	9.23 ±0.05 ^{aC}	9.64 ±0.11 ^{ajD}
	S2	NaCl %4	6.93 ±0.22 ^{aeA}	7.85 ±0.05 ^{adB}	8.86 ±0.06 ^{bC}	9.76 ±0.15 ^{aD}
	S3	Acetic Acid %4	6.88 ±0.07 ^{aAB}	6.96 ±0.31 ^{bAB}	6.82 ±0.06 ^{cA}	7.34 ±0.32 ^{bB}
	S4	Ascorbic Acid %4	6.97 ±0.03 ^{aeA}	7.12 ±0.10 ^{cA}	8.66 ±0.10 ^{dB}	8.15 ±0.15 ^{cC}
	S5	Sodium lactate %4	6.89 ±0.08 ^{aA}	7.89 ±0.05 ^{agB}	9.23 ±0.12 ^{aC}	9.14 ±0.06 ^{dC}
	S6	Citric Acid %4	7.01 ±0.06 ^{aeA}	7.73 ±0.05 ^{dgB}	8.57 ±0.13 ^{eC}	9.38 ±0.27 ^{ejD}
Dipping	D1	Sterile Water	5.70 ±0.03 ^{bA}	6.09 ±0.03 ^{eB}	6.12 ±0.05 ^{fB}	6.76 ±0.07 ^{fC}
	D2	NaCl %4	6.26 ±0.04 ^{cA}	6.04 ±0.05 ^{eB}	6.06 ±0.04 ^{fhB}	6.40 ±0.08 ^{gC}
	D3	Acetic Acid %4	5.71 ±0.04 ^{bA}	5.40 ±0.11 ^{fB}	5.79 ±0.07 ^{gA}	5.27 ±0.16 ^{hB}
	D4	Ascorbic Acid %4	5.74 ±0.08 ^{bAC}	5.41 ±0.04 ^{fB}	5.83 ±0.02 ^{gA}	5.51 ±0.15 ^{iCB}
	D5	Sodium lactate %4	6.07 ±0.07 ^{dA}	5.94 ±0.06 ^{eB}	6.01 ±0.07 ^{hB}	6.35 ±0.03 ^{gC}
	D6	Citric Acid %4	5.67 ±0.08 ^{bA}	5.44 ±0.08 ^{fB}	5.56 ±0.10 ^{iAB}	5.64 ±0.06 i^{A}
Untreated	UNT		7.03 ±0.06 ^{eA}	8.02 ±0.28 ^{gB}	9.15 ±0.05 ^{aC}	9.51 0.19 ^{jD}

* Uppercase letters on the same line show significant differences ($p < 0.05$) and lower case letters in the same column show significant differences ($p < 0.05$)

Considering that the inoculation level is 7.03 log cfu/g, sterile water treatment reduced the *A. hydrophila* load by spraying (6.97 log cfu/g) or dipping (5.70 log cfu/g), and dipped samples remained below the initial load even after 72 hours. Our study also showed that the dipping process provides mechanical reduction of the bacterial load by washing, enhancing the decontaminant effect. The inoculated *A. hydrophila* count (7.03 log cfu / g) decreased significantly ($P < 0.05$) after 72 hours in samples (D1) dipped in sterile water (Table 1). Treatment-related changes in the inoculated *A. hydrophila* count (7.03 log cfu/g) after 72 hours of cold storage are presented in Figure 1. Dipping treatment was found effective since the growth of *A. hydrophila* was inhibited by all dipping treatments and remained below the inoculation dose after 72 hours. Dipping in acetic acid (5.27 log cfu/g), ascorbic acid (5.51 log cfu/g), and citric acid (5.64 log cfu/g) were the most successful treatments, reducing the load of this pathogen. A study on oysters found that the use of five percent citric acid significantly reduced the bacterial population (Mahmoud, 2014). The dipping method is quite effective because all the food is covered with the dipping solution (Meredith et al., 2013).

Contrary to the results we obtained in our study, Smyth et al. (2018) reported that the total viable count did not change significantly in cod fillets dipped in 5% citric acid. In sprayed samples, acetic acid, and ascorbic acid were also more effective than other treatments (Figure 1). In the study by Dorsa et al. (1997), refrigerated beef carcasses were washed with a 3% acetic acid solution, and a significant reduction in total bacterial load was determined. Delmore et al. (2000) reported that different treatments, including acetic acid, effectively reduced the bacterial count on beef samples. Another study demonstrated that acetic acid reduced Listeria populations in fresh meat (Samelis et al., 2001). Carpenter et al. (2011) reported that the application of 2% acetic acid reduced the count of Salmonella and prevented residual growth of *E. coli* and *L. monocytogenes* in chickens. Gonzales-Fandos and Herrera (2014) observed a significant reduction in microbial counts after dipping chicken legs in acetic acid (1-2%) compared to control groups. Studies showing the effect of acetic acid on other pathogens in different foods support our results.

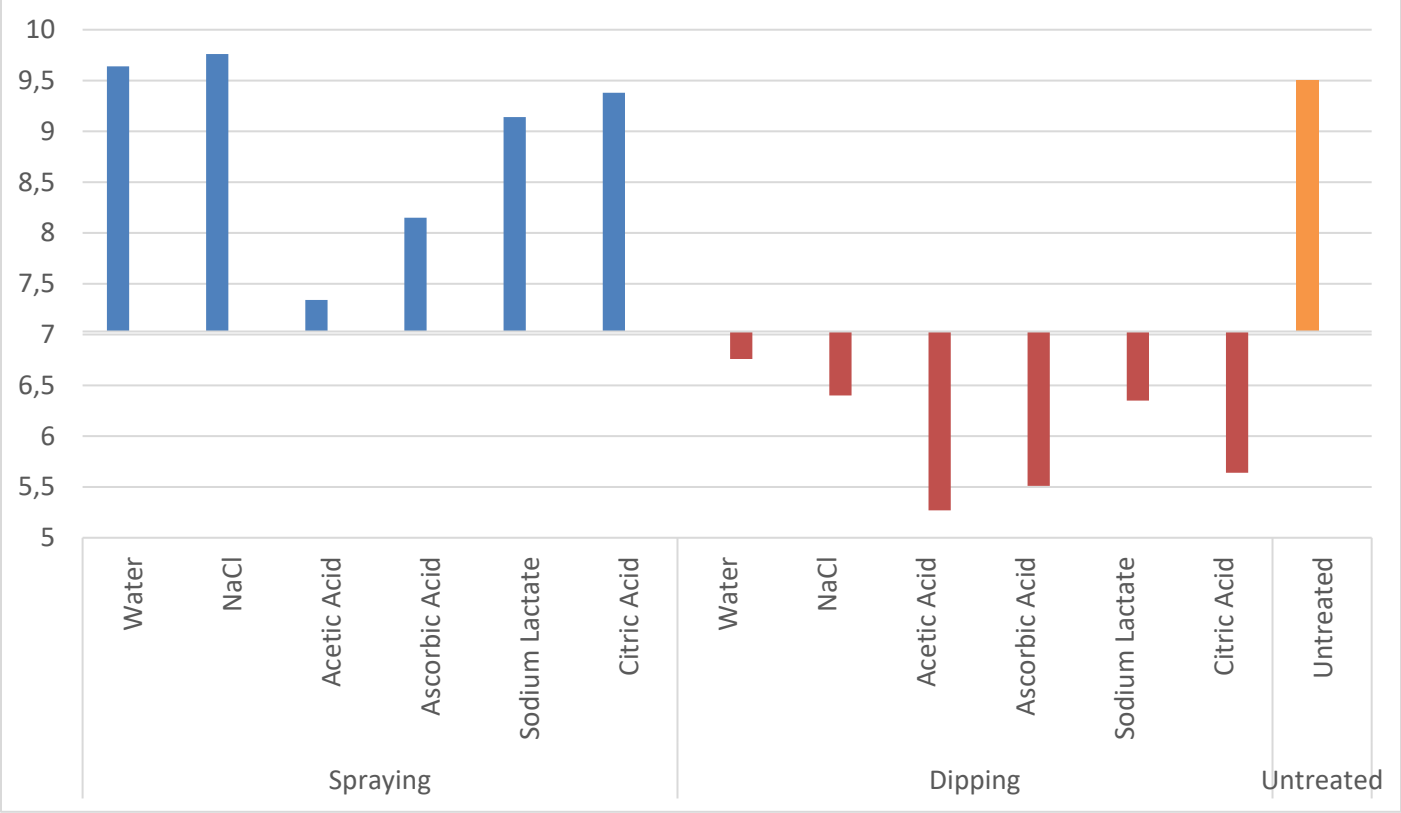

Figure 1. Reduction in *A. hydrophila* counts in bluefish samples after 72 hours.

Ascorbic acid is the other substance found to be effective in our study. Similarly, dipping chicken chunks in 1% ascorbic acid solution slowed microbial growth and increased shelf life (Arafata & Chen, 1978). Ouattara et al. (2002) indicated that ascorbic acid stabilized ground beef's total aerobic plate count. It has also been reported that the addition of ascorbic acid and/or citric acid could reduce bacterial load in food (Tajkarimi & İbrahim, 2011; Mahmoud, 2014). Bolton et al. (2014) reported that citric acid (1-5%) was effective against microbial growth in poultry products. Likewise, Doležalová et al. (2010) reported that 4% citric acid application effectively reduced microbial load on chicken skin. In the present study, the reduced load of *A. hydrophila* after dipping in citric acid is consistent with these studies.

Indigenous microbial flora can be inhibited by sodium chloride. Sodium chloride treatment decreases water activity and thus prevents the development of bacteria. Likewise, chloride ions are toxic for some bacteria that inhibit enzymatic systems (Leroi & Chevalier, 2000). In our study, sodium chloride treatment reduced the initial load of *A. hydrophila* by dipping and spraying treatments. However, dipping treatments resulted in more effectiveness in controlling the growth of *Aeromonas hydrophila* (Figure 1).

Sodium lactate has also been studied to prevent bacterial growth, and various products have reported different results. Although sodium lactate has been reported to be effective against bacterial growth in some foods (Sallam & Samejima, 2004; Seydim et al., 2006; Kilinc et al., 2009), it failed in preventing the growth of *L. monocytogenes* in minced beef during cold storage (Serdengeçti et al., 2006). Likewise, sodium lactate did not significantly suppress the growth of *A. hydrophila* in Bluefish in the present study.

Effect of Treatments on pH

It has been known that adding natural acids to food causes a decrease in pH (Bou et al., 2017). Since an undissociated portion of the acid molecule inhibits bacterial growth by creating an unfavorable environment, organic acids have antimicrobial effects (Hardin et al., 1994). Likewise, the treatments showing the highest antimicrobial effect on *A. hydrophila* have to lead to more pH reduction (Table 2). Doležalová et al. (2010) similarly reported a correlation between the decrease in pH and the antimicrobial effect of citric acid on chilled chicken skin. The growth and survival of pathogenic bacteria depend on a diversity of external factors, such as background flora and temperature, as well as internal factors, such as acidity and pH (Shekarforoush et al., 2007). A publication by Daskalov (2006) affirmed that a combination of low temperature and low pH decreases the growth of *A. hydrophila*.

Table 2. pH changes of bluefish samples treated with different organic acids under refrigerated storage

			Storage Hours			
Applications method	**Groups**	**Chemical solutions**	**0 hour**	**24th hour**	**48th hour**	**72th hour**
Spraying	S1	Sterile Water	6.55 ±0.00	6.88 ±0.03	6.32 ±0.03	6.79 ±0.02
	S2	NaCl %4	6.22 ±0.00	6.54 ±0.02	6.33 ±0.04	6.39 ±0.05
	S3	Acetic Acid %4	5.97 ±0.01	6.13 ±0.01	5.81 ±0.01	5.87 ±0.02
	S4	Ascorbic Acid %4	6.01 ±0.01	6.36 ±0.01	6.16 ±0.09	6.27 ±0.04
	S5	Sodium lactate %4	6.41 ±0.01	6.63 ±0.02	6.20 ±0.01	6.45 ±0.02
	S6	Citric Acid %4	5.64 ±0.01	5.85 ±0.01	5.84 ±0.10	6.15 ±0.02
Dipping	D1	Sterile Water	6.35 ±0.02	6.45 ±0.02	6.42 ±0.03	6.85 ±0.01
	D2	NaCl %4	6.22 ±0.00	6.43 ±0.03	6.41 ±0.02	6.30 ±0.10
	D3	Acetic Acid %4	4.56 ±0.01	4.81 ±0.06	5.81 ±0.01	5.13 ±0.04
	D4	Ascorbic Acid %4	5.39 ±0.01	5.54 ±0.02	5.43 ±0.06	5.94 ±0.01
	D5	Sodium lactate %4	6.34 ±0.01	6.53 ±0.01	6.43 ±0.01	6.56 ±0.02
	D6	Citric Acid %4	5.01 ±0.01	5.65 ±0.05	5.12 ±0.10	5.28 ±0.05
Untreated	UNT		6.45 ±0.01	7.28 ±0.12	7.18 ±0.19	6.68 ±0.07

Conclusion

The growth of *A. hydrophila* was inhibited by all dipping treatments and remained below the inoculation dose after 72 hours. Dipping in acetic acid, ascorbic acid, and citric acid (4%) was the most effective treatment to inhibit this emerging pathogen. Considering that the consumer is more worried about using artificial additives or treatments than the risk of pathogens, investigating consumer-friendly, natural substances to ensure food safety will provide significant benefits for the food industry.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

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Investigating the quality changes and shelf life of vacuum shrink-packaged raw and steam-cooked blue crabs under cold storage

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ABSTRACT

This study aimed to determine the quality changes and shelf life of raw and steam-cooked blue crab meat caught in the DALKO Fisheries Cooperative processing plant in the Köyceğiz Dalyan region. Both fresh and cooked crabs were taken from DALKO Fisheries Cooperative and brought to the laboratory under cold chain conditions. After the initial analyses (sensory, chemical, microbiological, and nutrient content) were made for the fresh and cooked blue crabs, the remaining samples were shrink packed. Packaged samples were kept under refrigerator (+/- 4ºC ±1) conditions and shelf-life analyses were carried out during cold storage.

The results determined fresh and cooked crabs' initial nutritional values (protein, lipid, moisture, ash) as 16.22%, 1.06%, 81.17%, 1.45%, and 17.13%, 0.94%, 79.88%, 1.88%, respectively. At the end of storage, these values were determined as 15.88%, 1.51%, 80.18%, 1.67%, and 17.83%, 1.06%, 78.65%, and 2.13% for fresh and cooked crabs, respectively. According to the sensory and microbiological analysis results, the consumable limit values were exceeded on the sixth day for fresh samples and the eighth day for cooked samples. When sensory and microbiological analyses were considered, it was determined that the fresh crabs have a shelf life of 4 days and cooked crabs have six days in vacuum-packaged refrigerator conditions.

Keywords: Blue crab, Shelf life, Vacuum shrink packaging

Introduction

The blue crab (*Callinectes sapidus*) is an allochthonous crab species originating from the Western Atlantic Ocean that was colonized in the coastal areas of Greece in 1940, especially in the Gulf of Thermaikos (Serbetis, 1959), also reported in the marine area of Rhodes Island in 1976 (Lewinshon, 1976). Blue crabs are harvested from estuarine and coastal waters. Factors more sensitive to the microbiological flora of crabs are usually environmental influences. (Balasaraswathy et al., 2008). Crabs are highly priced seafood products that are preferred in terms of edible meat quality and economic value, especially in developed countries (Dernekbaşı et al., 2021). Crab meat, rich in protein and mineral substances, is an important food in a balanced diet, especially calcium, iron, zinc, potassium and phosphorus, vitamins, and low-fat content (Gökoğlu and Yerlikaya, 2003; Erkan et al., 2008). Studies were carried out to determine the biochemical composition of blue crab meat obtained from different regions (Gökoğlu & Yerlikaya, 2003; Ayas & Özoğul, 2011; Khamassi et al., 2022; Tufan, 2023).

Crab meat is among the perishable seafood products. If adequate preservation methods are not applied, its quality can deteriorate rapidly. Cold storage is one of the most effective and accessible methods of preserving crabs. Due to crabs' meat value and susceptibility to rapid spoilage, research has identified the microbial flora responsible for spoilage (Balasaraswathy et al., 2008).

DALKO Dalyan Fisheries Cooperative was established by the people of the region in 1971 within the borders of Köyceğiz Lagoon, Dalyan town of Köyceğiz district in Muğla/Turkey. The cooperative was established in order to protect the small fishermen in the region, provide job opportunities, market the fishery products, continue the old lagoon fishery, and protect the environment and nature. DALKO cooperative stated that they have difficulty packaging the blue crabs offered fresh or steamed cooked to the consumers. Tearing vacuum bags during the packaging of shellfish products reduces the effect of packaging on the product's shelf life. Blue crabs in the cooperative are sold to the consumer in aluminum foils or wrapped in paper packages. The consumer has to consume the product that is bought either immediately or in a short time under refrigerator conditions. These punctures are thought to be prevented when shrink packaging is applied to these samples taken into the bowl. In addition, it is thought that the supply quality in the market will be increased by having some knowledge about the nutritional compositions of aquatic products produced by DALKO Fisheries Cooperative and marketed to the local and foreign markets.

This study aimed to determine the nutritional content and shelf life of shrink-packaged raw and steam-cooked blue crab caught by the DALKO Fisheries Cooperative fishermen in Köyceğiz Dalyan region and cleaned and steam-cooked (ready-to-eat) in the processing facility. This research is to contribute to our country's economy by providing added value to the product in the foreign market by promoting the consumption of our local products and extending the shelf life by packaging.

Material and Methods

Material

Blue crab caught from Köyceğiz Dalyan was used. Blue crabs (*Callinectes sapidus*) were brought to Muğla Sıtkı Koçman University, Faculty of Fisheries, Quality Control Analysis Laboratory from DALKO Fisheries Cooperative within 1 hour under cold chain conditions after the upper shell part was removed. The crabs were divided into two groups (first group had steamed crabs for 30 minutes, and the other group had freshly prepared crabs). 200 mature crabs were used in this study.

Method

Vacuum Packaging of the Samples

The raw and cooked crabs brought to the laboratory from DALKO Fisheries Cooperative were packed in plastic containers in 2 pieces, then packed with a vacuum shrink machine and stored in the refrigerator. Samples were analyzed periodically (0., 2., 4., 6., and 8. day) for microbiological, chemical, and sensory assessment of quality.

Nutritional Composition Analysis

In the raw and cooked blue crab meat, nutritional compositions analyses; % protein; according to AOAC (2006a, 984.13) by Kjeldahl method, the % lipid content of crab meats according to Bligh and Dyer (1959), % moisture; according to AOAC (2006b, 934.01) and % ash content analyses; according to AOAC (1990, 950.46) were carried out at the beginning and at the end of the storage.

Sensory Analysis

Ten trained panelists conducted sensory analysis for raw and cooked edible crabs on each sampling day. A hedonic scale test applied for raw crab was used in sensory analysis. Fresh crab meat was evaluated over 5 points (5: best quality, 0: poorest quality) in terms of color, texture, smell, appearance, and general appreciation criteria (Amerina et al., 1965). For

steamed crab meat, sensory analyzes were evaluated for odor, taste, and texture (clumping, firmness, juiciness, and consistency). It was scored between 0 and 8 using the hedonic scale and evaluated as 0 (best quality) and 8 (lowest quality). The general average of the scores was taken, and 6 was accepted as the acceptability limit (Anacleto et al., 2011).

Chemical Analysis

Over the 8-day period, chemical analyses were carried out. The pH value of crab samples was determined with a digital pH meter (InoLab pH Level 1 model, WTW, Weilheim, Germany) according to Manthey et al. (1988). The TVB-N analysis was carried out according to Antonocopoulus (1973). Homogenized crab samples were steam-distilled, and the distillate was collected in a 0.1 N HCl solution containing a beaker. Then, this solution was titrated with 0.1 NaOH solution. TVB-N value was expressed as mg nitrogen/kg of sample. TBA was determined as described by Tarladgis et al. (1960). Ten grams of crab sample homogenized was distilled with hydrochloric acid (HCl), then TBA reagent prepared with glacial acetic acid (90%) was added to the distillate. Distilatte incubated in a water bath, the mixture's absorbance was measured using a spectrophotometer (Shimadzu UV-1700, Japan) at 538 nm. TBA value was expressed as mg malonaldehyde/kg fish sample.

Microbiological Analysis

The following groups of microflora were monitored: total viable count (TVC) and psychotropic bacteria count (PBC). A sample of 10 g was removed aseptically from the filet using a scalpel and forceps, transferred to a stomacher bag containing 90 mL of sterile peptone water (PW) solution (0.1%), and homogenized at room temperature. Further serial decimal dilutions were prepared for each sample in PW solution (0.1%). The appropriate dilutions were subsequently used for the enumeration and differentiation of microorganisms. Total viable counts were determined using plate count agar (PCA, Code: 1.05463, Merck, Darmstadt, Germany) after incubation for 2 days at 37°C, and psychotropic bacteria counts were determined after incubation at 7°C for 10 days with the same medium (FDA/BAM, 2009).

Statistical Analysis

Experiments were performed in triplicate (n = 3) for three independent samples, and a completely randomized design (CRD) was used. Statistical analyzes were performed using the Statistical Package for Social Sciences v.21 Software Package (SPSS for Windows, SPSS Inc., Chicago, IL, USA). Data are given as mean values ± standard deviations, and a probability value of $P < 0.05$ was considered significant. Analysis of Variance (ANOVA) was applied to the obtained results, and the averages were compared with Duncan's multiple interval tests.

Results and Discussion

Nutritional Composition Analysis Results

At the beginning and the end of storage, nutritional composition analyses; protein, lipid, moisture, and ash analyses were made in the raw and steam-cooked crab meat. At the beginning (Day 0), protein, lipid, moisture, and ash was determined for raw and cooked crabs as 16.22%, 1.06%, 81.17%, 1.45% and 17.13%, 0.94%, 79.88%, 1.88%, respectively. At the end of storage, these values were determined as 15.88%, 1.51%, 80.18%, 1.67%, and 17.83%, 1.06%, 78.65%, 2.13% for raw and cooked crabs, respectively (Table 1). Protein and moisture content decreased slightly during storage. There were significant differences ($P < 0.05$) in the moisture, protein, fat, and ash contents of edible meat for raw and cooked crabs. The differences could be attributed to decreased moisture content during cooking. Zotti et al. (2016) find the moisture, protein, and ash values of blue crabs (*Callinectes sapidus*), 80.12%, 15.13%, and 1.63%, respectively, in their study in Acquatina Lagoon (SE Italy). The results are quite similar to the results in our study. Umer et al. (2021) determined the amount of lipid in commercial crab species; *P. pelagicus*, *P. sanguinolentus*, *S. serrata,* and *C. feriatus* in the range of 0.25-1.86 g/100 g. In the study of Anacleto et al. (2011**)** that investigated the shelf life of cooked *Cancer pagurus* at cold storage, moisture, protein, fat, and ash contents were determined as 76.9%, 18.0%, 0.6%, and 2.6%, respectively. As in our study, crabs have high protein and low lipid values. Balasaraswathy et al. (2008) observed a significant decrease in protein values of uncooked and cooked crab (*Portunus pelagicus*) meat under ice storage for 10 and 12 days, respectively. Unlike this study, no significant protein loss was observed in our study.

Table 1. Nutritional composition analysis results

Nutritional Composition (%)					
Storage Period	**Groups**	**Protein**	**Lipid**	**Moisture**	**Ash**
Initial (0. day)	**Raw**	16.22 ±0.28^{A}	1.06 ±0.04^{A}	81.17 ±0.26^{A}	1.45 ±0.04^{A}
	Cooked	18.05 ±0.60^{B}	1.46 ±0.02^{B}	76.88 ±0.11^{B}	1.88 ±0.01^{B}
End of Storage (8th day)	**Raw**	15.88 ±0.55^{a}	1.51 ±0.12^{a}	80.18 ±0.29^{a}	1.67 ±0.18^{a}
	Cooked	17.83 ±0.21^{b}	0.94 ±0.06^{b}	78.65 ±0.15^{b}	2.13 ±0.22^{b}

(Mean ± SD, n:4) Capital letters indicate the statistical difference between groups at the beginning, and lower letters indicate the differences between groups at the end of storage.

Sensory Analysis Results

According to the sensory analysis results, fresh samples' consumable limit values were exceeded on the sixth day. The odor and texture deteriorated after the fourth day, and the panelists evaluated these characteristics unfavorably. Color properties also deteriorated. The acceptability limit of 6 points for cooked crabs was exceeded on the eighth day for each sensory criteria. A statistically significant difference was found between the sensory analysis results of fresh and cooked crabs ($P < 0.05$). Lorentzen et al. (2014), in their study of determining the shelf life of red king crab (*Paralithodes camtschaticus*), sensory quality, especially odor and flavor parameters, deteriorated during chilled storage.

Chemical Analysis Results

While TVB-N values of fresh crabs were 12.42 mg/100 g at the beginning of storage, this value was much higher in cooked samples (Figure 1). A continuous increase was observed in TVB-N values of fresh samples during storage, while a fluctuation was detected in these values in cooked samples. During the storage, the TVB-N value of cooked crab samples exceeded the consumable limit of 25-35 mg/100 g according to the European Commission Regulation No 2074/2005 for fishery products (EC, 2005). In this study, the limit value of 35 mg/100 g for crab meat was exceeded on the eighth day for fresh samples. Higher results were obtained with cooked crabs than with fresh crabs throughout storage. The contents of TVB-N throughout storage were significantly different ($P < 0.05$) for fresh and cooked crab meat.

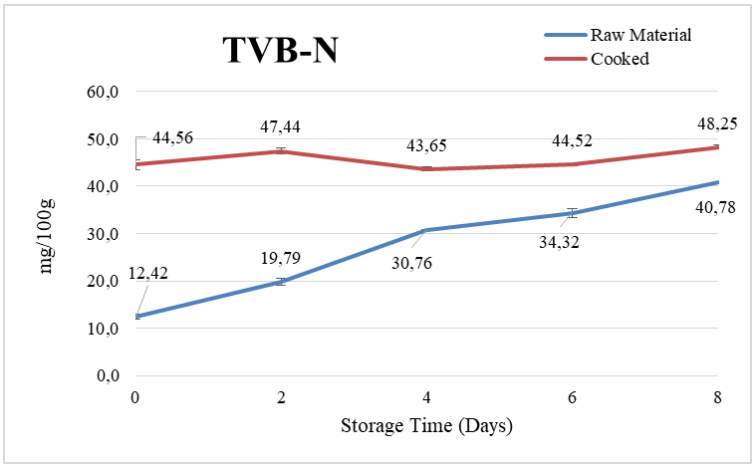

Figure 1. TVB-N analysis results

Lorentzen et al. (2016) studied the shelf life of snow crab stored at 0 and 4ºC in raw meat, the level of TVB-N was 20 mg/100 g from day 0, and it did not change during the storage period of 7 days. Sun et al. (2017), in their study about the effects of super chilling with modified atmosphere packaging on the shelf life of swimming crab, the TVB-N of air-packaged samples (without MAP) increased rapidly, and the value reached 30.64 mg N/100 g on the fifth day of storage at 4ºC. Similar results were obtained in our study for fresh samples. In this present study, higher TVB-N results were obtained in cooked crab samples than in fresh crabs. Anacleto et al. (2011) reported that TVB-N formation in cooked samples can increase by thermal breakdown during cooking. They found that TVB-N levels exceeded the consumable limits of 35 mg/100 g in their study of the shelf-life of cooked crab samples. Therefore, it was concluded that the TVB-N could not determine the chemical quality of steam-cooked blue crabs.

Generally, the pH value for fresh crabs increased continuously during storage, while for cooked samples, these values varied in equilibrium (Figure 2). It was reported that pH 7.80–7.95 is an acceptable critical limit for shrimps and prawns (Chung & Lain, 1979). In our study, only cooked crabs exceeded this value at the end of storage. One of the most obvious reasons for this increase may be the breakdown and deamination of tissue proteins. Especially during the deterioration of seafood, spoilage products such as ammonia and trimethylamine produced by endogenous enzymes and microorganisms are released (Finne, 1982).

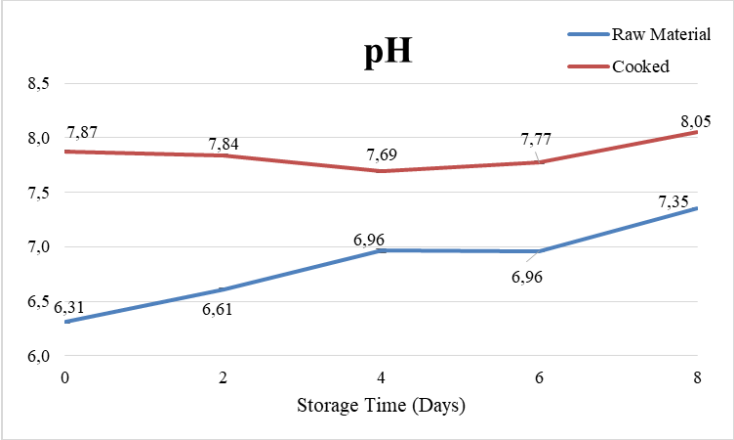

Figure 2. pH analysis results

Lorentzen et al. (2014) examined the shelf life of cooked red king crab at 4ºC, and the pH value was found to be between 7.3-7.9 during the 14-day study. Lorentzen et al. (2016), in the other study about the shelf life of snow crab stored at 0 and 4ºC, the pH of raw leg crab meat was 6.5 on day 2, and the final pH was 7.0 on day 7. Similarly, in this study, the pH increased from 7.0 to approximately 7.6 on day 2 in steamed crabmeat, independent of storage temperature. During refrigerated storage, Anacleto et al. (2011) found progressively increased pH in cooked crab (*Cancer pagurus*) samples. They reported that because of the higher content of non-protein nitrogenous compounds in crustaceans, crabs have a higher pH than fish and mammalian species.

TBA value increased slightly for fresh and cooked crabs during cold storage. There were no significant differences between these groups statistically (P>0.05) (Figure 3). During the storage, values were obtained entirely below the 8 mg malonaldehyde/kg limit for both samples. This value was not exceeded during storage.

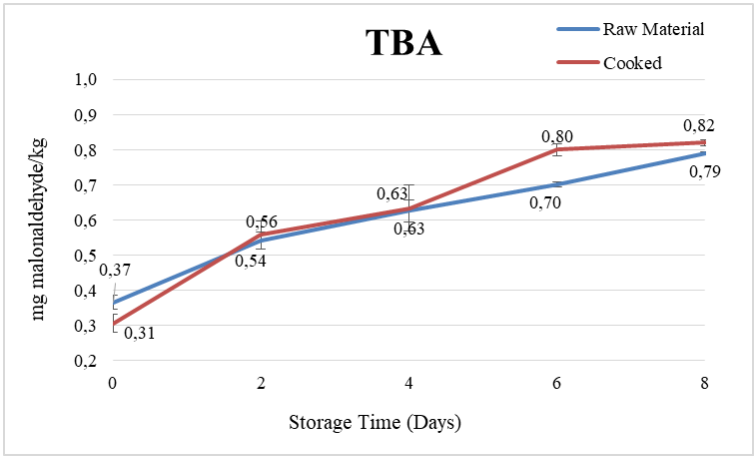

Figure 3. TBA analysis results

Sun et al. (2017), in the study that examined the effects of super chilling with modified atmosphere packaging on the shelf life of swimming crab, on day 4 of storage, the TBARS value reached 1.5 mg MDA/kg muscle for air-packed (without MAP) samples. Higher results were obtained than in our study.

Microbiological Analysis Results

Total Viable Count Results (TVC)

The total viable count was determined as 4.82 log CFU/g at the beginning of storage in fresh crabs and reached 7.44 log CFU/g on the sixth day of storage. An increase occurred during the storage of both samples. There was no microbiological growth for cooked crabs until the fourth day of storage; the total viable count was determined as log 4.4 CFU/g on the sixth day and log 7.41 CFU/g on the eighth day (Figure 4). Due to the cooking process, a lower microbiological load was determined in the cooked samples compared to the fresh samples. A statistically significant difference was found between each group in terms of total viable count until the sixth day of storage ($P < 0.05$). However, at the end of storage, this difference was found to be insignificant ($P > 0.05$). The recommended limit of $\log10^6$ CFU/g for refrigerated and frozen crab meat, according to ICMSF (1986), was exceeded on the sixth day for fresh crab and on the eighth day for cooked crab.

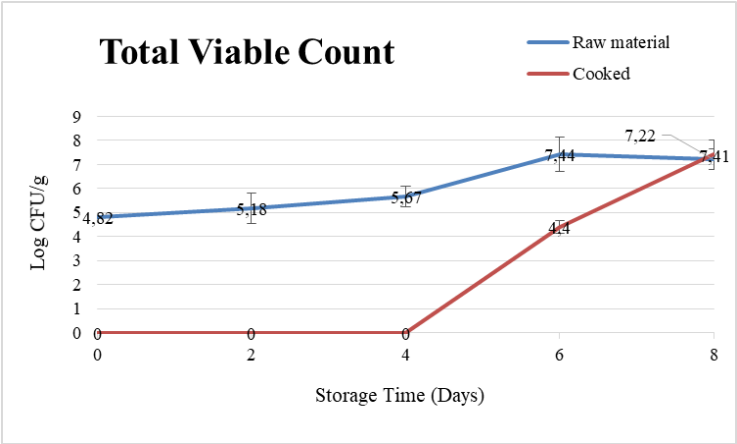

Figure 4. Total viable count results

The total number of psychrotrophic bacteria was determined as 3.35 log CFU/g in fresh crabs at the beginning of storage, and this value reached 7.64 log CFU/g at the end of storage. There was no psychrotrophic bacteria growth for cooked crabs until the fourth day of storage; it was log 2.75 CFU/g on the sixth day and log 5.72 CFU/g on the eighth day (Figure 5). An increase occurred during storage for both samples. The values of psychrotrophic bacteria throughout storage were significantly different ($P < 0.05$) for fresh and cooked crab meat.

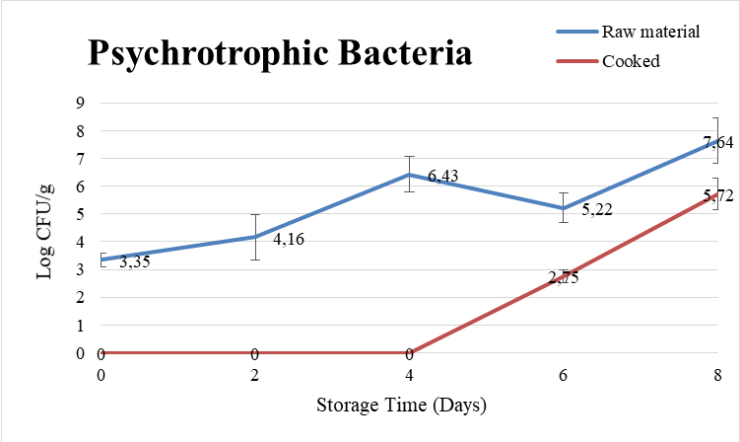

Figure 5. Psychrotrophic bacteria results

Anacleto et al. (2011) found that the TVC of cooked *Cancer pagurus* was below 4 log CFU/g until day 4 for the samples stored in the refrigerator. Lorentzen et al. (2014) examined the shelf life of cooked red king crab at 4ºC, the TVC value was found below the viable count up to 5 days, and it logged 4.4 CFU/g on the fifth day. Lorentzen et al. (2016), in the study of the shelf life of snow crab stored at 0 and 4ºC, up to day 4, the level of TVC was below consumable limit values of log1.7 CFU/g for cooked crab, and TVC increased to the maximum level of log 5.5 CFU/g at 4ºC on day 10. While the TVC level of fresh snow crab was approximately log 2.5 CFU/g at the beginning of storage, this value was reported to increase by approximately one unit during the next seven days of storage at 0ºC. Lower values were obtained than in our study due to storage at lower temperatures. Sun et al. (2017) in the study investigated the effects of super chilling with modified atmosphere packaging on the shelf life of swimming crabs; for air packed (without MAP) samples, the initial total aerobic plate count (TPC) was 3.96 log CFU/g for fresh crabs, and TPC reached log10^5 CFU/g at day 4.

Conclusion

This study applied shrink packaging to raw and cooked crabs, and shelf-life analyses were carried out during eight days of cold storage. As a result of the analyses, it was concluded that the shelf life of the packaged fresh and cooked crabs was 4 and 6 days, respectively, according to the results of sensory and microbiological analysis. The chemical analysis of the packaged crab samples determined that they preserved their quality properties during storage for up to 6 days. The high initial microbial loads of the samples prevented further shelf life extension. It is thought that pre-treatments such as cooking or disinfection before packaging to extend the shelf life will reduce the microbiological load and improve the product's sensory and chemical properties. In addition, it is suggested that compliance with the personnel and plant hygiene rules during the capture, processing, and storage of the product is important for future studies in terms of the quality of the product. It is thought that crabs, which are beneficial for human health due to being rich in protein, vitamins, and minerals and have low saturated and high unsaturated fatty acid content, by applying pre-treatments, cooking methods, and providing appropriate storage conditions under appropriate conditions will increase their consumption.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

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Disclosure: -

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Comparison of the efficacy of two phenotypic identification kits and classic PCR methods to identify *Aeromonas hydrophila* isolated from fish farms

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ABSTRACT

In recent years, phenotypic identification kits have been reported to give incorrect results in identifying *Aeromonas* species, whereas molecular identification is quite reliable. In this context, *Aeromonas hydrophila* strains, isolated from fish farms (9 strains), identified by polymerase chain reaction (PCR) method, and ATCC strain bacteria were used in the present study for the determination of the usability of API 20NE and Microgen GN-ID A + B panel test kits. All strains were determined as *A. hydrophila* in molecular methods. After phenotypic identification, a 100% accuracy rate was obtained for *A. hydrophila* with API 20NE. In the Microgen GN-ID A + B, these rates were 60% for the strains used in this study. Phenotypic identification for the ATCC strain in both kits was correct. This study showed that the API 20NE test kit had high validation for the rapid and correct identification of fish pathogenic *A. hydrophila*.

Keywords: Bacterial identification, API 20NE, Microgen ID tests

Introduction

Motile aeromonads, which are generally expressed as Motile Aeromonas Complex (MAC) are Gram negative and usually capable of movement with a single polar flagellum and the disease caused by these in fish is called Motile Aeromonas Septicaemia (MAS). Also, many bacteria that cause disease of fish in this group have been reported in different studies. Important species of this genus are *Aeromonas hydrophila*, *A. caviae*, *A. sobria*, *A. veronii*, *A. schubertii*, and *A. media* (Austin & Austin, 2010). The disease caused by the bacteria belonging to this group in fish is generally called Motile *Aeromonas* Septicaemia (MAS). It is possible to see hemorrhagic septicemia in acute cases of *Aeromonas* occurring in fish, and abscesses and large ulcers in chronic cases (Austin & Austin, 2010). In this group, *A. hydrophila* has been reported in different size ranges of various fish species. It has been reported that it causes blue sac syndrome in trout alevin (Kayış et al., 2015).

Identification of the disease agent is very important in understanding the disease process. Therefore, there is continuous improvement in pathogen identification. Various phenotypic, serologic, and molecular techniques are widely used for the identification of pathogenic fish bacteria. The accuracy level of these methods and the advantages and disadvantages of between each other are a matter of discussion. There may be differences between the results of these identification methods for the Motile Aeromonas Complex. However, it can be claimed that some methods are more sensitive than others in identification. In recent years, it has been reported that phenotypic identification kits may give incorrect results in identifying *Aeromonas* species, whereas molecular identification is quite reliable (Fernández-Bravo & Figueras, 2020). Even the classical PCR method is considered to be more unsafe than the whole genome sequencing method. So, identification studies by the whole genome method for *A. hydrophila* have been reported today (Jin et al., 2020)

Some studies compare or combine the molecular method and phenotypic identification methods related to *Aeromonas* species. API tests are very common among phenotypic kits used for this purpose. Significantly, studies for identifying *Aeromonas hydrophila* by API 20NE have been reported (Dubey et al., 2021; Toobaet al., 2024). Similarly, the other test kit, the Microgen ID test, was used to identify the fish pathogens *A. hydrophila* and *A. caviae.* (Gülaydın et al., 2018). However, it is stated in many studies that these tests can give different results for the same bacteria (Santos et al., 1993). For this reason, which tests can give more accurate results for which bacteria should be carefully examined under certain conditions? In recent years, it has been desirable to present and confirm all possible identification methods for the identification of pathogenic fish bacteria. So, researchers present molecular and phenotypic identification methods together in their studies. This study aimed to identify the *Aeromonas hydrophila*, defined by molecular methods (classic PCR), with API 20NE and Microgen GN-ID A + B Panel kits. In this way, comparing both methods and using two different identification kits for commercial use in the mentioned fish pathogen bacteria, *Aeromonas hydrophila* was determined.

Material and Methods

The bacteria used in the study were obtained from the Fish Diseases Laboratory of the Fisheries Faculty of Recep Tayyip Erdogan University. Detailed information about *Aeromonas hydrophila* strains is given in Table 1. Besides, an *A. hydrophila* strain of the American Type Culture Collection (ATCC), ATCC7966, which has a whole genome analysis report, was selected, and used in this study to compare and validate the results.

Table 1. Fish hosts of the bacterial isolates used in this study and their acceptance numbers in the National Centre for Biotechnology Information (NCBI).

Code	Host/Samples	Tissue	Acceptance Number
D7	*Capoeta banarescui*	Spleen	MT730008
D13	*Capoeta ekmekciae*	Spleen	MT730009
D17	*Squalius orientalis*	Spleen	MT730010
D22	*Squalius orientalis*	Spleen	MT730011
Y1	*Salmo* sp.	Egg	MT730013
Y21	Hatchery	Water	MT730014
Y28	Hatchery	Water	MT730015
K31	*Alburnus derjugini*	Kidney	MK548537
Y33	*Oncorhyncus mykiss*	Kidney	MT730016

For the molecular identification of the *Aeromonas* species, their genomic DNA was obtained by boiling method (Kayiş et al., 2015). The primers specific to the 16S rRNA region of eubacteria (27 Fwd 5'-AGA GTT TGA TCC TGG CTC AG-3', 1492 Rev 5'-GTT TAC CTT GTT ACG ACT T-3') were used. Then PCR reaction was carried out using bacterial genomic DNA and the given primers (Model Px2 ThermoHybrid; Thermo Electron Inc., Waltham, MA, USA). The 1465-bp amplified products were purified with a NucleoSpin PCR

purification kit (Macherey-Nagel) and sent for sequencing by double-sided reading (ABI PRISM 310 genetic analyzer, Applied Biosystems). Accession numbers of bacteria in the National Center for Biotechnology Information (NCBI) are given in Table 1. The API 20NE test kit (BioMerieux, France) and Microgen ID A+B (Microgen, UK) were used for the phenotypic identification of the bacterial strains. The tests and their differences included in both test kits are given in Table 2.

Pure bacterial cultures were inoculated into the kits as specified in the instructions of the test kits. API 20NE kits were incubated at 29 ±1ºC and 22 ±1ºC, while Microgen GN-ID A + B was incubated at 34 ±1ºC for 24 hours. Many researchers have stated that API tests need some modifications in them to use in fish pathogens (Popovic et al., 2014). The most important of these changes is perhaps the incubation temperature. Therefore, a low-temperature trial (22 ±1ºC) was also conducted, which is more suitable for fish pathogens. At the end of the incubation, different reagents determined for both kits were added to the tests. The codes of bacteria were formed according to the colour changes mentioned in the instructions, and the results were interpreted via the APIWEB and Microgen ID software systems for identification.

Kovac's reagent, VPI-VPII, Nitrate A and Nitrate B, and TDA reagent were added to the tests for Microgen, and Mineral oil, Nit1, and Nit2, Zn, and James solutions were added for API 20 NE. As a result of the reagents applied, the codes obtained according to the colour changes were uploaded to the licensed APIWEB and Microgen ID software systems, and the bacteria were identified.

Table 2. Comparison of the test contents of the kits used in this study.

API20 NE		**Microgen GN-ID A+B**		
NO_3	Potassium nitrate	OX	Oxidase	
TRP	L-tryptophan	MOT	Motility	
GLU	D-glucose (fermentation)	NIT	Nitrate	
ADH	L-Arginine	LYS	Lysine	
URE	Urease	ORN	Ornithine	
ESC	Esculin ferric citrate	H_2S	H_2S	
GEL	Gelatine	GLU	Glucose	GN-A
PNG	4-Nitrophenyl-β-D- glucopyranoside	MAN	Mannitol	
GLU	D-Glucose (assimilation)	XLY	Xylose	
ARA	L-Arabinose	ONPG	o-nitrophenyl-beta-D-galactoside	
MNE	D-Mannose	IND	Indole	
MAN	D-Mannitol	URE	Urease	
NAC	N-Acetyl glucosamine	VP	Voges Proskauer	
MAL	D-Maltose	CIT	Citrate	
GNT	Potassium gluconate	TDA	Triptofan	
CAP	Capric acid	GEL	Gelatine	
AD	Adipic acid	MAN	Malonate	
MLT	Malic acid	INO	Inositol	
CIT	Trisodium citrate	SOR	Sorbitol	
PAC	Phenylacetic acid	RHM	Rhamnose	
		SUC	Sucrose	GN-B
		LAC	Lactose	
		ARA	Arabinose	
		ADO	Adonitol	
		RAF	Raffinose	
		SAL	Salicin	
		ARG	Arginine	

Results and Discussion

The identification of bacteria in the NCBI database as a result of the molecular identification is given in Table 1. The results showed that all bacteria were *Aeromonas hydrophila* in both incubation temperatures. According to the API 20NE test results of bacteria in the Apiweb system, all strains, including the ATCC, were confirmed as *A. hydrophila* (Table 3). In the results of all Microgen ID test kits strains, 6 out of 10 different bacteria could be identified as *A. hydrophila* (Table 4). In addition, the ATCC strain was identified as *A. hydrophila*.

According to the data obtained from the study, reference strain *A. hydrophila* (ATCC7966) and D17, D22, Y28 Y21, and Y33 strains were defined as *A. hydrophila* in all three identification methods (molecular, API 20NE, and Microgen ID A + B). API 20NE codes of only three bacteria (D7, D22, and Y21) were identified as the same as the ATCC strain. On the other hand, in the Microgen ID system, none of the bacteria codes could be identified the same as the ATCC strain. In the API system, six tests (TRP, ARA, MNE, MAN, NAG, MAN, and CIT) differed with ATCC strains. All other tests were similar to the ATCC strain. The test with the most variability in the API system was determined as citrate. (Table 3 and Figure 1). On the other hand, only 11 tests were observed, similar to ATCC strains in the Microgen ID system. The most variable tests in the Microgen ID systems were VP, gelatine, mannitol, and hydrogen sulphide (Table 4 and Figure 2).

Molecular methods have been used frequently in identifying fish-origin bacteria for the last two decades (Altinok & Kurt, 2003). However, traditional phenotypic methods are still commonly used for bacteria. Many studies indicate that phenotypic identification methods for bacteria have some problems. Such as, some bacteria can be misidentified due to incubation temperature values and aquatic system differences (Popovic et al., 2004). For these reasons, the scientific authorities recommend the application of molecular techniques in the identification of bacteria. This question is an important detail that researchers ask; How is the compatibility of both methods? The presented study is a narrow answer to the accuracy of this approach. So, in the present study, *Aeromonas hydrophila* strains were identified with the classical PCR technique, and the two different test kits and the results were compared. According to the PCR technique, all bacteria were identified as *Aeromonas hydrophila*. Molecular identification was not performed on the ATCC strains used in the study. On the other hand, all strains were identified as *A. hydrophila* in the same bacterial group according to the API 20NE test. In contrast, six strains were defined as *A. hydrophila* according to Microgen tests. This study demonstrated that the API 20NE kit successfully identified *A. hydrophila*. On the other hand, it was determined that the Microgen ID system was more unsuccessful in identifying *A. hydrophila*. For *A. hydrophila*, all tests except citrate showed slight variation between the reference strain and isolates. In this sense, it can be said that the API 20NE test kit is quite successful in identifying *A. hydrophila.*

Table 3. Evaluation of the results of both test kits for *Aeromonas hydrophila*

Code	API 20NE	(%)	Bacteria	Microgen ID	(%)	Bacteria
K31	7576455	91.2	*Aeromonas hydrophila*	746622001	99.5	*Aeromonas sobria*
D7	7577755	99.3	*Aeromonas hydrophila*	644424000	95.9	*Burkholderia cepacia*
D13	5573754	99.8	*Aeromonas hydrophila*	706424123	99.7	*Vibrio fluvialis*
D17	7577754	99.9	*Aeromonas hydrophila*	777664123	98.2	*Aeromonas hydrophila*
D22	7577755	99.3	*Aeromonas hydrophila*	744660523	96.8	*Aeromonas hydrophila*
Y1	7574454	99.4	*Aeromonas hydrophila*	706424001	82.6	*Aeromonas sobria*
Y28	7577754	99.9	*Aeromonas hydrophila*	717624003	99.9	*Aeromonas hydrophila*
Y21	7577755	99.3	*Aeromonas hydrophila*	707624023	98.2	*Aeromonas hydrophila*
Y33	7574455	99.2	*Aeromonas hydrophila*	716624023	99.7	*Aeromonas hydrophila*
ATCC	7577755	99.3	*Aeromonas hydrophila*	754660101	98.7	*Aeromonas hydrophila*

Table 4. Similarities of the strain for ATCC strain in API 20NE tests.

Tests	**Bacteria**									
	K31	D7	D13	D17	D22	Y1	Y28	Y21	Y33	ATCC
NO_3	+	+	+	+	+	+	+	+	+	+
TRP	+	+	-	+	+	+	+	+	+	+
GLU	+	+	+	+	+	+	+	+	+	+
ADH	+	+	+	+	+	+	+	+	+	+
URE	-	-	-	-	-	-	-	-	-	-
ESC	+	+	+	+	+	+	+	+	+	+
GEL	+	+	+	+	+	+	+	+	+	+
PNG	+	+	+	+	+	+	+	+	+	+
GLU	+	+	+	+	+	+	+	+	+	+
ARA	-	+	+	+	+	-	+	+	-	+
MNE	+	+	+	+	+	-	+	+	-	+
MAN	+	+	-	+	+	+	+	+	+	+
NAC	-	+	+	+	+	-	+	+	-	+
MAL	-	+	+	+	+	-	+	+	-	+
GNT	+	+	+	+	+	+	+	+	+	+
CAP	+	+	+	+	+	+	+	+	+	+
ADI	-	-	-	-	-	-	-	-	-	-
MLT	+	+	+	+	+	+	+	+	+	+
CIT	+	+	-	-	+	-	-	+	+	+
PAC	-	-	-	-	-	-	-	-	-	-
OX	+	+	+	+	+	+	+	+	+	+

Table 5. Similarities of the strain for ATCC strain in Microgen ID tests.

Tests	**Bacteria**									
	K31	D7	D13	D17	D22	Y1	Y28	Y21	Y33	ATCC
OX	+	+	+	+	+	+	+	+	+	+
MOT	+	+	+	+	+	+	+	+	+	+
NIT	+	+	+	-	+	+	+	+	+	+
LYS	+	+	-	+	+	-	-	-	-	+
ORN	-	-	-	+	-	-	-	-	-	-
H_2S	-	-	-	+	-	-	-	-	+	+
GLU	+	+	+	+	+	+	+	+	+	+
MAN	+	-	+	+	-	+	+	+	+	-
XLY	-	-	-	+	-	-	+	+	-	-
ONPG	+	+	+	+	+	+	+	+	+	+
IND	+	-	-	+	+	-	+	+	+	+
URE	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	+	+	-	-	-	-	+
CIT	+	+	+	+	+	-	+	+	+	+
TDA	-	-	-	-	-	-	-	-	-	-
GEL	-	+	+	+	-	+	+	+	+	-
MAL	+	-	-	-	-	-	-	-	-	-
INO	-	-	-	-	-	-	-	-	-	-
SOR	-	-	-	-	+	-	-	-	-	-
RHM	-	-	-	-	-	-	-	-	-	-
SUC	-	-	+	+	+	-	-	-	-	+
LAC	-	-	-	-	-	-	-	-	-	-
ARA	-	-	+	+	+	-	-	+	+	-
ADO	-	-	-	-	-	-	-	-	-	-
RAF	-	-	-	-	-	-	-	-	-	-
SAL	-	-	+	+	+	-	+	+	+	-
ARG	+	-	+	+	+	+	+	+	+	+

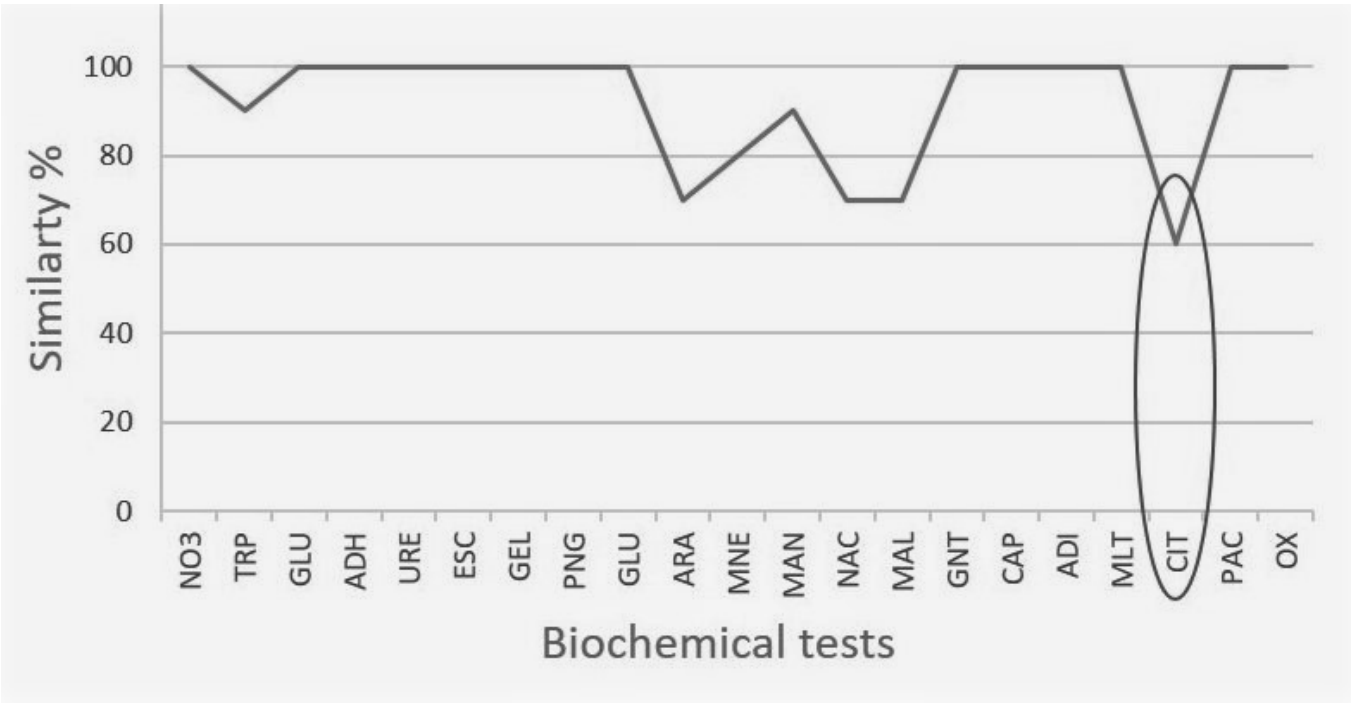

Figure 1. Similarities of the bacteria in API 20 NE tests for ATCC strain.

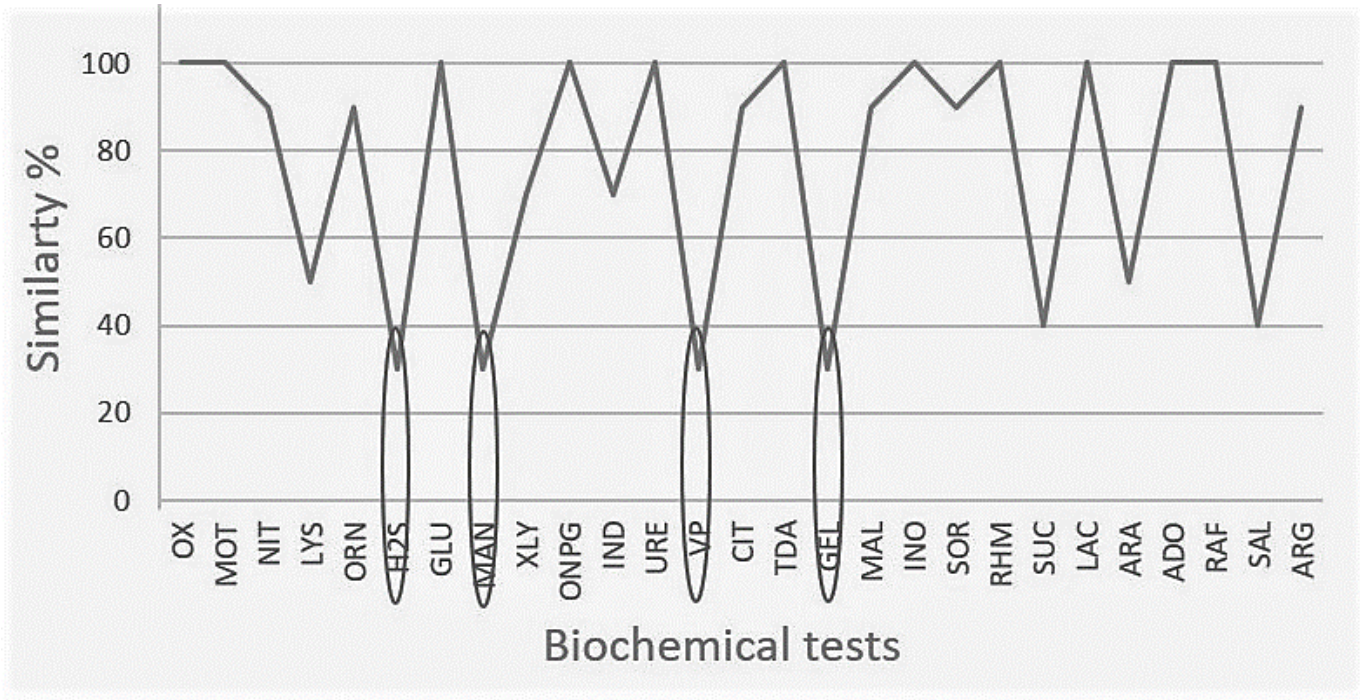

Figure 2. Similarities of the bacteria for ATCC strain in Microgen ID.

Microgen ID test kits are not as widely used as API tests in identifying pathogenic bacteria. However, it has been reported to be used in some studies. In a study conducted with bacteria isolated from sea turtle eggs, comparing API and Microgen ID tests with molecular identification methods contains quite detailed information. This study states that Microgen ID tests give the best result in identifying bacteria with Gram-negative rods (Awong-Taylor et al., 2007). Microgen ID kits were used to identify bacteria obtained from pearl mullet (*Chalcalburnus tarichi*) samples, and *A. hydrophila*, and *A. caviae* were identified in the mentioned study (Gülaydın et al., 2018). In the present study, it was observed that the Microgen ID test successfully identification of *A. hydrophila* isolates at 60% rates. The reasons for this situation (not completely successful in all isolates) should be investigated, and studies should be done to increase the reliability of the test. In particular, the incubation temperatures of the tests may have caused these false results. Successful identification of the reference strain ATCC isolate in the Microgen test indicates that the reliability of the test will increase after minor improvements. The kit database should be improved by adding more data on different strains of the same species. Additionally, different incubation temperatures can be studied.

Bacterial fish pathogens are known to prefer low incubation temperatures. However, the recommended incubation temperatures of commercial identification kits are relatively higher. Literature information indicates that these temperature preferences are a problem, especially in API tests. The present study reveals that the temperature difference does not differ in identifying *Aeromonas hydrophila* with these commercial kits.

Conclusion

The identification kits used in this study are generally designed for bacteria that are human pathogens. Therefore, it may give misleading results in the identification of pathogenic fish bacteria. However, with the studies to be done, the most accurate results can be achieved. With this study, it is understood that the API 20 NE test kit, which is frequently used, gives quite accurate results for *A. hydrophila*. Both kits were found as the test kit more suitable for identification with molecular methods and ATCC strains. Therefore, the use of these test kits can be recommended for the mentioned bacteria in the same conditions as the present study. However, the most correct approach is to study using both methods.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required for this study.

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Türkiye'nin kuzeyinde ova ve kentsel alanlardan geçen bir akarsuyun mikrobiyal kirlilik göstergelerindeki mekânsal-zamansal değişimlerin değerlendirilmesi

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ÖZ

Kentsel gelişim nedeniyle su havzalarının bozulması, yüzey sularının antropojenik kaynaklı kirleticilere karşı savunmasızlığını artırmaktadır. Yüzme sularının yakınından boşalan kentsel akarsular, su kalitesinin bozulmasına önemli ölçüde katkıda bulunabilir. Araştırmada, Terme Çayı'nın dört farklı noktasından örnekler alınarak akarsuyun mikrobiyal kalitesi mevsimsel olarak araştırılmıştır. Mikrobiyolojik analizler, membran filtrasyonu ile standart metotlar kullanılarak gerçekleştirilmiştir. Örneklerdeki fekal kontaminasyon koloni oluşturan birim (KOB) yöntemi kullanılarak belirlenmiştir. Terme Çayı su numunelerinde ortalama koloni sayısı toplam koliform, *Escherichia coli* ve *Clostridium perfringens* için sırasıyla 712 KOB/100 mL, 278 KOB/100 mL ve 64 KOB/100 mL olarak belirlenmiştir. Bu durum akarsuda fekal bir kontaminasyonun olduğunu göstermektedir. Su Kirliliği Kontrolü Yönetmeliği'ndeki bakteriyolojik parametrelere göre, Terme Çayı'nın toplam koliform bakteri sayısı bakımından II. Sınıf su kalitesinde olduğu tespit edilmiştir. Araştırmadan elde edilen sonuçlara göre Terme Çayı'nın mikrobiyolojik kalite yönünden çok kirli olmayan, fakat kirlilik baskısı altında değerlere sahip olduğu belirlenmiştir. Kirlik düzeyinin artmaması için akarsu çevresindeki yerleşim ve tarım alanlarından gelen evsel atıkların/atıksuların, hayvansal atıkların ve drenaj kanallarının doğrudan akarsuya karışması engellenmeli, gerekli önlemlerin alınması konusunda halk bilinçlendirilmelidir.

Anahtar Kelimeler: Fekal indikatör bakteriler, Fekal kirlilik, Kentsel ırmak, Su kalitesi, Terme Çayı

ABSTRACT

Evaluation of spatio-temporal variations in microbial pollution indicators of a river passing through lowlands and urban areas in Northern Türkiye

The degradation of watersheds due to urban development increases the vulnerability of surface waters to pollutants of anthropogenic origin**.** Urban streams draining near swimming waters can contribute significantly to the deterioration of water quality. In the study, the microbial quality of the Terme River was investigated seasonally by collecting samples from four different stream sites. Microbiological analyzes were performed using standard methods with membrane filtration. The colony-forming unit (CFU) method determined fecal contamination in samples. The mean number of colonies in Terme River water samples was determined as 712 CFU/100 mL, 278 CFU/100 mL, and 64 CFU/100 mL for total coliform, *Escherichia coli*, and *Clostridium perfringens*, respectively. This situation indicates that there was fecal contamination in the stream. According to the bacteriological parameters in the Water Pollution Control Regulation, Terme River was found to be of class II water quality in terms of total coliform bacteria count. The Research results determined that the Terme River is not much polluted in terms of microbiological quality but has values under the pressure of pollution. To not increase the pollution level, the direct mixing of domestic wastes/wastewater, animal wastes, and drainage channels from the settlements and agricultural areas around the river should be prevented. The public should be made aware of the necessary precautions.

Keywords: Fecal indicator bacteria, Fecal pollution, Urban River, Water quality, Terme River

Giriş

Akarsular hem antropojenik hem de doğal kirlenmeye en çok maruz kalan kaynaklardır (Ustaoğlu ve ark., 2021; Aydin ve ark., 2021). Kentsel gelişim nedeniyle su havzalarının bozulması, yüzey sularının antropojenik kaynaklı kirleticilere karşı savunmasızlığını da artırmaktadır. Özellikle fekal kirlilik, küresel olarak büyüyen bir sorundur (Reynolds ve ark., 2021). Dünya nüfusunun %55'i şu anda kentsel alanlarda yaşamaktadır ve bu oranın 2050 yılına kadar %68'e çıkması beklenmektedir (UN, 2019). Kentsel alanlardan geçen akarsuların yukarı havzadan aşağı havzaya doğru fekal kirlilikten fazla etkilendiği belirtilmektedir (Paule-Mercado ve ark., 2016; Taş ve ark., 2019; Reynolds ve ark., 2021).

Patojenik organizmalar tüm ekosistemlerin normal bileşenleridir. Ancak antropojenik aktivite sonrası artan fekal bakteri sayısının neden olduğu kirlilik, akarsularla havzalara ve denizlere taşınmaktadır. Suda patojenik bakterilerin varlığı, insan ve hayvan sağlığını tehdit eden önemli endişe kaynağını oluşturmaktadır (Şener ve ark., 2020). Kentsel akarsular insan faaliyetlerinin yoğun olduğu sucul ekosistemler olduğu için hem yapısını hem de işlevini etkileyebilecek bir dizi antropojenik kirleticiler içerir (Yüksel ve ark., 2021; Tepe ve ark., 2022). İnsan popülasyonundaki artışlar, potansiyel mikrobiyal patojen kaynaklarının sayısında artışa neden olur. Ayrıca, arazi gelişimi ile ilişkili peyzajdaki değişiklikler, artan konsantrasyon ve mikrobiyal patojenlerin mansap sularına taşınmasını sağlayabilir (Mallin ve ark., 2000). Doğal arazinin geçirimsiz yüzeylere dönüştürülmesi (yollar, otoyollar, kaldırımlar, otoparklar ve çatılar), arazinin doğal filtrasyon kabiliyetini ortadan kaldırır. Böylece arazi yüzeyindeki kirletici konsantrasyonunun artmasına izin verir ve kirleticilerin mansap su yollarına hızlı bir şekilde taşınmasını sağlar (Mallin ve ark., 2001).

Alt yapı sistemlerinin eksik ya da yetersiz olduğu şehir akarsularında kanalizasyon göstergelerine rastlanmaktadır (Kaushal ve Belt, 2012). Özellikle septik alanlardan deşarj, sızdıran kanalizasyon altyapısı, kanalizasyon taşmaları ve atık su arıtma tesisi atık suları gibi noktasal kaynaklardan akarsulara giriş vardır (Rosi-Marshall ve ark., 2015). Dağınık yerleşim alanlarındaki foseptik sızıntılar, ahır suları gibi noktasal olmayan kirleticiler de yüzey akışlarıyla kentsel akarsulara kirletici maddeler taşımaktadır.

Akarsuların içerdiği fekal kirleticiler denize taşındığında kıyısal alanın ve suyun kalitesi bozulur. Plaj gibi rekreasyonel olarak kullanılan suların kalitesini düşüren bu faktörlerin insan sağlığı ve ekonomik etkilerinin de olumsuz olması muhtemeldir. Kontamine kıyı suları insan sağlığı üzerinde ciddi risklere yol açar. Bu riskler, insanların patojenle kontamine sulara doğrudan, kontamine deniz ürünleri tüketimi yoluyla da dolaylı olarak maruz kalmasını içerir (Mallin ve ark., 2000).

Göreceli kullanım kolaylığı ve düşük maliyeti nedeniyle, bakteriyel indikatör organizmalar su kalitesini değerlendirmek ve su kalitesi kriterlerini belirlemek için kullanılır (Zhang ve ark., 2015). Koliform bakteriler, su kalitesinin geleneksel ve evrensel mikrobiyolojik göstergeleridir (Neill, 2004; Armah, 2014). Su kütlelerinde dışkı koliform bakterilerinin varlığı genellikle su kütlesinin kanalizasyonla kirlendiğinin bir göstergesi olarak kabul edilir (Wang ve Deng, 2019). Hem noktasal hem de noktasal olmayan kaynaklardan yüzey sularına boşaltılan organik ve biyolojik kirleticiler ve bu kirleticilerin yüklenmesi zamanla değişebilir. Yağmur suyu akışı, nokta kaynaklı olmayan organik ve biyolojik kirleticilerin yüzey sularına taşınmasının birincil yoludur (Henjum ve ark., 2010). Dolayısıyla, iklim de fekal koliform sayısının artmasını ve dağılımını etkileyebilir. Çevresel faktörlerin mevsime bağlı olarak sudaki kirletici yüklerini ve bakterilerin su kütlelerindeki yaygınlığını etkilediği bildirilmiştir (Wang ve Deng, 2019). Suyun doğal yapısının ve su kalitesinin bozulmasına neden olan tüm bu faktörler, yerüstü ve yeraltı sularının insanlar için su kaynağı olarak kullanımını kısıtlamakta ve halk sağlığını tehdit etmektedir.

Aşağı akış yönünde noktasal ve yayılı kaynaklardan gelen kirlilik baskısı altında olan akarsularda, bu baskı sadece antropojenik kaynaklı değildir. Doğal mekanizmalar (erozyon, yağış, akıntı gibi toprak ve hidrolojik faktörlerden kaynaklı) ve klimatik faktörler de akarsu ekosistemini değiştirebilmektedir. Özellikle her mevsim yağış alan, fakat etkili bahar yağışları olan Karadeniz Bölgesi gibi ılıman bölgelerdeki düzensiz rejimli akarsularda klimatik ve hidrolojik faktörler etkilidir (Taş ve ark., 2021).

Mikrobiyal kirliliğin belirlenmesi su kalitesinin değerlendirilmesinde kullanılan yardımcı araçlardan biridir. Şehir merkezlerinden geçen akarsuların su kalitesini belirlemek ve iyileştirme stratejileri geliştirmek için su kalitesinde mekânsal ve zamansal değişiklikleri etkileyen faktörleri anlamak esastır (Ustaoğlu ve ark., 2021). Yeşilırmak ve Kızılırmak havzaları içinde yer alan Samsun ili akarsuları kirlilik nedeniyle hızla bozulmaktadır. İlde öncelikli çevre sorunları arasında su kirliliği birinci sırada yer almaktadır (Taş ve Kolören, 2017). Orta Karadeniz'de Yeşilırmak Havzası'nın alt havzasında yer alan Terme Çayı'nda yapılan su ve sediment kalitesini belirleme çalışmalarında, akarsuyun yerleşim ve tarım alanlarının baskısı altında olduğu bildirilmiştir (Ustaoğlu ve ark., 2021; 2022). Bu çalışmada, Terme Çayı'nda

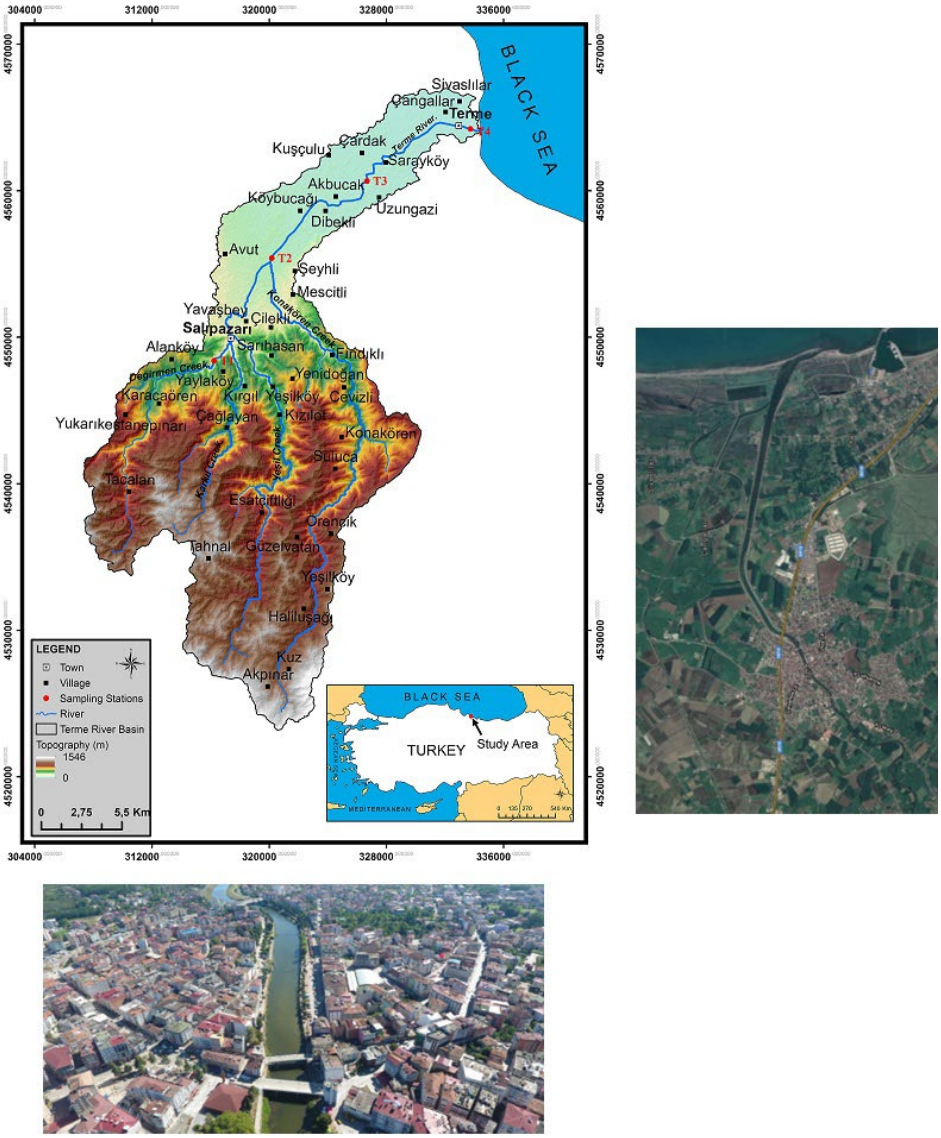
fekal indikatör bakteri (FIB) oranını belirlemek ve su kalitesi sonuçlarıyla mekânsal ve mevsimsel değişkenler arasındaki ilişkiyi incelemek amaçlanmıştır. Bugüne kadar, Terme Çayı'nda FIB kontaminasyonu hakkında rapor edilmiş bir araştırma bulunmamaktadır. Akarsuyun Karadeniz'e deşarj bölgesinde Miliç rekreasyon alanı bulunması nedeniyle bu çalışma halk sağlığı açısından önemlidir.

Materyal ve Metot

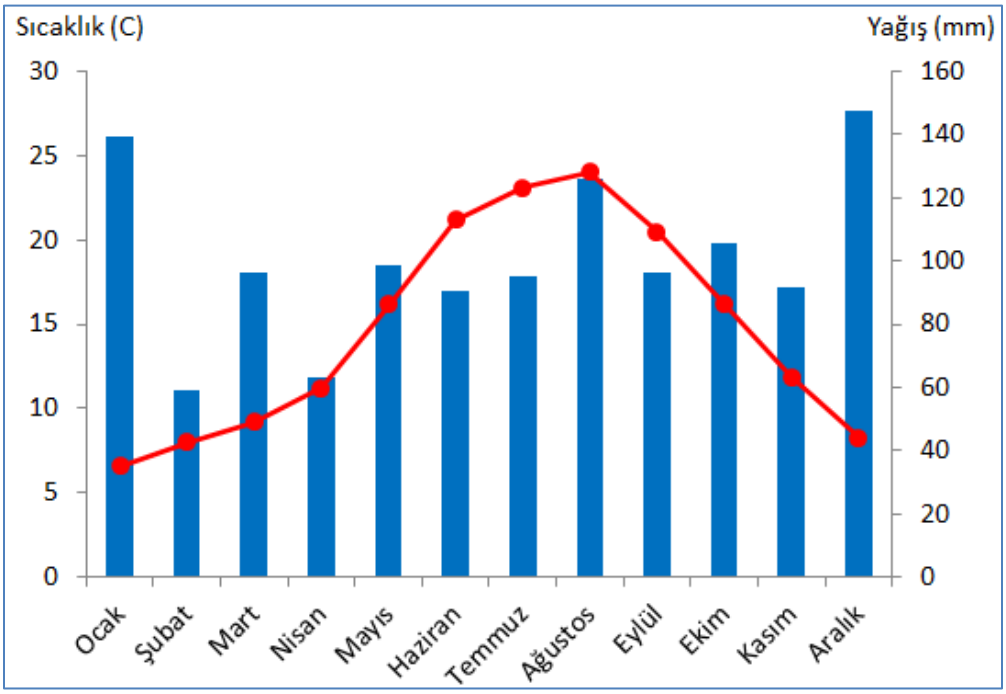
Çalışma Alanı

Çalışma, Karadeniz Bölgesi'nin Orta Karadeniz Bölümü'nde Samsun ilinin 58 km doğusundaki Salıpazarı ve Terme ilçelerinden geçen Terme Çayı'nda yapıldı (Şekil 1). Her mevsim yağışlı Karadeniz iklim özelliğine sahip alanda Salıpazarı'nın yağış alanı 74.5 km^2, Terme'nin yağış alanı 436.4 km^2'dir (Şekil 2).

Terme ilçesinde Karadeniz iklim özelliği görülür. Her mevsim yağışlı ve ılıman geçen çalışma alanında, 2014-2019 yıllarını kapsayan Terme–Kozluk meteoroloji istasyonu verilerine göre, aylık sıcaklık değeri 6.6 °C (Ocak) ila 24.0 °C (Ağustos) arasında değişmiş, ortalama sıcaklık ise 14.7 °C olarak hesaplanmıştır (Şekil 2). Çalışma alanında aylık toplam yağış ortalaması 59.15–147.23 mm aralığındadır. Ortalama aylık toplam yağış miktarı ise 100.7 mm'dir (MGM, 2020).



Şekil 1. Terme Çayı havzasının genel görünümü ve örnekleme lokalitelerinin konumu
Figure 1. The general view of the Terme River basin and the location of the sampling localities



Şekil 2. Araştırma alanının ortalama aylık yağış ve sıcaklık grafiği

Figure 2. Average monthly precipitation and temperature graph of the research area

Su Örneklerinin Toplanması

Terme Çayı boyunca Şekil 1'de görülen dört istasyondan (T1, T2, T3 ve T4) mevsimsel olarak (ilkbahar, Nisan 2019; yaz, Temmuz 2019; sonbahar, Ekim 2019; kış, Ocak 2020) toplam 16 su numunesi örneği toplandı. Numune alma kriterlerine uygun olarak steril 500 mL'lik koyu renkli, vida kapaklı steril cam şişe kullanılarak yüzeyden dibe doğru şişelere hava boşluğu kalmayacak şekilde suya daldırılarak numuneler alındı. Aynı gün içinde numuneler soğuk zincirle laboratuvara getirilerek analizler yapıldı.

Fekal İndikatör Bakteri (FIB) Analizi

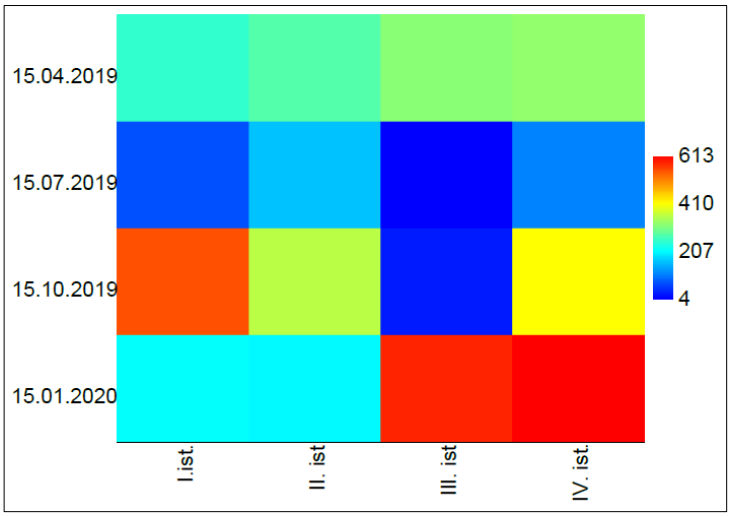
Bu çalışmada, toplam koliform (*TC*), *Escherichia coli* (*EC*) ve *Clostridium perfringens* (*CP*) varlığı araştırıldı. *TC* ve *EC* tespiti ve sayımı membran filtrasyon yöntemiyle standart çalışma prosedürü (TS EN ISO 9308-1, 2014) kullanılarak yapıldı. Çalkalanarak homojenleştirilen su numuneleri, filtrasyon sisteminde 0.45 µm'lik bir membran filtreden (47 mm diameter, Sartorious) süzüldü. Membran filtre kromojenik koliform agar (CCA) besiyeri üzerine yerleştirildikten sonra 36±2°C'de 21±3 saat inkübe edildi. ß-D-galactosidase pozitif koloniler (pembeden kırmızıya) muhtemel koliform olarak sayıldı. *Aeromonas* spp. gibi oksidaz pozitif bakterilerin neden olduğu yalancı pozitif reaksiyonu ayırmak için, muhtemel koloniler negatif oksidaz (oksidaz testi) reaksiyonu ile doğrulandı. Besiyerinde menekşe morundan laciverte kadar olan koloniler (ß-D galaktosidaz ve ß-D-glukuronidaz pozitif koloniler) *EC* olarak sayıldı. *TC* sayımı ise *EC* sayısı ile oksidaz negatif olan koliform bakterilerin toplamı sonucu elde edildi.

CP'nin tesbiti ve sayımı (sporlular dahil) membran filtrasyon metodu ile standard metoda göre yapıldı (TS EN ISO 6222, 1999). İncelenecek su numuneleri çalkalanarak homojenleştirildikten sonra, 0.22 µm'lik membran filtreden süzüldü. Membran filtre *Clostridium perfringens* agar (m-CP agar) besiyeri üzerine yerleştirilp anaerobik ortamda (anaero jar) 44±1°C sıcaklıkta 21±3 saat inkübe edildi. İnkübasyon sonunda opak sarı koloniler şüpheli *CP* olarak kabul edildi ve doğrulamaya alındı. Opak sarı koloniler 20–30 saniye amonyum hidroksit buharına tutulup pembe ya da kırmızıya dönen koloniler *CP* olarak kabul edildi.

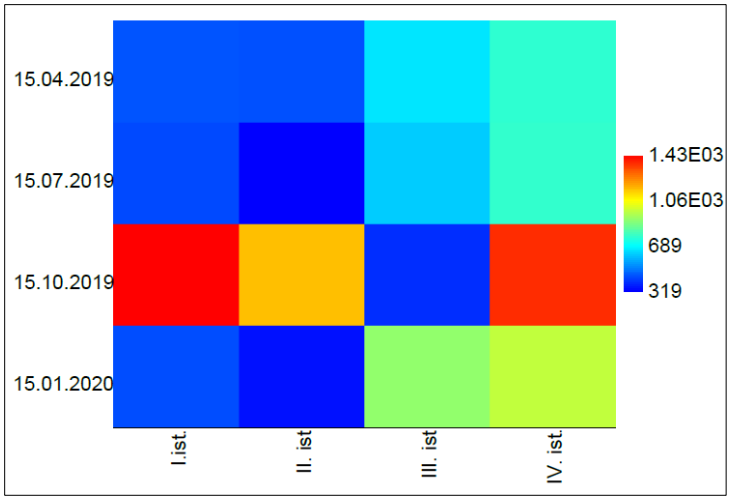
Bulgular ve Tartışma

Su kütlelerindeki dışkı kirliliği yaygın olarak FIB ölçülmesiyle belirlenir (Saxena ve ark., 2015). Şehir merkezlerinden geçen akarsuların su kalitesini belirlemek ve iyileştirme stratejileri geliştirmek için su kalitesinin zamansal ve mekansal

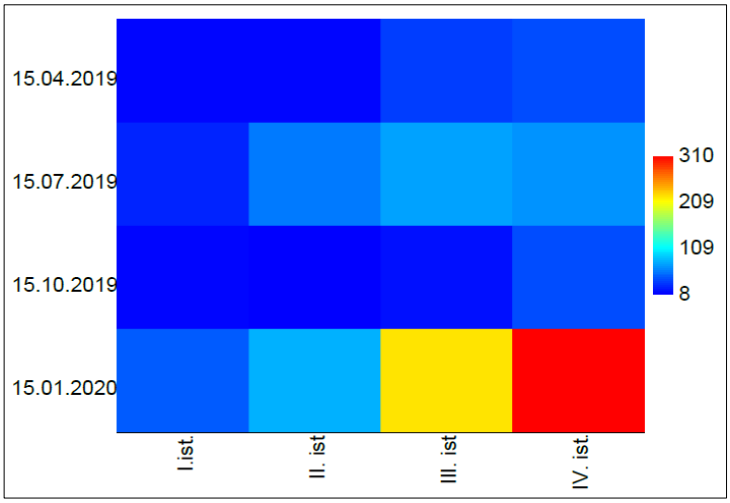
değişkenliğini etkileyen faktörleri anlamak gerekir (Lintern ve ark., 2018). Terme Çayı'nda yaptığımız FIB analizlerinin mevsimsel ve mekânsal sonuçları Tablo 1'de verilmiştir. Ayrıca, *TC*, *EC* ve *CP* hot plot grafikleri de Şekil 3–5'te görülmektedir. Hot plot grafiği, genellikle bir veri setindeki farklı özelliklerin etkileşimlerini görselleştirmek için kullanılan bir grafik türüdür. Bu grafik, bir ısı haritası şeklinde oluşturulur. Farklı renkler ve tonlar kullanılarak veri noktalarının yoğunluğu gösterilir. Zamana bağlı olarak istasyonlardaki bakteri yoğunluğunun gösterildiği grafikte, mavi renk bakteri yoğunluğunun az olduğunu, kırmızı renk bakteri yoğunluğunun fazla olduğunu ifade etmektedir. Terme Çayı'nda mevsimsel ortalama olarak FIB sayısı *TC*>*EC*>*CP* şeklindedir. Maksimum *TC* bakteri sayısı sonbaharda 1. istasyonda (1430 KOB/100 mL), maksimum *EC* (613 KOB/100 mL) ve *CP* (310 KOB/100 mL) sayısı kış sezonunda 4. istasyonda kaydedilmiştir.



Şekil 4. *E. coli* hot plot grafiği
Figure 4. *E. coli* hot plot graph



Şekil 3. Toplam koliform hot plot grafiği
Figure 3. Total coliform hot plot graph



Şekil 5. *C. perfringens* hot plot grafiği
Figure 5. *C. perfringens* hot plot graph

Koliform bakteriler insan ve hayvan bağırsağında bulunabileceği gibi çevresel ortamda da bulunabilir ve potansiyel fekal kirliliğin göstergesi olabilirler (Dindar, 2019). *TC* bakteri sayısı, su kalitesinin en güvenilir göstergesi olarak kullanılır. Terme Çayı'nda en yüksek *TC* bakteri sayısı sonbaharda 1. istasyonda (1430 KOB/mL), kış (970 KOB/mL), yaz (762 KOB/mL) ve ilkbahar (756 KOB/mL) mevsimlerinde ise 4. istasyonda gözlenmiştir (Şekil 3). *TC* bakteri yoğunluğu, dört istasyonda mevsimsel ortalama olarak sonbahar>kış>ilkbahar>yaz şeklindedir. Yüzey suyunun kalitesi genellikle çevre ve iklim koşullarına ve su toplama alanındaki

hayvan ve/veya insan dışkısının varlığına bağlı olarak dalgalanma gösterir (Shanks ve ark., 2006). Su kaynaklarının çoğu kırsal alanlardan beslendiği için, mevsimlik tarım uygulamalarından, hayvan hareketlerinden ve yağış gibi iklimsel faktörlerden kaynaklanan mikrobiyolojik kontaminasyon faktörlerinden etkilenebilirler (Koloren ve Kaya, 2012). SKKY (2004)'de bakteriyolojik parametreler içinde yer alan *TC* sayısına göre su kalite sınıfları şu şekilde sınıflandırılır: *TC*; <100 ise I. sınıf, 100–20000 ise II. Sınıf, 20000–100000 ise III. Sınıf, >100000 ise IV. Sınıf. Aynı yönetmeliğin göller, göletler, bataklıklar ve baraj haznelerinin ötrofikasyon kontrolü sınır değerleri tablosunda (Tablo 2), doğal koruma alanı ve rekreasyon alanı ile çeşitli kullanımlar için belirlenen alanlarda 1000 KOB/100 mL *TC* sayısı sınır değerdir. Yönetmeliğe göre, Terme Çayı II. sınıf su kalitesi özelliği taşımaktadır. Doğal koruma ve rekreasyon alanı için değerlendirildiğinde, Terme Çayı'nda sonbahar sezonunda *TC* sayısı sınır değeri aşmaktadır (>1000 KOB/100 mL). Bu sonuç ötrofikasyon kontrolü için alanın bakteriyolojik kirlenme baskısı altında olduğunu göstermektedir (SKKY, 2004).

Tablo 1. Terme Çayı'nda fekal indikatör bakterilerin zamansal ve mekânsal analiz sonuçları

Table 1. Results of temporal and spatial analysis of fecal indicator bacteria in Terme River

Mevsimsel Örnekleme	İstasyonlar	*TC*	*EC*	*CP*
İlkbahar (15.04.2019)	1	442	246	10
	2	436	274	10
	3	652	316	32
	4	756	324	38
Yaz (15.07.2019)	1	425	68	22
	2	319	158	56
	3	615	4	72
	4	762	108	66
Sonbahar (15.10.2019)	1	1430	550	10
	2	1153	355	8
	3	385	25	14
	4	1365	410	38
Kış (15.01.2020)	1	431	210	44
	2	343	203	78
	3	902	585	220
	4	970	613	310
	Ortalama (n=16)	712±362	278±191	64±83

EC, genellikle memelilerin bağırsak yollarında yerleşik bir kommensal bakteri olarak kabul edilir ve doğrudan dışkı veya idrar yoluyla ve dolaylı olarak arıtılmış atık su yoluyla çevreye atılır. Tartışmasız bir şekilde ve çeşitli antimikrobiyal gruplarına karşı hızlı direnç kazanması nedeniyle, *EC* tatlı sularda, topraklarda ve yiyeceklerde dışkı kirliliğini incelemek için bir gösterge organizma olarak benimsenmiştir (Li ve ark., 2015; Titilawo ve ark., 2015). Terme Çayı'nda, *EC* koloni sayısı 4–613 KOB/100 mL olarak tespit edilmiştir. En yüksek *EC* sayısı kış, sonbahar, ilkbahar ve yaz mevsiminde sırasıyla 613, 550, 324 ve 158 KOB/mL olarak tespit edilmiştir. Bu yüksek değerler sonbahar sezonu hariç (1. istasyon) diğer sezonlarda 4. istasyonda gözlenmiştir (Şekil 4). Terme Çayı'nda yıllık ortalama *EC* sayısı 278 KOB/100 mL olup, mevsimsel sıralama kış>sonbahar>ilkbahar>yaz şeklindedir.

Yerleşim ve tarım alanlarından geçerek Sinop'tan Karadeniz'e dökülen Sırakaraağaçlar Deresi'nde ve Karasu Çayı'nda yapılan mikrobiyolojik su kalitesi çalışmalarında, *TC* ve fekal koliform (*FC*) sayıları sırasıyla, 36-1264 (Sınıf I-II) ve 27-1020 (Sınıf II-III); 33-721 (Sınıf I-II) ve 25-627 kob/mL (Sınıf II-III) aralıklarında bulunmuştur. Her iki

akarsuda her mevsim ve her istasyonda tespit edilen *TC* ve *FC* en fazla yaz aylarında çoğalma göstermiştir. Mikrobiyolojik kontaminasyon kaynakları olarak akarsulara çevre köylerin kanalizasyon sisteminin karışması, tarım alanları ve mera alanları, bunların yanı sıra yaz aylarında bölgenin turizm faaliyetlerine bağlı olarak nüfusun artması gibi faktörlerin koliform bakteri artışlarında etkili olduğu bildirilmiştir (Gündoğdu ve Çarlı, 2020 a, b). Terme Çayı'nın yoğun tarımsal faaliyetlerin yapıldığı Terme Ovası'nın yanı sıra Salıpazarı ve Terme ilçelerinin merkezinden geçmesi dolayısıyla yoğun antropojenik baskı altında olduğu gözlenmiştir. Çalışma peryodu içinde Terme'de Haziran ve Ağustos ayında meydana gelen sel felaketi nedeniyle yaz aylarında beklenen bakteriyel artış gözlenmemiştir. Muhtemelen akarsuyun debisinin artması, su seviyesinin yükselmesi nedeniyle sudaki ve sedimentteki bakteriyel kontaminasyon denize doğru taşınmıştır.

Sularda dışkı kirliliği kaynaklarını doğru bir şekilde belirlemek için *EC* ile birlikte *CP* analizi yapmak değerli sonuçlar verebilir. *CP*, dışkıyla ilişkili Gram pozitif, spor oluşturan, biyofilm oluşturan, anaerobik ve patojenik bir türdür (Koo ve ark., 2020). *CP*, balıkların ve domuzlar ve ördekler gibi sıcakkanlı hayvanların bağırsaklarında; toprak, akarsular ve deniz çökeltileri gibi bazı enterik olmayan ortamlarda bulunabilir (Skanavis ve Yanko, 2001; Scott ve ark., 2018). Terme Çayı'nda *TC* ve *EC*'den daha az sayıda *CP* tespit edildi (8–310 KOB/100 mL; ort. 64 KOB/100 mL). *CP* ortalama koloni sayısının istasyonlara göre dağılımı genel olarak 4>3>2>1, mevsimsel sıralama ise kış>yaz>ilkbahar>sonbahar şeklindedir. Maksimum *CP* değeri 4. istasyonda kışın (310 KOB/100 mL), 3. istasyonda yazın (72 KOB/mL), 4. istasyonda ise ilkbahar ve sonbahar sezonlarında (38 KOB/ml) kaydedilmiştir (Şekil 5). *CP* ırmaklardaki ve diğer lotik sistemlerdeki atık su arıtma tesislerinden kaynaklanan nokta kaynaklı emisyonlar için mükemmel bir göstergedir. Hem noktasal hem de noktasal olmayan kaynaklardan etkilenen küçük akarsularda *CP* sporları, bakteriyolojik su kalitesini belirlerken nokta kaynağı kirliliğinin bir göstergesi olarak değerlendirilir (Sorensen ve ark., 1989). *CP,* doğrudan bağlantısı ve çevresel stabilitesi nedeniyle etkili bir insan kanalizasyon göstergesidir (Stelma, 2018). Terme Çayı'nda tespit edilen *CP* kontaminasyonu, atık suyla akarsuya giren mikroorganizmalar için hassas bir gösterge gibi görünmektedir. Aynı zamanda vahşi yaşamdan kaynaklı noktasal olmayan girdilerin de olduğunu göstermektedir.

CP sporları kirli sularda, özellikle tatlı sularda diğer FIB'lere göre daha uzun süre hayatta kalabilir. Sporlarının uzun süreli çevresel canlılığı, CP'yi uzun vadeli veya biriken dışkı kirliliğinin daha iyi bir göstergesi yapar. *CP* sporları, kirlilik giriş bölgesinden uzak yerlerde tespit edilebilir, bu da uzak veya eski fekal kirliliğin bir göstergesidir (Savichtcheva ve Okabe, 2006; Wang ve ark., 2012). Ayrıca, *CP* bolluğu her zaman diğer FIB'lerin bolluğu ile ilişkili değildir. Bu nedenle, *CP* ve diğer FIB'lerin birlikte kullanılması, patojenleri daha iyi tahmin edebilir (Li ve ark., 2021).

Terme Çayı'nda FIB kontaminasyonun varlığı kanalizasyon ve fosseptiklerin akarsuya karıştığını kanıtlamaktadır. Genel olarak, Terme Çayı'nda *TC*, *EC* ve *CP* sayıları yağışlı dönemde kuru döneme göre daha fazladır. Akarsuyun akış yönünde FIB konsantrasyonu da artmaktadır. Terme Çayı'nın fizikokimyasal su kalitesinin mekânsal ve mevsimsel olarak incelendiği kapsamlı çalışmada, yerleşim yerlerinin merkezlerinden ve tarımsal alanların ortasından geçen akarsuyun akış yönünde kirletici konsantrasyonunda artış, buna paralel olarak akarsuyun alt bölgelerinde su kalitesinin azaldığı bildirilmiştir (Ustaoğlu ve ark., 2021). Terme Çayı'nda mikrobiyolojik araştırma bulguları da benzer durumu göstermiştir. En yüksek FIB sayıları genellikle akarsuyun Karadeniz'e deşarj bölgesine yakın olan 4. istasyonda tespit edilmiştir. Giresun'dan Karadeniz'e dökülen Gelevera Deresi'nde de yerleşim merkezlerinin akarsu hatlarındaki bakteriyolojik kirliliği doğrudan etkilediği (kanalizasyon ve evsel katı atıklar) ve sistemde organik yük miktarının artmasına yol açarak Karadeniz ekosistemi için yüksek risk taşıdığı ifade edilmiştir (Akkan ve Çolaker, 2020). Güneydoğu Karadeniz kıyılarında yapılan incelemede *TC*, *FC* ve *FS* (fekal streptokok) seviyelerinin çok yüksek olduğu ve çoğunlukla değerlerin yüzme suyu için ulusal standartların zorunlu değerlerinin üzerinde olduğu bildirilmiştir (Kalkan ve Altuğ, 2020). Karadeniz'de deniz ekosistemi üzerinde antropojenik faaliyetler (tarımsal faaliyetler, endüstriyel toksik maddeler, kanalizasyon ve atıksu sorunları, denizcilik ve ulaşım, turizm, madencilik ve balıkçılık gibi) ciddi baskı oluşturmaktadır (Bat ve ark., 2018). Dolayısıyla, karasal kökenli akarsularla taşınan kirlilik faktörlerinin belirlenmesi ve gerekli önlemlerin ivedilikle alınması gerektiği yapılan su kalitesi çalışmaları ile görülmektedir.

Yüzey akışı ve erozyon toprak bakterilerini de akarsulara taşıyabilen süreçlerdir (Boithias ve ark., 2021). Birinci istasyon dağınık yerleşim alanları ve fındık tarım alanlarıyla çevrilidir. Bu bölgelerde bakteriyolojik kirliliğe yol açan en önemli potansiyel tehditler yayılı kaynaklardır (yerleşim alanlarının fosseptik sızıntıları, evsel atık/atıksular, vahşi katı atık depolama alanları, tarım alanlarına hayvansal gübre kullanımı, besi hayvanları ve yaban hayatı dışkı tortuları, hayvanların kullanıldığı tarım arazilerinden sızan sular). Yoğun şehir yer-

leşim alanlarında ise noktasal ya da kontrol edilmeyen antropojenik faaliyetler akarsuyun giderek kirlenmesine yol açmaktadır. Bölgenin her mevsim yağış alması ve zaman zaman yaşanan sel felaketleri noktasal ve/veya yayılı kaynakların yapısında mevcut olan kirletici yükleri alıcı ortama taşıyarak kirletici yükünü oldukça artırmaktadır. Aşırı yağış olayları, yerel ve küresel ölçeklerde ırmak akış rejimlerindeki yüksek değişkenlikler, su kaynaklı patojenlerin neden olduğu bulaşıcı hastalık risklerini arttırabilmektedir (Blöschl ve ark., 2019; Derx ve ark., 2023). Bol yağış alan Karadeniz Bölgesi'nde de su kaynaklı parazit patojenlerin halk sağlığını tehdit edebileceği belirtilmiştir (Kolören ve ark., 2011 a,b). Dışkı kaynaklı patojenlerin kirlettiği akarsular ve bunların karıştığı yüzme suları, insanlar için artan enfeksiyon riskini temsil etmektedir (Arnold ve ark., 2017; Kauppinen ve ark., 2017). Ayrıca, akarsuların fekal kontaminasyonu, rekreasyonel faaliyetler dışındaki amaçlar için kullanılan suyun (sulama suyu, içme suyu, balık yetiştiriciliği ve avcılığı gibi) değerlendirilmesini olumsuz etkileyebilir.

Terme Çayı'nın içinde bulunduğu Yeşilırmak Havzası'nda yapılan çalışmalarda, Yeşilırmak Nehri ve kollarında çevresel etkiler ve kirlilik gözlenmiş ve bu durumun su kalitesini olumsuz etkilediği bildirilmiştir (Başören ve Kazancı, 2015; Karaman ve ark., 2017a; Kolören ve ark., 2017). Tarımsal ve evsel atıkların neden olduğu kirliliğin kontrolü için su kalitesinin uzun süreli fizikokimyasal ve biyolojik parametrelerle izlenmesi son derece önemlidir (Başören ve Kazancı, 2015). Bakteriyolojik parametrelerin izlenmesi de su kirliliği kontrolü açısından önem arz etmektedir. Karaman ve ark. (2017b) Samsun ilindeki çevresel su örneklerinde parazit varlığını inceledikleri çalışmada; *Giardia* sp., *Cryptosporidium* spp., *Cyclospora* spp., *Microsporidia*, *Blastocystis* spp, *Entamoeba coli* kisti, *Dientemoeba*, *Chilomastix*, *Strongyloides* spp. ve kancalı kurt saptamışlardır. Bölgede hayvancılığın ve tarımın yaygın olarak yapılması ve akarsu etrafının otlak alanı olarak kullanılması, tespit edilen protozoonların belirli dönemlerde fazla görülmesinin başlıca nedenleri olduğunu belirtilmiştir. Aynı şekilde, Terme ve Kocaman Çayı havzasında yapılan bir araştırmada, *Cryptosporidium* spp., *Cyclospora* spp., *Strongyloides* spp., *Microsporidia* sporu, *Blastocystis* spp., *Chilomastix* spp., *Balantidium* spp., *Giardia* spp. ve kancalı kurt yumurtaları tespit edilmiştir. Çalışmada, tarımda, sanayide ve evsel kullanımda ihtiyaç duyulan suyun, potansiyel kullanım kriterleri doğrultusunda patojen mikroorganizmalardan arındırılmış olması gerektiği vurgulanmıştır (Karaman ve ark., 2017a). Yeşilırmak Nehri ve Tersakan Çayı'ndaki *Cryptosporidium* kontaminasyonunun incelendiği araştırmada ise, hayvan besiciliğinin, akarsulara çok yakın yerleşim yerlerine ait evsel ve tarımsal atık suların hiçbir işleme tabi tutulmadan Tersakan Çayı'na deşarj edilmesinin, bu akarsuda *Cryptosporidium* kontaminasyonunun daha fazla olmasına neden olduğu bildirilmiştir (Kolören ve ark., 2017).

Tüm bu çıkarımlar, Terme Çayı'nın, yukarı havzasında zirai faaliyetler, aşağı havzasında ise yerleşim alanlarından kaynaklı hem çeşitli parazitler hem de FIB kontaminasyonundan etkilendiğini göstermektedir. Dışkı koliform konsantrasyonlarının artışı tarım girdilerinin yoğunluğuyla açıklanabilir. Akarsu alanlarının hayvan girişinden yeterince korunmaması ve yetersiz çiftlik avlusu atık yönetimi nedeniyle dışkıyla kirlenmiş suların noktasal kaynak katkılarının akarsu kirlenmesine önemli ölçüde katkıda bulunduğu görülmüştür. Yine, akarsuyun çevresinde, akış yönünde besi hayvanlarının otlatılması ve besi hayvanı yetiştiriciliğinin etkisi ve organik atıkların (bulamaç ve gübre) havza topraklarına uygulanması, geniş alanları etkileyebilecek önemli miktarda dışkı koliform bakteri rezervuarı oluşturma potansiyeline sahiptir. Bu sonuçlar, bakteriyel su kalitesinin havza anlayışını iyileştirme gerekliliğini açıkça ortaya koymaktadır.

Kentsel yerüstü su kütleleri, içme suyu üretimi, sulama ve rekreasyonel su kullanımı için önemli kaynaklardır. Yüzey sularının suyla taşınan patojenlerle, dışkıyla kirlenmesi, potansiyel enfeksiyon risklerine ve suyla taşınan hastalık salgınlarına yol açabilir. Özellikle akarsuyun denize deşarj bölgesinde rekreasyon alanı olarak kullanılan alanlarda yüzme suyunda meydana gelen bakteri kontaminasyonu önemli sağlık problemlerine yol açabilir. Denizel rekreasyon alanlarında karasal kirlilik kaynaklarının bakteriyolojik kontaminasyona yol açarak halk sağlığı, ekoloji ve çevre açısından istenmeyen durumlar oluşturduğu (Çiftçi Türetken ve Altuğ, 2016), akarsuyun denize döküldüğü noktada tespit edilen bakteri konsantrasyonunun ise açık sudakinden daha yüksek olduğu bildirilmiştir (Hulyar ve Altuğ, 2020). Ayrıca, su kirliliğinin artmasıyla, Marmara Denizi'nde olduğu gibi diğer denizlerde de görülebilen ve önemli bir çevre problemi olan müsilajın, *EC* gibi bakteriler bakımından zenginleştiği, bunun da deniz yaşamını tehdit ettiği belirtilmiştir (Yümün ve ark., 2023).

Sonuç

Bu çalışma, Terme Çayı'nın mikrobiyolojik kalite değerlendirmesine odaklanmaktadır. Terme Çayı'nda mevsimlere ve istasyonlara bağlı olarak bakteri türlerinde ve yoğunluklarında mevsimsel dalgalanmalar gözlenmiştir. *EC* ve *CP* kış sezonunda yüksek iken, TK sayısının sonbaharda diğer mevsimlere göre daha yüksek olduğu tespit edilmiştir. Aşağı akış yönünde ise akarsuyun mikrobiyal kirliliğinin arttığı belirlenmiştir. Çalışma sonucunda, akarsuyun fekal kirlilikle kontamine olduğu gösterilmiştir. SKKY (2004)'ye göre bak-

teriyolojik parametre bakımından Terme Çayı II. Sınıf su kalitesinde "az kirlenmiş su" özelliği taşımaktadır. Deşarj bölgesinde FIB konsantrasyonunun artması rekreasyon için kullanılan plaj alanında risk teşkil edebilir. Bu durum Terme Çayı'nın üst havzasından alt havzasına kadar dışkı kirliliğinden korunma önlemlerine ihtiyaç duyulduğunu göstermektedir. Çünkü insanların akarsuyu doğrudan ya da dolaylı olarak kullanımı arttıkça, kontamine su ile temastan ve kontamine balıkları yemekten kaynaklanan su kaynaklı hastalıkların insidansı artacaktır. Noktasal ve yayılı kaynaklar nedeniyle yerüstü su kütlelerinin sürekli kirlenmesi göz önüne alındığında, özellikle tatlı su kaynaklarının korunması ve yönetilmesi gerekmektedir. Tüm insanlar güvenli su kaynaklarına ulaşma hakkına sahip olmalıdır. Temiz su, insan kullanımı ve dengeli ekosistem için gereklidir.

Etik Standartlar ile Uyumluluk

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

Etik kurul izni: Araştırma niteliği bakımından etik izin gerektirmemektedir.

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Açıklama: -

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Effects of environmental deterioration on Northeast Anatolia Trout (*Salmo spp*.) Populations

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ABSTRACT

In this study, the possible effects of overfishing pressure on local Salmonid populations and the critical environmental changes that have occurred in the wild habitats that have been deteriorating since the beginning of the 21st century were investigated in Northeast Anatolia. In the research, methods including quantitative and qualitative impact assessments were used to determine environmental changes and anthropogenic effects. In addition to numerical data, observations of the stream and its surroundings were recorded.

As a result, it has been determined that the natural trout populations, distributed in the limited habitats in the middle and upper parts of the studied rivers, have decreased considerably, especially since the number of individuals who have reached breeding age has reduced to a high level. It has been determined that the habitats in localities where the research was conducted have been under great pressure, since the end of the 20th century, due to infrastructure works, urbanization, and illegal fishing. It has been determined that the local trout populations, distributed in the limited habitats in the middle and upper basins of the studied rivers, are quite damaged. The number of individuals that have reached the breeding age has decreased significantly.

Keywords: Northeast Anatolia basin, *Salmo labrax*, Local trout populations, Overfishing, Habitat degradation

Introduction

The Black Sea salmon (*Salmo labrax,* Pallas 1814) inhabits the northern Black Sea coasts and inflowing rivers. There are anadromous, lacustrine, and resident river populations. This fish is a close relative of the brown trout. While it is the only native species of Salmonids present in the northern Black Sea basin, it may hybridize with (introduced) brown trout in the major rivers (Freyhof, 2013). Marine populations are currently found very rarely. But the resident river stocks are doing well (Froese & Pauly, 2019), just slightly better than marine populations. However, it is included in the Red List in European waters, and the IUCN status of the Caucasian and North-eastern Anatolian trout populations is NE (Nearly Extinct) (Mikeladze et al., 2021).

Today, it has been demonstrated by many studies that wild trout populations are exposed to great pressure as a result of natural ways and human activities (fishing, changing the living environment, pollution, etc.) (Crowx, 2002; Kratzer & Warren, 2003¸Zengin et al., 2017; Kirn, 2017; Şengül et al., 2018; Kalaç et al., 2019). *S. labrax* species shows a natural distribution in the Eastern Black Sea Region Rivers. This species prefers cold and fast-flowing streams. Adults migrate to the upper parts of the river to spawn, while young individuals migrate downstream to find more food and transfer to the sea. This situation then continues in reverse (Aksungur et al., 2011).

Regarding river potential, Northeast Anatolia is one of Turkey's most important geographical areas. Although the flow rate of the streams in the region is low, the flows periodically enter an increasing trend during precipitation periods (Zengin et al., 2017). After the 2000s, the rivers in the region were considered the most important potential resource in the policies to eliminate the energy deficit in Turkey. They started to be used intensively (Erdoğan, 2010r).

Local populations of Salmonid species with the same lineage group (Danube) show some morphological, ecological, and genetic differences, spread in the inland waters of Northeast Anatolia in Turkey (Çiftçi et al., 2007). *S. trutta* is represented by four subspecies in Türkiye (Berg, 1948; Balık & Geldiay, 1996). These are *Salmo abanticus,* Tortonese, 1954 is distributed in Lake Abant, *Salmo caspius,* Kessler 1877 is in the Caspian Sea region, *Salmo labrax,* Pallas 1814 is in the Black Sea region and *Salmo macrostigma,* Dumerill 1858 is in the Mediterranean region (Polat et al., 2011). However, according to recent genetic studies (Bernatchez et al., 2001; Bardakçı et al., 2006; Kalaycı et al., 2018), it has been stated that there is no molecular level difference between a large number of morphologically different trout populations in Turkey, which are considered different subspecies.

Despite the genetic findings, this taxonomic situation is still widely used (Solomon, 2000; Tabak et al., 2001; Kurtoğlu, 2002). *Salmo platycephalus* (Anatolian trout) is a trout species found only in the central south of Turkey, especially in the Seyhan River system. It was scientifically described for the first time in 1968 by a scientist named R. J. Behnke. The largest ones can reach 50 cm in length (Behnke, 1968). According to the findings obtained from the genetic studies carried out by Kalaycı et al. (2018) on the taxonomic differentiation of trout populations distributed in Turkish waters, they reported that Abant, Caspian, Black Sea, and Anatolian trout, which live in Turkish waters and are named according to the geographical regions where they have lived until today, are only an ecotype and each is an ecotype of *S. trutta,* which comes from the Danube lineage.

In this study, the possible effects of significant environmental changes in the natural habitats of the local populations of Salmonid species distributed in the Northeast Anatolian zoogeography of Turkey have deteriorated since the beginning of the 21st century on these populations were investigated.

Material and Methods

Trabzon Central Fisheries Research Institute carried out this study in consecutive periods with data from three different studies. The periods in which these projects and field studies are carried out are as follows; (1) "A Study on the Evaluation of Aquatic Ecosystems in and around Lake Çıldır in terms of Fisheries Management" carried out between 2011-2012 (Zengin et al., 2013). (2) "Investigation of Long-Term Cumulative Effects on the Ecosystem in the Solaklı Basin with an inSTREAM Agent-Based Model" conducted between 2015-2016 (Şengül et al., 2018), and (3) It was started in 2017 but canceled after two periods of fieldwork; "Studies on the Development of Black Sea Trout Fishing Populations on the Main Tributary and Tributaries of the Barhal Stream" (Çakmak et al., 2017).

Sampling Studies

Within the scope of the research, the samples of the trout populations in the river were carried out with the same standard sampling method using the 'electroshock' device. In addition, gillnets with different mesh sizes were used in the trout samplings in the Çıldır Lake. The locations where the research was carried out are shown in Figure 1. There are two ecotypes

of Caucasian trout in the Çıldır basin. These are the river ecotype and the lake (anadromous) ecotype. The height distribution of individuals in the lake ecotype is more significant than those in streams (Zengin et al., 2013). Caucasian trout migrate to some important streams (Doğruyol, Gülyüzü, and Gölebakan Streams) that discharge into the lake in the late spring and summer periods for spawning migration and migrate back to Çıldır Lake in late autumn and winter periods to feed (Zengin et al., 2013).

In the study, sub-locations, sampling periods, and the total number of samples related to the sampling studies carried out in each aquatic resource are shown in Table 1. Studies on all three aquatic resources are shown in Figures 2, 3, 4, and 5.

Table 1. Research stations, sampling periods, and total sample numbers

Research area	Stations	Sampling periods	Number of samples
Ardahan-Çıldır Lake and Streams	Lake Akçakale Stream Doğruyol Stream Çanaksu Stream Çıngıl Stream Gülyüzü Stream Gölebakan Stream Arpaçay/HEP Discharge	July 2011 September 2011 June 2011	99
Trabzon-Çaykara-Solaklı Stream	Haldizen Branch Karaçam Branch	August 2015 December 2015 April 2016 October 206	96
Artvin-Yusufeli-Barhal River	Barhal Main Branch Bıçakçılar Stream Altıparmak Stream	April 2017 July 2017	75

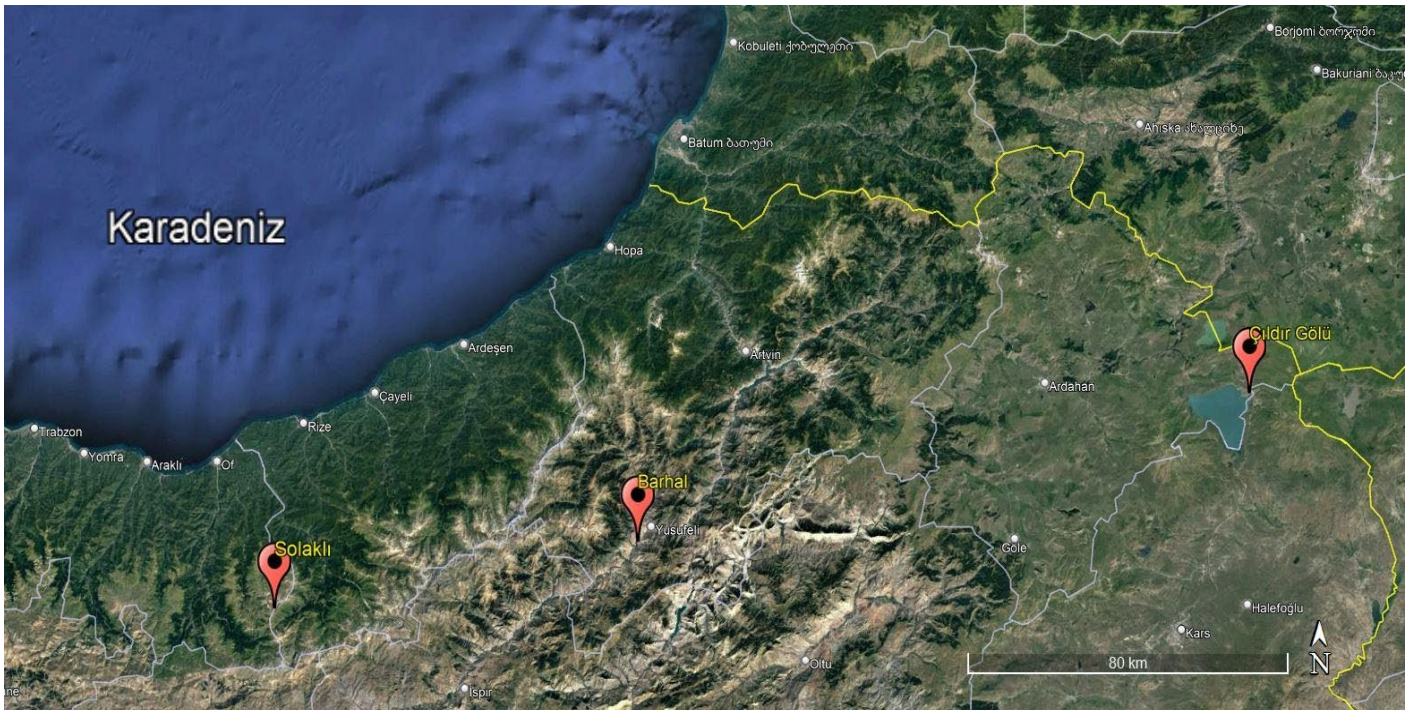

Figure 1. Aquatic areas in the Northeast Anatolia Region where the studies were carried out; Çıldır Lake (Ardahan, Arpaçay), Barhal Stream (Artvin, Yusufeli), and Solaklı Stream (Çaykara, Trabzon)

Figure 2. Samplings of Lake Çıldır were made with gillnets with different mesh sizes (July 2011)

Figure 3. Trout samplings from the streams discharging into Çıldır Lake (July 2011)

Figure 4. Trout sampling studies at stations in Trabzon, Çaykara-Solaklı Stream Karaçam and Uzungöl/Taşkıran tributaries (April 2016)

Figure 5. Sampling studies on the Altıparmak tributary of Barhal Stream (July 2017)

Biometric Studies

On fish samples, respectively, biometric measurements (mm total length, body weight measurements sensitive of 0.01 g and gonad weights), sex determinations, and laboratory studies for macroscopic determination of gonad development stages were performed (Holden & Raitt, 1974) (Figures 6, Figures 7). For age estimation, otoliths, an indicator of growth, were fixed and estimated from age rings under the electronic microscope (Jensen & Johnsen, 1982). With the data obtained, the analysis of parameters such as length-weight-sex-age-laying maturity, length-frequency, age-frequency distributions, and distribution of individuals who have reached sexual maturity will reveal the structure of the trout populations in each location, where made current structure of fish populations. Many reference points will reveal the effect of internal (natural) and external (environmental effects, anthropogenic, etc.) factors, such as whether the population has decreased or is it able to maintain itself at the optimum level. The most valuable and direct method that provides rational information to the researcher is to take the length and age distributions of the individuals in the population as a reference (Sparre et al., 1989).

Figure 6. Biometric studies on trout samples from Barhal Stream

Figure 7. Biometric measurements and determination of sex and gonad development stages on trout samples (Trabzon Central Fisheries Research Institute, Biology Laboratory)

Habitat Studies

Primary data of the study; retrospective, long-term hydrological data on rivers and water structures in the pilot regions, habitat observations, and ongoing investigations on stream beds. In the study, methods including quantitative and qualitative impact assessments were used for the determination of environmental changes and anthropogenic effects. In addition to numerical data, observations of the stream and its surroundings were recorded. Parameters related to environmental factors were divided into categories and standardized by giving a quantitative score/value to the variable under each category. The results were analysed and evaluated proportionally (Rosenthal et al., 2015).

The basic parameters listed below were considered for aquatic habitat analysis (Paffett et al., 2018; Rosenthal et al., 2015). These are, respectively, historical changes in the river flow regime, the structural features of the river bed, the past topographic and current state of the river, the settlement characteristics of the river basin, the depth, width, slope, bends/curves of the river bed, bottom profile, substrate type, and size, structure of the shore banks, islets, river reclamation/flood embankments type, erosion levels, in-river and coastal vegetation, creek basin land/soil use, hydrological structures and other activities (reservoirs, water sequestration), wastewater discharges, pollution status and levels, solid waste landfill, quarries, mining, bridge and road crossings, recreation areas, urbanization.

With all these collected data, answers to the following questions were sought. Does the previous and current evidence prove the migration of native trout across streams? Can species/ecotype detection be done safely? Are the proportions of the species known? Where are the spawning grounds? Where were the juveniles/fry seen on the stream? Is there evidence of their longest-distance migration on the river? Regarding hydrology/water quality, is there a smooth spring/summer flow? Is the depth in spawning areas sufficient? What is the average particle size in sediment transport? Is the water quality suitable for the upper streams as they are historically and currently potentially spawning areas for the species, or has it

changed? Are there any analytical plans to reduce the pollutants directly released into the stream? Can measures be taken to reduce the impact of new Hydroelectric Plants Power (HEPPs), which are implemented and planned on rivers, the river water regime, and the aquatic ecosystem/fish migration? (Gessner et al, 2000; Paffett et al., 2018).

Results and Discussion

State of Populations

Lake Çıldır: The length distribution of the *Salmo caspius* population sampled from the lake ecosystem was determined as 23.7-41.2 (31.2) cm, and the length distribution of the population sampled from the streams discharged into the lake was determined as 8.8-27.5 (10.3) cm (Figure 8). The first sexual maturity length of the population is 13.7 cm. When this size criterion is taken as a reference, 58.3% of the individuals distributed in the river environment consist of individuals who have not yet reached sexual maturity (Figure 9). Considering the age distribution, their populations mainly consist of smolt and young individuals (54.3%) in the 1st and 2nd age groups (Figure 10). Although individuals belonging to the 'lake ecotype' are biometrically larger, the age distribution of individuals belonging to the 'river ecotype' was found to be relatively higher. It is estimated that this growth difference is due to the fact that the feeding dynamics of the lake have a richer potential than the river. However, for both environments, overfishing pressure caused the populations to decline.

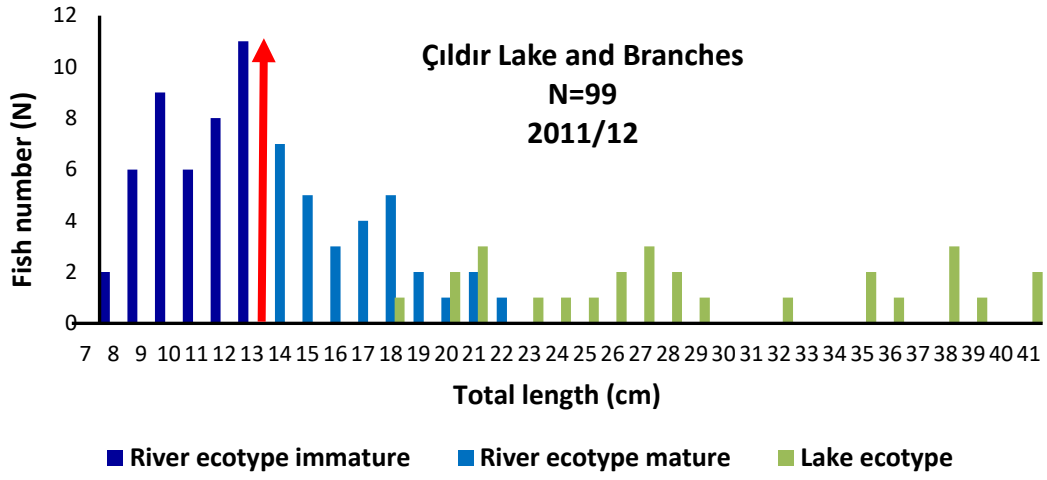

Figure 8. Çıldır lake; length-frequency distributions of river and lake ecotypes

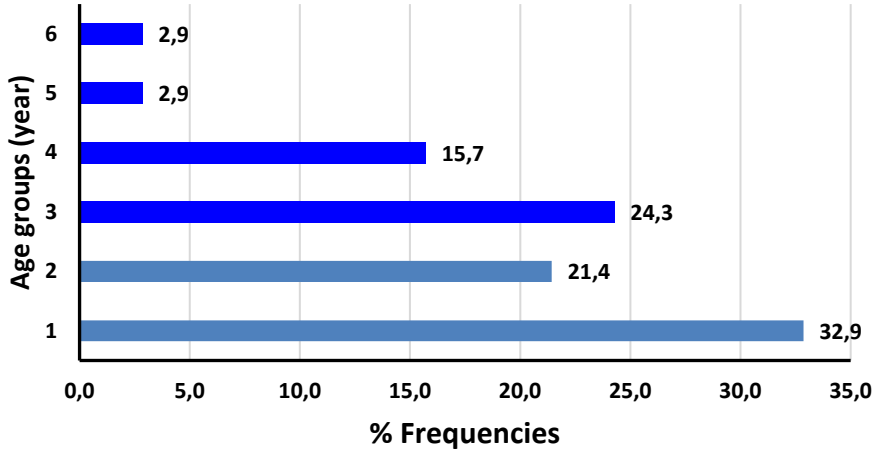

Figure 9. Distribution of age groups of *Salmo caspius* populations in Lake Çıldır

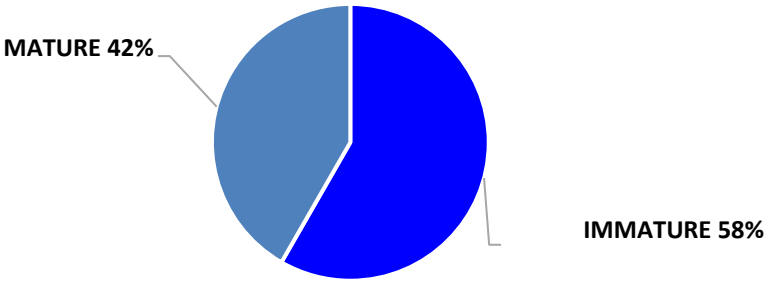

Figure 10. Sexual maturity distributions of *Salmo caspius* populations in the streams discharged to Lake Çıldır

Solaklı Stream: While the length distribution of *Salmo labrax* individuals sampled from the upper tributaries of the Solaklı Stream varied between 6.9-23.2 (13.2) cm, it was observed that the population disappeared completely in the main branch extending to the downstream of the creek in the center of Çaykara district (Figure 11). The age distribution of the Solaklı population ranges from 0 to 3. When the first sexual maturity size of *S. labrax* is taken as a criterion, 35.4% of the Solaklı population consists of adults, and 64.6% are juveniles/young individuals (Figure 12). Considering the first sexual maturity length of the population (13.7 cm), the majority of the individuals distributed in the river consist of individuals who have not reached sexual maturity yet. Considering the age distribution, their populations mostly comprise smolt and young individuals (65.7%) in 0 and 1 age groups (Figure 13). The findings reveal that the trout population living in this habitat has been overexploited. Urbanization along the Solaklı valley, tourism activities in Uzungöl, wastewater discharges, sand-gravel intakes, recreation in the riverbeds, and opening 14 HEPPs and regulators on the main and side branches of the same river have had an impact on this attrition, mainly since the 2000s.

Barhal Stream: The length distribution of the *Salmo macrostigma* population sampled on the upstream branches of the Barhal Stream, which constitutes the main branch of the Çoruh River, was found to be between 6.4-27.2 (16.8) cm (Figure 14), and the age distribution was between 0-6 (Figure 15). Considering the first maturity length of the population (13.7 cm), 35.7% of the fish distributed in the river environment consist of individuals who have not reached sexual maturity yet (Figure 16). The fact that the adult populations in this region are 65.3% higher than the other two localities indicates that the trout populations in the region are less overexploited. The reason for this is that the trout populations in the upper branches of the Barhal Stream are in a safer environment due to environmental factors and less separation of their habitats, compared to many aquatic environments of Türkiye. However, the Yusufeli Dam, which was recently built on the main branch of the Barhal Stream, poses a significant risk to these populations' breeding and feeding migration. As a result, it is likely that natural trout populations, which spread in limited habitats in the middle and upper parts of the studied rivers, will be damaged by this because there is no 'fish passage' planned on the HEPP system that the fish can use between the downstream and the upstream for breeding and feeding migration.

Environmental Impacts

The common results of multiple environmental parameter analyses conducted on stream habitats are shown in Figure 17. According to the findings, the effects of environmental impacts gathered under three main categories on aquatic habitats (Çıldır lake and its side streams, Barhal Stream and Solaklı Stream) are respectively; pollution 65%, hydrological changes 52%, biological effects 35%. Among these essential factors, the most important impact is eutrophication, solid waste storage, and nutrient discharges; in other words, it constitutes a group of urban, industrial, and agricultural wastes. On the other hand, the rate of hydrological impacts is still moderate, and this rate is around 50%. In this group, the effect of HEPPs established on riverbeds is prominent. In addition, the destruction of the structure of the riverbed is also essential. Below the medium level, the most negligible impact is excessive fishing pressure from biological origin, illegal fishing, and exotic species invasions.

At the beginning of environmental impacts, the effect of the Hydroelectric Power Plants, which started to operate at an increasing rate in the Eastern Black Sea Region since the beginning of the 2000s, has created significant risk for trout populations (Zengin et al., 2017). As a result of insufficient compensation of water, invertebrate and vertebrate fauna are adversely affected, as well as habitat loss in the stream bed. Depending on the increase in water temperature in summer

and the relative decrease in flow rate, algae colonization may occur in the localities from the tailwater discharge point to the downstream in HEPPs. These negative factors disrupted the water flow regime in the river system and the water quality, causing a rapid decrease in fish populations living in the region's rivers. On the other hand, due to the embankment in front of the reservoir (dam lake) of the HEPPs, the biological contact between the lower and upper sections is cut off. The temperature of the water accumulating in the reservoir rises. Due to the decay of organic materials accumulated in the reservoir, oxygen in the water decreases, and toxic gas accumulation increases. The flow of water in the stream decreases, especially in the summer months, and the amount of confirmed life in water cannot be fully achieved.

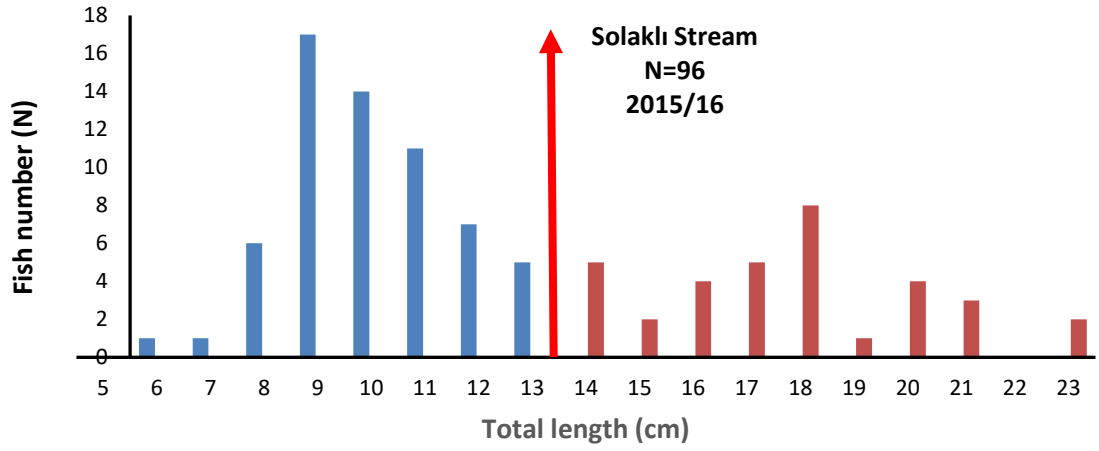

Figure 11. Length-frequency distributions of the *Salmo labrax* populations of Solaklı Stream

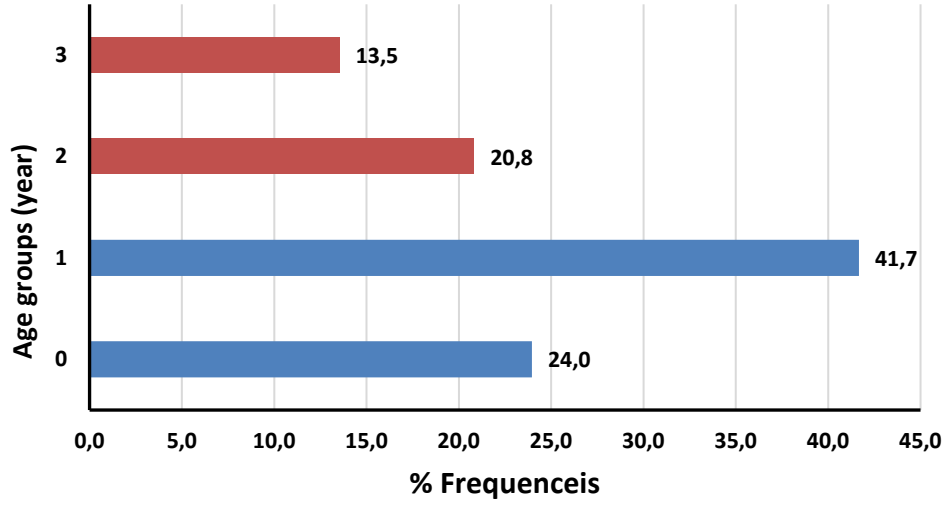

Figure 12. Distribution of age groups of *Salmo labrax* populations of Solaklı Stream

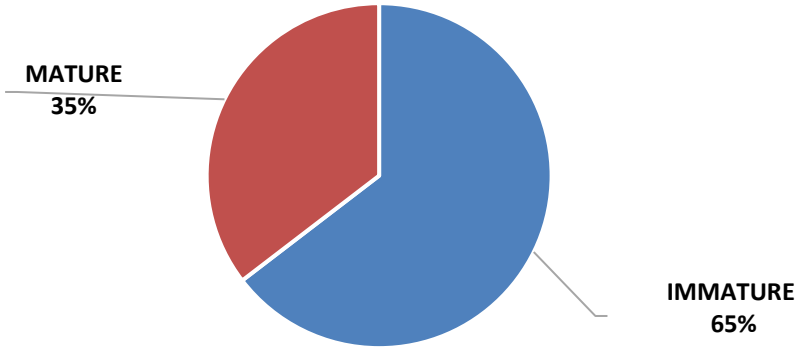

Figure 13. Sexual maturity length distributions of Solaklı *Salmo labrax* populations

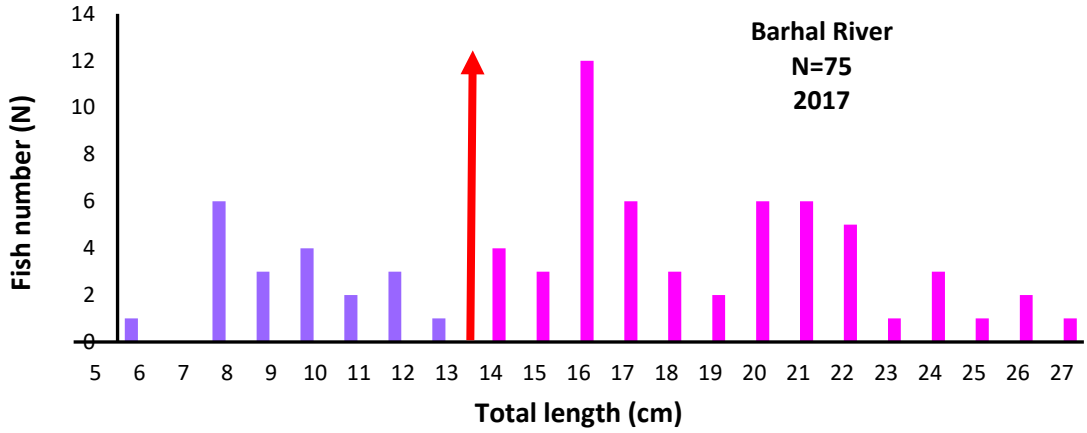

Figure 14. Length-frequency distributions of Barhal Stream *Salmo macrostigma* populations

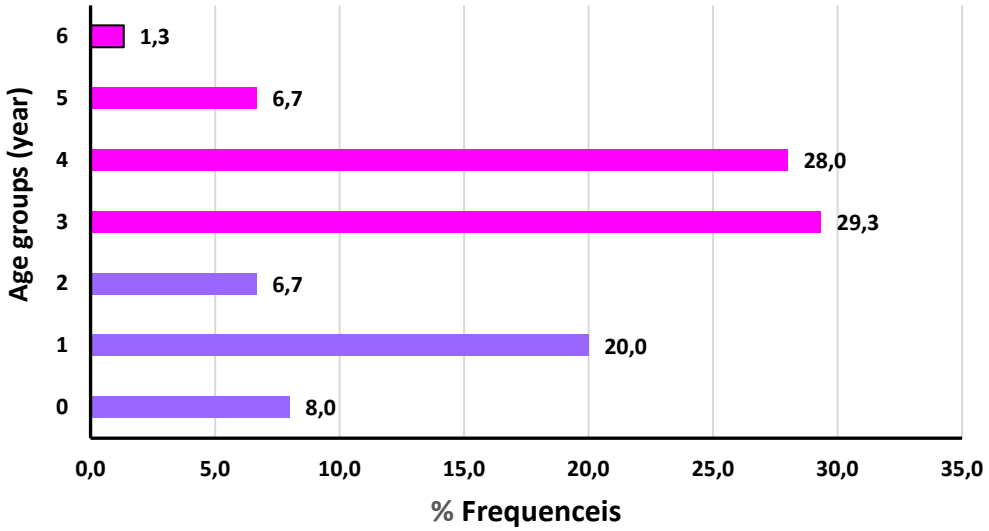

Figure 15. Distribution of age groups of *Salmo macrostigma* populations of Solaklı Stream

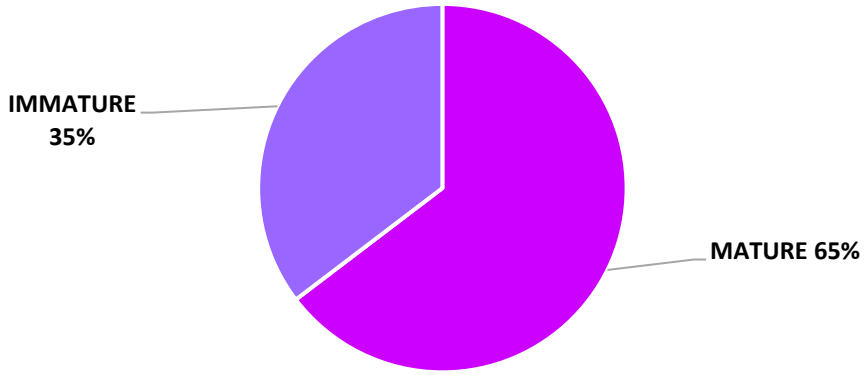

Figure 16. Sexual maturity length distributions of Barhal Stream *Salmo macrostigma* populations

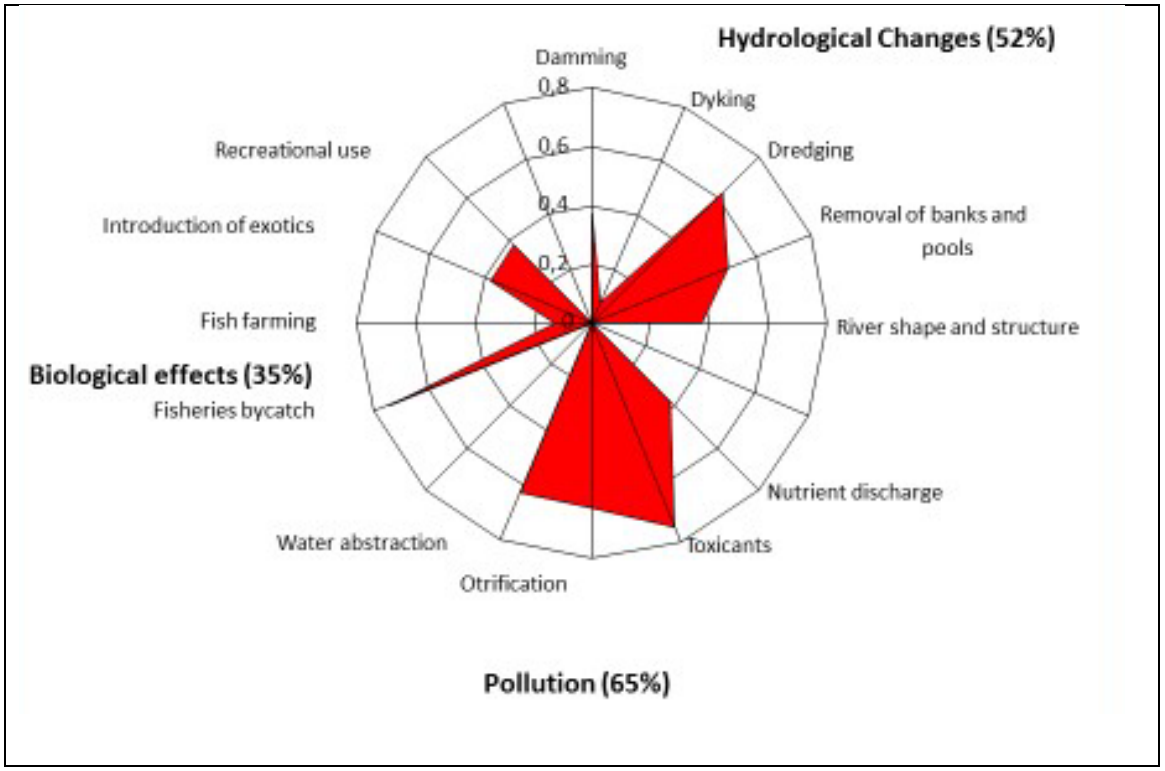

Figure 17. Categorical distribution of possible effects of environmental degradation on trout populations in Northeast Anatolia (Relative index values)

One of the most important risks in the creek's downstream regions is the riverbed's recreational activities carried out by the sand and gravel quarries and the local administrations. During and after these studies, the historical and natural structures of the stream and stream beds are completely changed. Trout individuals dispersed in downstream regions need a safe habitat for both feeding and breeding migration. Although there is no significant problem in this respect in the upper/upstream sections of the stream, where the breeding migration occurs to a large extent, the deterioration was observed relatively in the discharge/closer areas of the stream. In addition, due to the deterioration in these parts of the stream, Stream edge and inland aquatic vegetation could not develop. As a result of erosion/flooding, clay-shaft deposits have accumulated on the riverbank. The deposition has evolved into permanent substrate layers over time. The aquatic habitats in the shallow/marginal regions of the stream, where the current is stagnant, form the living areas of

all living creatures, aquatic insects, their adults, eggs, and larvae that feed the benthic macrofauna in the aquatic ecosystem, and therefore fish. For these reasons, stream modifications (such as retaining walls, road construction, and bridge) should be made within the framework of a certain plan.

During the interviews with some amateur fishermen from the local people who are interested in trout fishing in the area, It has been declared that approximately 25-30 fish are still caught in a day with fishing line, especially in the upper parts where the stream bed is less physically affected (in the foothills of the Kaçkar Mountains, in the small lakes and streams in the plateau and hamlet regions). However, it has been stated that the trout populations in the creek have decreased gradually in the 40 years since the 1980s. Undoubtedly, in addition to over and illegal fishing without control, the existence of an ineffective management strategy for amateur fisheries in our country is also an important factor. For example, when the irregular catch statistics in Lake Çıldır were examined, it was determined that the trout with carnivorous characteristics in the lake was at a fishable level at the beginning of the 1990s, but then it became almost extinct. Uncontrolled fishing is carried out by fishermen who are commercially caught lake trout that enter the lake in certain periods for feeding while spawning migration is carried out to some important streams that discharge into the lake in the spring and summer periods. Today, it is mentioned that about 8-10 lake trout are caught by each fisherman in the nets along with other target species during the fishing season in Lake Çıldır (Zengin, 2019). The development of the fish populations in the lake in favor of herbivorous and omnivorous species instead of carnivores has led to a decrease in the population in the following years due to reasons such as feeding and habitat sharing (Zengin et al., 2013). In the 2000s, the introduction of an exotic and invasive species of crucian carp (*Carassius gibelio*) and crayfish (*Astacus leptodactylus*); started to put potential pressure on the native fish fauna in the lake in response to the collapse of wild stocks in the lake since the early 1990s (Zengin, 2019). In addition, as a result of the increase in the operating capacity of the Arpaçay Hydroelectric Power Plant fed from Lake Çıldır, the coastal/shallow, reed, and sensitive areas that constitute the breeding and feeding areas of the local carp have been adversely affected.

Conclusion

It has been determined that the habitats in all three localities where the research was conducted have been under great pressure, especially since the end of the 21th century, due to infrastructure works, urbanization, and illegal fishing. It has been determined that in the downstream regions of the studied rivers, the trout populations have completely disappeared with habitat loss, the local trout populations that spread in the limited habitats in the middle and upper basins are quite worn out, and the number of individuals who have reached breeding age has decreased significantly.

If serious measures are not taken at the national level for aquatic ecosystems in the ongoing process, habitat loss and wild trout populations will be at greater risk. They will gradually enter the process of extinction as they cannot maintain their basic vital activities such as breeding, migration, and feeding. One of the most important threats in this process is the existence of hydroelectric power plants planned for energy purposes on rivers.

Like the trout populations distributed in other geographical areas of Türkiye, the trout population living in the Northeast Anatolian geography has decreased gradually as the environmental conditions deteriorate and the increasing illegal fishing cannot be prevented. In general, the stocks of the trout populations in our country's waters have come to the limit of extinction for many years, especially due to the deterioration of their habitats and overfishing with the market economy implemented since the beginning of the 1980s. Today, due to the significant population decline, it has no commercial importance. Despite the collapse of their stocks and their protection in fisheries management, we have left behind the first half of the 21st century, and there have not been enough promising developments in this regard yet.

Wild trout habitats (river, lake, and marine habitats) are exposed to three major pressures today. These are mainly (1) Domestic, agricultural, and industrial wastes, (2) Changes/modifications in riverbeds, and (3) Hydroelectric Power Plant activities. All factors cause habitat loss and insufficient water flow. As a result of insufficient compensation, water, invertebrate, and vertebrate fauna are adversely affected, as well as habitat loss in the stream bed.

In this study, the relations of natural trout populations in North-eastern Anatolian rivers with the environment are evaluated; These stocks, which remained relatively stable until the 1980s, deteriorated gradually, especially from the beginning of the 20th century. Today's trout populations consist of younger and smaller individuals. Small age classes characterize the age distribution of individuals in natural breeding and feeding areas. This situation shows that the populations have decreased significantly in the last 20-30 years as a result of the disruption of the habitats in the natural trout habitats and uncontrolled fishing. The long-term viability of Northeast Anatolian wild trout river populations will require protecting and developing forested coastal areas, diverse, complex, and interconnected aquatic habitats, and maintenance and im-

provement of suitable water temperatures, natural water quality, and natural hydrology. With the impact of environmental regulatory procedures, policies, and practices of the Ministry of Agriculture and Forestry, the highest responsible and administrative unit in this regard, can positively affect the protection and development of critical habitat requirements of this important ecological fishery resource. In addition, training, social outreach, and technical assistance should be provided to private landowners and people in local settlements.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required.

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AQUATIC RESEARCH

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First record of the benthopelagic fish John dory *Zeus faber* (Linnaeus, 1758) in the Black Sea coasts of Türkiye

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ABSTRACT

In this study, a male specimen of John dory (*Zeus faber*) with 31.4 cm in total length and 365.43 g in body weight was caught with a trammel net at a depth of 15 m from Fener Island in the province of Fatsa (Ordu, Black Sea). This record is the first verified report suggesting that John dory expanded its distribution in the Mediterranean towards the Geographical Sub-Area 29 (Black Sea).

Keywords: Zeidae, Geographic expansion, New record, Ichthyofauna, Black Sea, Türkiye

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Introduction

The Zeidae family (Rafinesque, 1815) is an important ecological component of global marine and brackish waters in muddy substrates. Zeidae is widely distributed near or directly over the sea floor but occasionally in midwater. The family comprises large, showy, deep-bodied marine fishes occurring in benthopelagic waters of oceans, including a limited number of species and assembling low biomass (Grande et al., 2018). The family includes just six species in two genera (Nelson, 1994), and only two are found in the Mediterranean (Froese & Pauly, 2022).

The John dory, *Zeus faber* (Linnaeus, 1758), is widely distributed Atlantic, Indian, and Pacific Oceans and along the entire West African coast (Wheeler, 1969; Dunn, 2001; Vrgoč et al., 2006; Choi et al., 2011; Iwamoto, 2015). This species spreads to a depth of 400 m but commonly occurs between 25-160 m (Iwamoto, 2015). The John dory has a laterally compressed body, but it is distinguishable from the other species by a large round black spot on its body sides. The body of the John dory is covered with such small rudimentary scales that it appears naked (Quéro, 1986). This species' maximum size and age are 90 cm (Iwamoto, 2015) and 18 years (İşmen et al., 2013), respectively. It is usually caught by bottom trawl net, long line, or trammel net close to the bottom in the fisheries areas (Jardas, 1996) but is mainly considered a valuable by-catch (Dunn, 2001; İşmen et al., 2013; Kim et al., 2020). This species is used in commercial fisheries for human consumption, fish meal, and oil (Iwamoto, 2015).

There are various studies on the distribution of John dory in Turkish territorial waters on the shores of the Mediterranean (Başusta & Erdem, 2000; Çiçek et al., 2006; Başusta & Başusta, 2021), Aegean Sea (Torcu & Aka, 2000; Akyol, 2001; İşmen et al., 2013; Bilge et al., 2014) and Marmara Sea (Keskin & Eryılmaz, 2010; Karadurmuş, 2022). The occurrence of John dory on the Turkish coasts of the Black Sea is reported in various marine fish checklists (Bilecenoğlu et al., 2002; Keskin, 2010; Bilecenoğlu et al., 2014) referring to Ninni (1923). In the last case, there is no evidence of its existence on the Turkish coasts of the Black Sea, and local fishermen have verified the first existence of this species. In this paper, we report the first documented record of *Z. faber* on the Turkish coasts of the Black Sea, providing detailed capture and biological data and discussing its mechanisms of introduction.

Material and Methods

A male specimen of *Z. faber* was sampled on January 27, 2023, in the Fatsa Gulf (41°03'42.5" N − 37°31'00.3" E) (Figure 1) located in General Fisheries Commission for the Mediterranean (GFCM) Geographical Sub-Area 29 (the Black Sea). The sampling location was 520 m away from Fener Island in the central province of Fatsa, Ordu. The specimen was captured at a depth of 15 m during an experimental biodiversity survey using an artisanal trammel net with 80 mm inner and 280 mm outer panel mesh size. During sampling, water salinity and surface sea temperature were measured at 17.9 ‰ and 11.2 °C, respectively. The specimen was initially identified using the taxonomic keys of Fischer et al. (1987), and its scientific name was checked from FishBase (Froese & Pauly, 2022). Total length (TL) was measured using an ichthyometer with 0.1 cm precision, while body weight (W) was weighed using a scale with 0.01 g precision. Sex distinction was made according to the shape and color of gonads (Gunderson, 1993).

Results and Discussion

John dory is distributed in the Indian Ocean, the eastern Atlantic from Norway to South Africa, the Mediterranean Sea, the South and East China Seas, and the western Pacific in southern Japan, New Zealand, Australia, and Korea (Wheeler, 1969; Jardas, 1996; Yoneda et al., 2002; Choi et al., 2011). Several studies refer to its wide-range occurrence in the Adriatic Sea (Vrgoč et al., 2006), the entire West African coast (Yoneda et al., 2002), and off Mauritania (Iwamoto, 2015). This paper reports the first documented record of *Z. faber* on the Turkish coast of the Black Sea. The current record essentially expands the species' distribution area and discusses its potential for spread. In recent years, the Black Sea's biological, chemical, and physical properties have been changing through the impact of global climate change and the Red Sea and the Suez Canal. This situation defines the "Mediterraneanization" of the Black Sea (Oğuz & Öztürk, 2011). It is estimated that this change will contribute to the inclusion of John dory in the Black Sea ecosystem and support its subsequent spread.

The TL and W of the specimen were measured as 31.4 cm and 365.43 g, respectively (Figure 2). The sex was identified as male based on the macroscopic observation of the gonads – flat, white-cream in color, and soft-textured. John dory reaches maturity at 4-5 years of age (Jardas, 1996; İşmen et al., 2013) and between 26 cm and 37 cm in length (Dorel, 1986; Dunn, 2001; Vrgoč et al., 2006). The existence of a mature individual of this size seems promising for the potential continuity of stocks in the Black Sea. Maravelias et al. (2007) reported the bottom sea temperature, water depth, and latitude as the species' spatial aggregation determinants in all

seasons. John dory lives up to a depth of 400 m, although abundance is most significant in shallow (<80 m) and warmer waters (>16.5°C) characterized by weak hydrographic activity (Maravelias et al., 2007). It prefers warm waters for spawning (Akyol, 2001). The Turkish coast of the Black Sea, which represents the main shelter area for many species with coastal dunes, wetlands, reefs, and islands, is host to diverse and rich habitats (Öztürk et al., 2013). With an annual average surface water temperature of 16.3°C (quoted from the Turkish State Meteorological Service) and nutrient abundance (Öztürk et al., 2013; Zengin, 2019), the Black Sea can provide suitable conditions for the life of the species. The presence of hydrogen sulfide at a depth of more than 150 m in the Black Sea is considered a limiting factor for the distribution of this species (Algan et al., 2002).

The specimen was dissected according to Hyslop (1980) to analyze the stomach contents of the fish. The sample's stomach was fully empty, so we cannot infer its feeding behavior at the sampling site. John dory is an opportunistic piscivorous predator that takes advantage of abundant and diverse prey items, from cephalopods to Pisces (Choi et al., 2011; An et al., 2012; İşmen et al., 2013; Kim et al., 2020). John dory is capable of stalking enough to consume selected prey fish in a group or school (Ressell, 1983). Prey items cover the entire water column, and their diet varies by region, habitat, and size (Ressell, 1983; Stergiou & Fourtouni, 1991; Kim et al., 2020). The Black Sea is very rich in Pisces, such as whiting, sardines, horse mackerel, anchovy, picarel, bogue, and gobies (Aydın & Karadurmuş, 2012; Gücü et al., 2017; Karadurmuş et al., 2021a), which are the main prey of John dory. (Silva, 1999; Kim et al., 2020). The variety and abundance of preys in the Black Sea can provide a vital feeding opportunity for the species. The presence of crustaceans, cephalopods, and anthozoa species that the species prefers will also support the species' feeding. Its main predators are sharks (Mendonça, 2009) and large bony fish (Morte et al., 1997). Cartilaginous fish, sturgeon, and some bony fish found in the Black Sea can be considered possible predators of John dory.

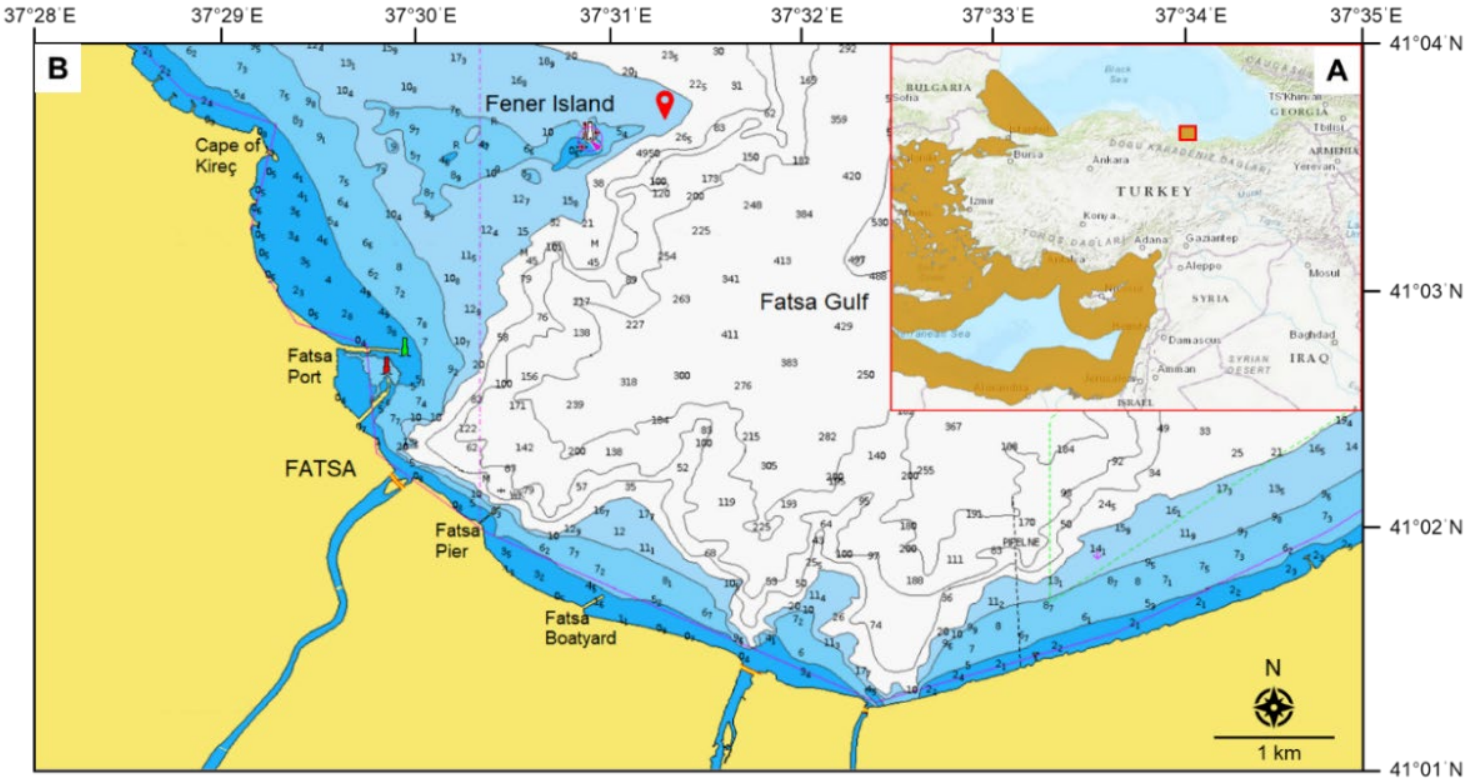

Figure 1. Study map. The extant residence of *Zeus faber,* compiled by IUCN (continuous orange areas in part A), represents the sampling region (the orange area in the red frame in part A), geographic location of the sampling point (red mark in part B)

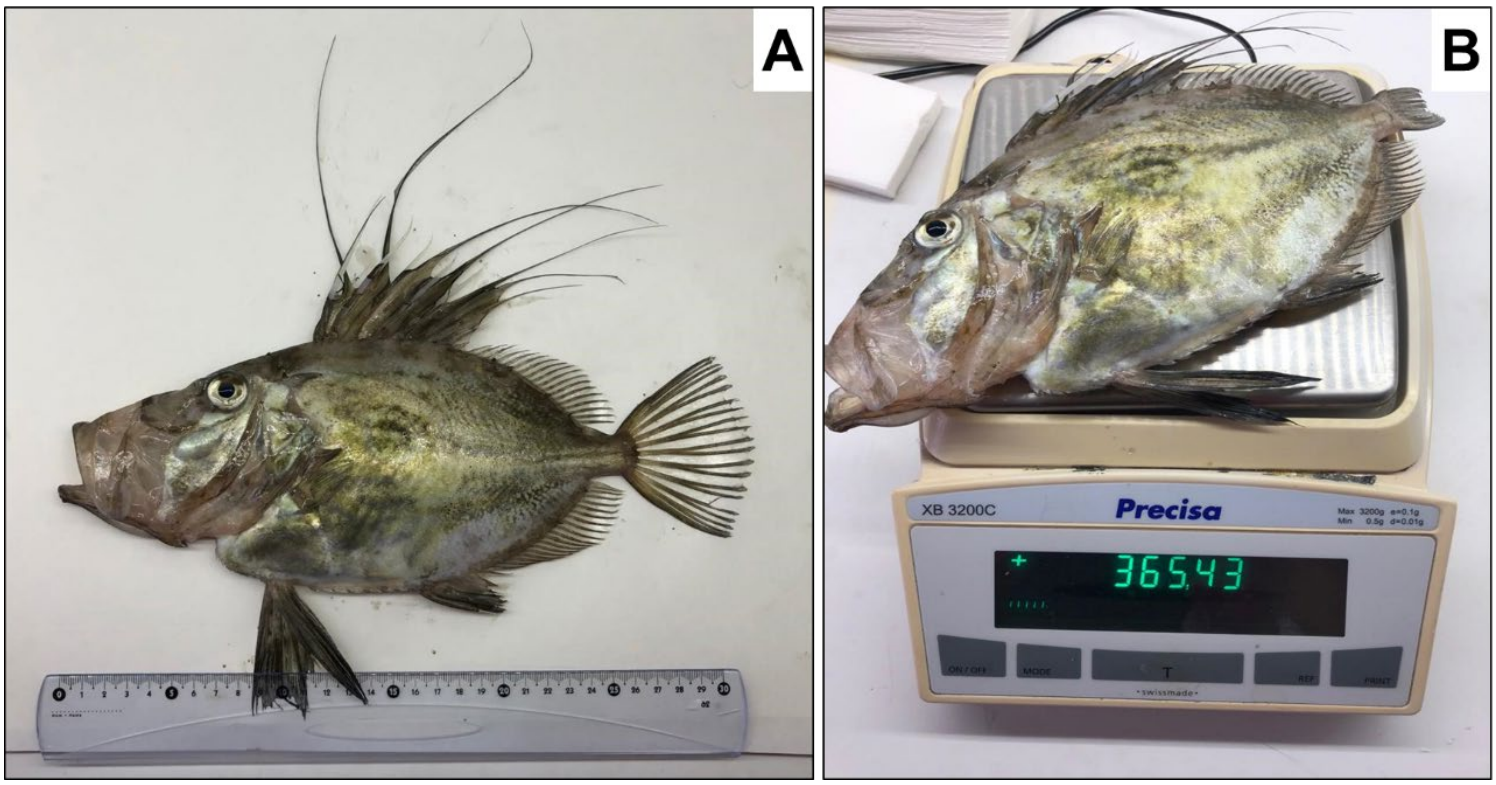

Figure 2. *Zeus faber*, 31.4 cm in total length (A) and 365.43 in body weight (B), was sampled from Fatsa Gulf on January 27, 2023.

John dory is vulnerable to bottom trawling and longline fishing (Fischer et al., 1987; Jardas, 1996). The species is under pressure due to overfishing and inefficient fishing regulations in the Sea of Marmara. For this reason, Bilecenoğlu et al. (2016) have evaluated John dory in the list of bony fish species that need immediate conservation action in the Sea of Marmara. It is also caught by-catch in commercial shrimp fishing with beam trawl (Karadurmuş, 2022). Researchers (Jukic-Peladic et al., 2001; Vrgoč et al., 2006) point to decreased biomass indices in the Mediterranean and the Adriatic Sea due to overfishing. This species is classified as the "Least Concern" for the Mediterranean in the red list of endangered species reported by the International Union for Conservation of Nature (Iwamoto, 2015). There are no species-specific conservation actions in place for John dory; however, the range for this species coincides with several marine protected areas (Iwamoto, 2015).

Conclusion

In recent years, fish species that settled in the Black Sea ecosystem with the effect of global climate change are in constant change (van der Voo, 1990; Aydın & Sözer, 2016; Aydın & Bodur, 2018; Zengin, 2019; Aydın, 2020; Karadurmuş et al., 2021b). John dory can increase the biomass level on the Turkish coast of the Black Sea and become a sustainable fishery resource within a productive habitat in the long term. In this context, we advise local fishermen to be sensitive to the conservation of John dory individuals.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required.

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- Assess the effects of their journal policies on author and reviewer behavior and revise policies, as required, to encourage responsible behavior and discourage misconduct
- Ensure that any press releases issued by the journal reflect the message of the reported article and put it into context

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Contribution to Editorial Decisions: Peer review assists the editor in making editorial decisions and, through editorial communications with the author, may also assist the author in improving the paper.

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Confidentiality: Any manuscripts received for review must be treated as confidential documents. They must not be shown to or discussed with others except as authorized by the editor.

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2. Drafting the work or revising it critically for important intellectual content; AND
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Full Name(s) and Surname (s) of author(s)

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Authors complete correspondence Address (es) of affiliations and e-mail (s)

Abstract

Keywords (indexing terms), usually 3-6 items

Introduction

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Material and Methods

Results and Discussion

Conclusion

Compliance with Ethical Standards

- **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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- **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", "Conclusion", "Compliance with Ethical Standard**" and **"References" sections.**

Statistical analysis to support conclusions is usually necessary. International statistical reporting standards must conduct statistical analyses. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section, and the statistical software used during the process must be specified.

Units should be prepared by the International System of Units (SI).

Review Articles: Reviews prepared by authors with 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. The journal may even invite these authors. Reviews should describe, discuss, and evaluate the current knowledge level of a research topic 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 subheadings in between those sections.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking features of a previously published article. Articles on subjects within the journal's scope 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", "Conclusion", "Compliance with Ethical Standard"** and **"References"** 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

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Tables

Tables should be included in the main document and 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 in the tables should be defined below them 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 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 support the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted through the submission system in main document WORD files (in JPEG or PNG format). 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 primary 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 numbered consecutively in the order they are referred to within it.

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

References

Reference System is APA 6th Edition (with minor changes)

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 publication date 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.

....(Bhujel, 2014).

....(Mol & Erkan, 2009).

....(Alofa et al., 2023).

....(Mol & Erkan, 2009; Bhujel, 2014; Alofa et al., 2023).

Citations for a Reference Section:

An article

Alofa, C.S., Olodo, I.Y., Chabi Kpéra Orou Nari, M., Abou, Y. (2023). Effects of the fresh and dried housefly (*Musca domestica*) larvae in the diets of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758): growth, feed utilization efficiency, body composition, and biological indices. *Aquatic Research*, 6(1), 1-10. https://doi.org/10.3153/AR23001 **(if DOI number has)**

A book in print

Bhujel, R.C. (2014). A manual for tilapia business. CABI Nosworthy Way Wallingford Oxfordshire OX10 8DE UK, 199 p. ISBN 978-1-78064-136-2. https://doi.org/10.1079/9781780641362.0000 **(if DOI number has)**

A book chapter

Craddock, N. (1997). Practical management in the food industry A case study. In Food Allergy Issues for the Food Industry; Lessof, M., Ed.; Leatherhead Food RA: Leatherhead, U.K., pp 25-38. ISBN: 4546465465

A webpages

CDC (2020). Rift Valley Fever | CDC. https://www.cdc.gov/vhf/rvf/index.html (accessed 20.08.2020).

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 canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

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