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To achieve open access to scholarly journal literature, we recommend two complementary strategies.

I. Self-Archiving: First, scholars need the tools and assistance to deposit their refereed journal articles in open electronic archives, a practice commonly called, self-archiving. When these archives conform to standards created by the Open Archives Initiative, then search engines and other tools can treat the separate archives as one. Users then need not know which archives exist or where they are located in order to find and make use of their contents.

II. Open-access Journals: Second, scholars need the means to launch a new generation of journals committed to open access, and to help existing journals that elect to make the transition to open access. Because journal articles should be disseminated as widely as possible, these new journals will no longer invoke copyright to restrict access to and use of the material they publish. Instead, they will use copyright and other tools to ensure permanent open access to all the articles they publish. Because price is a barrier to access, these new journals will not charge subscription or access fees, and will turn to other methods for covering their expenses. There are many alternative sources of funds for this purpose, including the foundations and governments that fund research, the universities and laboratories that employ researchers, endowments set up by discipline or institution, friends of the cause of open access, profits from the sale of add-ons to the basic texts, funds freed up by the demise or cancellation of journals charging traditional subscription or access fees, or even contributions from the researchers themselves. There is no need to favor one of these solutions over the others for all disciplines or nations, and no need to stop looking for other, creative alternatives.

Open access to peer-reviewed journal literature is the goal. Self-archiving (I.) and a new generation of open-access journals (II.) are the ways to attain this goal. They are not only direct and effective means to this end, they are within the reach of scholars themselves, immediately, and need not wait on changes brought about by markets or legislation. While we endorse the two strategies just outlined, we also encourage experimentation with further ways to make the transition from the present methods of dissemination to open access. Flexibility, experimentation, and adaptation to local circumstances are the best ways to assure that progress in diverse settings will be rapid, secure, and long-lived.

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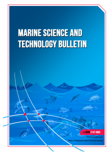

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RESEARCH ARTICLE

A techno-environmental and energy efficiency investigation of marine dual-fuel engines

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ABSTRACT

The ship-based greenhouse gas emissions along with the volumetric growth in maritime transportation have increased significantly over the years. International Maritime Organization (IMO) has tightened the emission limits by putting new regulations into effect to overcome the environmental impacts and therefore, the maritime industry has focused on energy-efficient ship design and operation, recently. Regarding the latest developments, dual-fuel engines operated with different fuels have been installed and new technological developments in emission control have been implemented onboard ships. In this context, the selection of engine systems where there are many options available has been a substantial problem in the design process of a ship, recently. The latest marine engines are capable of operating with various types of fuels at different emission control modes, therefore, energy efficiency and emission performance of the prime movers should be analyzed in detail. In this study, VLSFO, methanol, LPG, LNG and MDO-fueled engines with the same power output are investigated and the NO_X reduction device integrated engines' technical specifications are compared. Then, the selected dual-fuel engines are thermodynamically analyzed and the environmental impacts are evaluated under different engine loads, Tier II, Tier III modes and ambient conditions. Moreover, EEDI calculations are conducted under the case study of powering a medium-range tanker and engine options are evaluated in terms of energy efficiency. Finally, a sensitivity analysis of engine performance is carried and the results are validated. According to the results, the energy efficiency of the ship can be increased by up to 20% by selecting the LNG-fueled engine as the prime mover while it requires more space and equipment compared to other engines.

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Introduction

Ship design is a multidisciplinary and complicated field consisting of iterant analysis and optimization steps. A typical ship design project includes concept, preliminary, contract and detail design phases where hull form design, arrangements, maneuverability, stability, strength, resistance and power characteristics are determined (Evans, 1959; Turan & Akman, 2021). The design optimization in the process is substantial to improve energy efficiency and decrease a ship's fuel consumption and emissions. It was reported that maritime-based greenhouse gas (GHG) emissions in 2018 had a 2.89% share in global GHG along with sulphur oxides and nitrogen oxides from ships accounting for 13% and 15% of total NO_X and SO_X emissions, respectively (Han et al., 2019; IMO, 2020). International Maritime Organization (IMO) put regulations into force as Ship Energy Efficiency Management Plan (SEEMP) and Energy Efficiency Design Index (EEDI) along with the emission limits defined in MARPOL Annex VI (IMO, 2013). Currently, the Tier III NO_X emission limit per cycle is 3.4 g/kWh for the slow-speed engines (<130 rpm) which reduces the NO_X emissions approximately by 76% compared to the Tier II limit (IMO, 2013). Moreover, the sulphur rate in marine fuel oil is 0.1% in terms of mass inside the Emission Control Areas (ECAs) and 0.5% outside the ECAs determined in Reg. 13 of MARPOL Annex VI (IMO, 2019). Besides, IMO has enlarged the scope of MARPOL and enacted Energy Efficiency Existing Ship Index (EEXI), Carbon Intensity Indicator rating (CII) and enhanced Ship Energy Efficiency Management Plan regulations which have been implemented as of 1 January 2023 (IMO, 2021). In addition, to determine maritime-based fuel consumption and emission; Monitoring, Reporting and Verification (MRV) in European Union (EU) and IMO Data Collection System (DCS) have been implemented since 2017 and 2018, respectively (DNV, 2022). The current regulations aim to decrease the ship-based GHG by 40% as of 2030 and by 50% as of 2050 baselining the 2008 level of GHG (IMO, 2020). Therefore, various measures have been taken in the design and operation to increase the energy efficiency of ships and comply with the regulations. The hull and bow form, the aft body with propeller and rudder optimizations, weight reduction, low resistance hull coating, and highly efficient machinery system selection are the options in the design stage of a ship (JASNAOE, 1980; MAN, 2016a; Vidović et al., 2023). On the operation side, speed reduction, ballast and trim optimization, route optimization, on-time cleaning and maintenance, just-in-time operations and cold ironing are conducted for ships in service (JASNAOE, 1980; Johnson & Styhre, 2015; Köseoğlu et al., 2021; Vidović et al., 2023). Furthermore, there are novel systems available to increase energy efficiency and emission performance. Waste heat recovery (Akman & Ergin, 2019; Konur et al., 2022), carbon capture and storage (Güler & Ergin, 2021), air lubrication (Vidović et al., 2023), rotor sails (Wärtsilä, 2022) and performance monitoring systems (Wang et al., 2018) have been used onboard ships.

Main engines, auxiliary engines and boilers are the major fuel consumers and also the power suppliers onboard ships. According to the latest GHG report (IMO, 2020), the world fleet's main engines of containers, bulk carriers and oil tankers consume much more fuel than the engines of other types of ships. Moreover, HFO or LSFO fuel oil was reported to be the major fuel type followed by MDO and LNG in the commercial fleet (IMO, 2020). Fuel type, on the other hand, identifies the ship's energy efficiency and operational performance levels based on different chemical and combustion characteristics. Carbon fuels; HFO, LFO, LSFO, MDO, MGO, LNG, LPG, methanol (MeOH) and ethanol have been used in prime mover power generation and zero carbon fuels; ammonia and hydrogen are shown as future marine fuels (Bureau Veritas, 2022). Stiff limitations on sulphur and NO_X emissions directed shipowners and engine manufacturers to the fuel-switching option that can be conducted on dual-fuel engines. Liquid or gas fuels can be burned with pilot fuels in these engines and according to the fuel type Tier II or Tier III NO_X limitations can be complied with or without before and after treatment systems.

Regarding the importance of the topic, many studies have been conducted on the thermal, emission and economic performances of marine fuels. Spoof-Tuomi & Niemi (2020) environmentally and economically analyzed LNG, LGB (liquified biogas) and MDO-fueled Ro-Pax ferry. They found that the total operation cost of the ferry fueled with LNG is about 41% and 64% less compared to that fueled with MDO and LBG, respectively. Perčić et al. (2021) investigated the life cycle and costs of various marine fuels including diesel, LNG, methanol, hydrogen, ammonia and electricity for inland vessels. According to their assessments, electric-powered propulsion is the most cost-effective solution for inland passenger vessels while diesel is the most economical option for the selected dredger. Law et al. (2021) compared HFO, natural gas, solar and biomass-based marine fuels in terms of lifecycle energy and cost. They stated that methanol from biomass is favourable considering cost, energy and technology readiness level while hydrogen and ammonia are referred to as the worst

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among all fuels in terms of energy and cost. Liu et al. (2022) investigated the thermal performance of diesel, biodiesel, hydrogen, methane, methanol and ammonia fuels in a zero-dimensional model of the Millet-Sabathe cycle. They stated that the marine diesel engine's brake thermal efficiency can be enhanced by up to 53.09%. Feng et al. (2022) investigated the future SCR systems of marine engines for marine applications. They suggested that the decomposition of the catalyst with SO_2 and H_2O is challenging for the future SCR system of marine engines. Feng et al. (2022) environmentally analyzed the impacts of alternative fuels for ships and they stated that LNG is a feasible fuel for reducing SO_X and PM along with the NO_X by lean combustion. Napolitano et al. (2022) experimentally studied the SCR technology for ships and they pointed out that NO_X reduction efficiency can be increased by increasing the dosing ratio of additives. Law et al. (2022) investigated the various fuels for maritime by considering the ship type, cargo and voyage. According to their results, biodiesel has the best environmental score among HFO, ammonia, bio-methanol, LNG, hydrogen and electricity. Lu et al. (2022) conducted an optimisation study for two-stroke marine engines integrated with exhaust gas recirculation (EGR). According to their results, the proposed engine model complies with the Tier II limitations and by adjusting the EGR rate 22% and %36 Tier III limits can be fulfilled. Huang et al. (2022) performed a life cycle assessment of GHG emissions caused by fuels and a case study for a very large crude carrier. They pointed out that solar-driven methanol production from hydrogen can cause almost zero carbon and engines using pilot fuel cannot achieve zero carbon emissions. Livaniou et al. (2022) compared the emissions of LNG and MDO fuels burned in different types of ships based on the real data obtained from different databases. According to their results, using LNG reduces CO_2 and NO_X emissions about by 20.7% and 83.6% compared to MDO, respectively. Elkafas et al. (2022) analyzed the LNG, diesel and methanol fuels in terms of environmental, technical and economic perspectives. They pointed out that LNG is more environmentally friendly while methanol is a more economical fuel compared to diesel. Zou & Yang (2023) conducted life-cycle assessments of methanol, LNG, hydrogen and ammonia fuels for various-sized ships by considering shipowners and the public. They stated that HFO with scrubbers is the most economical short-term option for container ships while methanol is the most favourable solution when the social costs of the pollutants are objective.

The internal combustion engines as main prime movers directly affect the thermo-environmental and economic performance of the ship which is aimed to be operated efficiently as pointed out. Moreover, there are many options for alternative fuels complying with the regulations with emission control technologies integrated into marine engines, therefore, suitable engine selection has been a substantial question in the design stage of a ship, recently. Hence, parallel to the IMO regulations and emission targets, detailed analyses are needed on engine systems in terms of thermodynamic, technological and environmental performances. Regarding the latest studies and developments, this study reveals and compares the energy efficiency performance, technological properties of emission control systems and technical specifications of VLSFO, MeOH, LPG, LNG and MDO-fueled engines with the same power output. The selected dual-fuel engines are thermodynamically analyzed and the environmental impacts are evaluated under various engine loads, Tier II and Tier III emission control modes. After obtaining the techno-environmental data, a case study is conducted for a medium-range tanker's EEDI calculations. In addition, a sensitivity analysis is performed to point out the effect of ambient conditions on engines' performances. Apart from the literature where economic and environmental analysis of alternative fuels have been commonly studied, this study focuses on the marine dual-fuel engines operated with alternative fuels and aims to contribute literature with the technical assessments and operational condition-based thermo-environmental results that can be used for the decision-making in the powering and engine selection stages of a merchant ship design.

The workflow of the study is summarized as follows: Analyses start with the methodology to explain the mathematical model and used tools on evaluating the thermo-environmental performances of dual-fuel engines and the energy efficiency of the tanker. The next section details and compares specifications with the technological properties of emission control devices integrated into the selected engines. Then the obtained results related to thermo-environmental and EEDI are evaluated. After evaluations, sensitivity and validation studies are performed and finally, the concluding remarks are presented.

Material and Methods

Methodology

The techno-environmental investigation and evaluation consist of six steps; the determination of the engine group based on the medium-range tankers' particulars, data collection of the determined dual-fuel engines using CEAS, analysing of the

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collected data in terms of emission control technology integrated into the dual-fuel engines, thermal and environmental impact analyses of the engines under various engine loads and Tier II – Tier III emission control modes, a case study on engine selection for a medium-range tanker to reveal the hull to wake emissions and preliminary EEDI calculations for the tanker and finally, a sensitivity and validation study. The summary of the analysing steps is shown in Figure 1.

The data collection step is based on medium-range tankers (45000 – 55000 DWT) built after 2013 and the main particulars include length, breadth, draft, design speed, cargo capacity and power generation systems. The equations regarding tankers' particulars are obtained from previous publications (Akman & Ergin, 2019) and the latest technical reports (MAN, 2021, 2022a; MEPC.308(73), 2018) regarding the propulsion of tankers as shown in Table 1. The obtained regression-based equations show that if the main dimensions and capacity are known, engine main and auxiliary engines' power requirements can be estimated. Using a typical length of around 180 m and 49990 DWT cargo capacity engine power requirements are determined for the tanker as shown in Table 1. It is worthy of note that there may be deviations in the values of about 5-10% compared to those of the actual ship. The tanker's propulsion system consists of a single main engine with a fixed-pitch propeller, and two service generators without a shaft generator. It is assumed that there is no energy efficiency-increasing device onboard the ship.

The estimated power of the main engine is used for the selection of available dual-fuel engines. The Computerized Engine Application System (CEAS) (MAN, 2023) and technical guides (MAN, 2022b) are used for obtaining the parametric and technical data of the engines.

The CEAS database consists of two-stroke engines ranging from 35 cm to 95 cm bore with 5 to 12 cylinders and 2475 kW to 82440 kW brake power capacity. The primary fuel type, engine power capacity, number of cylinders, bore and stroke, ambient conditions including scavenge air and cooling water (coolant) temperatures and NO_X reduction technology can be changed in the CEAS. The ambient conditions are ISO, tropic and specified cold, where the air temperature ranges from 10°C – 45°C and the cooling water temperature ranges from 10°C – 36°C. Then the obtained data is used in the determination of the sensitivity of the engines.

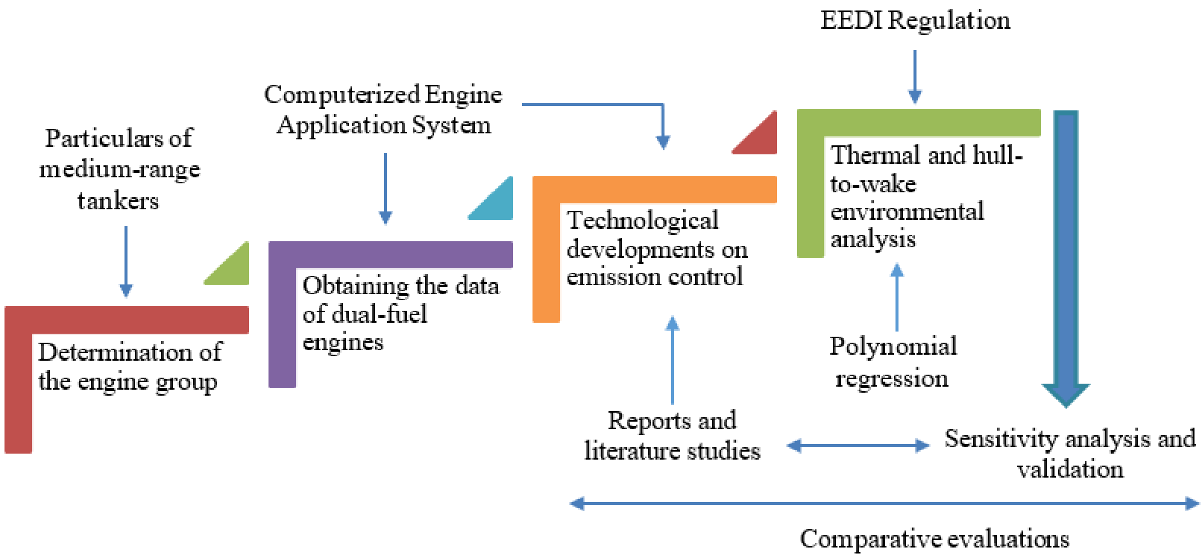

Figure 1. Techno-environmental analysis steps and tools used in the analyses

Table 1. Main particulars of the medium-range tanker (Akman & Ergin, 2019; MAN, 2021, 2022a; MEPC.308(73), 2018)

Properties	Value	Unit	Regression-Based Estimations
Length between perpendicular (L_{PP})	183	m	$L_{PP} = F_{\text{des}} \cdot DWT_{\text{scant}}/(B \cdot T_{scant})$
Moulded breadth (B)	32.2	m	$B = F_{\text{des}} \cdot DWT_{\text{scant}}/(L_{PP} \cdot T_{scant})$
Scantling draught (T_{scant})	13.0	m	$T_{scant} = F_{\text{des}} \cdot DWT_{\text{scant}}/(L_{PP} \cdot B)$
Design speed (V)	14.5	knot	14.5
Capacity (DWT)	49990	ton	$DWT = L_{PP} \cdot B \cdot T_{scant}/F_{\text{des}}$
M/E power (full load)	8600	kW	$0.0652 \cdot DWT + 5960.2$
A/E power (full load)	840 (x2)	kW	$0.05 \cdot MCR_{ME}$

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The polynomial regression is applied to the parametric data and thermodynamic performances with environmental impacts of the engines are calculated using the equations as follows:

The brake thermal efficiency of dual-fuel engines can be calculated by,

$$\eta_{th}{}^{i,j} = \frac{P_b}{Q_{LCV}{}^{i,j} \cdot \dot{m}_{fuel}{}^{i,j}} \quad (1)$$

where P_b is the main engine's (ME) brake power. The superscripts i and j indicate the engine load and fuel type specific to the engine, respectively. The parameter $\dot{m}_{fuel}$ shows the fuel consumption in terms of kg/s. The low calorific values (LCV) of VLSFO, MeOH, LPG, LNG and MDO are 41700 kJ/kg, 19900 kJ/kg, 46000 kJ/kg, 48000 kJ/kg and 42700 kJ/kg, respectively (EPA, 2014; MEPC.308(73), 2018). The CO_2 emitted by the dual-fuel engines, $R^{i,j}$ in t/h can be estimated by

$$R^{i,j} = \frac{SFC^{i,j} \cdot P_b}{10^6} \cdot C_{F_j} \quad (2)$$

where SFC is specific fuel consumption and $C_{F,j}$ is the factor used to convert of fuel consumption to CO_2. The C_F of VLSFO, MeOH, LPG, LNG and MDO are 3.15, 1.375, 3.015, 2.75 and 3.206, respectively (EPA, 2014; MEPC.308(73), 2018).

$$EEDI_k = \frac{P_{ME} \cdot C_{F_{ME,j}} \cdot SFC_{ME,j} + P_{ME} \cdot C_{F_{ME,j}} \cdot SPOC_{ME,j} + P_{AE} \cdot C_{F_{AE}} \cdot SFC_{AE}}{f_i \cdot f_c \cdot DWT \cdot f_w \cdot V_{ref}} \quad (3)$$

where SFC of main and auxiliary engines is taken at 75% MCR. The specific pilot oil consumption ($SPOC$) is taken at the same engine load of 75% MCR and MDO is used as pilot oil for gas fueled engines. The auxiliary engines are assumed to be fueled with MDO and SFC_{AE} is taken as 215 g/kWh (MEPC.308(73), 2018). The f_i, f_c and f_w are the capacity factor, cubic capacity correction factor and factor for speed reduction at sea, respectively. The factors are taken as 1 and the average reference speed is 14 knots (MEPC.308(73), 2018). The VLSFO, MeOH, LPG and LNG fuels are assumed to be the primary fuels for the ship for each EEDI calculation.

Dual-Fuel Engines and Emission Control Technologies

Analyzed main power generation systems are capable of burning liquid and gas fuels, low-speed and two-stroke dual-fuel engines generating 8.6 MW brake power (P_b) at full load as can be seen in Figure 2. The engines have a single turbocharger (T/C), 5 cylinders, 500 m bore and 2500 mm stroke, classified as ultra-long stroke. The engines are also electronically controlled and named "G-type" which have a low bore-to-stroke ratio (0.2) and lower rpm requiring a large diameter of the propeller which decreases fuel consumption by up to 7% (MAN, 2016b). The engines' dimensions are the same and length *x* width *x* height are about 5.8 m x 3.6 m x 9.8 m, respectively. The dry mass of the gas-fueled engines is 215 tons and it is 211 tons for the VLSFO-fueled engine. The mass of the NO_X reduction device varies; high-pressure SCR and EGR systems weigh 4 tons and 12 tons, respectively. The total mass of oil and water in the engines is 1.6 tons.

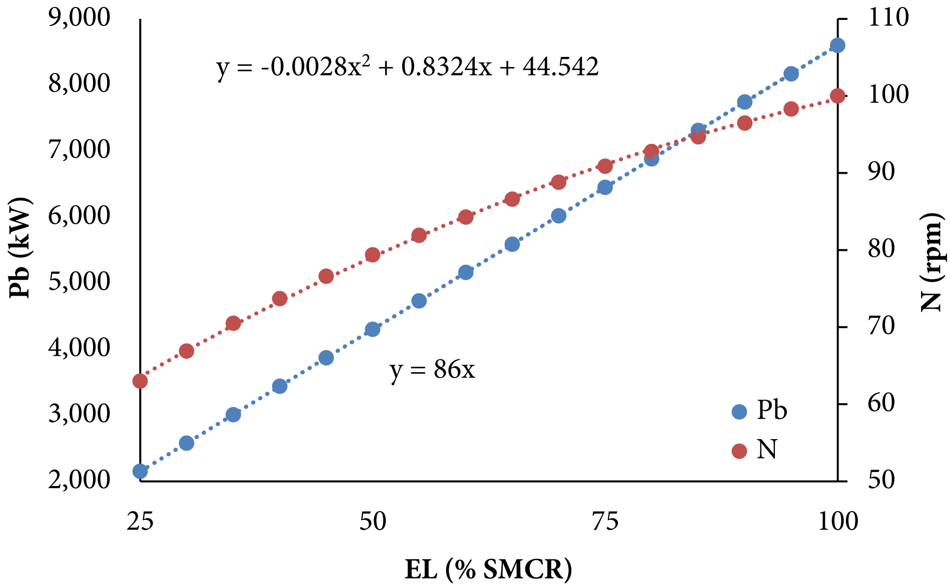

Figure 2. Brake power output change of the engines concerning RPM and engine load (EL)

Table 2. The properties of the engines' fuel systems

Engine Model	Main Fuel	Pilot Fuel	Fuel System Design Pressure (bar)	Fuel Supply Pressure (bar)	Sealing oil consumption (l/24h)	Tier III Technology
5G50ME-C9.6	VLSFO	-	6	4	-	SCR
5G50ME-C9.6-LGIM	MeOH	MDO	16	13	24	EGR
5G50ME-C9.6-LGIP	LPG	MDO	65	53	24	SCR
5G50ME-C9.6-GI	LNG	MDO	350	300	6	SCR

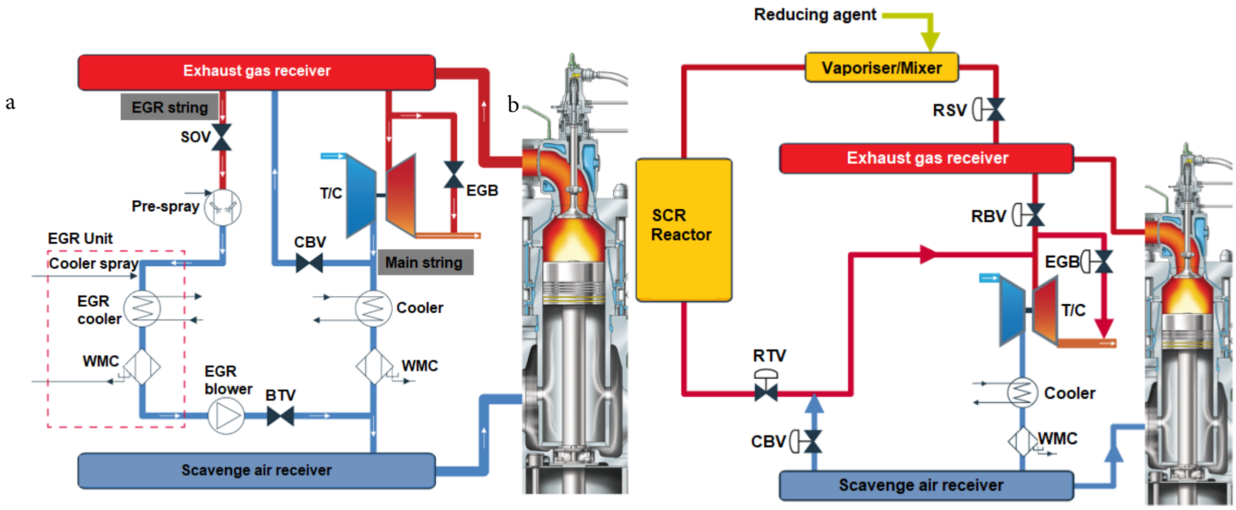

Figure 3. The diagrams of the EGR (a) and high-pressure SCR (b) systems (MAN, 2022a)

The fuel system properties of the engines are shown in Table 2. According to tabulated data, engines are operated under main and pilot fuel which prevents knocking and misfiring during the combustion of MeOH, LNG or LPG (Woodyard, 2004). On the other hand, the design and supply pressures of fuel systems are remarkably different. LNG-fueled engines require more complex and costly infrastructure and it is reported that LNG engines occupy about 3-4 times while methanol-fueled engines occupy about 2 times as much space as marine gas oil-fueled engines (Harris et al., 2022b). According to the obtained data, LNG, LPG and MeOH engines' fuel tanks are 1.7, 1.3 and 2.4 times higher relative to marine gas oil fuel tank size (MAN, 2014). Moreover, it is stated that the new-building cost of a ship increases about by 22% and 10% when LNG and MeOH-fueled engines are used as prime movers (Harris et al., 2022a). However, the LNG-fueled engine's sealing oil consumption which prevents the gas oil to penetrate the hydraulic oil of the valves is quite less compared to that of other engines.

The gas and methanol-fueled engines meet the Tier II NO_X limits and Tier III NO_X limitations are fulfilled by EGR or SCR systems (MAN, 2022b). The schematic diagrams of EGR and SCR systems are shown in Figure 1 (MAN, 2022a). The exhaust gas bypass (EGB) valve in EGR and the reactor bypass valve (RBV) in the SCR units control the switching of Tier II or Tier III modes. The NO_X reduction units are integrated into the exhaust gas line and operated when NO_X control is needed. It is reported that EGR and SCR systems can decrease NO_X emissions by 80% to 90% (MAN, 2016c). On the other hand, compared to EGR the capital cost of SCR is less for engines under 15 MW; however, the operating cost of SCR is higher than the EGR system (MAN, 2015).

Part of the exhaust gas in the EGR system of the MeOH-fueled engine is cleaned, cooled and mixed with the scavenge air to decrease the O_2 content by using CO_2 before the combustion. The heat capacity of the scavenge air is increased to reduce the combustion peak temperature which is the main factor in the formation of NO_X (MAN, 2022a). In Tier II mode, the EGR valve and blower throttle valve (BTV) are closed and the recirculation starts with the opening of these valves in Tier III mode. After pre-spraying, the EGR blower forces the flow to enter the scavenge air cooler and the water mist catcher (WMC) which avoids reaching the liquid water into the cylinders.

The SCR system consists of an SCR reactor, vaporiser/mixer and dosing units. As can be seen in Figure 1 (b), the SCR unit is deactivated by the reactor throttle valve (RTV) and reactor

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sealing valve (RSV). When the RBV is opened the exhaust gas is forwarded to T/C. The SCR unit of the selected engines is working on the high-pressure which means that the process is maintained with exhaust gas before T/C. When the exhaust gas is bypassed to the SCR line, the reducing medium is injected into the vaporiser/mixer by the dosing system and the medium is vaporised and mixed with the exhaust gas. Then the mixture is sent to the reactor for reducing NO_X. The SCR system is operated at high exhaust temperatures; therefore, the temperature of exhaust gas should be kept 50-175°C (MAN, 2022a) higher compared to that on the low-pressure side. Moreover, during the engine is in Tier III mode the SCR system is adjusted to operate above the sulphuric acid condensation temperature limit of 200°C (MAN, 2022a). An automatic heating system is positioned on the SCR system to keep the temperature above the specified limit. In addition, the soot-blowing system is operated against the clogging of the reactor by soot particles.

Results and Discussion

The results and discussions are presented in subsections; thermal and environmental performance evaluations and, sensitivity analysis with the validation of the obtained results. The load and emission control mode-depended performance parameters including fuel consumption, brake thermal efficiency, CO_2 emission and EEDI are evaluated and discussed. Then, the incremental effects of ambient conditions and engine load on performance parameters are analyzed. Finally, the results are validated using the literature studies.

Thermal Performance

The dual-fuel engines with the same power output and dimensions have remarkable differences in terms of fuel consumption. Figure 4 shows the specific fuel (a) and pilot oil (b) consumption of the engines under various engine loads at Tier II mode. The point data is also given in polynomial functions where engine load is variable. According to the plots, methanol-fueled engines consume approximately 50% more fuel compared to other engines. The main difference is based on the specific energy content (kJ/kg) and density (kJ/m³) of methanol which is about 53% and 58% less compared to MDO (MAN, 2014). The fuel consumption of LNG-fueled engines is less in comparison with that of the other engines based on the same reasons that the energy content of LNG is about 12% higher than MDO. Besides, the fuel consumptions at the light and heavy engine loads are higher compared to the normal continuous rating of 85% MCR for each engine. On the other hand, the MDO as pilot oil consumption decreases by the engine load. The methanol-fueled engine's SPOC is about 60% and 220% higher than that of LPG and LNG-powered engines, respectively. On the other hand, the fuel consumption increases at Tier III mode approximately by 0.73%, 0.72%, 0.76% and 3.58% for LNG, VLSFO, LPG and MeOH-fueled engines, respectively. In addition, the fuel consumption at light loads rises from 1% to %5 based on the fuel type. The MeOH-fueled engine has the highest fuel consumption at Tier III mode based on the EGR activation. The reason for the fuel increase is related to the combustion characteristics at Tier III mode where the combustion temperature is higher and firing pressure is lower compared to those at Tier II mode (ABS, 2020).

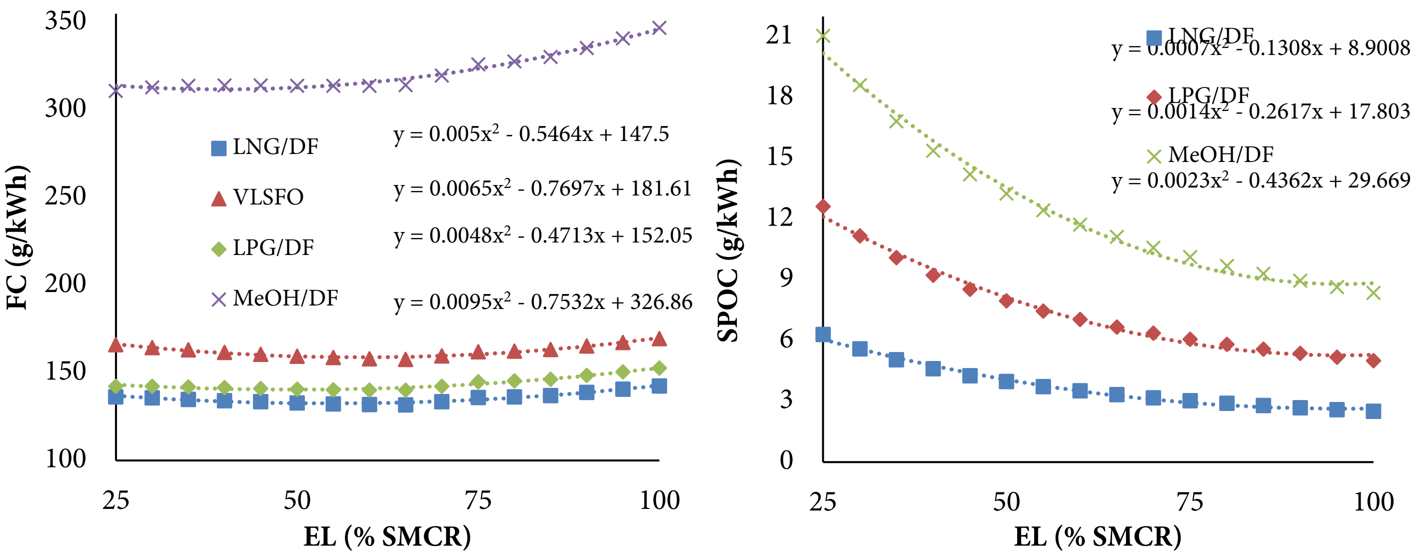

Figure 4. The change of specific fuel (a) and specific pilot oil consumptions (b)

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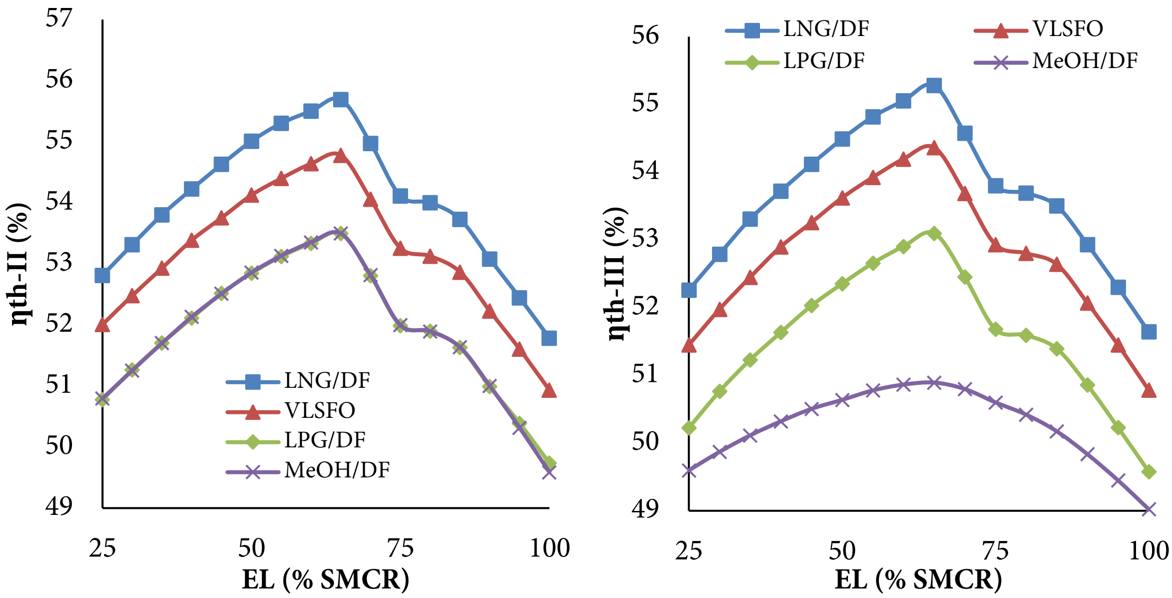

Figure 5. The change of thermal efficiencies under Tier II (a) and Tier III (b) modes

The thermal efficiency of the engines under different engine loads and Tier II (a) and Tier III (b) modes are indicated in Figure 5. According to the ISO ambient conditions, LNG-fueled engines are more efficient in terms of thermal performance. In Tier II mode, the calculated maximum thermal efficiencies are 55.7%, 54.8%, 53.4% and 53.5% for LNG, VLSFO, LPG and MeOH-powered engines, respectively. Switching the engine from Tier II to Tier III mode, the thermal efficiency of the engines decreases by 0.5% to 4.9%. NO_X reduction devices are operated in Tier III mode which requires more fuel for producing the same power output as Tier II mode. Furthermore, according to the plots, the thermal efficiency of the engines is remarkably low at the light and heavy loads; therefore, the dual-fuel engines should be operated at medium loads (between 60% - 70% MCR) for energy efficiency. The reason behind this phenomenon is related to the combustion characteristics depending on the load. The analyzed engines are turbocharged and at light loads scavenge air pressure drops which cause low combustion efficiency and increase fuel consumption and carbonization (Garcia et al., 2014). Moreover, the formation of carbon deposits due to the lack of sealing can increase and the steam production capacity from economizer can decrease at light loads for two-stroke engines (Dere et al., 2022). Fuel consumption also substantially increases at higher loads based on more power demand.

On the other hand, the brake thermal efficiency plots show that approximately 50% of fuel energy is lost by the cooling and exhaust gas of the engines. Such two-stroke dual-fuel engines have jacket cooling, scavenge air cooling and lubrication oil cooling loads which correspond to about 20% - 25% of the total fuel energy and the exhaust gas has a share of about 25% in total (Akman & Ergin, 2021, 2022; Singh & Pedersen, 2016). Moreover, switching the engine mode from Tier II to Tier III increases the cooling load and exhaust gas heat potential. Therefore, the remaining part of the heat energy after the combustion process can be harvested for increasing the energy efficiency of power generation systems onboard ships.

Environmental Performance and EEDI

The estimated CO_2 emissions released by the engines at ISO ambient conditions are shown in Figure 6. Raising the engine load substantially increases fuel consumption and CO_2 emissions. According to the results, VLSFO-fueled engine emits more CO_2 which is calculated as 3.82 ton/h CO_2 at 85% MCR and Tier II mode, and at the same operating conditions LNG, LPG and MeOH-fueled engines emit 2.82 ton/h, 3.35 ton/h and 3.53 ton/h, respectively. The CO_2 emissions increase in Tier III mode that VLSFO, LNG, LPG and MeOH engines at 85% MCR release 3.84 ton/h, 2.83 ton/h, 3.37 ton/h and 3.64 ton/h, respectively. Activation of SCR or EGR at the Tier III mode significantly lowers the NOx emissions which comply with the regulations where Tier III NOx is at least 76% less than that at Tier II. However, the Tier III mode has higher fuel consumption resulting in more CO_2. Therefore, load optimization is significant in both NO_X and CO_2 reduction. As discussed in the previous sections, medium loads ranging from 50% to 75% MCR seem more efficient in CO_2 and NO_X reduction.

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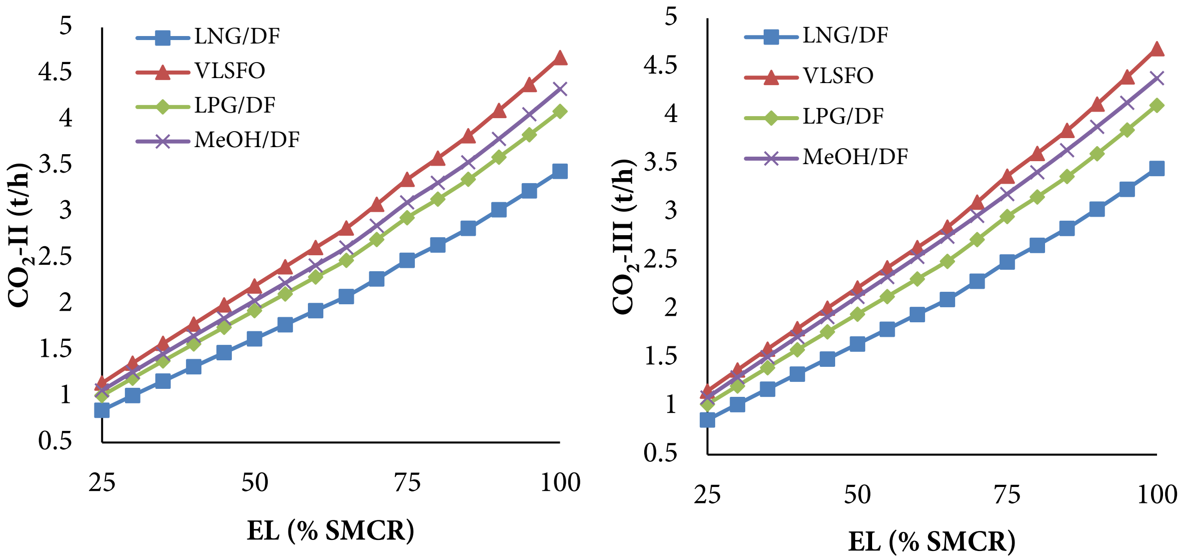

Figure 6. The estimated hull-to-wake CO_2 emissions under Tier II (a) and Tier III (b) modes

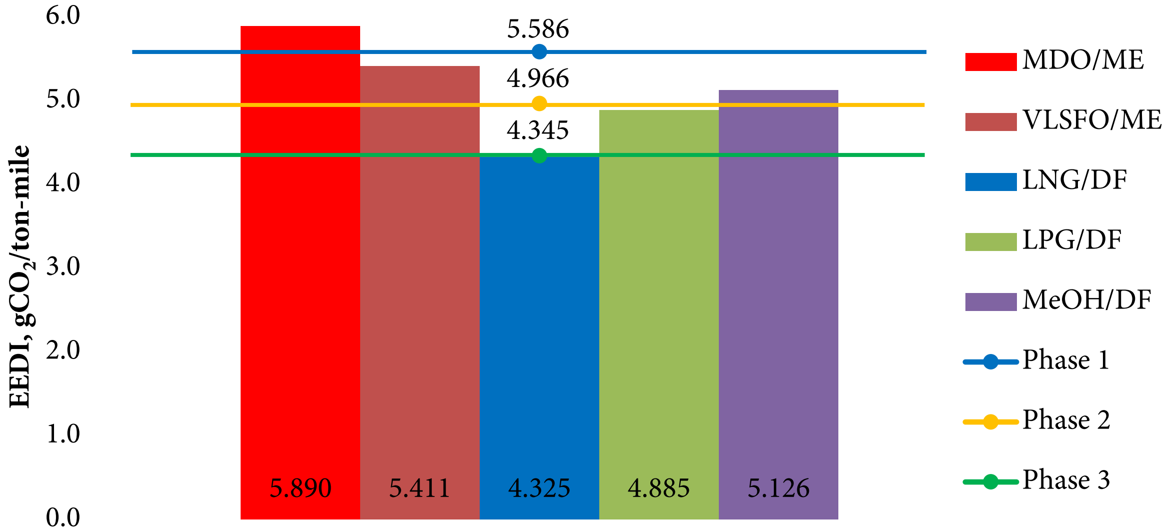

Figure 7. The estimated EEDI values of tanker installed with different main engines

The Energy Efficiency Design Index for ships is defined as the mass of CO_2 emission per unit of transport work and depends on the fuel consumption of main and auxiliary engines with boilers. Main engines are the major fuel consumers; therefore, the primary fuel has a significant role in EEDI. During calculations, only main and auxiliary engines are considered and the reference engine load is taken as 75% MCR. Figure 7 shows the reference and attained EEDI of a medium-range tanker installed with different dual-fuel engines. The reference EEDI value varies regarding ship type and for a tanker, it is 6.206 g-CO_2/ton-mile (IMO, 2012). According to the regulation (IMO, 2012), EEDI reduction factors by baselining reference EEDI are given as 10%, 20%, and 30% for Phase 1, Phase 2 and Phase 3, respectively.

According to the preliminary calculations, the ship with an MDO-fueled engine complies with none of the EEDI phases. Even though the ship with VLSFO and LPG-fueled engines complies with sulphur regulations, the attained EEDI of this engine-integrated ship is above Phase 3. LNG and LPG-fueled engines comply with the current regulation and methanol-fueled engines can be an option considering the optimized design. However, it should be noted that methane slip is a problem in LNG-fueled engines which increases the ship-based GHG emissions and for the analyzed engine slip is about 0.2 g/kWh (MAN, 2021a). Besides, as discussed in the previous section, medium loads are energy efficient both in Tier II and Tier III modes and enable to obtain of low EEDI. Moreover, the thermal performances show that the majority of the fuel energy is lost by exhaust gas, engine scavenge air, jacket water and lubrication oil cooling; therefore, integrating the energy efficiency-increasing devices such as waste heat recovery systems onboard ships can reduce the attained EEDI.

Sensitivity Analysis and Validation

The analyzed two-stroke dual-fuel engines show different performances at various ambient and loading conditions. Table 3 shows the fuel consumption of the engines at specified cold, ISO and tropical air and coolant temperatures at Tier II mode. The ambient conditions are defined in CEAS (MAN, 2023) and fuel consumptions are tabulated at varying engine loads. The obtained data show that fuel consumption depends on not only the engine load but also the intake air and freshwater temperatures. Hence, for the evaluation of the engines' performance sensitivity, loads and temperatures are increased by one unit. According to the results, increment of ambient temperatures by 1°C, the fuel consumption increases approximately by 0.05%. Mean deviations are calculated by baselining ISO condition at 75% MCR and results show that the MeOH-fueled engine has higher tolerance on ambient conditions compared to other engines as can be seen in Table 3. On the other hand, when the engine load is increased by 1% MCR, fuel consumptions increase about by 1.5%. The light loads (<50% MCR) have higher tolerances rising to 3.5% as stated in the engine manufacturer's performance reports (MAN, 2023).

Table 3. Engines' fuel consumption at various ambient and loading conditions

Ambient Conditions	**Engine Load (%)**	**Fuel Consumption by Engine Type (g/kWh-Tier II)**			
		VLSFO-DF	**MeOH-DF**	**LPG-DF**	**LNG-DF**
Specified	25	162.1	311.0	134.5	136.4
Air temperature: 10°C	50	155.5	313.5	135.6	132.8
Coolant temperature: 10°C	75	157.4	324.5	140.4	135.2
	100	164.5	344.5	148.6	141.7
ISO	25	164.1	315.3	136.4	138.1
Air temperature: 25°C	50	157.4	317.7	137.4	134.4
Coolant temperature: 25°C	75	159.4	328.7	142.2	136.9
	100	166.4	348.9	150.5	143.5
Tropic	25	165.8	319.1	138.0	139.7
Air temperature: 45°C	50	159.1	321.4	139.0	135.9
Coolant temperature: 36°C	75	161.0	332.4	143.8	138.4
	100	168.3	352.8	152.1	145.0
Mean deviation by baselining ISO Condition (%)	75	1.129	1.202	1.195	1.169

Validation of the results is conducted by comparing the available literature studies where the same calculation methodology is used. Grljušic et al. (2015) calculated the efficiencies of a ship power plant including a marine two-stroke diesel engine with 18660 kW and integrated waste heat recovery system under different engine loads. The calculated brake thermal efficiencies at 50%, 75%, 85% and 100% MCR are about 51.3%, 51.4%, 51% and 49.7% in the reference study. Compared to the results obtained in this study where the thermal efficiency of VLSFO fueled engine is shown in Figure 5 (a), there is a maximum 5% deviation between the results. The difference can be related to using of different ambient conditions and engine models during calculations.

Conclusion

Main engines available for a medium-range tanker and capable of burning VLSFO, methanol, LPG, LNG and MDO are investigated to compare the technical and emission-control-based technological features of the engines. The dual-fuel engines are thermodynamically analyzed and the CO_2-based environmental impacts are presented under different engine operating conditions. Then, EEDI calculations are conducted for a medium-range tanker using the analyzed dual-fuel engines. The following conclusions are drawn that can be used in the decision-making processes of a ship design where there are research gaps in suitable engine selection based on techno-environmental and energy efficiency assessments:

- The engines have the same size and brake power output but burn different types of fuels which chemical and physical properties are different. LNG is stored and supplied at about 5- and 20-times higher pressures compared to LPG and MeOH, therefore LNG fueled engines with tanks and equipment occupy about 50% more volume onboard compared to MeOH-fueled engines.
- The available NO_X reduction systems for the analyzed engines are EGR or SCR for complying with the Tier III limits. The systems are operated as before or after treatment emission control. The system selection depends on the engine size and cost but the EGR system seems feasible for engines with high power output (>15MW).
- The main and pilot fuel consumption of LNG-fueled engines in terms of ton/h is approximately 16.6%, 8.6% and 140% less compared to that of VLSFO, LPG and MeOH-fueled engines. Therefore, LNG-powered engines have higher brake thermal efficiency under various engine operating conditions.
- Regarding the carbon content and fuel consumption, the VLSFO-powered engine has the highest hull-to-wake CO_2 emissions while the LNG-powered one has the least at the same engine operating conditions.
- EEDI calculations show that LNG-powered engines seem more favourable compared to other engines. The attained EEDI can fulfil the Phase 3 level when LNG is used as the primary fuel.
- Sensitivity analysis indicates that engine load is the dominant parameter of the engine performance compared to intake air and cooling water temperatures. Medium loads are feasible in terms of efficient operation.
- Detailed well-to-wake assessments can vary the optimal fuel order; however, LNG and methanol-powered dual-fuel engines seem the midterm option for IMO GHG targets.
- The brake thermal efficiency of the engines at different loads and Tier modes shows that there is a significant amount of waste heat to recover. In future studies, analysis can be expanded by integrating the waste heat recovery devices into the engines to increase the thermal efficiency of the power generation systems and EEDI performance of the ships powered with alternative fuels.
- In future studies regarding the selection of optimal marine engines operated with alternative fuels, a comprehensive well-to-wake thermo-economic analysis can be conducted. During analysis, ship types which have different operational profiles can be included and evaluated in terms of economic sustainability.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

All data generated or analyzed during this study are included in this published article.

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Nomenclature

Abbreviations

A/E	Auxiliary engine
CEAS	Computerized Engine Application System
CO_2	Carbon dioxide
DF	Dual-fuel
DWT	Deadweight tonnage
EEDI	Energy Efficiency Design Index
EGR	Exhaust gas recirculation
EL	Engine load
F	Factor
FC	Fuel consumption
GHG	Greenhouse gas
IMO	International Maritime Organization
LCV	Lower calorific value
LNG	Liquified Natural Gas
LPG	Liquified Petroleum Gas
MEPC	Marine Environment Protection Committee
MCR	Maximum Continuous Rating
MDO	Marine Diesel Oil
M/E	Main engine
MeOH	Methanol
NO_X	Nitrogen oxide
PGS	Power generation system
SCR	Selective catalytic reduction
SPOC	Specific pilot oil consumption
SFC	Specific fuel consumption
T/C	Turbocharger
VLSFO	Very low sulphur fuel oil

Symbols

C	Coefficient
f	Factor (for EEDI)
η	Efficiency
$\dot{m}$	Mass flow rate (kg/s)
N	Speed (rpm)
$\dot{Q}$	Heat flow (kW)
P	Power (kW)
R	Emission (ton/h)

Subscripts

b	Break
des	Design
pgs	Power generation system
ref	Reference
scant	Scantling
th	Thermal

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RESEARCH ARTICLE

Site analysis of maritime transportation infrastructures by using the Coastal Vulnerability Index approach: The case of Bodrum Peninsula

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ABSTRACT

In this research, site locations of marina-type maritime transportation infrastructure (MTI) in the Bodrum Peninsula were analyzed using the coastal vulnerability index (CVI) approach. For the calculation of CVI, six parameters; coastal slope, relief, relative sea level change, shoreline erosion/accretion, mean tide range, and mean wave height were used in accordance with the method. After the data collected from different data sources were transferred into a geo-database, basic geographical information systems analyses were applied (reclass, buffer, subset, slope, overlay, classify, count, map algebra, etc.). CVI results have been presented as maps and tabular values using a scale of 1 (Very Low) to 5 (Very High). Thus, the vulnerability level of the MTI site locations was determined. According to the determined results, it was founded that, Ortakent and Turgutreis were Very High (red-5); Yalıkavak, Milta and Kale MTIs are High (orange-4); Cruise Port, Gumbet and Bitez were found to have Moderate (yellow-3) CVI values. In this research, the CVI approach was applied by evaluating the physical site location characteristics of marina-type MTI, for the first time applied in the Bodrum Peninsula, where there is a high density of marina. For adaptation strategies for existing MTIs, more investigations should be realized from the functional, economic, social, and ecosystem points of view. From the managerial point of view, it can be said that small marinas or municipal berthing facilities with a state ownership model are advised to work together with other marinas in the region if they exist. The CVI-methodology should also utilize for the site selection of any type of maritime transportation infrastructure.

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Introduction

Maritime transportation infrastructures (MTIs) are among the most important physical infrastructures built to meet the expected usage demands from coastal areas. In general, an MTI differs according to its usage purpose, ownership model, type of service it provides, type of vessel, and cargo it serves (DLH, 2010a, 2010b). The MTIs constitute coastal infrastructures for transport, maritime tourism, and other purposes such as fishing and military. The MTIs including commercial cargo ports (container ports, general cargo ports, chemical terminals, etc.), passenger ports, and special purpose ports (such as fishing) are defined as "seaports" which are logistic and industrial transportation nodes (Notteboom et al., 2022). Depending on their functions, the number and density of MTIs in a region and the site location characteristics in the coastal area may differ from each other. For example, although the number of seaports is less than the number of marina-type MTIs in a region, the area surrounding a seaport and its hinterland needed for trade operations requires more space than a marina-type MTI.

In the field of vulnerability assessment, climate change risks, planning, and policies for adaptation, studies have been conducted for seaports, especially for major commercial ports (Becker et al., 2010, 2012, 2016; Messner et al., 2013; IPCC, 2014; Monioudi et al., 2018; Christodoulou et al., 2019; Hanson & Nicholls, 2020; Sweeney & Becker, 2020; UN, 2020; Izaguirre et al., 2021) because of a strategic role in the global trade system. However, marina-type MITs including modern marinas, yacht ports, municipal berthing facilities are often overlooked despite their high economic value (Lazarus & Ziros, 2021). However, there is a crucial gap in the literature in the field of coastal vulnerability analysis of marina-type MTIs which host recreational and maritime activities as well as valuable physical assets (Lazarus & Ziros, 2021).

In course of time, the physical characteristics of the selected site for an MTI can also create challenges or convenience for its existence and operational activities. The coastal zone of Bangladesh is a good example of this. Because, according to Minar et al. (2013), Bangladesh has an extremely flat, low-lying coastal type, and it has been determined that in the event of a one-meter SLR, about 18% of the country's total land area and all MTIs could be inundated. MTI sites can range in size from a small dock with a few ten boats or small crafts to a very large port of over hundreds of yachts or ships. The major physical site characteristics include coastal landforms, land use, bathymetry, tidal range, wave, relief, slope, climate, availability of land transportation, etc. (DLH, 2010b; Golestani & Amiri, 2021). Both seaports and marina-type MTIs are built in coastal zones due to their functions. Coastal areas are considered highly vulnerable hot points by climate change worldwide (IPCC, 2019; Fox-Kemper et al., 2021). It is commonly accepted that the most important observed and expected physical impacts of climate change on the coastal areas are SLR; the permanent inundation of low-lying urban areas and physical infrastructures; augmented flooding because of extreme weather conditions such as storm surges; saltwater intrusion to coastal groundwater; high erosion on coastlines, beaches, cliffs, and wetlands (Fox-Kemper et al., 2021).

Many populated cities, residential areas, and transportation infrastructure in the world are located on low-lying coastal plains, and these coastal areas are among the regions that can be directly affected in the case of SLR because of climate change. Therefore, knowing the coastal areas' vulnerability because of SLR is of great importance in terms of taking necessary precautions and developing policies to deal with risks. Gornitz (1991) and Gornitz et al. (1994) developed the coastal vulnerability index (CVI) approach as a "site" analysis in order to determine vulnerability in the case of SLR. This method has been used by many researchers to evaluate the vulnerability of coastal areas in the world. According to Koroglu et al. (2019) and Cogswell et al. (2018), approximately 30% of the analyses about coastal vulnerability utilized the CVI method. Different tools such as geographical information systems, computer models, mapping tools and index-based approach as CVI have been used at different spatial and temporal scales to analyze vulnerability in coastal areas (Ramieri et al., 2011) Generally, the CVI-based approach has been developed for fast assessment and visualization in different areas and spatial scales based on available datasets to quantify and analyze the level of vulnerability of coastal areas to the effects of SLR.

In this research, site locations of marina-type MTI in the Bodrum Peninsula were analyzed using the CVI approach. The aim of the research is to determine the vulnerability level of the site locations with existing MTIs according to the CVI. Thus, the analysis was carried out according to the internationally accepted CVI method to determine the vulnerability level of the sites where marina-type MTIs are located, which is seen as a deficiency in the literature. For the calculation of CVI, six parameters were used in accordance with the method. These parameters are; coastal slope, relief, relative sea level change, shoreline erosion and accretion, mean tide range, and mean wave height. After the data collected from different data sources were transferred into a geo-database, basic GIS analyses were applied (reclass, buffer, subset, slope, overlay, classify, count,

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map algebra, etc.). CVI results have been presented as maps and tabular values using a scale of 1 (Very Low) to 5 (Very High). Thus, the vulnerability level of the MTI site locations was determined. According to the determined results, MTI locations at each CVI level were analyzed, evaluated, and recommendations were developed.

Material and Methods

Research Area

Bodrum Peninsula was chosen as the research area (Figure 1). It is located in the South Aegean region of Turkey and is a world-famous tourism area. Bodrum has a very rich marina-type MTIs including modern marinas, yacht ports and berthing facilities with small and large mooring capacities for various size of boats and a cruise port. Apart from these, there are quite small and local piers of various sizes. In addition, there are shipyards in Icmeler and Yalıkavak that specialized in manufacturing, maintaining and repairing touristic boats and yachts. While the total resident population of Bodrum district in 2022 is 187.284, during the summer seasons, especially during the peak season for tourism, the total population can sometimes exceed 1.500.000 (TUIK, 2022).

In order to conduct site location analyses based on the CVI method, a peninsular coastal area rather than a mainland coastal area was considered for the first time, where there is a high density of marina-type MTIs. So, the Bodrum Peninsula can be seen as a pilot study in the application of the CVI approach for the field of MTIs. The marina-type MTIs included in the site analyses are Bodrum Cruise Port, Bodrum (Kale) Yacht Port, Milta Marina, Gumbet Yatch Port, Bitez Yatch Port, Ortakent Marina, Turgutreis Marina, and Yalıkvak Marina, which are located throughout the Peninsula (Figure 1).

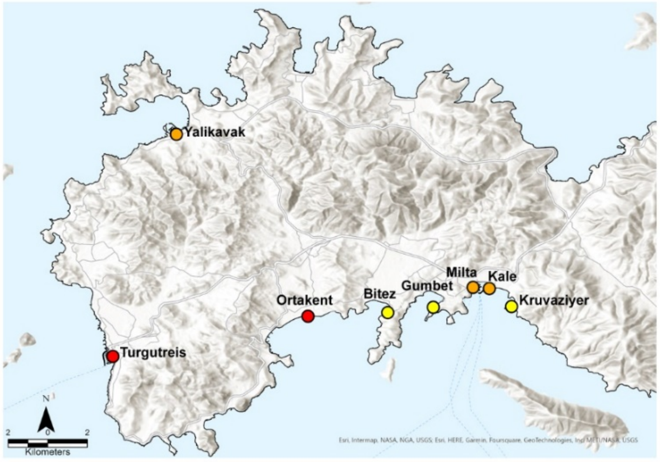

Figure 1. Research area. Bodrum Peninsula

Data and Methods

In this research, the variables, data types, and data sources used to determine the CVI and evaluate marina-type MTIs sites are shown in Table 1.

Space Shuttle Radar Topography (SRTM) data set (SRTM, 2018) (1 arc-second, 30 meters) was used to determine the coastal relief and coastal slope variables (USGS, 2020).

In order to determine the shoreline erosion/accretion variable, the 8th band images of the Landsat 8 satellite on two different dates (16.09.2013 and 27.10.2022) were used with a resolution of 15 meters.

The Mean Tide Range variable was determined, according to Turkish National Sea Level Monitoring System (TUDES, 2022) observations, and near-time literature such as Sayre et al. (2018, 2021), and Ecological Coastal Units (ECU) (2022).

Table 1. CVI variables and data sources

Variables (a_n)	**Data Source**
Coastal slope	Processing from Shuttle Radar Topography Mission (SRTM) dataset
Relief	From SRTM (NASA JPL, 2013; SRTM, 2018)
Relative sea level change	3.3±1.1 mm/yr from Kuleli (2010), Caldwell et al. (2015), Zlotnicki et al. (2019), CMS (2021), TUDES (2022), SLE (2022)
Shoreline erosion / accretion	-1 - +1 m/yr from Landsat 8 images from USGS (2022)
Mean tide range	0.2-0.3 m from TUDES (2022), Sayre et al. (2018, 2021), ECU (2022)
Mean wave height	1.1-2.0 m from Özhan & Abdalla (2002), Sayre et al. (2018, 2021), CMS (2022), ECU (2022)

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According to Özhan & Abdalla (2002), the mean wave height variable was retrieved from three samples point which is very close to the research area. The geographical coordinates (Lat-Long) of these points are; 27.10 E-37.25 N, and 27.40 E-37.25 N, and 27.40 E-37.00 N. Also, cross check was performed using data of 1km global coastline segments and segment midpoints database (ECU, 2022). The workflow diagram of the methodology is shown in Figure 2.

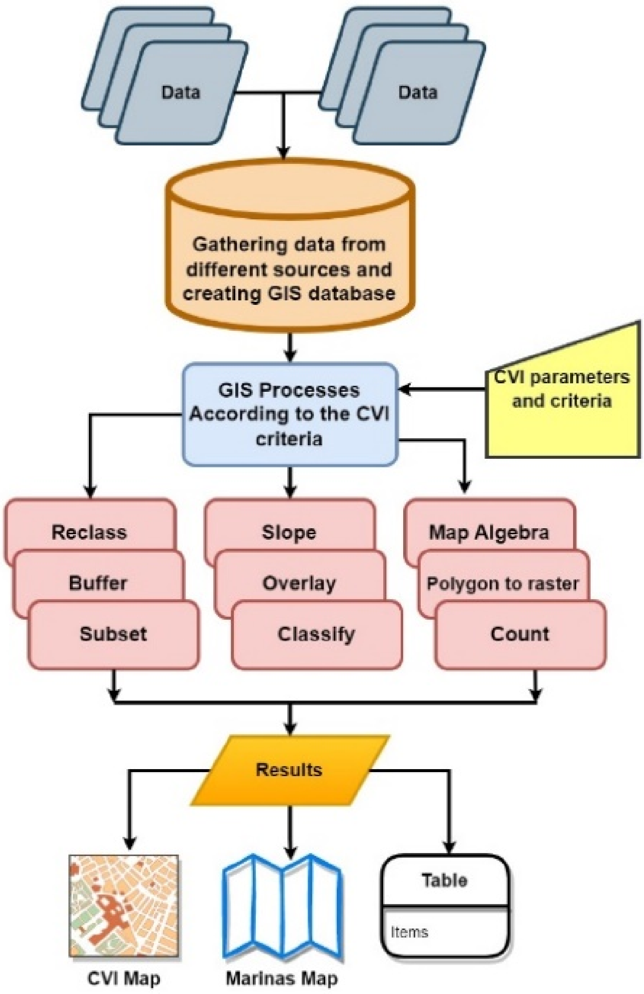

Figure 2. Workflow diagram of the methodology

As a first step, a geo-database was created in which all the data obtained from different sources were gathered together in order to make the data suitable for analysis. A GIS software was used for geo-database creation.

The second step is the preparation of the CVI variables. Coastal Vulnerability Index (CVI), which was also used in this research, is the most common index-based assessment method used to determine SLR-inducted coastal vulnerability. Many of the CVI methods commonly used for assessing coastal zone vulnerability were adapted from Gornitz (1990, 1991), which evaluated the US coastline on a national scale (Koroglu et al., 2019). Thereafter, Shaw et al. (1998) and Gornitz (1991) added the geology variable to the CVI calculations. In this research, the CVI values have been calculated with the following variables (a_n) according to Eq. (1);

$$CVI = \sqrt{\frac{a_1 \times a_2 \times a_3 \times a_4 \times_5 \ldots \times a_n}{n}} \quad (1)$$

a_1: Coastal slope
a_2: Relief
a_3: Relative sea level change
a_4: Shoreline erosion / accretion
a_5: Mean tide range
a_6: Mean wave height

Variables are classified from 1 to 5 by dividing them into 5 equal intervals as follows, and the ranking table of variables of the selected method was given in Table 2; 1: Very Low, 2: Low, 3: Moderate, 4: High, 5: Very High.

According to the CVI variables scale, the method of obtaining the values of each variable by using GIS software has been explained in detail below.

Grids for analysis: Grid resolution directly affects CVI analysis results. For this reason, it is necessary to determine the grid size that represents the variables in the most accurate way

Table 2. Coastal Vulnerability Index (CVI) ranking scale from Gornitz (1990, 1991)

Variable	Unit	Very Low 1	Low 2	Moderate 3	High 4	Very High 5
Coastal slope	%	>12	8-12	4-8	2-4	<2
Relief	m	>30	21-30	11-20	6-10	0-5
Relative sea level change	mm/yr	<-1	-1.0-0.9	1.0-2.0	2.1-4.0	>4.0
Shoreline erosion / accretion	m/yr	>2.0	1.0-2.0	-1 - +1	-1.1 - -2.0	<-2
Mean tide range	m	<1.0	1.0-1.9	2.0-4.0	4.1-6.0	>6.0
Mean wave height	m	<1.1	1.1-2.0	2.0-2.25	2.25-2.60	>2.60

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and gives the most accurate analysis result. In the literature, it is seen that many different sizes are used for grid resolution and there is no standard for grid resolution. For example, Gornitz et al. (1994), who introduced the CVI method, used 0.25-degree grids in a coastal hazards database study for the US Gulf Coast in 1994. In determining the grid resolution, the resolution of the data used and the length of the coastal area should be considered and an expert opinion should be taken into account. In this research, a grid network of 30×30 m in size, 250 m inward from the coastline, was created to cover the shoreline in the research area to evaluate the coastal vulnerability (Figure 3). The data of all the variables were extracted by the grids to calculate the CVI using Gornitz (1990) and Gornitz (1991) method.

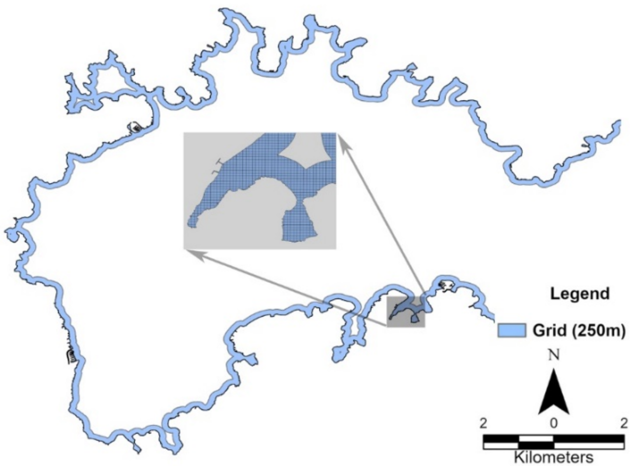

Figure 3. Grids for analysis

Coastal Slope: Using SRTM data, first the slope was calculated, then the slope map was reclassified according to the ranges shown in Table 2, and a new slope raster image consisting of values ranging from 1-5 was obtained. Percent slope Eq. (2) as follows (DeYoung, 2022);

$$Slope\% = \frac{Rise}{Run} x100 \quad (2)$$

In this equation, *Rise* is land elevation, and *Run* is distance.

Relief: Relief refers to the coastal land elevation. SRTM coastal land elevation data was reclassified according to the ranges shown in Table 2, and a new relief raster image consisting of values ranging from 1-5 was obtained.

Relative sea level change: The data acquired from the data sources specified in Table 1 were converted to raster format and a new relative sea level change raster image consisting of values ranging from 1-5 was obtained.

Shoreline erosion/accretion: To detect shoreline changes in the research area the 8th band images of the Landsat 8 satellite on two different dates (16.09.2013 and 27.10.2022) were used with a resolution of 15 meters. By using the automatic detection of shoreline change method (Kuleli et al., 2011), the Normalized Difference Water Index (NDWI), the areas with change were determined by overlaying the shorelines of two different dates. The Normalized Difference Water Index (NDWI) Eq. (3) as follows (Xu, 2005);

$$NDWI = \frac{(Band3 - Band5)}{(Band3 + Band5)} \quad (3)$$

Using this Eq. (3), the images were made suitable for separating the land and sea areas. Thus, the coastline and the sea border were clarified and it was examined whether there was a difference between the obtained coastlines.

Mean tide range: The data acquired from the data sources specified in Table 1 were converted to raster format and a new mean tide range raster image consisting of values ranging from 1-5 was obtained.

Mean wave height: The data acquired from the data sources specified in Table 1 were converted to raster format and a new mean wave height raster image consisting of values ranging from 1-5 was obtained.

Calculation and mapping of CVI value: According to the formula in Eq. (1), the raster maps of each variable were calculated using the map algebra function. The obtained CVI map was reclassified according to values between 1 and 5. Equal interval formula Eq. (4) is used in classification (Campbell & Shin, 2011).

$$Interval = \frac{Range\_of\_Data}{Number\_of\_Classes} = \frac{(HighestValue - LowestValue)}{Number\_of\_Classes} \quad (4)$$

For example, if 5 classes will be created for values ranging from 0 to 50, the class ranges will be as Table 3;

Table 3. Example for equal interval classification

$$\boldsymbol{Interval = \frac{(50-0)}{5} = 10}$$

Value	**Class**
1	0-10
2	11-20
3	21-30
4	31-40
5	41-50

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After the CVI map and its statistical distribution were created, the marina-type MTI sites were overlapped with the CVI map and the sites were evaluated according to their CVI classes. The results are presented as maps, graphs and tables.

Results

In this research, according to the CVI method, site analysis was performed for eight marina-type MTIs in the Bodrum peninsula. These are Bodrum Cruise Port, Bodrum (Kale) Yacht Port, Milta Marina, Gumbet Yatch Port, Bitez Yatch Port, Ortakent Marina, Turgutreis Marina, and Yalıkvak Marina. The CVI values of the marina-type MTI sites in Bodrum were determined by using coastal slope, relief, relative sea level change, shoreline erosion and accretion, mean tide range and mean wave height data, and various GIS methods and formulas. The results of the analyses were presented separately on the basis of variables, CVI values, and MTI sites.

First, the analysis results obtained in terms of relief and slope were explained. The slope can be evaluated as a distance-dependent function of the terrain height (Eq. 2). If the terrain elevation suddenly rises over short distances, the slope also rises. Such land areas are evaluated as steep terrain. Conversely, if the land elevation gradually rises over long distances, the slope decreases. Such land areas are evaluated as flat or low plains. Relief and slope maps classified according to the CVI scale in Table 2 were determined as in Figure 4 and Figure 5. It has been found that MTIs in the research area are generally located naturally in low plains and low-sloping areas.

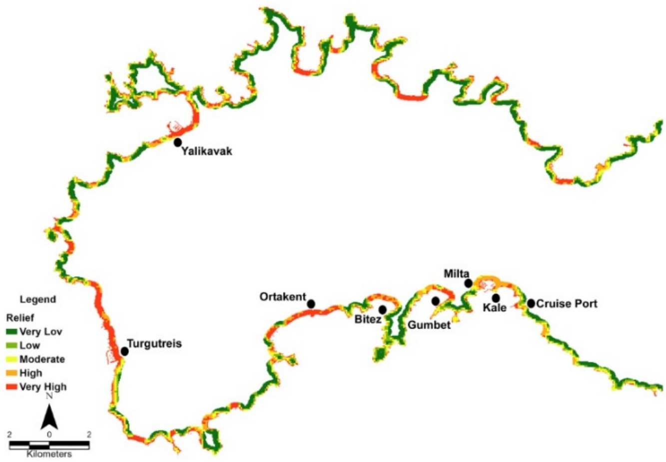

Figure 4. According to the CVI scale, relief map in the research area

In order to better understand site location characteristics of marina-type MTIs in the research area in terms of relief and slope, the slope and relief values of the site are given in Table 4. According to these results, Gümbet, Bitez, and Cruise Port are the three marina-type MTIs located in the highest coastal elevation and steepest slopes, respectively. On the other hand, the first three MTIs located in the lowest coastal elevation and flat slopes are Ortakent, Turgutreis, and Yalıkavak, respectively.

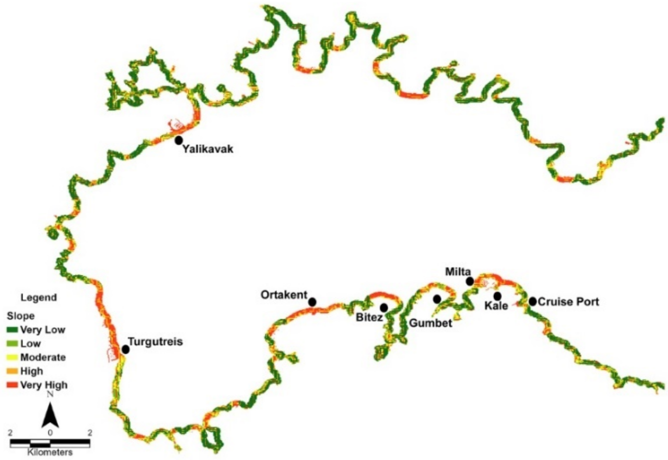

Figure 5. According to the CVI scale, slope map in the research area

Table 4. The distance of reaching a 10m land elevation from the coastal line to the land side, and the average ground slope for each marina-type MTI site

MTI	Land Elevation (m)	Distance From Coastline (m)	Slope (%)
Gümbet	10	23	43.48
Bitez	10	30	33.33
Cruise Port	10	65	15.38
Milta	10	251	3.98
Kale	10	564	1.77
Yalıkavak	10	632	1.58
Turgutreis	10	764	1.31
Ortakent	10	1140	0.88

Relative sea level change (RSLC): According to the data sources in Table 1, the RSLC value in the entire research area was determined as 3.3±1.1 maximum. This RSLC value corresponds to the High (4) value according to the CVI classification (Figure 6). Therefore, the RSLC value in the CVI for the entire sites where marina-type MTIs are located, was evaluated as High (4).

Shoreline erosion/accretion (SEA): SEA was determined by using satellite images of two different dates noted in the method section. In the last ten years, it has been determined that the shoreline in the research area is stable and there is no erosion or accretion. According to the CVI scale table (Table 2), the Moderate (3) value was used for areas with stable shorelines

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such as the research area (Figure 7). Therefore, the SE-A value in the CVI for the entire sites where marina-type MTIs are located, was evaluated as Moderate (3).

Figure 6. According to the CVI scale, relative sea level change map in the research area.

Figure 7. Maps showing CVI values for shoreline erosion/accretion.

Mean tide range and ***Mean wave height:*** According to the data obtained from the ECU (2022) and other sources in Table 1, the MTR and MWH distribution maps in the research area was created Thus, lower and upper limits were determined for the values that MTR and MWH can take according to the CVI scale. According to the CVI scale, it was determined that the MTR distribution was <1.0 and had a Very Low (1) value, the MWH distribution was between 1.1-2.0 and had and Low (2) value. Therefore, the MTR value in the CVI for the entire sites where marina-type MTIs are located, was evaluated as Very Low (1) (Figure 8), and the MWH value was evaluated as Low (2) (Figure 9).

Figure 8. Maps showing CVI values for mean tide range

Figure 9. Maps showing CVI values for mean wave height

Results of Calculation and mapping of CVI value for MTI: The CVI value was calculated for the entire research area by using all six variables and Eq. (1). It was seen that relief (elevation) and slope, two of the six variables used in calculating CVI for the entire research area, were the most determinant. The calculated raw CVI values were found to range between 2 and10. According to the CVI ranking/classification, raw CVI values were reclassified between 1 and 5 by using an equal interval procedure in Eq. (4), and the result CVI map was obtained (Figure 10). It was observed that marina-type MTI sites in the research area were distributed in regions with red (Very High), orange (High) and yellow (Moderate) CVI values.

When the CVI result map and the marina-type MTI site map were overlapped, the CVI values corresponding to the sites where the marina-type MTIs are located were also determined (Figure 10). According to this overlay analysis, Ortakent and Turgutreis were Very High (red); Yalıkavak, Milta and Kale MTIs are High (orange); Cruise Port, Gumbet and Bitez were found to have Moderate (yellow) CVI values (Table 5).

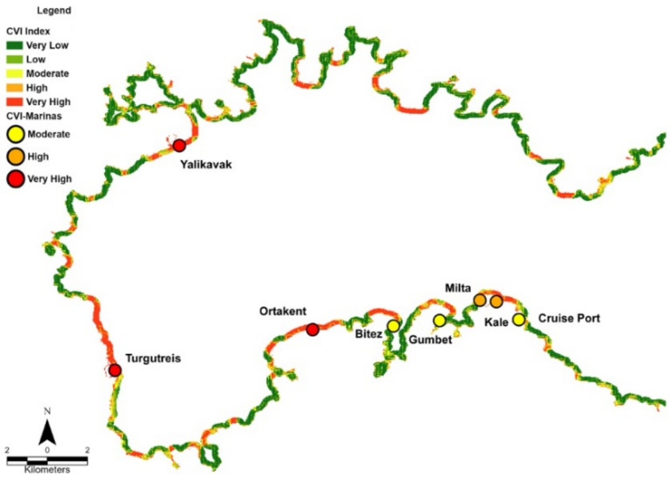

Figure 10. Distribution of the CVI value for the whole peninsula and CVI values of site location of the marine-type MTIs

Table 5. CVI values of the site location of MTI

Maritime Transportation Infrastructure	CVI Value
Cruise port	3 (Moderate)
Gümbet	3 (Moderate)
Bitez	3 (Moderate)
Kale-Bodrum	4 (High)
Milta	4 (High)
Ortakent	5 (Very High)
Turgut Reis	5 (Very Heigh)
Yalıkavak	5 (Very Heigh)

As the overall result of the site location analysis of maritime transportation infrastructure according to CVI, Ortakent, Turgutreis and Yalıkavak which have "very high vulnerability" values in terms of size and location are among the MTIs in the Bodrum peninsula; those with "high vulnerability" values are Milta, and Bodrum-kale; Bitez, Gümbet and the cruise port are the ones with "moderate vulnerability" value.

Discussion

In this research, the site vulnerability of marina-type MTIs including modern marinas, yacht ports, municipal berthing facilities and cruise ports located in Bodrum Peninsula was analyzed and evaluated based on the CVI method. The overall result of the site location analysis of marina-type MTIs based on the CVI method showed that Ortakent and Turgutreis Marinas were the marinas located in the most vulnerable sites. Relative to them, the site location of Bodrum (Kale) Yatch Port, Yalıkavak and Milta Marina were found the second most vulnerable. Besides, Bodrum Cruise Port, Gumbet, and Bitez Yatch Ports were the MTIs located in the least vulnerable sites, relative to the other marina-type MTIs in Bodrum. It has been observed that the most decisive variables on the vulnerability levels of the marina-type MTIs in the Bodrum Peninsula are slope and relief. According to the slope and relief variables, Bodrum Cruise Port, Gumbet, and Bitez Yacht Ports are located in steep terrain locations. So, sites where they are located noteworthy were moderately vulnerable and relatively resistant based on their CVI values. In addition, Milta Marina can be considered relatively less vulnerable against the SLR-induced effects in terms of its higher slope, comparing to Bodrum (Kale) Yacht Port and Yalikavak Marina, which are located in highly vulnerable sites. On the other hand, Ortakent Yacht Port and Turgutreis Marina are relatively the most disadvantaged MTIs due to being built on a flat (low plain) location. Therefore, Ortakent and Turgutreis would be exposed to relatively the greatest physical effects due to climate impacts such as coastal flooding and extreme SLR, waves, and winds, storm surges, etc.

Unlike commercial ports, for marina-type MTIs, effective transportation between the commercial centers in its hinterland and the marina is not crucial. In order for a marina-type MTIs to fulfil its functions, it needs a sheltered and sufficient boat mooring capacity at sea, and a certain dock area in the land area depending on the demand for maintenance-repair works and wintering or other services. Most municipal berthing facilities do not even have any land space but have marina power, lightning, and water supply box-like devices. In the course of time, expanding a seaport hinterland can be needed depending on global trade volume. To expand a seaport in order to obtain a larger handling capacity, step terrain sites would increase construction costs. However, to fulfil its function, a marina needs more maritime space than a hinterland. Therefore, building or being located in low plain sites would not be the priority for marina-type MTIs. In general, while choosing a location for any type of MTI, it is preferred that the hinterland is as flat as possible, has less slope and has a transportation connection (UN, 1992; Murphy et al., 1992; Glatte, 2015). However, according to the evaluation realized with the CVI-approach, the areas where the MTIs are located with low land and low slope hinterland would be more fragile and more vulnerable against the SLR.

The CVI-based investigation is critical for existing MTIs. It determines how vulnerable a marina is based on its own site location characteristics. It also provides information to shed light on adaptation processes. In this research, CVI-based methods were used to evaluate the site location of existing marina-type MTIs in Bodrum Peninsula. It was seen that Gumbet and Bitez Yacht Ports were built in an advantageous

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site, however, Ortakent would be needed adaptation solutions. The cruise port was built on a steep terrain site, which may limit expanding of the port but provide the port resistance against SLR-induced coastal vulnerability. For large marinas located in a low-lying coastal area such as Turgutreis, coastal vulnerability is very high so adaptation solutions should be taken as soon as possible. Climate and vulnerability analysis in order to provide information for policymakers who will take adaptation strategies for existing MTIs can be utilized the literature on commercial ports, but should take into account the characteristics specified to marina-type MTIs, such as yacht ports (Lazarus & Ziros, 2021). Some applications have been developed to increase the resistance to inundation and flooding of areas where MTIs are located (Messner et al., 2013). Elevating, defending, and relocating or retreating are the three major adaptation solutions (Cheong, 2011; Aerts et al., 2014). When determining which climate adaptation measures will be taken for existing coastal systems, using a combination approach site and situation characteristics for each MTI should be considered.

There is a need for more researches to determine adaptation strategies for marina-type MTIs which are located in low-lying coastal areas and therefore vulnerable to climate impacts. For a more adaptable marina by implying one of the adaptation solutions (protect, elevate, or relocate) which involves near and long term planning for hard and soft interventions requires to make a cost-benefit analysis, utilizing site and situation specific characteristics of MTIs. For marinas, yacht mooring facilities, and cruise ports, which are located in SRL-vulnerable sites based on CVI, one or a combination of more adaptation solutions can be adopted depending on site location characteristics, port characteristics based on its function and capacity, and its socio-economic importance in its region. Future studies may also evaluate the site location of a new location using the CVI approach for site selection of MTIs. According to Nguyen et al. (2021), decisions are mostly given by politic-based decision makers, and an assessment of the physical variables of the selected site would be carried out after that. However, for the benefit of the region physical site characteristics should be taken into account for a sustainable MTI's site selection (PIANC, 2019; Nguyen et al., 2021; Taneja & Oosterwegel, 2022). It is expected that this study will encourage authorities and policy-makers to include the CVI methodology in the site selection criteria.

The limitation of this research is derived from the nature of the CVI method. The simple and widely used first version of CVI approach includes only physical variables. The first version of CVI approach does not include socio-economic variables such as the number of the affected population, number of buildings, infrastructure, economic sectors or economic costs (Gornitz et al., 1993; Cooper & McLaughlin, 1998; ETC/ACC, 2010a, 2010b). Therefore, the CVI approach, first used by Gornitz (1991), has continued to be developed, with some researchers incorporating socio-economic variables into the formula (Cutter et al., 2003; Kleinosky et al., 2007; Ergin et al., 2008; Tate et al., 2010; Guillard-Gonçalves et al., 2015; Tragaki et al., 2018). The CVI-method for site location analysis of existing or new building decisions of marinas utilizing socio-economic variables can be modified. Future studies are advised to apply CVI-method to the MTIs considering marina dynamics such as mooring capacities, number of services provided, and socio-economic dynamics such as transportation facilities such as roadways and population adjacent to the marina.

Conclusion

Yachting and cruising activities represent a large part of marine and coastal industry which constitute the significant economic value for the most Mediterranean countries. Marinas and cruise ports are essential facilities for these activities. Marinas and cruise ports facilitate marine and coastal tourism. Marinas-like MTIs are critical coastal infrastructures located on shorelines so are highly vulnerable to coastal risks, and host valuable assets which are yachts. Coastal risks could affect marina facilities such as slipways, boatyards, stores, chandlery, shops and areas adjacent to marinas such as roadways. The vulnerability and risk profile of each marina-type coastal infrastructure would be different based on its physical and functional qualities. In this research, the CVI approach was applied by evaluating the physical site location characteristics of marina-type MTI, for the first time applied in the Bodrum Peninsula, where there is a high density of marina. For adaptation strategies for existing MTIs, more investigations should be realized from the functional, economic, social, and ecosystem points of view. From the social point of view, for large and complex marinas disruption of roads to the marina or harbor could affect the movement of people and service movements around the marina. From the ecosystem point of view via floodwaters, contaminants existing at various sites, such as slipways, and maintenance-repair areas within the marina could flow into the sea or contaminate rising groundwater. From the managerial point of view, it can be said that small marinas or municipal berthing facilities with a state

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ownership model are advised to work together with other marinas in the region if exist. The CVI-methodology should also be utilized for the site selection of any type of maritime transportation infrastructures.

Compliance With Ethical Standards

Authors' Contributions

Please provide contributions of authors for the paper. Use first letters of name and surnames. See below for an example.
TK: Manuscript design, Field sampling, Laboratory experiments, Draft checking.
ŞB: Writing, Statistical analyses, Draft checking, Reading, Editing.
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

1) Relief and digital elevation data:

- SRTM data: NASA JPL (2013). NASA Shuttle Radar Topography Mission Global 1 arc second number [Data set]. NASA EOSDIS Land Processes DAAC. Accessed 2022-11-29 from https://doi.org/10.5067/MEaSUREs/SRTM/SRTMGL1N.003

- SRTM 1 Arc-Second Global elevation data offer worldwide coverage of void filled data at a resolution of 1 arc-second (30 meters) and provide open distribution of this high-resolution global data set (https://doi.org/10.5066/F7PR7TFT).

2) Mean tide range and mean wave height data:

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3) All other data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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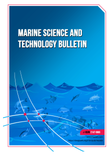

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RESEARCH ARTICLE

Molecular and phenotypic characteristics of short-beaked garfish *Belone svetovidovi* Collette and Parin, 1970 in a new location, the Sea of Marmara

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ABSTRACT

There are two species of garfish belonging to the genus *Belone* (garfish *B. belone* and short-beaked garfish *B. svetovidovi*) in the coastal regions of Türkiye. It is known that *Belone belone* is distributed all around the Turkish coasts, and *Belone svetovidovi* is distributed only in the Mediterranean and Aegean coasts of Türkiye. These species' morphological similarities and mainly overlapped morphological characters led to confusions for years. The present study represents the first detailed morphological and molecular analysis including twenty-two body portions and DNA barcode analysis of *B. svetovidovi* and reports the newly established populations in the Sea of Marmara with molecular and morphological evidences.

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Introduction

The Belonidae family, represented by a total of 10 genera and 44 nominal species in marine and freshwater ecosystems, constitutes one of the important groups of the ichthyofauna of the Eastern Atlantic, the Mediterranean and the Black Sea (Fricke et al., 2022).

In the last checklist of the marine fish species of Türkiye, the existence of three species is mentioned; *Belone belone* (Linnaeus, 1761), *Belone svetovidovi* Collette & Parin, 1970 and *Tylosurus acus* (Lacepède, 1803) (Bilecenoğlu et al., 2014). According to this checklist, while *Belone svetovidovi* and *Tylosurus acus* species are distributed only in the Mediterranean and Aegean coasts of Türkiye, it is known that *Belone belone* is distributed in all around Turkish coasts (Bilecenoğlu et al., 2014).

The short-beaked garfish *Belone svetovidovi* was first described by Collette & Parin (1970) from Galicia, Spain then was reported by Collette & Parin (1986) in the north-eastern Atlantic (Southern Ireland, Spain and Portugal), in the eastern Mediterranean (Israel) by Golani (1996), and in the Aegean Sea by Meriç & Altun (1999). After its Aegean Sea record, only one

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specimen of *B. svetovidovi* was recorded from the north-eastern Levantine Sea by Dalyan & Eryılmaz (2006). According to Collette & Parin (1970), *B. svetovidovi* differs from *B. belone* by having more closely spaced and smaller teeth on the jaws, by lacking vomerine teeth (mostly present in adult *B. belone*), and by having more numerous gill-rakers (Dorman, 1987; Meriç & Altun, 1999).

Although there are a couple of previous studies using limited metric and meristic characters (Dorman, 1987; Meriç & Altun, 1999), there is no integrative study that includes molecular and morphological analysis that can shed light on molecular and phenotypic characteristics of this commercial species.

The aim of this paper is to represent the first detailed morphological and molecular analysis and report the newly established populations of *Belone svetovidovi* in the Sea of Marmara with both molecular and morphological evidences.

Material and Methods

Samplings

B. svetovidovi specimens were collected directly from the local fishing boats in the Sea of Marmara, which used trammel nets (Figure 1). Ten specimens were sampled from the Northern coast of the Sea of Marmara (the Bosporus/Istanbul) and nine specimens were sampled from Southern coast of Sea of Marmara (Gemlik District). Pectoral fin clips were fixed in 96% ethanol for genetic analyses and then the specimens were fixed in 4% formalin solution for morphological analysis. The collected specimens were deposited in the Fish Collection Centre of İzmir Katip Çelebi University (IKC PIS 1271-1289).

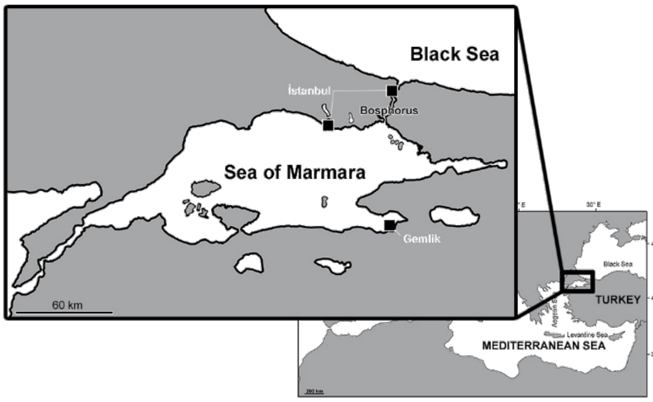

Figure 1. Sampling locations of short-beaked garfish *Belone svetovidovi* in the Sea of Marmara

Morphological Analysis

Morphological methods follow Zorica & Čikeš Keč (2011) with some additional characters (SL% of body depth at anal fin pelvic fin and dorsal fin origins; head depth, interorbital distance, counts of gill-rakers and teeth, the teeth count within a section of the middle of the upper jaw equalling the eye diameter) were added (Figure 2). Totally 22 different body proportions were used (7 of which were related to head length) in morphological analysis (Table 1).

Table 1. Morphometric and meristic characters of *Belone svetovidovi* (n=19)

Characters	**Range (mean±S.D.)**
SL (cm)	26-35.7 (30.03±3.2)
SL%	
HL/SL	20.4-35.3 (28.5±4)
Pre-dorsal/SL	76.4-82.9 (79.9±1.6)
Pre-pelvic/SL	61.1-69.4 (65.7±2.3)
Pre-anal/SL	74.5-80.4 (77.8±1.4)
Pre-pectoral/SL	21.5-36.3 (29.7±4.3)
Pelvic to anal/SL	10.5-14.3 (12.1±1.1)
Pectoral length/SL	5.2-7 (6.0±0.5)
Pelvic length/SL	10.5-14.3 (12.1±1.1)
Caudal fin length/SL	9.3-12.6 (10.5±0.9)
Caudal peduncle depth/SL	1.8-2.7 (2.0±0.2)
Depth at dorsal fin orj/SL	4.6-6.4 (5.5±0.4)
Depth at pelvic fin orj/SL	4.8-6.6 (5.7±0.5)
Depth at anal fin orj/SL	4.6-6.5 (5.6±0.5)
Dorsal fin base/SL	11.5-14.8 (13.1±0.9)
Anal fin base/SL	13.6-16.9 (15.3±0.8)
Pectoral fin base/SL	5.2-7 (6.0±0.5)
Pelvic fin base/SL	3.4-5.3 (4.4±0.5)
HL (cm)	5.6-10.6 (8.6±1.6)
HL%	
Head depth/HL	13.2-23.8 (16.8±3.4)
Pre-orbital/HL	48.2-74 (63.8±7.4)
Post-orbital/HL	18.3-37.5 (24.9±5)
Eye diameter/HL	8.4-15.5 (11.0±2.4)
Interorbital/HL	6.5-12.7 (8.9±1.9)
Meristic Counts	
Dorsal fin rays	15-19 (16.7±1.1)
Anal fin rays	18-23 (21.1±1.3)
Pectoral fin rays	11-15 (12.3±1)
Pelvic fin rays	6-7 (6.1±0.3)
Gill-rakers	40-49 (43.5±3.2)
Teeth count within a section of the middle of the upper jaw equalling the eye diameter	14-20 (16±1.94)

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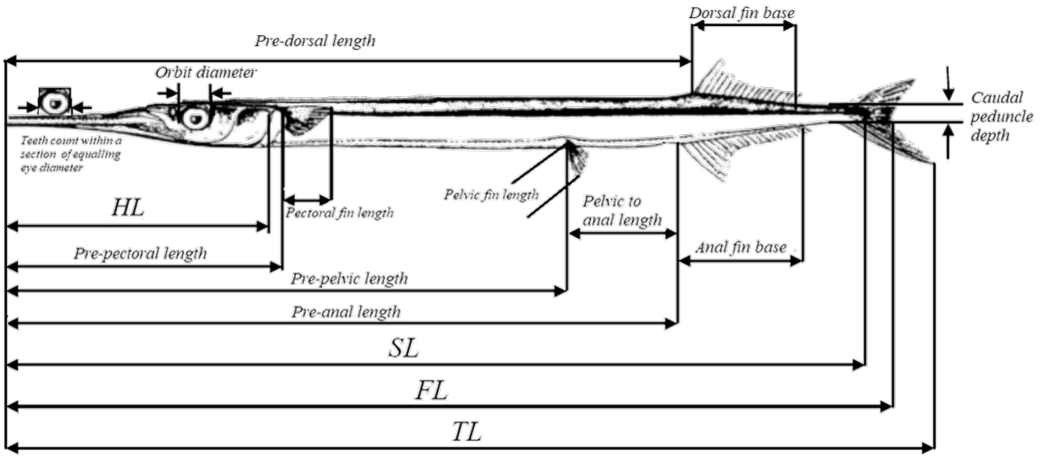

Figure 2. Drawing of body proportions of short-beaked garfish modified from Zorica & Čikeš Keč (2011)

Molecular Analysis

Total genomic DNA was isolated from the fin clips according to the manufacturer's instructions with a DNA isolation kit (AMBRD Laboratories, Türkiye). FishF2/FishR2 primers described by Ward et al. (2005) were used to amplify Cytochrome c Oxidase I, which is also known as the barcode region using the 5x FIREPol Master Mix (Solis Biodyne; www.sbd.ee). The thermocycler profile consisted of a step 1 min at 94°C, five cycles of 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min, followed by 35 cycles of 94°C for 30 s, 54°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were checked with 2% agarose gel. Sequencing was carried out by Macrogen Inc., Seoul, South Korea. Nucleotide sequences were aligned using ClustalW (Thompson et al., 1994) implemented in MEGA version X (Kumar et al., 2018) and edited with BioEdit (Hall, 1999). Aligned sequences of Sea of Marmara samples were submitted to GenBank with accession numbers: OQ329406- OQ329424. Maximum Likelihood (ML) and Neighbour Joining analysis were performed with the MEGA version X using the T92 (Tamura-3) + Gamma model due to the lowest Bayesian Information Criterion (BIC) values. Uncorrected *p* and T92 distances were used to compare the barcode data. A bootstrap test with 1000 replicates was performed to verify the robustness of the tree.

Results

Morphological Analysis

Nineteen specimens of *B. svetovidovi* have been obtained of which 12 were male and seven were female. All measured specimens varied in total length from 28.4 to 39 cm (mean±SD: 32.9±3.2 cm). The total length of males varied between 30.6-39 cm (mean±SD: 34.5±3 cm), whereas for females varied between 28.4-33.1 cm (mean±SD: 30.3±1.6 cm). Meristic counts and the results of the morphometric proportion of the body measurements expressed in percentages are given in Table 1. All examined specimens lacked vomerine teeth and featured 14-20 teeth within a section of the middle of the upper jaw equalling the eye diameter.

Genetic Analysis

A total of 625 bp of COI fragments of 31 short-beaked garfish were analysed COI clades of the specimens were well supported in the ML and NJ phylogenetic analysis (Figure 3, Figure 4). Since there are no COI barcode data of *B. svetovidovi* available in both GenBank and BOLD system, the Aegean Sea sequences of this species (which were also generated in this study) were used to compare the data. The sequence of the COI fragment, presented here for the first time for *B. svetovidovi.* Both uncorrected *p* and T92 distances have shown low intra-specific distance (0.4% SE=0.001) between analysed sequences. The interspecific distance between *B. svetovidovi* and *B. belone* species is 3% SE=0.006 (for both uncorrected *p* and T92 distances).

Discussion

There are two species of garfish belonging to the genus *Belone* (garfish *B. belone* and short-beaked garfish *B. svetovidovi*) on the coastal regions of Türkiye (Bilecenoğlu et al., 2014). While the garfish *B. belone* is distributed over a wide area of the Atlantic Ocean from Iceland and Norway in the north, the western coast of Africa in the south and also in the Mediterranean Sea, the short-beaked garfish *B. svetovidovi* is distributed North-East Atlantic (Southern Ireland, Spain,

Figure 3. Maximum Likelihood tree of analysed *Belone* species. The tree is drawn to scale, with branch lengths (above the branches) measured in the number of substitutions per site Numbers next to branches indicate bootstrap values for 1000 replicates

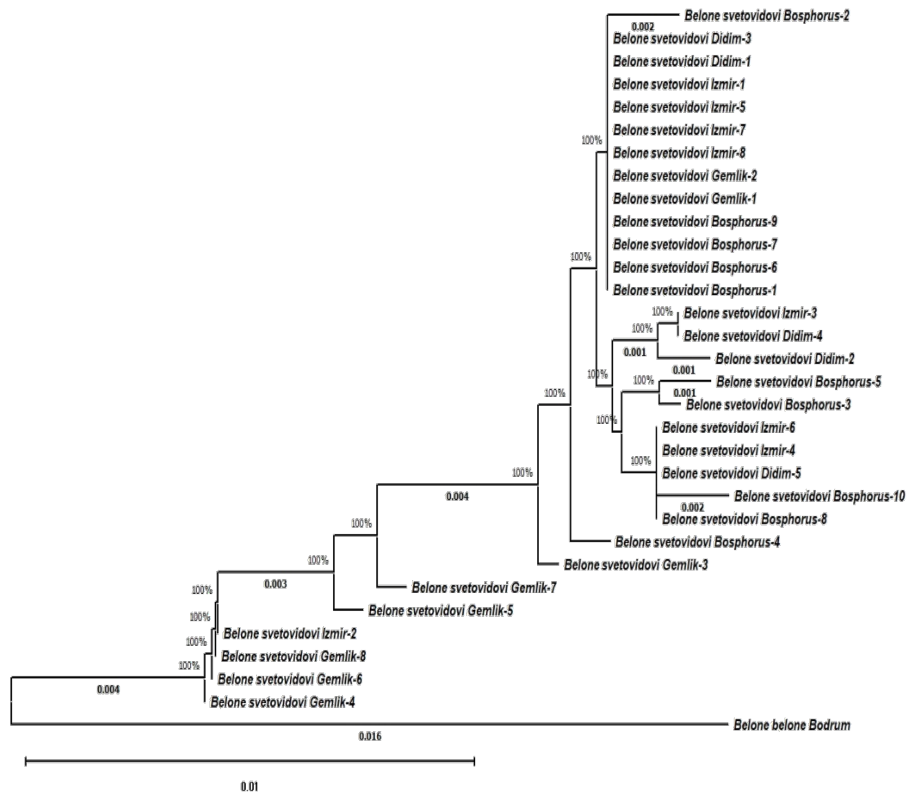

Figure 4. Neighbour joining tree of analysed *Belone* species. The tree is drawn to scale, with branch lengths (above the branches) measured in the number of substitutions per site Numbers next to branches indicate bootstrap values for 1000 replicates

Portugal) and in the Mediterranean Sea (Collette & Parin, 1986; Golani, 1996; Meriç & Altun 1999; Dolgov & Zabavnikov, 2021). These species' very similar appearance and mainly overlapped morphological characters led confusions for years (Dorman, 1987; Meriç & Altun, 1999; Dolgov & Zabavnikov, 2021). *B. svetovidovi* differs from *B. belone* by having more closely spaced and smaller teeth on the jaws, by lacking vomerine teeth (mostly present in adult *B. belone*); and by having more numerous gill-rakers (Dorman, 1987; Meriç & Altun, 1999). Additionally, Meriç & Altun (1999) also remarked that the teeth count within a section of the middle of the upper jaw equalling the diameter of its eye can distinguish these two species. Due to this, they reported that while *B. svetovidovi* has 11-21 teeth in this section, *B. belone* has 5-10 teeth within a similar-sized area. In this study, *B. svetovidovi* featured 14-20 teeth within a section of the middle of the upper jaw equalling the eye diameter. The number of the gill-rakers of *B. svetovidovi* was reported as a mean of 45.20 and 48.7, respectively, by Dorman (1987) and Meriç & Altun (1999) while it is determined as 43.5±1.8 in this study. These counts are noticeably higher than those reported for *B. belone* [mean 32.22 (Meriç & Altun, 1999; mean 35.6 Dorman, 1987)].

In addition to morphological analysis, DNA barcoding technique was used to confirm the identification of *B. svetovidovi*. DNA barcoding technique has a proven efficacy for species identification throughout fish taxa (Ward et al., 2005, 2008; Hubert et al., 2008; Steinke et al., 2009). According to the DNA barcoding concept; if the inter-specific distance is greater than intra-specific distance and there is no overlap between these thresholds, this would indicate a taxonomic identity among samples (Hebert et al., 2004; Wiemers & Fiedler, 2007). Genetic distances between *B. svetovidovi* and *B. belone* were on average 7.5-fold higher than within species. The results of the barcoding analysis represented that *B. svetovidovi* is clearly distinct from analysed *B. belone*. This study showed that barcoding analysis in proximate and mixed species is a powerful aid in making taxonomic distinctions.

Conclusion

Present study represents the first detailed morphological and molecular analysis of *Belone svetovidovi*. The sequence of the COI fragment, presented here for the first time and findings of morphological analysis highlighted that the number of the gill-rakers and the teeth count within a section of the middle of the upper jaw equalling the diameter can distinguish these two species. This study also reports,*Belone svetovidovi* has extended its distribution of from the Aegean Sea to the Sea of Marmara in Turkish coasts.

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Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

The author confirms that the data supporting the findings of this study are available within the article.

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RESEARCH ARTICLE

Effects of different carbon sources on growth and some innate immune responses of Russian sturgeon (*Acipenser gueldenstaedtii*) in biofloc systems

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Fish

ABSTRACT

The Russian sturgeon (*Acipenser gueldenstaedtii*) species is in high demand owing to its valuable caviar. Therefore, it is in danger of extinction. Since the Russian sturgeon reaches sexual maturity late in its life cycle, this species has a high economic cost for farmers. However, this high cost can be reduced with an environmentally friendly system called biofloc technology. This study compared the growth performance and health indicators of biofloc groups using different carbon sources such as starch (BS), molasses (BM) and dextrose (BD). In the 60-day study, fish with an average initial weight of 106.44±5.79 g were stocked in tanks at a density of 21 fish/tank (0.4 m^3/tank). The water temperature was set at 19 °C degrees throughout the study. On the 30th and 60th days of the experiment, fish were weighted to measure the growth parameters and sampled for immune indices. No mortality was observed in any group throughout the study. A between group comparison of weight gain revealed that BS and BM (105.51±2.26; 100.50±2.18) performed better than the control (BC, without external carbon sources) and BD groups (95.90±2.09; 87.36±2.18) ($P<0.05$). Furthermore, FCR and SGR were calculated from the data obtained at the end of the experiment, and the data shows that the BS and BM groups were statistically more effective than the other groups. Moreover, a comparison of NBT, lysozyme and myeloperoxidase enzyme activities indicated that all BFT groups had a stronger immune system than the control group ($P<0.05$). According to the results, the immune-enhancing effect of BFT for sturgeon was determined, and it was reported that BS and BM are more suitable for use in this species in terms of FCR and SGR, as they result an economic and environmentally friendly production.

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Introduction

Aquaculture is understood to be an important nutrient source, providing essential macro and micronutrients, as well as having a better protein conversion efficiency, lower carbon emissions and carbon footprint than other animal production systems. However, the ever-increasing demand for aquaculture in recent years has led to more intensive production per unit area. Since this situation may cause some environmental and economic problems, it has become crucial to develop new production techniques. The number of studies on BFT, a new production technology, have increased using these criteria.

Biofloc technology (BFT) is an aquaculture technique in which limited water exchange results in an increase in the number of microscopic organisms, such as bacteria, zooplankton, nematodes, fungi and, algae (Dauda et al., 2018; Khanjani et al., 2022). In this technology, the nutrients in the water are consumed by the aquatic organisms, and as a result, the number of microorganisms in the water is increased and they can be consumed as food by the fish. Increasing the number of microorganisms in the water increases the water quality because they consume the fish feces and feed waste that is in the environment (Zhao et al., 2022).

BFT has low investment and maintenance costs for intensive production (Avnimelech, 2015). Intensive aquaculture techniques produce high levels of inorganic nitrogen, ammonia (NH_3) and converted nitrite (NO_2) (Ogello et al., 2021). Bacteria in BFT systems remove nitrogen from the water for protein production (Avnimelech, 2015). This system has several benefits, such as maximizing feed conversion, increasing biosecurity, reducing water use through zero or minimal water changes, and reducing the environmental impact of wastewater (Avnimelech, 2015).

Today, the importance of protecting the environment and eliminating potential waste at its source has increased even more. Therefore, environmentally friendly approaches are supported in the aquaculture sector. Since BFT was understood to be a sustainable method in aquaculture, studies have generally followed water quality. In intensive aquaculture with zero water exchange, high nutrient input encourages the densification of microorganism communities. While some of the nitrogen (N), carbon (C) and phosphorus from the feed is used by aquatic organisms, some of it is released into the aquatic environment (Mugwanya et al., 2021). Heterotrophic bacteria in water incorporate organic pollutants into their biomass by adjusting the appropriate C and N ratio (Emerenciano et al., 2017). Bacteria-consuming communities developed from dissolved organic matter from fish and crustaceans, the water continues to maintain its quality without water exchange in the system (Pérez-Rostro et al., 2014).

Increasing aquaculture production will increase the dependence on fish feed (Liu et al., 2019). This situation returns as feed cost for farmers rises and pollution in the environment increases. In this context, BFT systems, which have increased their popularity recently, keep the wastes in the culture and transform it into biofloc as a food (Khanjani et al., 2022). The presence of heterotrophic bacteria, which is the driving force of BFT, improves the weight gain and feed conversion ratio (FCR) of fish (Bossier & Ekasari, 2017).

For aquaculture to be sustainable, the aquaculture environment must be healthy and free from diseases. Aquaculture has an economic loss of approximately 50% due to fish diseases (Assefa & Abunna, 2018). This loss negatively affects the producer directly and the consumer indirectly. Based on sustainable production, it is necessary to focus on "prevention" instead of "treatment" (Jeney, 2017). For this purpose, BFT systems emerge as an innovative strategy especially for disease resistance.

Shrimp, tilapia, and carp are the most commonly used species in BFT (Mugwanya et al., 2021). The most crucial point here is that it is possible to produce the world's two most cultivated species with sustainable aquaculture. The fact that this system has not been tested for other species and successful results have not been obtained has left this method undeveloped. However, new species have been continuously tried with BFT.

Sturgeon is an anadromous fish species that usually lives in the Sea of Azov, the Black Sea, and the Caspian Sea, with a 200–250-million-year history. Natural sturgeon populations have declined because of environmental pollution and excessive demand for their meat and caviar. As a result, the International Union for Conservation of Nature (IUCN) listed all commercially used sturgeon species worldwide in Appendix II of the CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) regulations and prohibited their fishing in 1997. After this regulation, sturgeon aquaculture studies started to meet the worldwide demand, and successful results were obtained quickly. Although production has not reached the desired level, meat production has increased threefold, and caviar production has increased approximately fourfold between 1998 and 2017 (Sytova, 2017).

The fact that approximately 20% of the globally produced caviar is obtained from Russian sturgeon has brought this species to a very crucial position in terms of the aquaculture sector (Bronzi et al., 2019). Due to the production difficulties of being an anadromous species, its cultivation is carried out in completely controlled conditions. Temperature and feeding protocol are vital in breeding of this species, and sperm and egg are usually taken with hormone application in spring (Vasilyeva et al., 2019).

Although wide range of production techniques, including recirculating aquaculture system (RAS) and cage are used in the production, the most widely used method is the production in flow-through system with 36% (Bronzi et al., 2019). However, the inability to provide optimum water conditions throughout the year leads the species to grow with RAS, but in this case high electricity and operating cost arise.

RAS has the additional advantage of the helping the species reach sexual maturity 8 years earlier. On the other hand, studies have reported that the taste and quality of the caviar obtained from the fish produced with RAS is suboptimal as a disadvantage (Korchunov, 2012). Therefore, studies on new and combined production techniques are needed. With biofloc technology, an innovative new production model, feed cost can be reduced, feed efficiency can be increased, fish health can be improved, and environmental impact can be minimized because water use will be reduced. Therefore, in this study, the effects of different C sources on growth and some innate immune responses of Russian sturgeon (*Acipenser gueldenstaedtii*) in biofloc systems BFT were investigated.

Material and Methods

Experimental Design and Set-Up

The studies were carried out in the Trabzon Central Fisheries Research Institute (SUMAE). Russian sturgeon produced in SUMAE were used as material. A total of 252 fish grown in a flow-through system (fresh water) in concrete ponds were taken and placed in a total of 12 tanks (0.4 m^3/tank), consisting of 3 replications, with 21 fish in each, after an adaptation period of approximately 10 days. Fish were obtained with an average weight of 106.44±0.63 g. Throughout the studies, the temperature was fixed at 19 ± 1°C. Temperature, oxygen, and pH were recorded daily with the Hach HQ40d probe. During the experiment, all groups were fed twice day (08:00-16:00) with diet containing 45% crude protein as 3% of their total biomass. The feed was obtained from a commercial fish factory (manufactured by Sürsan Aquaculture Company), and the nutritional values of the feed are shown in Table 1. The experiment was conducted for 60 days.

Table 1. Proximate composition of the experiment diet

Diet Content	Amount
Crude protein (%)	45
Crude lipid (%)	20
Ash (%)	6
Vit A (IU)	8800
Vit D3 (IU)	1600
Vit E (mg/kg)	200
Calcium (%)	1.75
Sodium (%)	0.25
Total phosphorus (%)	1

Note: Diet was manufactured by Sürsan Aquaculture Company

Four treatments were considered for the present study, including a control group (BC, without external C sources) and three biofloc treatments with different external C sources (BM: molasses; BS: starch; BD: dextrose). The experiment was carried out according to the principles of the animal experimentation ethics committee of SUMAE.

Floc Formation

In order to accelerate flocculation, 3 separate tanks were prepared for each type of C source. After adding 25 mg/l N to each preparation tank, molasses, starch, and dextrose were added separately to the tanks as a C source. After the biofloc level in the tanks reached 20 ml/l, floc was inoculated into the treatment tanks to support the formation of floc, and the experiment was started later. Oxygen for the fish was provided by an air blower; 3 lines were drawn for each tank, and cylindrical air stones were used.

The daily amount of external C sources (once a day) for each group was determined, as reported by Avnimelech (1999). The C:N ratio was considered 15:1. During the experiment, the biofloc density in the tanks was monitored with the Imhoff funnel. No complete water changes were made; we only added water to replace the water lost by evaporation.

Growth Parameters

The fish weights were measured at the beginnings, day 30, and the end of the experiment. Growth parameters; weight gain (WG), specific growth rate (SGR), survival rate (SR), and feed conversation ratio (FCR) were calculated as reported by Khanjani et al. (2017).

Formulas for determining growth parameters;

$$SR(\%) = 100 \times \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \quad (1)$$

$$WG(g) = Final\ weight - Initial\ weight \quad (2)$$

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$$SGR(\%) = 100 \times \frac{\ln(Final\ weight\ (g)) - \ln(Initial\ weight\ (g))}{Time\ (Exp\ days)} \quad (3)$$

$$FCR = \frac{Feed\ provided\ (g)}{Weight\ gain\ (g)} \quad (4)$$

Blood Sample Analysis

In days 30 and 60, 7 fish were taken from each tank for blood sample collection and analyses. The fish were stunned with clove oil (dose: 30 mg/l), a widely used natural product. After thoroughly cleaning the posterior part of the anal fin with alcohol to prevent blood from mixing with mucous, blood was drawn from the caudal fin by inserting a 5 ml plastic syringe without harming the fish. Collected blood samples were placed in K3EDTA and gel serum tubes and immunological analyses were performed.

Immunological Analyses

Respiratory burst activity

The respiratory burst activity of neutrophils and monocytes was determined by NBT (nitro blue tetrazolium) activity in blood. For NBT analysis, 100 µl of blood samples were incubated for 30 minutes with NBT solution. 50 µl of this mixture for each sample, was added to tubes containing N, N-dimethyl formamide. Subsequently, the centrifuged tubes were read at 540 nm in a 1 ml spectrophotometer cuvette. NBT activity was calculated as mg NBT formazan/ml (Siwicki et al., 1993).

Lysozyme activity

In order to determine the lysozyme activity, phosphate buffer solution (PBS) was added at the same rate to 100 µl of the serum sample. *Micrococcus lysodeicticus* was added to this mixture and read in a multiscan microplate reader at 530 nm at 0.5 for 4.5 minutes. Analysis results were calculated as µg/mL (Ellis et al., 1990).

Myeloperoxidase activity

For myeloperoxidase activity (MPO), 10 µl of the serum sample was diluted with 90 µl of hanks balanced salt solution (HBSS) solution. Afterwards, a solution containing 3,3′,5,5′-tetramethylbenzidine dihydrochloride and hydrogen peroxide was added to this mixture, and the reaction was stopped with 35 µl of sulfuric acid after 2 minutes, and readings were taken at 450 nm in a multiscan microplate reader (Quade & Roth, 1997).

Data Analysis

In this study, analyses of variance were used to evaluate the relations between the experimental groups, and Tukey multiple comparison tests were used for the differences between the groups using the SPSS v22 statistical program.

Results

At the end of the treatment, the best FCRs were found as 2.18±0.01 and 2.16±00 in the BS and BM groups, respectively ($P<0.05$). The highest SGRs were also in the BS and BM groups ($P<0.05$), as were the highest degrees of weight gain ($P<0.05$). The group with the highest feed consumption was BS, whereas the groups with the lowest were BM and BD ($P<0.05$), as shown in Table 2.

As shown in Table 3, Figure 1, Figure 2 and Figure 3, the results of the NBT analyses on 30th and 60th days indicate that the biofloc groups had a higher NBT than the BC ($P<0.05$), with the BS group having the highest NBT of all biofloc groups (1.39±2.13;2.53±0.05). In another analysis examining the effects of different C sources on lysozyme and MPO, as immunological parameters, the BS group also had the highest lysozyme and highest MPO ($P<0.05$) (Figure 1, Figure 2). Despite showing no difference between them, the biofloc groups were significantly different from the BC (P<0.0.5) in terms of MPO and lysozyme.

Table 2. Growth performance of fish reared in biofloc system with different carbon sources

Parameters	BS	BD	BM	BC
Initial weight (g)	106.73±1.26	105.20±1.25	108.11±1.27	105.72±1.26
Final weight (g)	212.24±1.70^{a}	192.57±1.69^{c}	208.61±1.62^{a}	201.62±1.62^{b}
Weight gain (g)	105.51±2.26^{a}	87.36±2.18^{c}	100.50±2.18^{a}	95.9±2.09^{ab}
FCR (g)	2.18±0.01^{a}	2.46±0.01^{c}	2.16±0.00^{a}	2.39±0.00^{b}
SGR (%)	1.14±0.02^{a}	1.00±0.02^{b}	1.09±0.02^{a}	1.07±0.11a^{b}
Feed Intake (g)	77.44±0.33^{a}	72.23±0.28^{b}	72.71±0.29^{b}	76.44±0.22^{c}

Note: Values are provided as mean ± standard error (n=21). Each treatment has three replications. Values with no superscripts in horizontal row are not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C)

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Table 3. Immunological indices of Russian sturgeon reared in biofloc system

		BS	BD	BM	BC
	Initial	0.87±1.97[a]	0.82±0.03[a]	0.85±0.00[a]	0.86±0.06[a]
NBT	30th day	1.39±2.13[a]	1.36±0.05[a]	1.36±0.07[a]	0.88±0.06[b]
(OD at 540 nm)	60th day	2.53±0.05[a]	2.40±0.09[a]	2.46±0.13[a]	0.89±0.06[b]
	Initial	20.20±0.85[a]	20.09±0.84[a]	20.08±0.84[a]	20.3±0.85[a]
Lysozyme	30th day	30.30±1.27[a]	30.14±1.27[a]	30.13±1.27[a]	20.7±0.95[b]
(µg/ml)	60th day	36.06±1.52[a]	35.87±1.51[a]	35.85±1.51[a]	20.9±0.73[b]
	Initial	39.18±1.27[a]	38.97±1.64[a]	38.97±1.64[a]	39.38±1.66[a]
Myeloperoxidase	30th day	46.66±1.51[a]	46.41±1.96[a]	46.40±1.96[a]	39.58±1.67[b]
(U/l)	60th day	50.48±1.65[a]	50.21±2.12[a]	50.19±2.12[a]	40.17±1.69[b]

Note: Data are presented as means ± SEM. values with different superscripts in horizontal are statistically different (P<0.05, *n* = 7). Each treatment has three replications. BS: starch, BD: dextrose, BM: molasses, BC: control

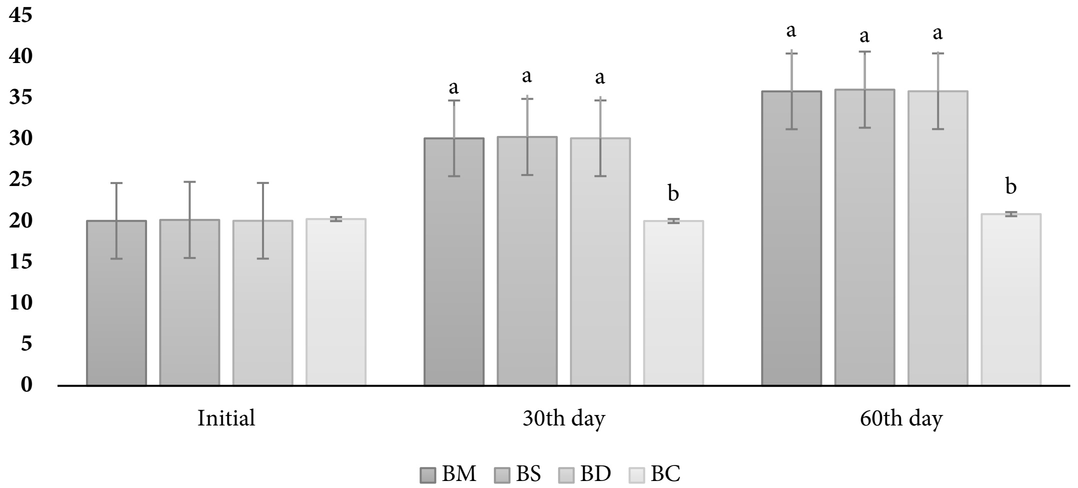

Figure 1. Lysozyme activity of Russian sturgeon reared in different biofloc systems (Error bar showing standard deviation of three replicates (*n*=7). Significance between different groups (P<0.05) marked with asterisk. Error bar with no superscripts is not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C))

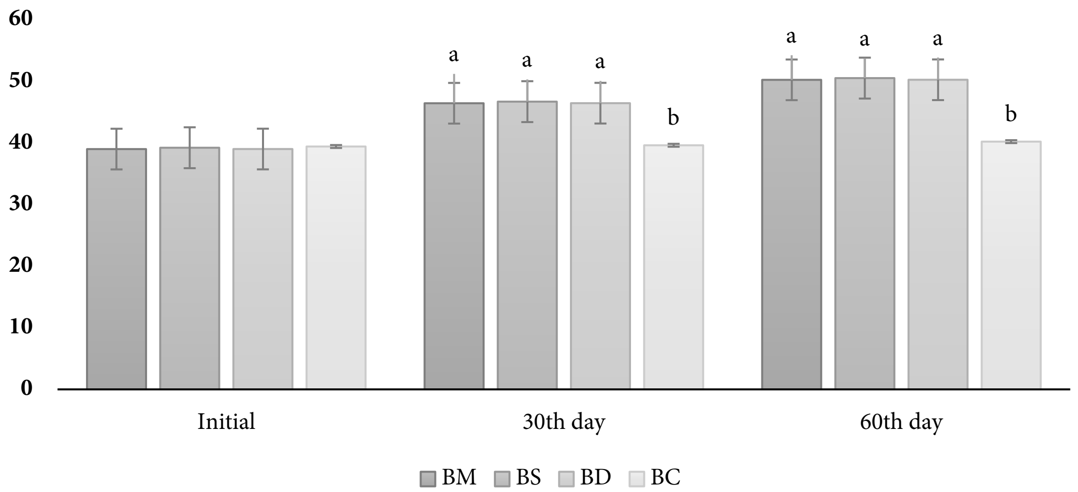

Figure 2. Myeloperoxsidase activity of Russian sturgeon reared in different biofloc systems (Error bar showing standard deviation of three replicates (*n*=7). Significance between different groups (P<0.05) marked with asterisk. Error bar with no superscripts is not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C))

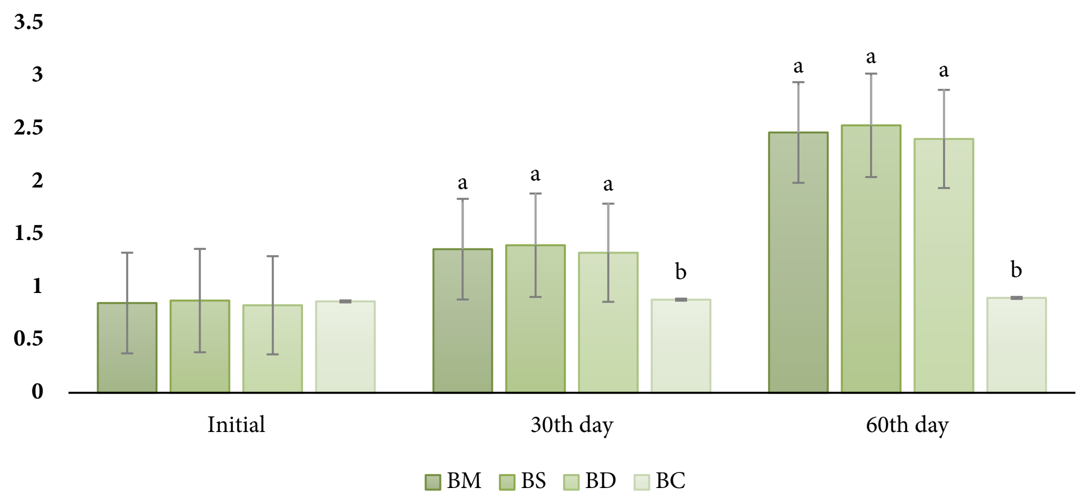

Figure 3. NBT analysis of Russian sturgeon reared in different biofloc systems (Error bar showing standard deviation of three replicates (*n*=7). Significance between different groups (P<0.05) marked with asterisk. Error bar with no superscripts is not significantly different (P>0.05). BS: starch, BD: dextrose, BM: molasses, BC: control (without external C))

Discussion

In this study, they identified other C sources that affect the sturgeon. No studies have reported comparable growth parameters of Russian sturgeon BFT using various C sources. Therefore, it is necessary to compare the growth parameters of the current study with those of Aghabarari et al. (2021). Using only a single C source in the beluga sturgeon (*Huso huso*) and Khanjani et al. (2021) studied the effect of different C sources on the growth performance of Nile tilapia (*Oreochromis niloticus*). Aghabarari et al. (2021) cultured beluga sturgeon (120 fish, 3% feeding rate) with an average initial weight of 168.2±2.9 g in 4 m^3 liter fiberglass tanks for 8 weeks. In another study, Khanjani et al. (2021) cultured Nile tilapia fingerlings (average weight: 1.7 ± 0.1 g, 160 fish/tank, 6% feeding rate) in 160-liter tanks for 30 days. The current study is a small-volume aquaculture model and was carried out in 0.4 m^3 tanks in a controlled environment (21 fish/tank, 3% feeding rate). The protein ratio of the feed used in the present study is 45%. Khanjani et al. (2021) gave the protein content of the feeds as 35% in his research, while the protein ratios of the feeds were not reported in the study of Aghabarari et al. (2021).

Biofloc contains nutrients such as fatty acids, amino acids, and minerals that improve the growth performance of aquatic organisms (Ju et al., 2008; Najdegerami et al.,2016; Ahmad et al., 2017; Panigrahi et al., 2019; Adineh et al., 2019). Khanjani et al. (2021) reported the group using corn, starch, molasses and barley as C sources in Nile tilapia achieved better weight gain than the control group. According to the same study, the group that used molasses and starch had significantly higher survival rates than the other group that used corn and barley. Likewise, Durigon et al. (2020), Hoang et al. (2020), Khanjani et al. (2020) and Mirzakhani et al. (2019) reported that the growth performance was better in BFT in tilapia. In contrast to previous studies, Aghabarari et al. (2021) found no significant difference between biofloc and control groups in terms of final weight and survival rate in beluga sturgeon. Although, present study using molasses, starch and dextrose as a C source, the C/N ratio was optimized to be 1/20, Aghabarari et al. (2021) did not report the C source and the C/N ratio. In terms of weight gain, unlike Khanjani et al. (2021), it was found that only the starch performed better in the current study. Upon closer examination of the details, it should be noted that the current study used tanks with a water volume of 0.4 m^3 and surface area of 1 m^2. The study conducted by Aghabarari et al. (2021) used larger tanks (4 m^3) in a greenhouse, which could induce the development of algae and zooplankton resulting in, additional nutrient sources in the water beyond the biofloc. Furthermore, Khanjani et al. (2021), conducted a study for 30 days, providing twice the daily nutrient input compared to the current study, indicating a higher nutritional input for Nil tilapia compared to the current study. Although the molasses and control groups showed similar differences in the current study compared to Aghabarari et al. (2021), a full comparison could not be made due to the lack of reported C source used by the researcher. Despite the same duration of the study (8 weeks) difference may be explained by the lower water volume (160-400 liters) and larger fish size used in the study by Aghabarari et al. (2021) as well as the higher initial bodyweight of the great sturgeon compared to the Russian sturgeon used in the current study.

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These differences between studies suggest that weight difference could be considered.

FCR is an important parameter used to determine the effectiveness, suitability, and acceptability of specially prepared feeds for fish. Aghabarari et al. (2021) reported an FCR as 1.50±0.16 for control and 1.20±0.33 for BFT, with a survival rate of 100% in each group. Since there were no mortalities in the current study, it can be said that the studies are similar. FCR in the current study were as follows: BS: 2.18±0.01, BD: 2.46±0.02, BM: 2.16±0.00, F: 2.39±0.00, depending on the groups. both studies had a feeding rate of 3%, but objective comparison is not possible, considering that Aghabarari et al. (2021) did not report the protein content of the feed used in the study. The FCR of beluga sturgeon in both the biofloc and control groups was lower than in the current study. Differences may be due to initial fish weight, protein ratio of the feed, and system differences, as discussed above. Therefore, FCR must be supported by other critical parameters such as stock density and survival rate, as it is insufficient to measure efficiency without comparing it with other parameters.

Water temperature, feeding rate and fish size are crucial growth factors for fish (Şener et al., 2006). The current study found that the best final weight results and SGR were in the BFT groups using starch and molasses, with no significant difference observed between the dextrose and control groups. Similar findings were reported in other studies conducted on tilapia (*Oreochromis mossambicus*) (Avnimelech, 2007), freshwater shrimp (*Macrobrachium rosenbergii*) (Asaduzzaman et al., 2008), and western white shrimp (*Litopenaeus vannamei*) (Xu et al., 2012), which showed that the biofloc groups had better results than the control group. Although Aghabarari et al. (2021) reported no significant difference in final weight between the groups, SGR was found to be better in BFT. Therefore, the high growth indexes of BFT can be attributed to the presence of bacteria in the water, which acts as a factor that improves environmental conditions.

Many studies have been published on improving the immune system. Researchers have found that various supplements can increase immunity in fish, including tulsi (Das et al., 2015), levan (Gupta et al., 2008), levamisole (Maqsood et al., 2009) and β-glucan (Misra et al., 2006). Additionally, increasing the number of beneficial bacteria in the water has been shown to elevate the presence of components such as carotenoids, Poly b-hydroxybutyrate (PHB), chlorophylls, and polysaccharides in the water, this strengthens the immune system of fish, and also helps to reduce viral, bacterial, and pathogenic effects In the current study, the effects of different C sources on the immune system were examined, and when the results were evaluated, no difference was found between C sources. The results indicate that it is possible to grow sturgeon in the BFT, and this system positively affected non-specific immune indicators such as NBT. This accords with the findings from Ekasari et al. (2014), Komara et al. (2022), and Ahmad et al. (2017).

MPO and lysozyme are known as enzymes that defend against bacterial infections in aquatic organisms. The detection of the highest MPO and lysozyme activity in the BFT explains that BFT improves the immune system in sturgeon fish and creates a stronger defense system against disease factors. The increase in MPO and lysozyme activity is similar to previous studies by Mansour & Esteban (2017) in *O. niloticus*, Ahmad et al. (2017) in Indian major carp (*L. rohita)*, and Zhang et al. (2018) in gibel carp (*Carassius auratus gibelio)*.

Conclusion

The growth performance and health characteristics of sturgeon, which are in danger of extinction, were compared in the present study using different C sources in BFT, a new aquaculture technology. It has been determined that BFT saves water by minimizing the water content change, and thus, ecosystem damages that occur in aquaculture activities carried out in traditional methods have been significantly reduced. In this study, where different C sources were used, growth parameters such as weight gain, SGR and FCR were positively affected in the groups using molasses and starch, compared to the control and dextrose groups. On the other hand, results showed that each different C source used strengthened the immune system.

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Compliance With Ethical Standards

Authors' Contributions

SB: Conceptualization, Supervising
TÖ: Conceptualization
İSY: Writing, Review, Editing
All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The protocol was approved by the Animal Use and Care Committee of Central Fisheries Research Institute (SUMAE), Republic of Türkiye Ministry of Agriculture and Forestry via document No. Etik-2023/14.

Data Availability Statements

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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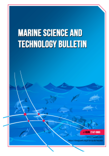

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RESEARCH ARTICLE

Investigation of the effect of anti-fouling systems on meeting energy efficiency regulations

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ABSTRACT

The operational efficiency of marine vessels should be kept as high as possible to achieve sustainable development goals in the maritime field. However, a lot of factors such as resistance components reduce the operational efficiency of the ship. Frictional resistance is the biggest resistance component for the power needed on ships and its coefficient increases due to the biofouling as long as the ship interacts with seawater. The increased total resistance of the ship causes extra power needed and excessive fuel consumption to reach service speed. The increase in both fuel consumption and power will create an obstacle to meeting the EEXI and CII reference values which became mandatory after January 1, 2023. That's why the utilization of effective anti-fouling systems is quite critical in maritime applications. The purpose of this study is to reveal anti-fouling systems' effect on EEXI, CII, and CII ratings by utilization of the container ship operated in liner shipping. That's why, high, medium, and low effective anti-fouling system scenarios have been created since the effect of each anti-fouling will not be the same on the container ship. According to the results, the required EEXI and CII reference values will have been met respectively when the effect of ship biofouling is ignored. However, the reduction ratios and biofouling effect have created quite a challenge in meeting EEXI and CII in the following years. Although the required EEXI value has been met for 2024 and 2025 by the high-effective anti-fouling system and reference value has not been met by the low-effective anti-fouling system in the following years. Any anti-fouling system utilized in this paper won't be sufficient to meet the reference value at the end of 2023 because attained CII of the container ship is very close to the reference value of CII in 2023. The CII rating of the container ship will have been at C level until the end of 2026 when the biofouling effect is ignored. However, it decreased to D and E levels in the following two years depending on the best and worst scenarios. This study will be a valuable resource for scientists, researchers, experts, and maritime stakeholders who want to investigate the effect of EEXI, CII, and CII rating of antifouling systems.

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Introduction

International Maritime Organization (IMO) works to reduce marine pollution originating from ships as much as possible to ensure sustainable development goals (IMO, 2023a) since more than 80% of global transportation is carried out by maritime transportation (UNCTAD, 2023). Biofouling is one of the obstacles to realizing sustainable and efficient operations in maritime transportation. Biofouling occurs when aquatic organisms growing quite rapidly attach to the ship's hull or are contained in ballast water (Levin, 2013). The first organic molecule initiates attaching to the ship hull in one minute as soon as the ship's hull submerges into the water and starts the fouling process. Growing aquatic organisms have simply been transferred from one region to another utilizing large-scale maritime transportation. Aquatic organisms in one area can be invasive to transferred local areas and they result in the degradation of aquatic life and biodiversity (Davidson et al., 2016; IMO, 2023b). The ever-developing maritime trade has triggered the effect of biofouling and that's why the Marine Environment Protection Committee (MEPC) took a decisive step and published a guideline for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species (IMO, 2003) as well as researchers, managers, and policymakers have highly concerned translocated invasive organisms (Davidson et al., 2016). Many coating, paint, surface treatment, or device systems have been utilized to prevent unwanted organisms that have the potential to attach to the ship's hull (Wartsila, 2023). Lime and arsenic were used in early ship operations and then tributylamine (TBT) which was the most well-known antifouling painting in modern times had been utilized to protect biofouling. however, studies revealed that tributyltin has highly poisonous effects on sea life and causes irreversible damage to the environment and economy. That's why the International Convention on the Control of Harmful Anti-Fouling Systems on Ships (AFS Convention) has been adopted to ban the use of harmful organisms in antifouling systems by IMO (IMO, 2003). Moreover, the utilization of Cybutryne in anti-fouling systems has been banned as of January 1, 2023 (DNV, 2022).

On the other hand, biofouling especially occurred in ship hulls has also adversely affected the ship's hydrodynamic performance due to creating additional resistance (IMO, 2003). More power is needed to reach service speed in the fouled hull compared to the clean hull (IMO, 2023b). Small-scale ship hull fouling leads to increases of up to 40% in fuel consumption, and even this rate reaches 50% depending on the vessel speed (IMO, 2003; IMO, 2023b).

The age and condition of the ship utilized anti-fouling coating systems, service speed, water temperature as well as operation profile which refers frequency of idling, transit, and maneuvering are critical factors in the growth of biofouling. Moreover, prolongation of the ship's spending time at anchor accelerates the growth rate. Rudder, propellers, and propeller shafts are among the most susceptible areas to biofouling. The frequency of anti-fouling coating system maintenance intervals postpones the formation of fouling as much as possible (IMO, 2012).

The propulsion dynamics effects of hull fouling are one of the primary concerns in the maritime sector (Davidson et al., 2016). A ship with clean hull cruises more with less power because friction resistance, which is one of the biggest resistance components on the ship, is minimized (IMO, 2003). Extra power needed increases fuel consumption and fuel-based emissions in the ship operations with the fouled hull (IMO, 2023b). In addition to the adverse effect on the ship's performance, fouling increases the formation of corrosion and causes clogging of the necessary water inlets for the emergency fire pump, scrubber system, sea chest, and more (IMO, 2012).

One of the sustainable development goals is climate action since global warming has constantly been increasing which will have devastating consequences such as flooding, drought, poverty, inability to fundamental needs, and more. The increase in global warming is 1.1°C above the pre-industrial period which is the highest increment experienced in recent years. Almost a quarter of global energy-related CO_2 emissions are caused by transport and approximately 2% of the energy-related CO_2 emissions are attributable to international shipping (IEA, 2009; United Nations, 2023).

The IMO under the GHG strategies aims to reduce carbon emissions by 40% and 70% rates by 2030 and 2050, respectively compared to 2008 (IMO, 2023c). IMO has announced the energy efficiency existing index (EEXI) and carbon intensity indicator (CII) ratings to reduce the CO_2 emission from ships (Bayraktar & Yüksel, 2023; Konur et al., 2023). These metrics require the efficient operation of marine vessels to lower the fuel consumption of the vessel. Decreased performance of the vessel due to the fouling can cause it not to meet these requirements. EEXI has been created under the greenhouse gases (GHG) emission reduction strategy to increase the energy efficiency of ships step by step. Ships above the EEXI reference line will have been out of service over time.

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This study examines the effect of the fouling by employing various fouling factors on a reference container vessel to observe the fuel consumption increase. The impact of the increased fuel consumption on the EEXI, CII, and CII ratings have been examined and discussed. The paper structure consists of literature review, research gap and contribution, methodology, results and discussion, and conclusion sections.

Literature Review

The main purpose of antifouling systems is to minimize the impact of the settlement of fouling species on drag resistance as much as possible (Munk et al., 2009). Various fouling prevention systems have been utilized and investigated in research papers. Turan et al. (2016) investigated the fouling effect on the LNG tanker by using three plates that have different frictional resistance coefficients. Artificial barnacles have been utilized on plates with 5%, 10%, and 20% coverage to create frictional resistance. The frictional resistance coefficient has doubled in the third plate corresponding to 20% coverage and substantial drag characteristics have been experienced on the remaining plates. Needed effective power has increased by 22.5%, 41.3%, and 59.7%, respectively, due to plates covered with artificial barnacles compare to operations of the LNG tanker with a clean hull at speeds of 20 knots. Performance reduction rate may differ in real ship applications since artificial barnacles have homogeneously been distributed on the plate. Hakim et al. (2017) have conducted towing test to explore the marine fouling effect using sandpapers that have different roughness values. Ship hull having irregular roughness has caused a 41.88% resistance increment to compare the smooth ship hull model. Hakim et al. (2017) recorded the full consumption rate of ferries throughout 11 months after dry-docking. The fuel consumption rate increased by 20% at the end of the period due to fouling.

Hakim et al. (2019) have investigated two different anti-fouling coating applications in terms of fuel consumption. Approximately 20% fuel consumption increase has been recorded at the end of the 9.5 months in the first sea operations using the regular antifouling paint. Before the second operation, the ship entered the dry docking to polish and repair of ship hull and propeller. The second operation of the ship in which higher quality antifouling paint has been used on the hull and propeller has been monitored for 11.5 months period. The fuel consumption has only raised 5% at the end of the period after dry-docking with small fluctuations. Notti et al. (2019) have utilized a fluoropolymer-based anti-fouling paint with a biocide-free feature on a Mediterranean bottom trawler for fuel consumption reduction. The fluoropolymer-based anti-fouling coating has provided fuel consumption saving and carbon emissions. Hull fouling effects have been examined under two different operation modes trawling and cruising throughout approximately three years. Traditional antifouling paint has been applied and measurements regarding Vessel speed and fuel consumption have been recorded for one year before the ship hull has been cleaned. After that fluoropolymer-based anti-fouling paint have been applied over the following two years on trawler ships to compare antifouling systems. A fluoropolymer-based anti-fouling paint has provided almost 10% and 3% fuel savings in Trawling and Cruising operations, respectively compared to the ordinary anti-fouling system.

Farkas et al. (2020) have created different Fouling Conditions in which the roughness length scale is calculated by the height of the largest barnacles and the percentage of the surface covered with barnacles. Container ships, Very Large Crude-oil Carriers, and Bulk carriers equipped with KP505, KP458, and WB propellers, respectively, have been utilized to assess different fouling conditions. The results have revealed that the total resistance coefficients have increased even more than 100% according to the ship type in the most severe pollution conditions. In addition, at least a 50% change in the total resistance coefficients has been observed even at the lowest fouling condition. Erol et al. (2020) have investigated the fouling impact on ship performance deterioration by continuous monitoring. speed loss of around 6% has been recorded on the ferry at the end of the nine months. Laurie et al. (2021) have analyzed performance deterioration due to hull fouling combined with machine learning techniques and data logging systems. Ship hull and propeller fouling have caused a 5.2% change in power requirement in one year.

The ship maintenance intervals must be optimized to avoid the fouling impact most effectively (Farkas et al., 2020). The utilization of effective anti-fouling paint postpones the dry-docking of the ship which not only reduces maintenance costs but also extends the duration of ship operation (Notti et al., 2019) and vice versa (Davidson et al., 2016). Effective anti-fouling systems have also enabled faster ship operations with constant power or meet needed service speed with low power (Turan et al., 2016) that provides a reduction of greenhouse gas emissions and economic efficiency throughout the ship operations (Davidson et al., 2016; Hakim et al., 2017). Apart from fouling, shipload conditions, weather, waves, engine degradation, and aging have also affected fuel consumption rates (Hakim et al., 2017).

Research Gap and Contribution

The literature review indicates the effect of various fouling conditions (Hakim et al., 2019; Notti et al., 2019; Farkas et al., 2020), anti-fouling usage, and various anti-fouling techniques on ship resistance, and fuel saving (Turan et al., 2016; Erol et al., 2020; Laurie et al., 2021). This study aims to fill a research gap by investigating the impact of different fouling conditions on compliance with EEXI and CII requirements. The main motivation is to demonstrate the importance of the fouling effect and possible undesirable efficiency decreases which result in increased carbon tax rates due to low CII ratings or not meeting the EEXI required value. To achieve the objective, a container vessel and its sample route have been determined as the case study. Different anti-fouling techniques that result in various ship resistance increases have been assessed and the impact of the efficiency decreases on EEXI and CII ratings have been depicted and discussed. This research paper can contribute to the literature by demonstrating the importance of the fouling effect from a different perspective and can be useful for academics and maritime sector workers in the related field.

Material and Methods

Methodology

The methodology section has been divided into two sections which are system description and modeling. In the system description subsection, the specifications of the ship and its route have been indicated. The modeling section depicts the mathematical background and assumptions of the calculations. Figure 1 illustrates the flow chart of the methodology used in the paper.

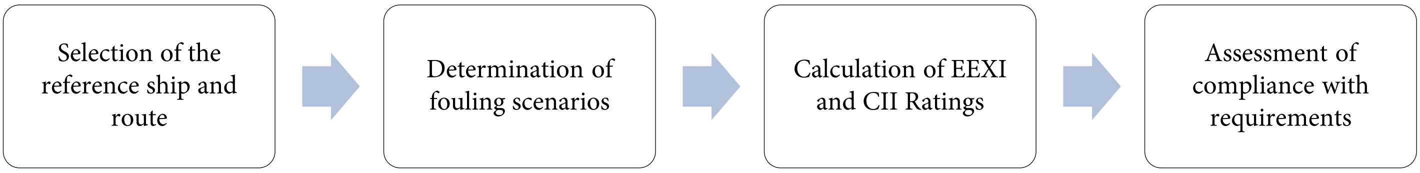

Figure 1. Flowchart of the methodology

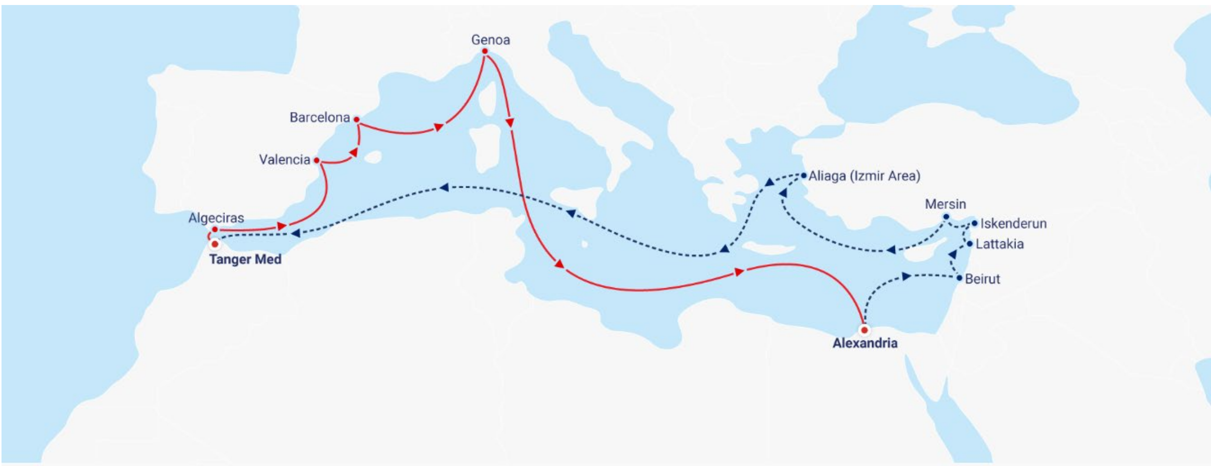

Figure 2. The route of the selected container ship (CMA CGM, 2023)

Table 1. Specifications of the reference container vessel

Parameters	Value	Unit
Gross tonnage	27,910	t
Summer DWT	39,479	t
Length × Beam × Draught	222 × 30 × 11	m^3
Service speed & Max. speed	22 & 24	KN
Capacity	2.824	TEU
Main engine	25,228	kW
Specific fuel oil consumption	174	g/kWh

System Description

Since the biggest percentage of CO_2 emissions in the world fleet came from container ships (European Commission, 2023), a container ship has been selected as a reference vessel for analysis to reveal the effect of ship hull and propeller fouling on EEXI, CII, and CII ratings. The route of the reference container vessel is a liner shipping line and shown in Figure 2. The illustrated route is the real course followed by the reference vessel during its service time.

There are eleven ports of call in the eastbound and westbound routes which are completed in 28 days. The total line length is approximately 6000 nautical miles. The specifications of the container ship have been listed in Table 1.

Modeling

The EEXI Concept formula described in Equation 1 has been used for the calculation (ClassNK, 2021).

$$EEXI\ \left[\frac{g}{ton*mile}\right] = \frac{CO_2\ Conversion\ factor*SFC\left[\frac{g}{kW}*h\right]*Engine\ Power[kW]}{Capacity\ [ton]*V_{ref}} \qquad (1)$$

where *SFC* is specific fuel consumption, *capacity* is the deadweight (DWT) of the ship and V_{ref} is the reference speed of the vessel. Required *EEXI* which is the upper allowable limit for the vessel, can be calculated using Equation 2.

$$Required\ EEXI = \left(1 - \frac{X}{100}\right) * EEXI\ Reference\ Line \qquad (2)$$

The reference value of EEXI calculated in 2023 will have been decreased linearly by applying the reduction factor (X) in the following years. By 2030, there will have been a 20% reduction for the selected vessel in total compared to the initial value (ClassNK, 2021). The reference line of EEXI will have lowered at a specific rate each year according to ship types to provide ongoing advancement in maritime transportation. The calculation of the reference EEDI line for a container ship can be conducted by employing Equation 3.

$$EEDI\ Reference\ Line\ = DWT^{-0.201} x\ 174.22 \qquad (3)$$

Attained CII is calculated by employing Equation 4 based on fuel oil type, carbon factor, the capacity of the ship, and total distance (ClassNK, 2021).

$$Attained\ CII\ = \frac{FC_j * C_{f_j}}{Capacity * D_t} \qquad (4)$$

In Equation 4 the subscript j is the fuel type, FC represents fuel consumption in g, D_t stands for the traveled distance in nautical miles, and C_f is the carbon factor of the fuel. CII will have also been lowered reference value the following year as in EEXI. Unless any improvement is performed on the ship to lower the attained CII value, the CII rating will decrease to lower levels each following year (IMO, 2021a). Required CII is computed by utilizing Equation 5.

$$Required\ CII\ = \left(1 - \frac{Z}{100}\right) * CII_{Ref} \qquad (5)$$

Z refers to the reduction factor relative to the 2019 CII Reference value (CII_{Ref}). The reduction value Z is increasing as 5, 7, 9, and 11 every year throughout 2023-2026 (IMO, 2021a). The increasing rate of reduction every year triggered a decrease in the CII rating. CII_{Ref} is calculated using Equation 6 based on the dimensionless coefficients and Capacity shown in Table 2 for a container ship (IMO, 2021b).

$$CII_{Ref}\ =\ aCapacity^{-c} \qquad (6)$$

Table 2. Parameters to calculate the reference CII and to decide the CII rating boundaries

Capacity	a	c	d1	d2	d3	d4
DWT	1984	0.489	0.83	0.94	1.07	1.19

Using the values (d1 to d4) CII rating boundaries can be decided for a container vessel. The CII rating scale is illustrated in Figure 3. The negative effect of the hull fouling on the ship resistance power curves directly affects the attained Energy Efficiency Existing Ship Index (EEXI), Carbon Intensity Indicator (CII), and CII rating. EEXI has become mandatory as of January 1, 2023, to reduce greenhouse gas emissions (DNV, 2023). The significant increase in fuel consumption due to hull fouling is a difficult obstacle to meeting both EEXI and CII reference values. Biofouling Management is one of the methods recommended by IMO to comply with CII reference values since the amount of fuel consumption is the most critical parameter in both EEXI and CII calculations (ClassNK, 2021).

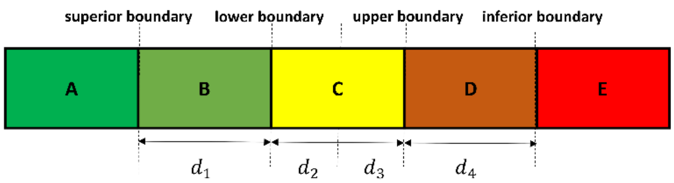

Figure 3. Rating limits according to dd vectors (IMO, 2021c)

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The performance of the ships has varied according to the efficiency of the anti-fouling systems used on the ship hull and propeller. In this study, 3 different anti-fouling systems which are low, medium, and high effective have been applied on container ships Hull, and propeller fouling factors that increase the fuel consumption by 5%, 10%, and 20% to maintain the service speed of a container ship have been considered regarding the results of the literature research. EXXI, CII, and CII rating changes over two years have been analyzed based on 3 different performance changes due to fouling. The following assumptions have been performed during the calculations.

- An average speed of 14.4 knots has been acquired when the main engine has been operated at approximately 40% load.
- Auxiliary engines have used MDO up to 10% of the total fuel consumption of the main engine.
- Any application has not been performed to prevent ship hull and propeller fouling for the following two years after anti-fouling applications were performed on the containership.

Results and Discussion

Calculated EEXI and CII values of the reference container ship have been described for 2023 and depicted in Table 3. The reference value of EEXI calculated in 2023 will have decreased linearly in the following years. There will have been a 20% reduction in total compared to the initial value.

Table 3. EEXI and CII values of container ship

Reference EEXI	Attained EEXI	CII_{Ref}	Attained CII
20.76	19.53	11.22	10.61

The change in the Reference Value of EEXI for the reference container ship has been illustrated in Figure 4. Any performance degradations such as hull and propeller fouling and any improvements performed on container ships have not been considered for the calculation of values shown in the figure.

EEXI reference values of the vessel will have met international regulations until the end of 2025 in the base scenario. EEXI values have been calculated considering different anti-fouling applications for the following two years in Table 4.

Table 4. EEXI Values under different anti-fouling systems

Conditions	EEXI Values	
	2024	2025
High effective anti-fouling system (I)	20.02	20.52
Medium effective anti-fouling system (II)	20.5	21.53
Low-effective anti-fouling system (III)	21.48	23.63

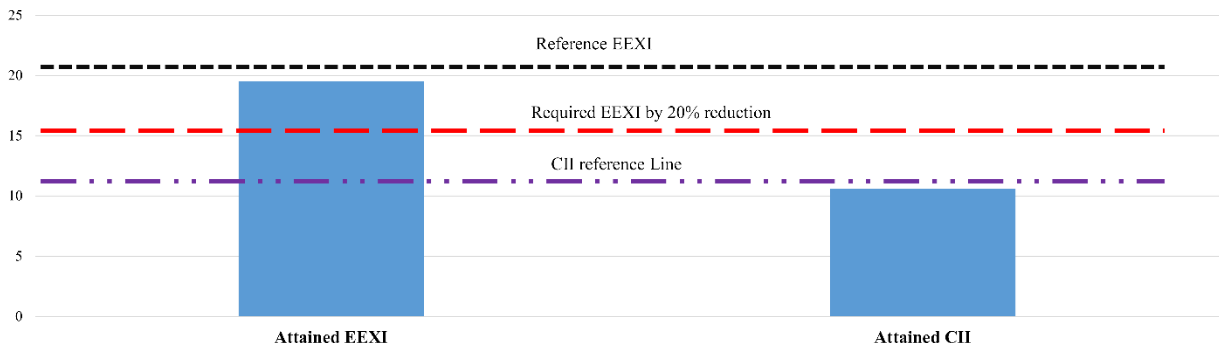

Figure 4. Required and attained EEXI values of the reference container vessel

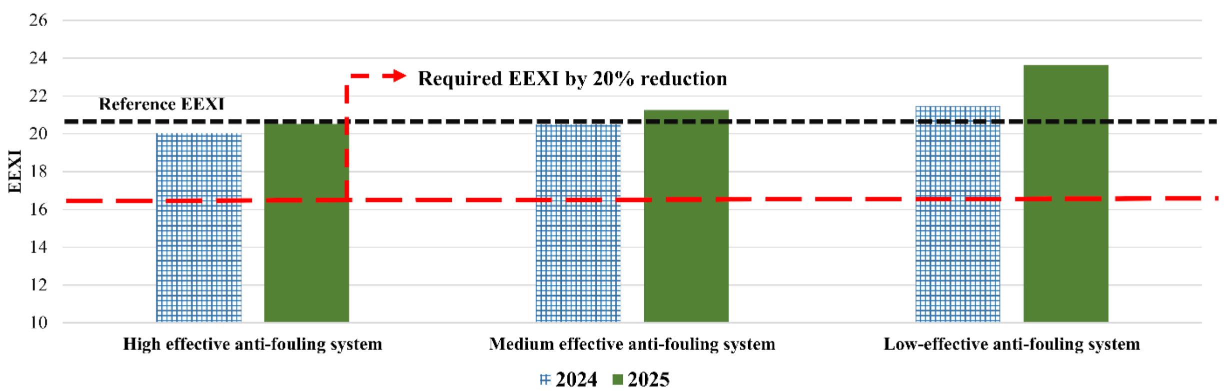

Figure 5. States of meeting EEXI reference values under different anti-fouling applications for container ship

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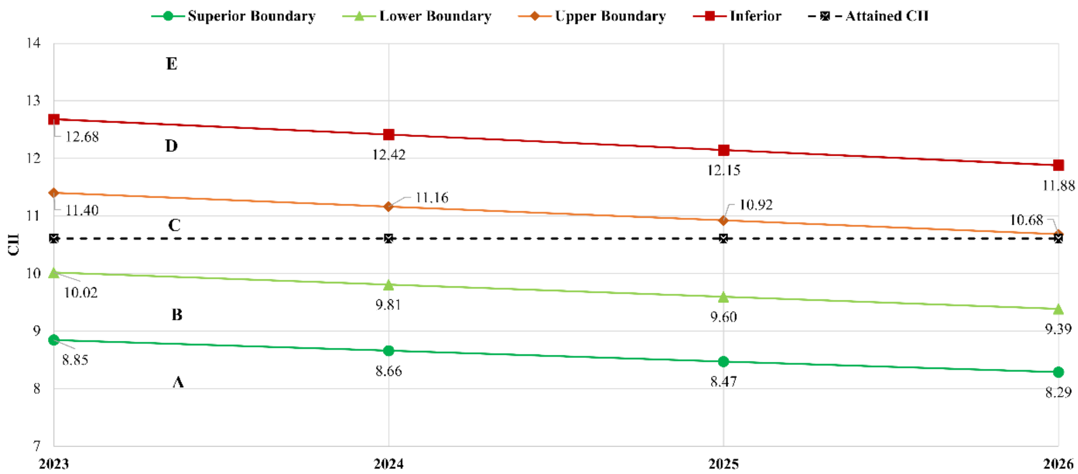

Figure 6. CII boundary limits and attained CII of the reference vessel

Performance decrease and increased fuel consumption due to fouling formation have affected the EEXI values of the reference vessel. Figure 5 indicates whether different fouling applications meet international regulations according to the required EEXI values specified in Table 3.

Superior, lower, upper, and inferior boundaries' values have been calculated for future years by using the coefficients shown in Table 2 for the following years. The boundary limits utilized in CII rating determination have been expressed in Figure 6.

CII ratings of container ships have been calculated by ignoring the hull and propeller fouling effect on the container ship. Different anti-fouling applications' effects on the CII ratings have been described in Table 5.

Table 5. CII ratings under different anti-fouling applications for the reference ship

Condition	I		II		III	
Year	2024	2025	2024	2025	2024	2025
Attained CII	10.87	11.15	11.14	11.70	11.67	12.84
Rating	C	D	C	D	D	E

Energy efficiency measures have been taken to provide sustainable maritime transportation by maritime authorities. An effective anti-fouling system which is one of the energy-saving methods both protects the hull of the ship and prevents performance degradation. after the ship is left from dry docking in which the ship's hull and propeller are cleaned, operational performance is at the highest level. The effective anti-fouling system minimally affects the ship's performance and provides the ship to sustain the highest level of performance. This helps ships to pass the EEXI and CII thresholds that became mandatory after 1st January 2023. EEXI, CII, and CII ratings have been calculated by utilizing logging performance data getting from ships. Therefore, the high-effective anti-fouling system has minimized ship performance fluctuations throughout two dry docking periods.

The EEXI value of the selected container ship has met international regulations by staying below the EEXI reference value on the determined route when the bio-fueling effect on the ship's hull and propeller has been ignored. After the end of 2023, the effect of fouling on the EEXI value of the container ship has been examined by utilizing 3 different anti-fouling systems. While the container ship equipped with the high-effective anti-fouling system has met the EEXI reference value, the required EEXI of 20% reduction will not be met by the high-effective anti-fouling system. On the other hand, the EEXI reference value will have been met until the end of 2024 by the medium effective anti-fouling system. However, the EEXI reference value will not have been met when the year 2026. In addition, the EEXI reference value has not been met by a low-effective anti-fouling system at any time. Attained CII of the container ship has met international regulations by the end of 2023 staying under the CII ref value when the bio-fouling effect is ignored. Moreover, the Container ship has been C level under the CII rating considering throughout the boundaries condition 2019 to 2026. However, the CII rating of the container ship is C for 2024 and D for 2025 in high and medium effective anti-fouling system applications when the bio-fueling is considered on the ship's performance. it is D for 2024 and E for 2025 in low-effective anti-fouling system applications.

Therefore, new applications should have been performed to meet the EEXI reference value and CII_{Ref} following years besides an effective fouling system. The utilization of alternative or renewable fuels such as LNG, methanol, ammonia, biofuels, hydrogen, nuclear, solar, wind, and wave power has come to the fore in complying with the EEXI and meeting CII_{Ref} in maritime transportation. Moreover, the utilization of Shaft power limitation (SHaPoLi) and engine power limitation (EPL)

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on ship propulsion systems have provided advantages meeting EEXI and CII reference values. When all these applications have been used in combination on the ship, they will also ensure that CII ratings are maintained at A level for a long time.

Conclusion

A significant part of international transport has been carried out by maritime transportation. Therefore, the operational efficiency of ships in maritime transport has a global impact, both environmentally and economically. the operational efficiency of the ships has deteriorated by many factors such as the ship's crew experience, weather conditions, sea state, and more. Biofouling is one of the factors that reduce the ship's performance in terms of both fuel consumption and power. Increased fuel consumption and added power needed due to biofouling is a major obstacle to achieving EEXI, and CII reference values getting even lower over the years. The performance of the ships has been maintained at a high level for a long time with the high-efficient anti-fouling systems after launching from the dry dock in which the ship's hull and propeller are cleaned. However, the ship's performance-enhancing applications should be performed as well as maintaining the existing performance to comply with the new regulations. The utilization of alternative and renewable fuels, SHAPoLi, and EPL applications have stood out within the scope of energy efficiency measures.

This study will help reveal the anti-fouling systems' impact on EEXI, CII, and CII ratings that can be beneficial for academicians, marine engineers, and ship surveys whose working fields are related to the fouling-related fuel consumption increments. Future studies can cover the analysis based on recorded speed and fuel consumption data to observe the impact of the biofouling growth, the effect of the antifouling techniques on the EEXI, CII, and CII ratings, or an experimental study to observe the biofouling effect and timing on the hull or propeller surface.

Compliance With Ethical Standards

Authors' Contributions

MB: Conceptualization, Investigation, Formal analysis, Methodology, Writing.
OY: Conceptualization, Investigation, Visualization, Methodology, Writing.
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

All data generated or analyzed during this study are included in this published article.

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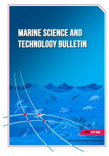

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RESEARCH ARTICLE

Protective role of bio-based coating of ultrasound-improved trout (*Oncorhynchus mykiss* Walbaum, 1792) waste protein hydrolysate for bonito (*Sarda sarda* Bloch, 1793) fillets at storage at -18±1°C

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ABSTRACT

A novel and rich protein source was utilized in bonito fillets to prevent/ delay deterioration during frozen storage at -18±1°C. Accordingly, trout wastes protein hydrolysates (PH); produced enzymatically traditional protein hydrolysate (TPH) and ultrasound–treated protein hydrolysate (UPH) containing 86.40 g/100 g and 86.75 g/100 g protein respectively used as a coating. Hydrolysates were mixed with glycerol (2:1) to form coating materials. Three groups of fillets were prepared as control fillets (C) without coating, TPH coated fillets (TPHCF), and UPH coated fillets (UPHCF). pH, color, TVB-N, TBA, and TMA, and sensory analyzes were performed in all groups. The L* value of all groups reached a maximum the highest value at 6 months and was 54.56±0.27, 53.74±0.23, and 54.83±1.26 for C, TPHCF, and UPHCF, respectively. TVB-N was 18.08±0.10, 17.71±0.09, and 17.36±0.12, for C, TPHCF, and UPHCF, respectively, in the first month of storage. The values reached 32.18±0.29, 26.61±0.12, and 25.72±0.08 at 7th month for C, TPHCF, and UPHCF, respectively. TBA value of the C group samples of the frozen bonito fillets reached 7.53 in the 7th month, it remained within the consumable limits, and it remained within the consumable limits in the coated groups. Significant increases occurred in TMA values of all groups between months during the seven-month storage period. Accordingly, the TMA values for C, TPHCF, and UPHCF were 2.56±0.04 mg/100 g, 2.12±0.04mg/100 g and 2.16±0.06mg/100 g, respectively, at the 7th month. The mean values of sensory parameters were 9.15±0.08; 9.51±0.12 and 9.46±0.13 for C, TPHCF, and UPHCF, respectively, at the 1st month of storage. While they were 5.29±0.09, 6.23±0.06 and 6.24±0.09, in THE same order, respectively. Results showed that TPHCF and UPHCF have a potential as a coating for bonito fillets at frozen conditions, prolonging the shelf life.

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Introduction

Freezing is extensively and effectively applied for the conservation of fishery products. It prevents or minimizes undesirable chemical changes in fresh fish to preserve seafood and its properties.

Although microbial spoilage can be prevented to a large extent by freezing, due to temperature fluctuations caused by physical and biochemical changes and repetitive freeze-thaw processes during frozen storage, the distribution of water in muscle tissue and water holding capacity were found to be affected. The changes occur as color changes and tissue destruction by protein denaturation and lipid oxidation (Sriket & Laongnual, 2018).

In frozen fish, edible coatings can reduce quality losses with their features such as control of moisture, prevention of oxygen permeability, prevention on flavor, and can extend shelf life (Dehghani et al., 2018).

In recent years, as the people's awareness increased about the utilization of synthetic additives has potential risks both for health and environmental issues, the food sector is trying to use science and technology to add value to animal by-products to produce natural agents for prevention purposes on food (Aliotta et al., 2019).

As the range of processed products in seafood has increased, there have been significant increases in by-products and wastes, which are often become trash or utilized for animals such as feed, fertilizer and etc. However, having a significant nutritional value. Using innovative technologies, the evaluation of waste as functional components is an increasing focus of attention. (Al-Khawli et al., 2019).

Fish protein hydrolysates (FPH) are produced by breaking down proteins into small chain peptides and amino acids by chemical or biological means. Chemical hydrolysis is generally applied in industrial applications. On the other hand, biological hydrolysis used preferred by using enzymes for it results in products with high functionality and nutritional value (Krisstinson & Rasco, 2000). Essential amino acids are occurred with functional properties that can be evaluated in the fields of food and pharmacy. Due to their high content of essential amino acids, these products can be used as protein enhancers, as a substitute for milk powder, as a fat binder in meat products, as an emulsifier, and as an emulsifier with a high water binding capacity in foods (Nikoo et al., 2014; Karnjanapratum & Benjakul, 2015; Shen et al., 2022).

Some studies were conducted on FPH and peptides used as a coating in frozen foods. They have been applied as antioxidant and antimicrobial agents in the prevention of quality losses during storage (Gokoglu, 2019). FPH forms a physical barrier, that prevents the product from physical, chemical, and microbiological effects (Loi et al., 2019).

The aim of present research to evaluate the potential of ultrasound-improved trout wastes protein hydrolysates as coating for frozen trout fillets stored at -18±1 ±1°C for 7 months.

Material and Methods

Materials

TPH and UPH were produced from *Oncorhynchus mykiss* wastes enzymatically and characterized previously. The biochemical composition and antioxidant activity of PHs were determined in Balcik Misir & Koral (2019a). Provided from a local fishery market in Trabzon, Türkiye, a total of 30 bonito (totally 25 kg, with average weight and length 832.21±42.17 g and 38.94±1.08 cm, respectively) were prepared. Food-grade glycerol (99.96% purity W252506, Sigma-Aldrich, Steinheim, Germany) and analytically pure chemicals and reagents were used in the research.

Methods

Bio-based coatings were prepared from TPH and UPH. The preparation of the coating solution was given in Figure 1.

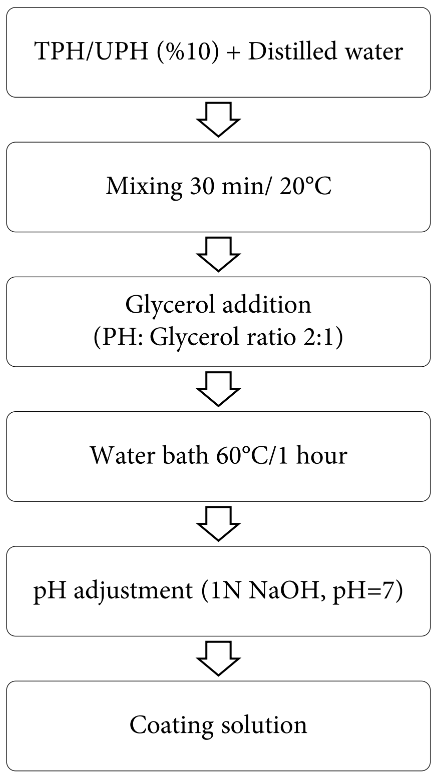

Figure 1. Preparation of coating material from protein hydrolysate (PH)

Bonito fillets were immersed in coating solution under aseptic conditions. After draining on the grill fillets were separated into three: 1) (C) without coating, 2) CH (10% w/v) coated fillet (TPHCF), and 3) UH (10% w/v) coated fillet (UPHCF). The coating process is given at Figure 2.

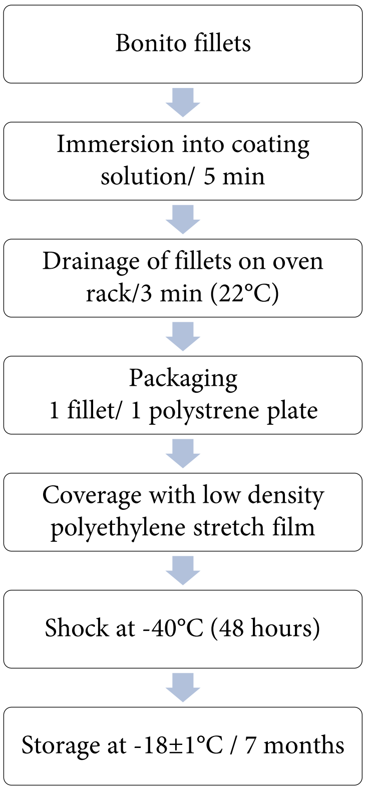

Figure 2. Coating of bonito fillets

Physical Analysis

pH measurement was made by immersing the probe of device (Mettler-Toledo AG, Seven Compact pH meter, 8603 N, Switzerland) into the homogenate (Koral., 2012).

The water activity was determined with the Aqualab 3TE (0.100- 1.000 ± 0.003, Aqualab, USA) working at 25°C (±0.2°C). The amount of water activity in the samples placed in the measuring cups of the device was automatically read and recorded.

Before measuring the L*, a* and b* of the fillets, the device (Konica Minolta, CR 10, Tokyo, Japan) was standardized using a white plate and values recorded from 3 points.

Chemical Analysis

TVB-N analysis was done according to the Lücke & Geidel (1935) method modified by Antonacopoulus and reported by Inal (1992). TVB-N results were given as mg/100g.

TBA values, were estimated using the method described by Tarladgis et al. (1960), results were expressed as mg malonaldehyde/kg.

TMA-N values were analyzed according to Boland & Paige (1971), results were given as mg/100g.

Chemical analyzes were applied as described in detail in Balcik Misir & Koral (2019b). Analyzes were done in triplicate. All reagents were analytical grade.

Sensory Analysis

Six trained panelists performed the sensory changes during the storage of bonito fillets. Panelists gave scores out of 10 based on odor, texture and appearance and average for raw fillets. 10-9 excellent, 8-7 good, 6-5 moderate, 4 acceptable limits, 3.9-1 unacceptable. Sensory parameters were evaluated according to Fisheries Regulation of Türkiye (URL-1, 2011), and Varlık et al. (1993).

Statistical Analysis

One-way ANOVA was utilized for all data. Tukey and Mann Whitney U test was applied if significant differences are found, (data not provided in the normality of assumptions) under the program called JMP5.0.1 (SAS Institute Inc., Cary, NC, USA) and SPSS 18.0 (SPSS Inc., Chicago, IL, USA) (Sokal & Rohlf, 1987). A significance level of 95% ($P < 0.05$) was used for all the analyses.

Results and Discussion

pH value of fresh bonito fillets was measured as 6.16. This value followed a fluctuating course during frozen storage conditions as seen in Figure 3. C showed a significant decrease in the 2nd month this decrease can be attributed to the release of inorganic phosphate, a product of lactic acid formation and ATP breakdown during anaerobic glycolysis. After that with a continuous increase it reached 6.58±0.04 in the 7th month. Similar values were obtained for the TPHCF and UPHCF at the 2nd, 3rd and 4th months. The increase can be dependent on the increase of some elements such as ammonia and trimethylamine due to autolytic and microbial activities. Considering the pH 7 is taken as the limit for consuming, all groups were lower than this limit during the storage.

Kaba et al. (2013) investigated the shelf life of fish meat ball produced with smoked bonito was during refrigeration conditions (4°C). They stated that the pH value of fresh bonito meat was 6.09 at the beginning of the study. Morachis-Valdez et al. (2017) coated carp fillets with chitosan and stored at -18±1°C and monitored the changes in their biochemical, physicochemical, textural, microbiological and nutritional characteristics. The researchers worked with an uncoated control group and a chitosan-coated group. In the study in which five months of storage was carried out, significant decreases were detected in the pH of the control, coated group up to the 2nd month. Karsli et al. (2021) found that the pH fluctuated barely for all groups during the 180-day study, and the pH of the control group were above the coated groups. They attributed this situation to acetic acid and aspartic acid, which they used together with chitosan in the coating material.

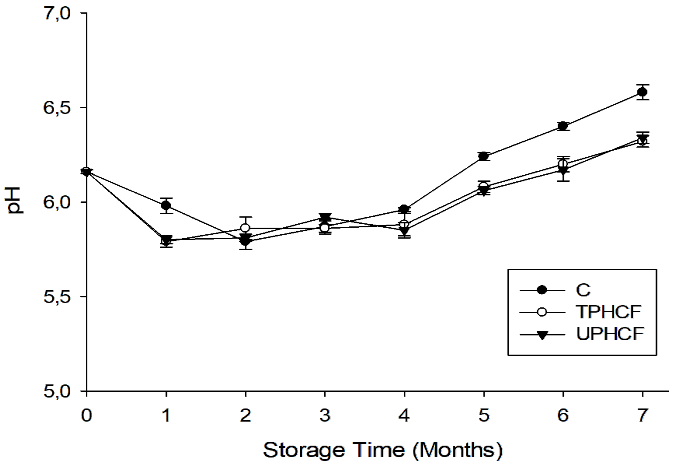

Figure 3. pH values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Color index (L*, a*, b*) of bonito fillets during storage at -18±1°C shown in Figure 4. At 0 day the L* value was 46.48±1.13; it was 51.42±0.24, 51.90±0.47 and 52.13±0.13 for C, TPHCF and UPHCF, respectively. There is no statistical difference between the values on the 1st month of storage ($p<0.05$). While statistically similar values were detected in the 2nd, 3rd, 4th and 7th months in the C group, a significantly lower value was found in the 5th month and a significant increase in the 6th month ($p<0.05$). The L* of all groups reached the highest value at 6th and it was 54.56±0.27, 53.74±0.23 and 54.83±1.26 for C, TPHCF and UPHCF, respectively.

While a* value of fresh bonito fillets was 7.80±0.88, it decreased to 4.94±0.05 4.85±0.06 and 4.75±0.17 for the 1st month C, TPHCF and UPHCF, respectively. The a* value of the UPHCF showed a similar increase and decrease trend with the TPHCF. C, TPHCF and UPHCF reached the highest a* values as of 6.86±0.42, 6.20±0.19 and 6.34±0.13, respectively, in the 7th month.

The b* of fresh bonito was measured as 11.06±0.63. This value increased in all groups at 1 month and was 12.00±0.08 for the groups covered with C, TPHCF and UPHCF, respectively; It was measured as 12.90±0.08 and 12.78±0.13. The highest values for all groups were obtained in the 7th month and were 17.86±0.07, 17.46±0.06 and 17.50±0.08 for C, TPHCF and UPHCF, respectively.

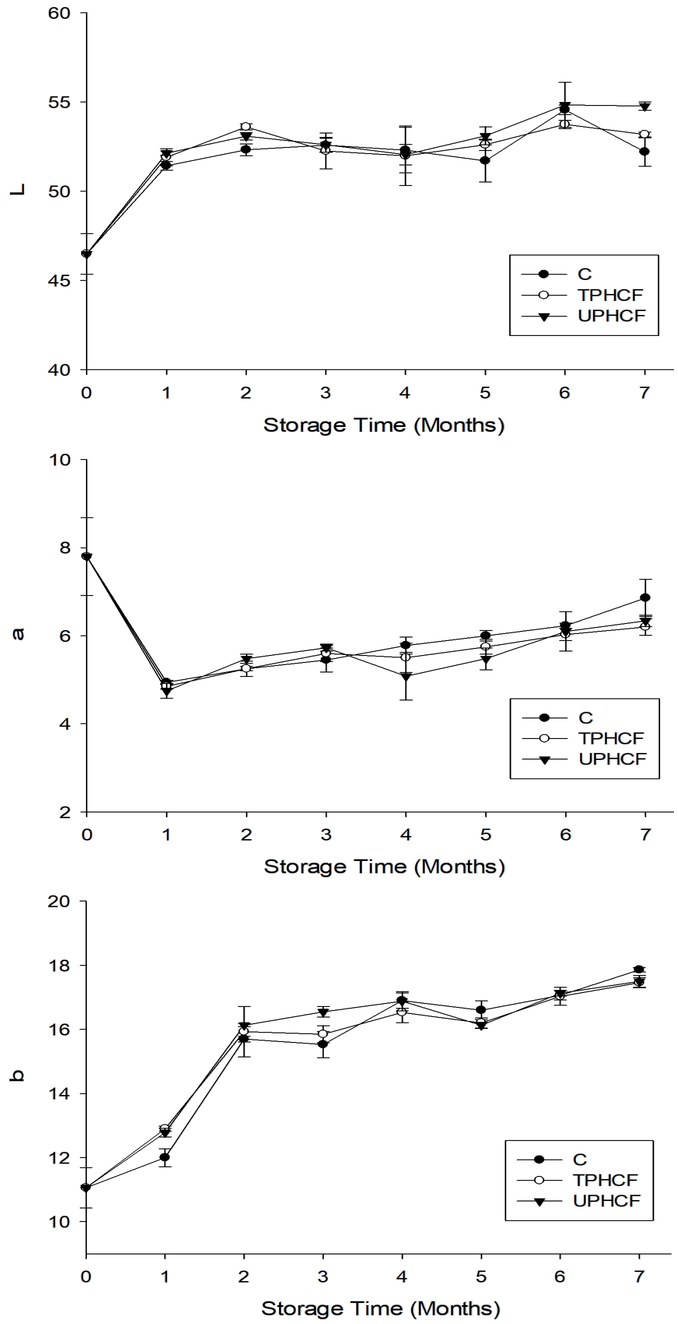

Figure 4. Color index (L*, a* and b*) of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Karsli et al. (2021) examine the effects of chitosan on catfish fillets stored at -20°C, and they prepared a control group and 3 different chitosan coating groups. They reported insignificant change in L* values during storage, small fluctuations were determined in the study. They obtained similar results in a* values ($p>0.05$). Regarding the b* value they reported that the b* value was higher after 6 months of frozen storage compared to day 0 in all fillets except the control group.

Rodriguez-Turienzo et al. (2012) searched the impact of ultrasound-treated whey protein coatings on frozen Atlantic salmon and determined the L* value as 45.37 from the color index. They measured the a* value of the fillets as 19.49 and the b* value as 18.10.

Wang et al. (2022) compared the effect of sodium alginate coating or chitosan on the large yellow croaker after a 3-day frozen storage. The coatings showed better muscle color acceptability.

Figure 5 illustrated the TVB-N values of C, TPHCF and UPHCF during the storage of bonito fillets stored at -18±1°C.

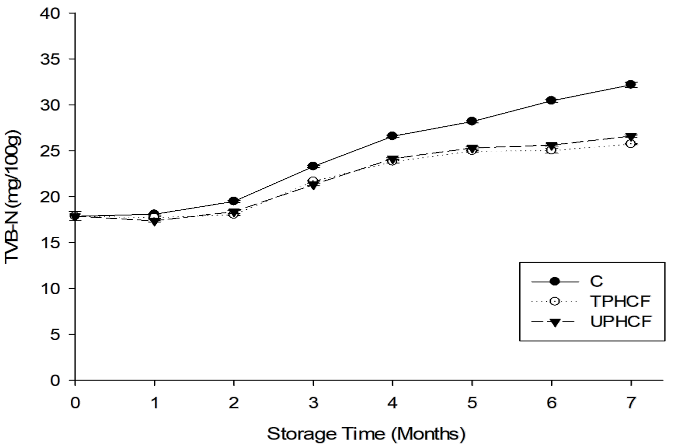

Figure 5. TVB-N values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Bonito fillets had 17.86 mg TVB-N/100 g at 0 day. TVB-N value of fresh bonito was calculated as 15.33±1.12 mg/100 g in research (Gargacı Kınay & Duyar, 2021). In the present study TVB-N values represented a statistically significant increase during the whole time of the storage for all three groups ($p<0.05$). They were 18.08±0.10, 17.71±0.09 and 17.36±0.12, for C, TPHCF and UPHCF, respectively at the first month of storage. The values reached to 32.18±0.29, 26.61±0.12 and 25.72±0.08 at 7th months of storage for C, TPHCF and UPHCF, respectively. TVB-N values around 25 mg/100 g in coated groups indicate that the products are in consumable condition. The results were in accordance with the results of pH. Although the more increasing values was found for both pH and TVB-N values of C than TPHCF and UPHCF, these values were not exceeded the consumable limits at the end of the storage. It can be said that the coating materials prepared with TPH and UPH may be effective in the microbial deterioration of proteins and other nitrogenous compounds, therefore, the coating material used in addition to the temperature during freeze storage may also be effective in keeping the TVB-N of bonito fillets under control.

Supporting results were collected by previous investigations. Luo et al. (2018) stored mackerel fish treated with nisin, chitosan and phytic acid at -18±1°C for 1 year and inspected the effects of coating materials on the quality of the fish. According to the results obtained, TVB-N of the coated groups were below the control group, within acceptable limits, during storage. Kulawik et al. (2019) analyzed the effects of a film (FUR/HGEL) consisting of active bilayer furcellaran/gelatin hydrolysate and Ala-Tyr peptide system on fresh Atlantic mackerel stored at -18±1°C. In the study, it was reported that the TVB-N value of the control group increased significantly after the first month and showed an unfluctuating regime in the following months, no significant increase was observed in the film-coated groups during storage. Literature shows that the low TVB-N indicates that the coating materials protect the fish fillets better than the control groups during cryopreservation. On the other hand, it can be concluded that depending on the factors such as the coating material used, the type of fish, the microbial load that may arise from contamination at the time of fishing or during processing, the storage time and temperature, it may affect the deterioration rates of fish fillets and thus the protection rates of the coating materials.

Lipid oxidation, which causes quality loss in fish during frozen storage, is higher in oily fish species than in lean fish. One of the problems that occur during the frozen storage of oily fish is rancidity due to lipid oxidation. The rancidity causes the expiration of the shelf life of the frozen product (Parvathy et al., 2018; Lee et al., 2019). Figure 6 represents the change of TBA values of bonito fillets during the storage.

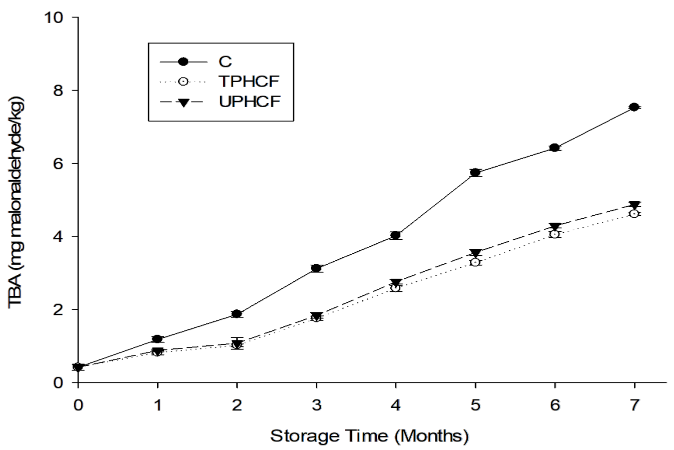

Figure 6. TBA values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated

Protein Hydrolzate Coated Fillet during the storage of bonito fillets stored at -18±1°C

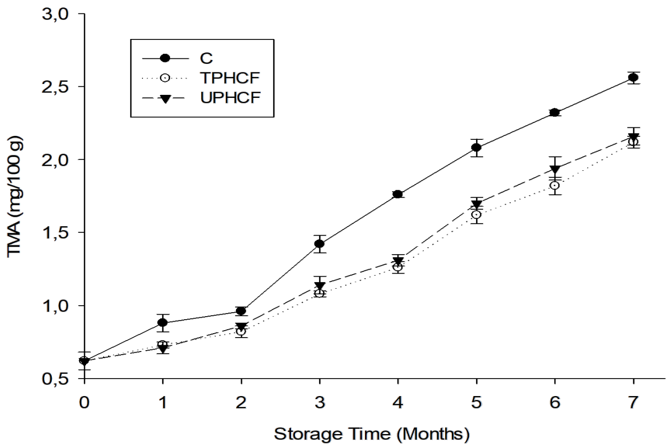

Figure 7. TMA values of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

TBA was 0.42 mg MA/kg at the beginning of the storage. Çorapçı (2018) calculated TBA value of fresh bonito meat as 0.95 mg MA/kg. Similar to TVB-N values, TBA values increased in all groups during the storage. TBA value of the K was 7.53±0.02 mg MA/kg in the 7th month, as exceeding the acceptable limit, they were 4.88±0.06 and 4.61±0.04 mg MA/kg for TPHCF and UPHCF, respectively at the same period. It is seen that TPHCF and UPHCF, are below the consumable limits in terms of TBA values until the last month of storage. This situation is compatible with the literature and can be explained as the coating materials used have good antioxidant properties and protect the products against oxidation. The differences between the TBA values of the TPHCF and UPHCF in the last 3 months of storage were assessed to be statistically significant ($p<0.05$). TBA values UPHCF were lower than the TPHCF. This situation can be interpreted as ultrasound application may be effective in providing more effective protection against oxidation in the product. Also, it could be said that ultrasound application creates a difference in the peptide sequences of protein hydrolysates, so amino acids with high antioxidant activity can come to the fore (Elias et al., 2005; Marchioni et al., 2009). It could be also emphasized that the coating materials could delay the lipid oxidation of the fish, so natural coating materials can be effective alternative materials for the preservation of the freshness of oily fish stored by freezing at -18±1°C. Rodriguez-Turienzo et al. (2012) demonstrated that ultrasound-treated protein-based coating materials preserve fish fillets better than non-ultrasound coating materials. The researchers reported that the TBA values of the ultrasound-treated groups were significantly below than the other groups. TMA analyzed monthly during storage in the study remained within consumable limits in all months (Figure 7).

In the study, the inhibition of the rise of microbial load by the effect of temperature in the freezer was effective in lowering TMA. In addition, the increases in the C group were higher than the TPHCF and UPHCF groups ($p<0.05$). Since protein hydrolysates show antimicrobial and antioxidant properties, it can be said that TMA values remain at lower levels with the inactivation of bacteria. TMA for consumption should be <10–15 mg/100 g. Varlık et al. (1993) stated that values above this value considered as deteriorated.

Vale et al. (2020) demonstrated that chitosan coating ensured low levels of TMA-N. Aref et al. (2018) investigated the role of transglutaminase enzyme, chitosan and rosemary extract and their different combinations on the quality parameters of fish fingers produced from catfish in frozen storage. Although there was an increase in TMA values during storage in all groups in the study, TMA value was lower in all coated groups compared to the control group. In addition, the rate of increase in the coated groups was also lower than control group.

Appearance, texture, odor and overall sensory scores are presented in Figure 8. Frozen storage protects foodstuffs from undesirable sensory and chemical deteriorates influenced by microorganisms, but not able to prevent degradation completely. Especially the reactions originating from proteins and lipids have an impact on sensory properties and cause bad smell, taste, and texture. Researchers have reported that sensory characteristics are the most crucial parameters that identify the quality of food, supposing a product that cannot be acceptable sensory cannot be offered for consumption (Erkan & Gökoğlu, 1999).

In this study, all three groups of bonito fillets remained within the acceptable limits over the study period, but the C group values were lower than the coated groups according to the scores given by the panelists to the sensory quality parameters. Therefore, the coating materials created a barrier against the passage of oxygen between the external environment and the fillets, and contributed to the prolonging of the storage period by affecting the stability of parameters such as odor and color, which are released as a result of rancidity due to lipid oxidation. In addition, by preventing textural deterioration due to microbial activities, coatings contributed to the tightness of the tissue and a smoother general appearance.

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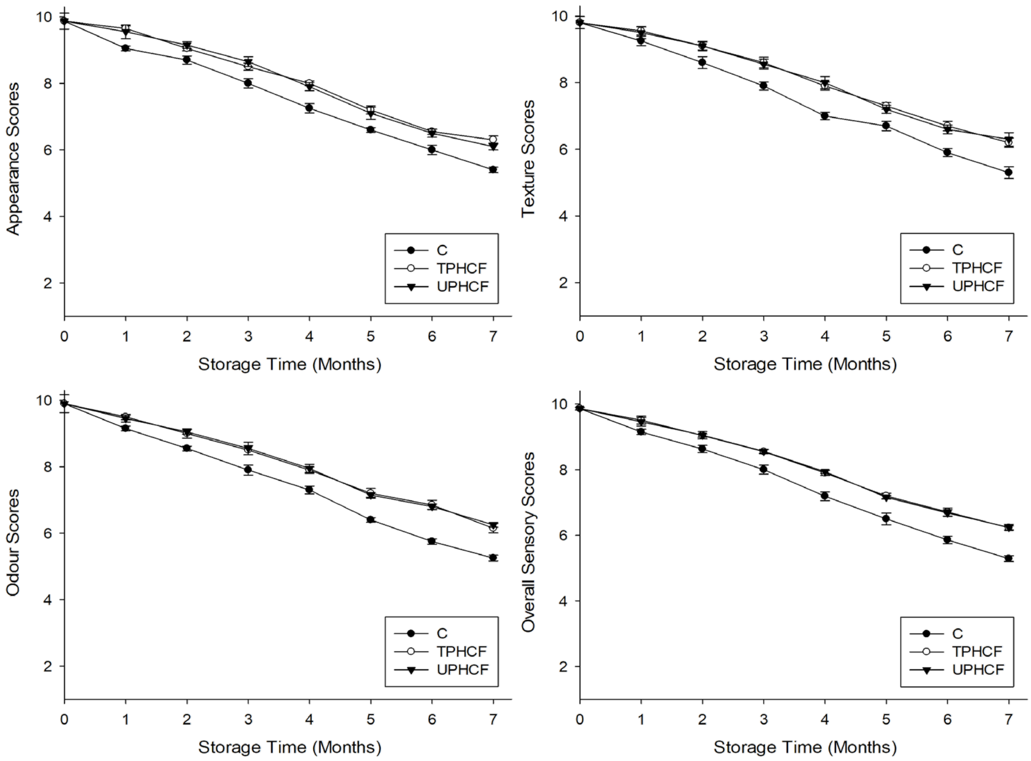

Figure 8. Appearance, texture, odor and overall sensory scores of C: Control; TPHCF: Traditional Protein Hydrolysate Coated Fillet; UPHCF: Ultrasound-treated Protein Hydrolysate Coated Fillet during the storage of bonito fillets stored at -18±1°C

Conclusion

This research evaluated that traditional and ultrasound-treated enzymatic protein hydrolysate coatings on the quality properties of the frozen bonito fillets. It is thought that using protein hydrolysates obtained from fish wastes as a coating in aquatic products, the products can be protected physically and chemically effectively. However, the expectation that UPH might have superior properties compared to TPH was not met in the study. If FPH is to be used as a coating material, it has been revealed that the ultrasound application used in the study conditions is unnecessary. However, in the functional property analyses made, it can be recommended to use UPH as an improver of the functional properties of the product in emulsion-form seafood products, especially the superior and functional properties of UPH.

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Compliance With Ethical Standards

Authors' Contributions

GBM: Conceptualization, Methodology, Software, Investigation, Resources, Writing original draft.
SK: Methodology, Software, Review & Editing, Supervision.
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

All data generated or analysed during this study are included in this published article.

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RESEARCH ARTICLE

Financial implications of the COVID-19 pandemic on the container ship time charter business

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ABSTRACT

This study examines the financial implications of the COVID-19 pandemic on the container ship time charter business. In this context, the container charter transactions were derived from the Clarksons Research Database, which included the ship types, daily charter fees, ship ages, and total charter days. The empirical analysis employed the K-Means Algorithm to cluster the observations in which the elbow curves revealed three cluster centers in the pre-COVID period and four in the post-COVID era, respectively. Based on the industry-wide used threshold definitions, the clusters were then named according to the mean value of given features. In addition, the relative weight of each cluster was disclosed based on the number of transactions falling into the respective cluster. Accordingly, the pre-COVID period clusters were described as intermediate-rated middle-termed young-aged intermediate-TEU container ships; low-rated middle-termed middle-aged feeders; and intermediate-rated long-termed middle-aged upper intermediate-TEU container ships. As for the post-COVID era, the cluster definitions were determined as intermediate-rated middle-termed young-aged feeders; intermediate-rated middle-termed old-aged feeders; high-rated long-termed middle-aged intermediate-TEU container ships; and high-rated short-termed middle-aged intermediate-TEU container ships. The findings suggested that the pandemic boosted the demand for relatively lower TEU container ships such as the feeders in which the criterium of ship age lost its importance due to availability reasons in the market. In addition, the pandemic led to higher charter rates which was a prioritized factor over the charter period.

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Introduction

The socio-economic effects of the COVID-19 virus, which was seen only as a threat to health when it first appeared, reached much greater dimensions all over the world. The COVID-19 pandemic, which was declared by the World Health Organization on March 11, 2020, continued more than 2 years. The effects of the global decline in economic activity as a result of the pandemic on supply chains and international transport are still being felt (Gençer, 2022). A large part of world trade is carried out by maritime transport. More than 50% of maritime transport in terms of the value of cargo is carried out by container shipping (Clarksons Research, 2021). Container shipping is one of the most preferred transport types in international trade, thanks to the safe and intermodal transport opportunities it provides.

It can be said that the most important fact in container shipping during the COVID-19 pandemic period is the excessively rising freight rates. In Figure 1, it is seen that the daily rates and earnings of container ships have increased clearly compared to the beginning of the pandemic. Clarksons Container Ship Time Based Chartering Index reached 400, Clarksons Average Container Ship daily earnings approaching 80000 USD (United States Dollar). Similarly, as seen in Figure 2, the Shanghai Container Freight Index reached its highest levels since its establishment in 2009.

Fluctuations in freight rates affect the profitability liner carriers. Therefore, liner carriers may prefer charter ships on time charter basis instead of buying ships, as they cannot foresee how the freight will progress in the future (Munim, 2022).

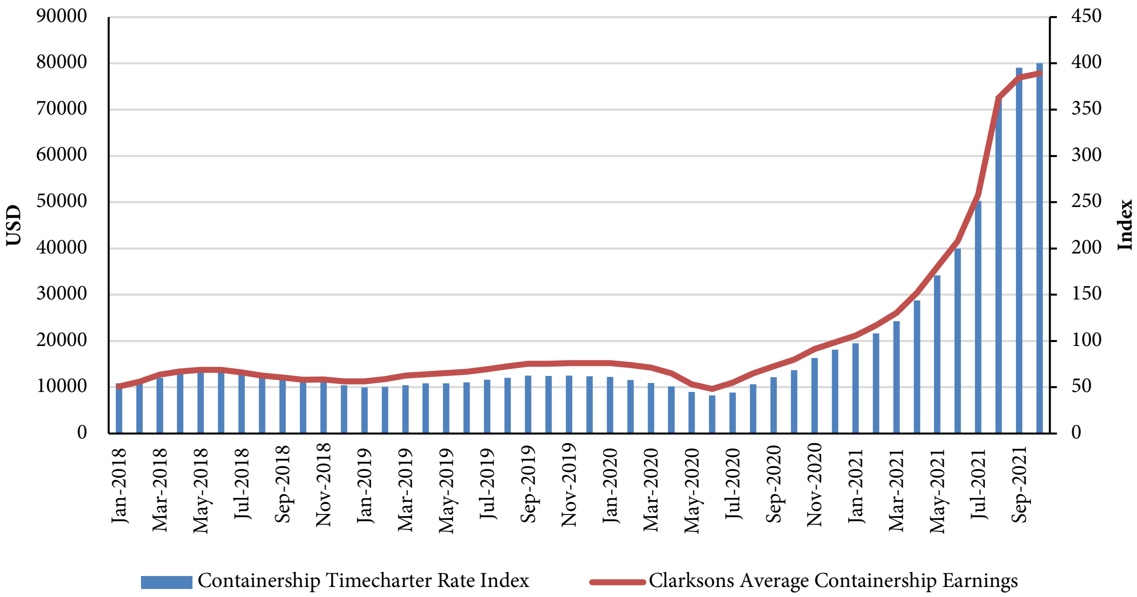

Figure 1. Clarksons container ship time charter rate index and average container ship earnings

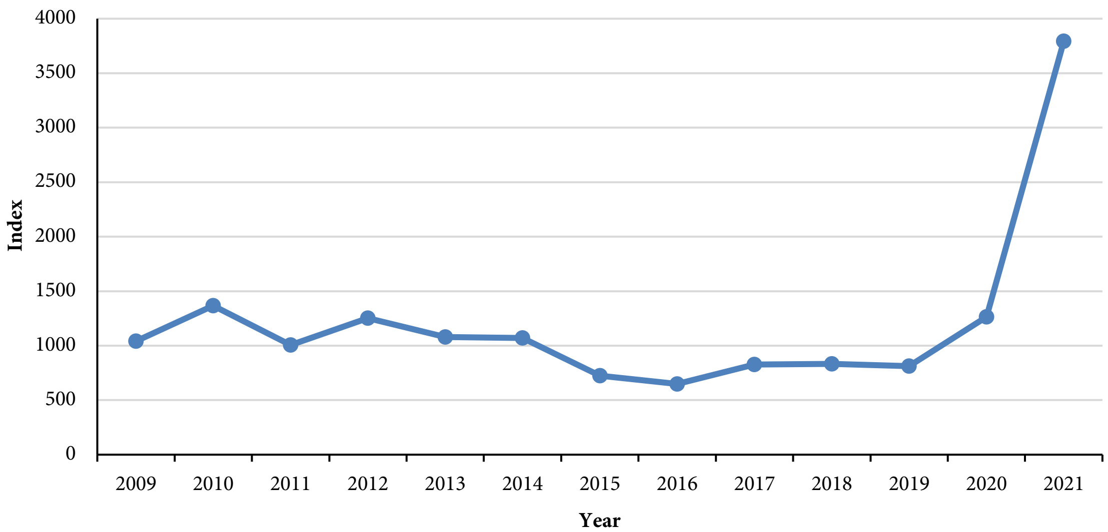

Figure 2. Shanghai container freight index

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Although large ships provide economies of scale, they can carry high risks due to high operating costs, especially when freight rates are low. On the other hand, smaller ships such as feeders can be easily adapted to the region and market in which they operate. These types of ships are less affected by the fluctuations in the industry, as they can also carry other types of cargo besides containers, such as project cargoes. Considering that the use of feeder type ships as general cargo ships is preferred during the pandemic period, when there is a shortage of containers, it is better understood why the demand for this type of ships has increased.

This study examines the financial implications of the COVID-19 pandemic on the container ship time charter business. In this context, the container charter transactions were derived from the Clarksons Database from January 2018 to October 2021, which included the ship types according to their size in terms of TEU, daily charter fees, ship ages, and total charter days.

Literature Review

Due to the COVID-19 pandemic, global supply chains have faced various disruptions due to government restrictions, limited working hours, delayed shipments due to limited staff shortages, longer transit times, shortages of raw materials. During this period, many logistics companies, especially ship owners, operators and ports, sought ways to increase their operational efficiency and global competitiveness (Noteboom et al., 2021). However, the expected impact at the beginning of the pandemic was not as great as feared and the maritime transport sector managed to get out of the crisis (UNCTAD, 2021). In addition, despite all the interruptions and disruptions, maritime transport has once again demonstrated its importance and flexibility by ensuring the continuity of the supply of food, medical products and basic materials (Charbonnaux et al., 2020; Weerth, 2020).

Nowinska & Schramm (2021) examined more than 2700 ship chartering deals in container shipping industry. Their study reveals important findings for decision makers in an environment of uncertainty in the sector. Guerrero et al. (2022) examined the effects of the COVID-19 pandemic on container shipping routes and connections between ports. Researchers have shown a reduction in global seaway connections, demonstrating that large ports and densely interconnected smaller ports are better able to resist the pandemic. Jin et al. (2022) investigated China's international regular container line transport connections during the pandemic period. In the study, it was stated that there were large fluctuations in freight flows, especially between China and the USA, and it was revealed that China's international regular container line transport connections increased even though the number of main ports frequented by ships decreased.

In the literature, there are various studies on the estimation of freight rates and charter rates in maritime transport depending on crisis situations. Munim (2022) proposed Seasonal Autoregressive Integrated Moving Average (SARIMA), Seasonal Neural Network Autoregression (SNNAR) and the state-space TBATS models for forecasting container freight rates. It has been seen that the results of the models can provide promising predictions. Monge (2022), Monge covered the analysis of the evolution of international trade after COVID-19 by examining the shipping industry and the impact of bunker fuel cost. Mo et al. (2022) proposed a neural network model for the prediction of monthly time charter rates of dry bulk ships. The empirical findings of their study show that the model is significant in estimating the short-term time charter rates. Saeed et al. (2023) introduced the Prophet model for estimating container freights, taking into account the factors of congestion, peak demand, policy, price up, overcapacity and coronavirus. Their study reveals that the proposed forecasting model will help policy makers and practitioners to implement strategies to reduce the risks associated with the variability of freight rates and supply chain costs.

In the time charter rates, period of the charter, the state of the industry and characteristics of the ships come to the fore. In the literature, no study has yet been found on the clustering of container ships according to the characteristics of time charter contract.

Material and Methods

The dataset used in this study was acquired from the Clarksons Database which is the global provider of shipping and sea trade data (Clarksons Research, 2021). The dataset encompassed the charter container ship data for the period between the beginning of 2018 and October 2021, which described the charter date, ship name, built year, capacity in TEU (twenty-foot equivalent unit), charterer description, laycan date, length of the charter period, the daily charter rate in USD and the ship owner, respectively. In the instances where the charter time was presented in a continuous period, such as 9-12 months, the upper bound was taken as the respective value and all values were converted into days. Whereas the total data accounted for 7,174 observations, 624 of them were eliminated

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due to missing daily rate values. Hence, the final dataset consisted of 6,550 observations. The dataset was then divided into two sub-groups to represent the pre-COVID and post-COVID periods. The key date for the split was taken as 31.12.2019 in which December 2019 corresponded to the acknowledgment of the outbreak in China.

As for the first step, exploratory data analysis was carried out to investigate the descriptive statistics in both periods to gain insights comparatively. Accordingly, daily charter fees, ship ages, and total charter days were analyzed by container ship types. The container ship types were classified into six groups in the dataset based on TEU capacity thresholds, which were between 100-999; 1,000-1,999; 2,000-2,999; Narrow Beam 3,000+; Wide Beam 3,000-5,999; and 6,000+ TEU, respectively. While carrying out the visual analysis, boxplots were used. Boxplots, often called as the five-number summary, depict the minimum value, the maximum value, the median, and the first and third quartiles that reveal the variability in a distribution. The box in the plot represents 50% of the observations in a distribution where the line in the box is the median. The points below or above the whiskers (lines stretching out the box) are potential outliers that are beyond the 1.5 interquartile range (Agresti & Franklin, 2013). Figures 3 and 4 presented below display the boxplots of daily charter fees by fixture types in the pre-COVID and post-COVID periods.

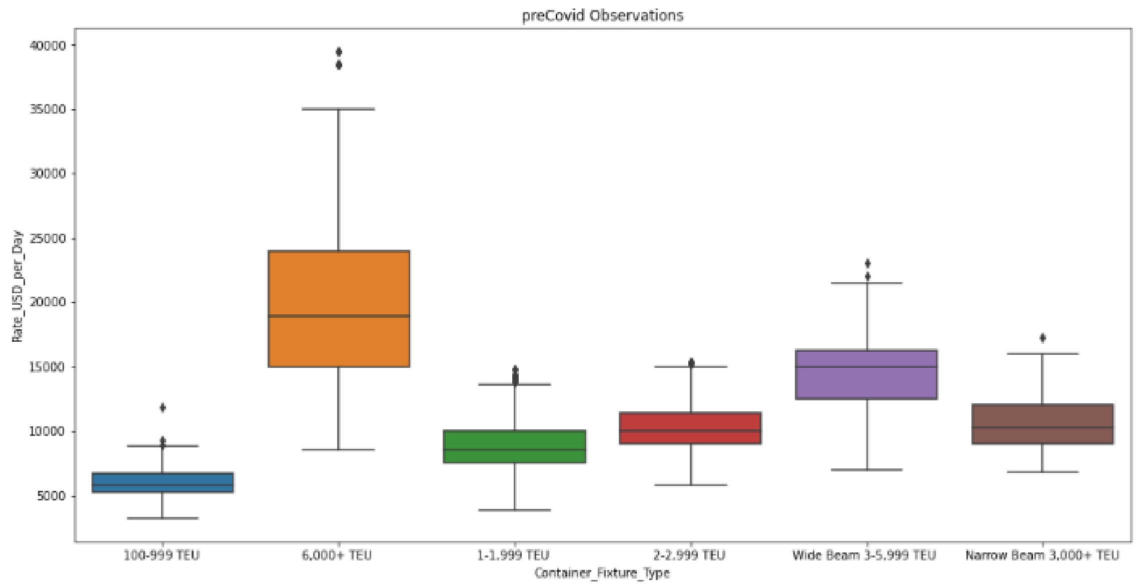

Figure 3. Boxplot of daily charter fees (USD) by container ship fixture types in the pre-COVID period

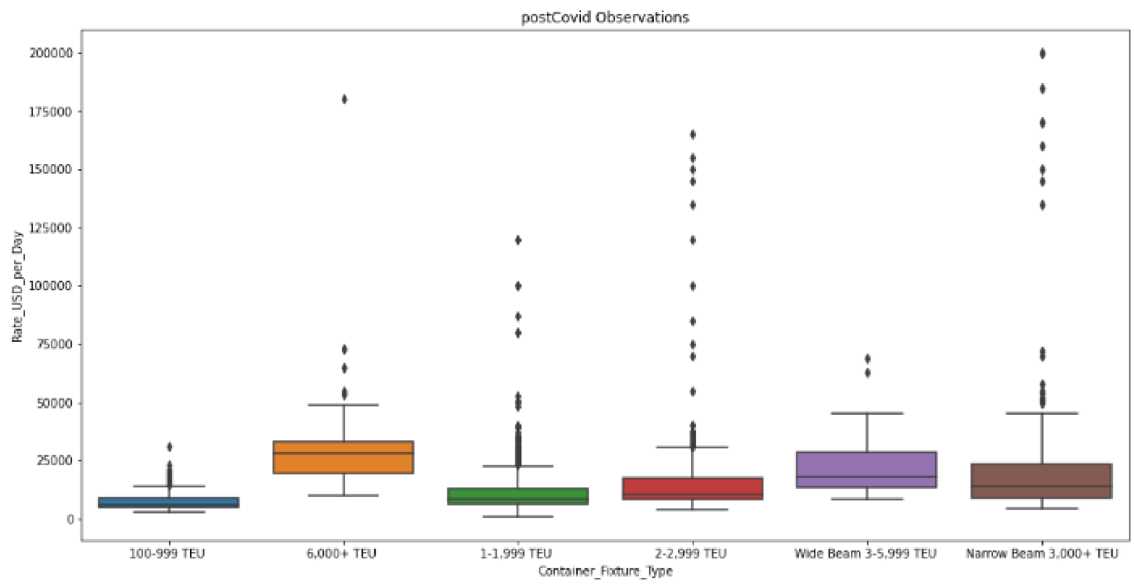

Figure 4. Boxplot of daily charter fees (USD) by container ship fixture types in the post-COVID period

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The comparison of the boxplots in Figures 3 and 4 revealed that the median of the daily charter rates increased for almost all container ship types in the post-COVID period compared to the pre-COVID time which was comparatively more evident in container ships with larger TEU. In addition, the average daily charter rates increased by 24% (100-999 TEU); 30% (1,000-1,999 TEU); 47% (2,000-2,999 TEU); 90% (Narrow Beam 3,000+ TEU); 49% (Wide Beam 3,000-5,999 TEU) and 44% (6,000+ TEU) between the two periods. Looking at the point observations stretching out the whiskers, it can be concluded that the number of outliers increased in the post-COVID period. Indeed, the variability in the distributions rose in the post-COVID period. Apart from the rise in the daily charter fees, it can be asserted that shipping companies had to incur higher charter costs in particular instances which could be a result of instant urgent demand. The visual analysis continued with the ship ages by fixture types, which are illustrated in Figures 5 and 6.

The average ship age in the pre-COVID period varied from 11 to 13 years, whereas the variation was between 12 to 16 years in the post-COVID period. The average ship age rose by 9% (100-999 TEU); 20% (1,000-1,999 TEU); 12% (2,000-2,999 TEU); 17% (Narrow Beam 3,000+ TEU); 14% (Wide Beam 3,000-5,999 TEU) and 18% (6,000+ TEU) between the two periods. Given that no new ships were introduced and no others retired, the increase in the average ship age would be expected as a percentage between 8% to 10% due to normal aging. However, the increase rates stated above suggest a higher variation. This could be explained by the increased demand for charter ships in the post-COVID period in which the ship age factor played a comparatively lower role.

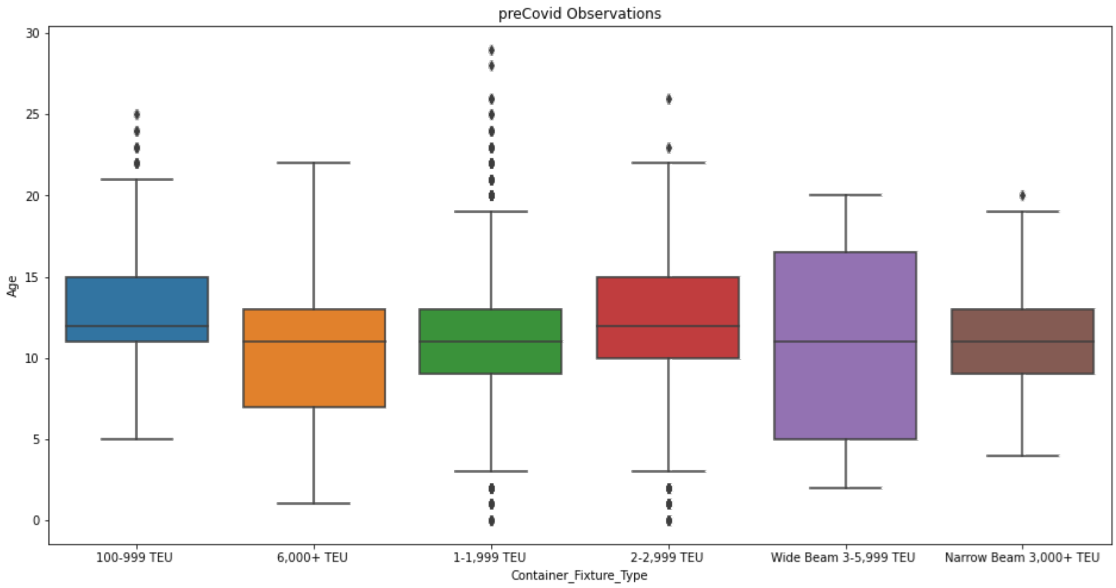

Figure 5. Boxplot of ship age by container ship fixture types in the pre-COVID period

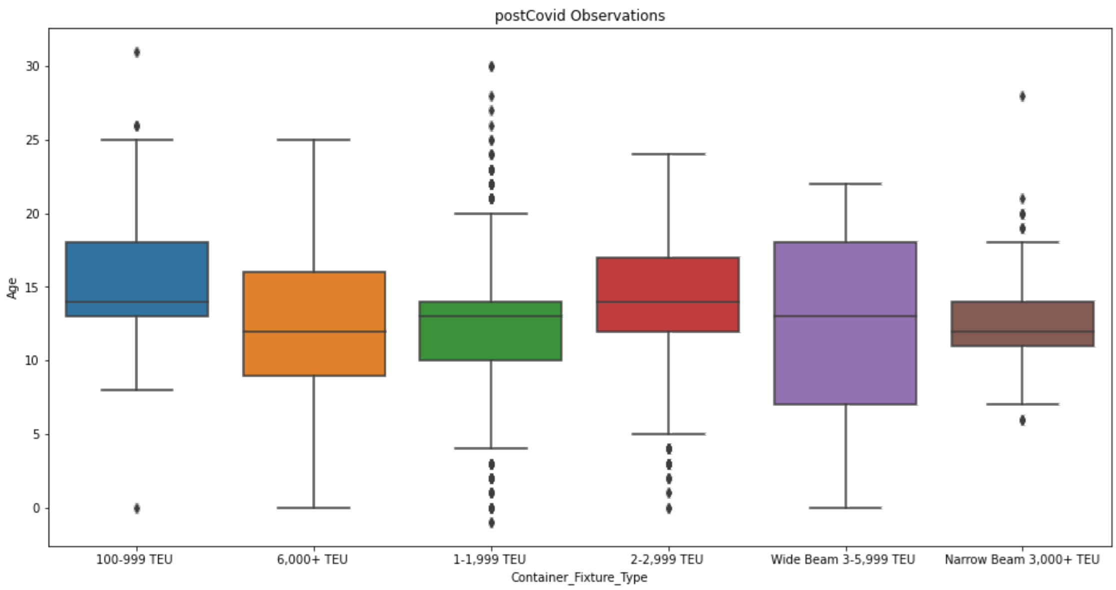

Figure 6. Boxplot of ship age by container ship fixture types in the post-COVID period

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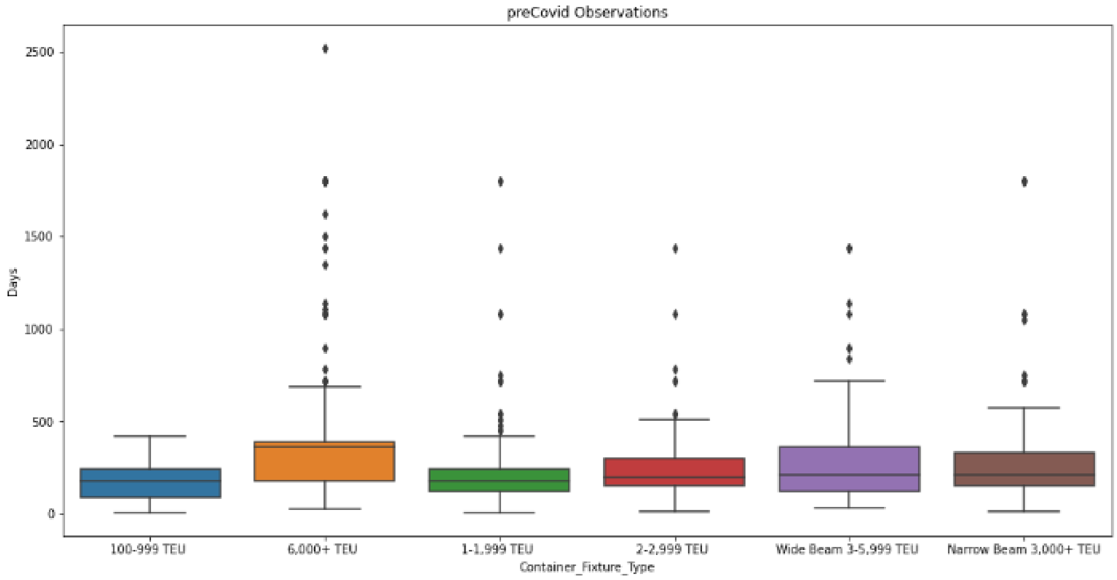

Figure 7. Boxplot of charter days by container ship fixture types in the pre-COVID period

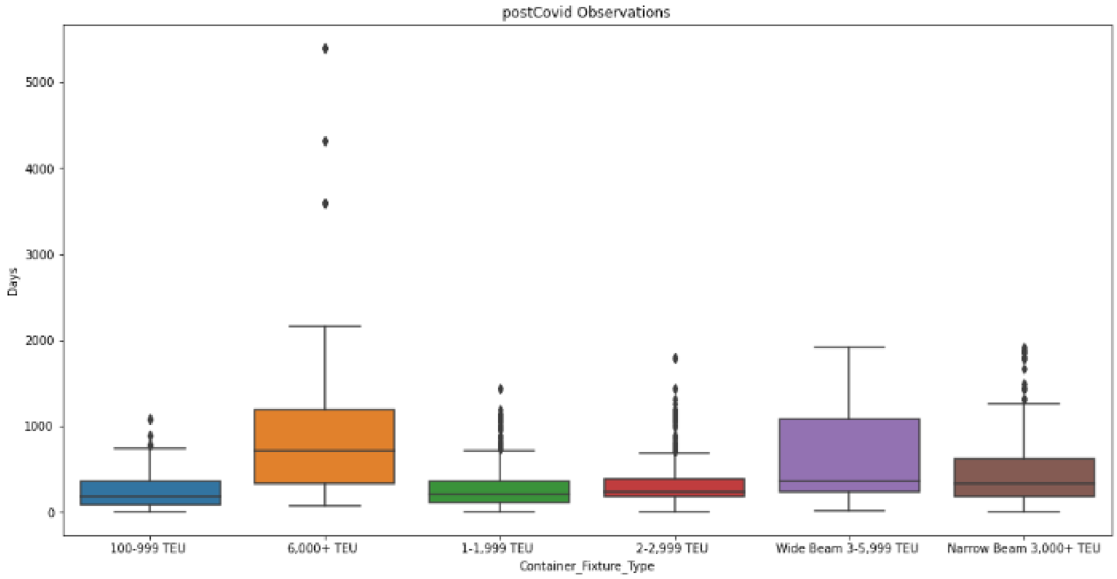

Figure 8. Boxplot of charter days by container ship fixture types in the post-COVID period

Thirdly, the charter days by container fixture types are compared in the pre-COVID and post-COVID periods, respectively. The boxplots in Figures 7 and 8 depict this analysis.

The average charter days rose significantly in the post-COVID period for all container ship fixture types substantially. The rate of increase was 51% (100-999 TEU); 36% (1,000-1,999 TEU); 65% (2,000-2,999 TEU); 122% (Narrow Beam 3,000+ TEU); 89% (Wide Beam 3,000-5,999 TEU) and 120% (6,000+ TEU) between the two periods. In addition, the variability in the charter days for all fixture types increased considerably in the post-COVID period. Overall, it can be suggested that the duration of the charter period rose and fluctuated following the outburst of the pandemic.

When it comes to the empirical analysis that was carried out in this study, the applied method was clustering. Clustering encompasses the grouping of data into classes in which those grouped are similar to each other and dissimilar from the rest of the classes based on given qualities. In other words, clustering algorithms detect and group the observations that are similar to each other compared to the rest of the groups (Jayashree & Chithambaramani, 2020). In addition, clustering methods do not require a target or dependent variable. Hence, the number of the groups is defined at the beginning of the analysis, stays constant and the observations are grouped under the previously set number of clusters (Rather & Bala, 2020). Widely used in knowledge discovery in empirical research, clustering methods have evolved into numerous different models in which we can detect publications with more than a hundred diverse clustering algorithms in the literature (Rather & Bala, 2020). Among different clustering algorithms, the K-Means algorithm is a widely used clustering method in which

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clusters are classified by their centroids that implies the average value of given observations. The algorithm has an iterative approach in which the predetermined number of cluster centers initially emerge at random, and the recalculation goes on as long as the error function no longer decreases (HajKacem, et al., 2019; Chander, 2020; Giordani et al., 2020). Since the number of clusters is not initially known, the elbow method can be applied to determine the number of clusters. In this method, the within-cluster sum of squares is plotted against the number of clusters. Accordingly, the point at which the elbows vanish leading to a considerable decline in the within-cluster sum of squares can be offered as the number of clusters to be integrated into the K-Means algorithm (Giordani et al., 2020).

Results

The empirical analysis started with the determination of the number of clusters in the pre-COVID and post-COVID datasets, respectively. In this sense, the elbow curve for the pre-COVID period observations depicted the first bump with 3 clusters. On the other hand, the first bump appeared with 4 clusters in the post-COVID observations plot. The elbow curves are illustrated in Figures 9 and 10.

The execution of the K-Means algorithm on the pre-COVID observations classified 38.6% of the observations in the first, 52.5% in the second, and 8.9% in the third cluster. As for the post-COVID period, the percentage of the observations that fell into four different clusters was 30.1%, 55.7%, 13.3%, and 0.8%, respectively. Besides the number of observations corresponding to each cluster, the cluster characteristics were revealed by the mean value of given features in each cluster. The mean values of TEU, daily rate, days, and age for each cluster in the pre-COVID and post-COVID periods are summarized in Table 1 and Table 2.

To define the clusters, the mean value for each feature was compared with the generally accepted industry-standard classifications. As for container ship classes, the TEU is grouped as a feeder (up to 3000 TEU); intermediate-TEU (between 3,000 and 6,000 TEU); upper intermediate-TEU (between 6,000 and 8,000 TEU); neo-panamax (between 8,000 and 12,000 TEU); upper neo-panamax (between 12,000 and 17,000 TEU); and post-panamax (from 17,000 TEU onwards). Daily rates per day (USD) are classified as low-rate; intermediate-rate; and high-rate based on the thresholds of up to 10,000 USD; between 10,000 and 25,000 USD; and more than 25,000 USD, respectively. Charter days fall into three categories which are short-term; middle-term; and long-term with up to 180 days; 180-360 days; and more than 360 days. Lastly, ship age can be summarized into three groups which are young-age (up to 10 years); middle-age (between 10 and 15 years); and old-age (from 15 years onwards). In light of the industry definitions and

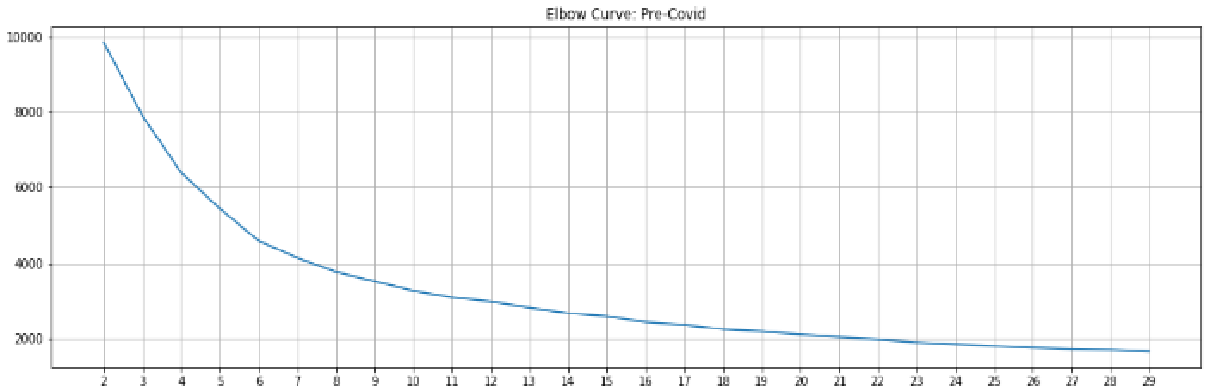

Figure 9. Elbow curve for the pre-COVID observations

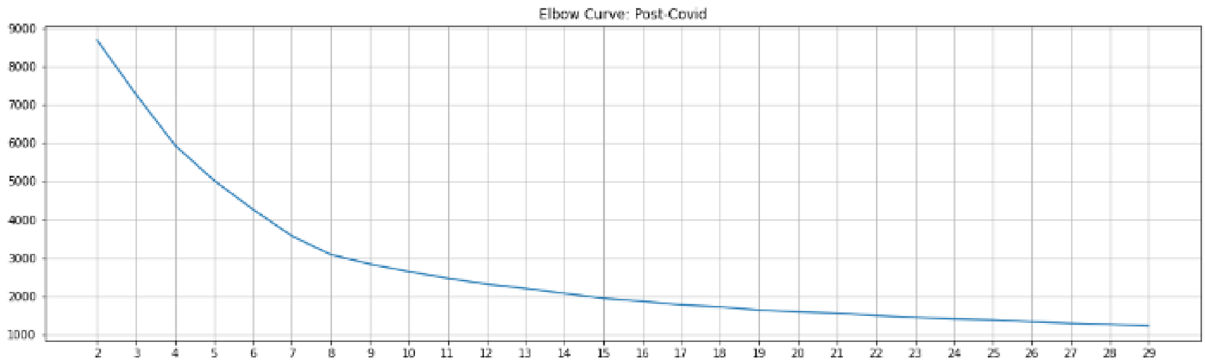

Figure 10. Elbow curve for the post-COVID observations

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cluster centers, the first pre-COVID cluster was defined as intermediate-rated middle-termed young-aged intermediate-TEU container ships. The second cluster in this period was low-rated middle-termed middle-aged feeders. The third cluster was intermediate-rated long-termed middle-aged upper intermediate-TEU container ships. On the other hand, when it came to the post-COVID period, the first cluster was intermediate-rated middle-termed young-aged feeders. Secondly, we had intermediate-rated middle-termed old-aged feeders. As for the third cluster, high-rated long-termed middle-aged intermediate-TEU container ships were described. As for the last cluster, high-rated short-termed middle-aged intermediate-TEU container ships were named. The definitions of the clusters including the weight of the observations in each cluster for each period are summarized in Table 3 and Table 4.

Table 1. Mean value of features by clusters, pre-COVID period

Cluster Label	TEU	Daily Rate	Days	Age
0	3,074.53	11,075.69	229.14	7.99
1	1,958.26	8,338.39	186.29	14.09
2	7,435.07	19,708.42	508.24	11.32

Table 1. Mean value of features by clusters, post-COVID period

Cluster Label	TEU	Daily Rate	Days	Age
0	2,519.26	11,693.20	267.61	8.29
1	1,923.96	10,495.97	273.84	15.71
2	5,441.83	30,466.06	1,106.71	12.47
3	3,333.24	137,480.00	128.60	12.16

Table 2. Definition and weight of pre-COVID clusters

Cluster Label	Cluster Weight	Definition
0	38.6%	Intermediate-TEU Intermediate-Rate Middle-Term Young-Age
1	52.5%	Feeder Low-Rate Middle-Term Middle-Age
2	8.9%	Upper Intermediate-TEU Intermediate-Rate Long-Term Middle-Age

Table 3. Definition and weight of post-COVID clusters

Cluster Label	Cluster Weight	Definition
0	30.1%	Feeder Intermediate-Rate Middle-Term Young-Age
1	55.7%	Feeder Intermediate-Rate Middle-Term Old-Age
2	13.3%	Intermediate-TEU High-Rate Long-Term Middle-Age
3	0.8%	Intermediate-TEU High-Rate Short-Term Middle-Age

Discussion and Conclusion

This study examined the changing trends in the time charter business of container ships due to the COVID-19 pandemic from the financial perspective. The charter instances in the pre-COVID and post-COVID periods were clustered considering the capacity in terms of TEU, daily charter rates, charter period, and the age of the container ships. By using the generally accepted industry descriptions, the revealed clusters were defined and compared with each other in two periods to provide insights into the implications of the pandemic on the industry. Accordingly, the comparison of the defined clusters disclosed the following findings. Firstly, whereas three main container ship classes were observed in the pre-COVID period, this observation was dropped to two in the post-COVID period. The feeders, for which the weight of the instances was 52.5% in the pre-COVID period rose to 85.6% in the post-COVID period. The weight of the charter instances of intermediate-TEU ships fell from 38.6% to 14.2% in the post-COVID period. In addition, the clusters in the post-COVID period did not include upper intermediate-TEU ships which were 8.9% in the pre-COVID period. The increase in the demand for relatively lower TEU container ships can be explained by their comparatively higher availability and relatively lower operating costs in an uncertain economic environment. Secondly, as for the daily charter rates of container ships, the low-rate cluster which dominated the market with 52.5% vanished in the post-COVID period. Indeed, the post-COVID period resulted in the emergence of high-rate charter fees which was not the case in the pre-COVID period. The weight of the intermediate-rate

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charter instances rose considerably from 38.6% to 85.8% in the post-COVID period. Hence, the pandemic resulted in higher charter rates in the industry, which can be mainly due to increased demand for container shipping. Thirdly, as for the charter periods, it can be stated that the middle-term charter period, which was the common practice in the pre-COVID period seemed to have lost its weight in favor of long and short charter periods in the post-COVID periods. Considering the daily rates and the charter periods of the clusters together, it can be asserted that container shipping companies had a preference of daily rates or income over the charter period. Lastly, the analysis of ship age showed that the old-age class ships dominated the market with 55.7% in the post-COVID period, which was not observed in the clusters of the pre-COVID period. In addition, the ship type of this class was feeders. Apart from the availability of the feeders, this follows that the ship age seemed to have played a minor role in the charter decisions in the post-COVID era in which the clients opted for what was available in the market.

During the pandemic period, global container liner carriers made frequent changes to their mother ships' schedules, canceling some hub port calls and making calls to other unplanned ports when necessary. In particular, it can be said again that there is not much competition on the main routes where the global container liner carriers, united in the form of alliances, determine the freights and ship schedules (Gençer, 2022; Ksciuk et al., 2023). Accordingly, this study reveals that one of the main competitive elements that make a difference in container shipping is the smaller ships operating in regional transportation.

Smaller ships such as feeders can be easily adapted to the region and market in which they operate. These types of ships are less affected by the fluctuations in the industry, as they can also carry other types of cargo besides containers, such as project cargoes. Considering that the use of feeder type ships as general cargo ships is preferred during the pandemic period, when there is a shortage of containers, it is better understood why the demand for this type of ships has increased.

Future Research

Clustering approach, which is considered in this study, is a very useful method for the rapid and efficient identification, analysis and determination of the current needs of the maritime industry during fluctuations or crisis periods. Therefore, it can be applied to different ship types and can be used as an important decision-making method regarding what size, age and characteristics of ships are in demand, depending on the situation in the industry. In addition, it can also be used to shape the ship portfolios of companies with fleets of different ship types. Nevertheless, due to the largeness, diversity and complexity of the maritime industry, keeping track of all developments and collecting data can be challenging. Therefore, as new data come in the coming periods, there will be much more research in the maritime industry.

Compliance With Ethical Standards

Authors' Contributions

HG: Designed the study, Wrote the literature review, Analyzed the results.
TT: Conducted statistical analyses, Analyzed the results.
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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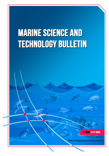

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RESEARCH ARTICLE

Risk analysis, assessment and management for local fishing ports in North-eastern Mediterranean, Türkiye

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Risk analysis

ABSTRACT

This study aimed to analyze the risks of activities in fishing ports in the province of Hatay. Due to these activities, the fishing port is under health, good, labor, and environmental risks. In this study, risks were identified and evaluated at the four fishing ports. For this purpose; (1) A preliminary survey was conducted with stakeholders on the current situation and the functions of the fishing ports (2) brainstorm meetings were organized in three sessions in one day- three sections with 12 invited participants throughout the year 2016, (3) as a result of these meetings, risk assessments were carried out in terms of the risks identified, loss of health, good, labor, and environment for each fishing port. The risk matrices were presented graphically in terms of the risk effect and the frequency value, and risk tables were created for each risk. Approximately 20 (17-22) risks were identified for each fishing port. The risks identified for the fishing port were found similar these were different impact levels and frequency values. These differences were generally related to the location. The differences in the settlement structure of the fishing port caused advantages and disadvantages to risks of the fishing port risk. When risks were grouped and classified as administrative, structural, pollution, and security, the lack of management and supervision was striking. Risk analysis outputs were evaluated by using the "L type matrix" method with issuing by numerical and statistical comparisons for different fishing ports. The reason for the risks was due to incompatible legal regulations and control mechanisms rather than a benefit for fishing ports than regional and personal shortfalls with the current situation and expected. Finally, these assessments were the result of a regional study, but they were important both nationally and internationally fishing port risk lack of management. This article attempts to provide a range of knowledge, which is compulsory for managers.

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Introduction

Fishing port is one of the most contributing factors that providing logistical support for fisheries and other maritime activities. Additional services carried out buy the past such as port is area where there is facilitation for repair and maintain for fishing vessels. In this direction, these areas were a workplace with a wide variety and high activities as marine tourism, different character service vessel, private yacht (Akar et al., 2022). The role of a fishing port can be thought of as the interface between logistics provide to fishing essential services landing of catch and measure point to prevent, deter, eliminate illegal, unreported and unregulated fisheries (Flothmann et al., 2010; Huntington et al., 2015). Despite this important role, there were absences about the management of fishing ports worldwide (Scheffczyk, 2010). The limited availability of international academic publications, studies and guidelines for fishing ports were the biggest indication of the shortage compared to other similar fishing and maritime issues (Akar, 2017).

In many parts of the world, there were differences in the construction and operation of the fishing port, where vary in countries as well as regions. FAO divided fishing ports into three classes according to their sizes those needed for fishing ports as artisanal beach landings to coastal fishing ports, offshore fishing ports and distant water fishing ports. The small settlement areas have separated shelters for coastal and offshore fisheries (Sciortino, 2010).

Commercial fishing is a profession that is characterized by the long-term high risk of death (Mitchell et al., 2001; Atay & Cengiz, 2022; Cengiz, 2022). In fishery health and safety inspection, fishing was described as a dangerous occupation (Ross, 2015; Soykan, 2021). Naturally, the potential important risk is at different levels for the fishing port. Consequently, any studies on risk analysis for fishing ports may be necessary at the first glance. Nevertheless, in addition to traffic in fishing ports lack authority and intensive activities in human including environmental risks also been added to traffic in fishing ports. Moreover, these risks have reduced the benefits gained in terms of fishing port infrastructure and workforce.

The natural consequences of modern management understanding are to identify possible risks that may come about in a sector (McNeil et al., 2015; Haimes, 2015). In international port management, there are some basic concepts such as planning, organizing and defining the management responsibility (MSANZ, 2004; Lam & Notteboom, 2014).

The fishing port regulations in Türkiye have been regulated by the Ministry of Agriculture and Forestry. The fishing port is described in the regulation as: "*provide service to all kinds of fishing vessels and provide sufficient pool, backwater and sheltered vessels with water area and depth to ship movement, loading, unloading and docking berths with water, electricity, the administrative building, pre-cooling and docking area, fishermen's harbor according to their sizes and possibilities, coastal structures called harboring place or docking place*" (MAF, 2015). In Türkiye, there are 385 fishing ports that have different sizes and extended along the wide coastline. This may be different from the demands of the fishing port according to the distance of the city center, other maritime activities, etc. However, management of any fishing port must have been required by Circular of Coastal Structures in the Ministry of Transport, Communication and Maritime (UBAK, 2013). Unfortunately, in practice, the majority of the fishing ports were not able to the conditions provided in the circular.

Although Iskenderun Bay has an important fishing area (Gezmen et al., 2015; Demirhan et al., 2020): it has intensive maritime activities such as transportation and tourism in the East-Mediterranean (Mazlum et al., 2019; Yılmaz et al., 2019, 2022; Akar et al., 2021). Although there are seven fishing ports, four of these ports are active (Akar et al., 2022).

In this study, the risks of these four active fishing ports were determined and analyzed by health, goods, labor and environmental losses. The outputs of the risk analyses were evaluated by numerical and statistical comparisons for each fishing port. Although these assessments were the result of a regional study, they provide significant contributions to decision-makers for the development of fisheries management strategies and policies at the national level due to the absence of fishing port risk analysis and management in local fisheries management, Besides, it has a high potential for application at the international level.

Material and Methods

The study assessed the risk analyses and management of four fishing ports in the Iskenderun Bay, the eastern Mediterranean, Türkiye. The region has served the local port-based fishing, maritime activities, heavy industry facilities small or large fishing vessels repairs, fish handily, power plants, suppliers of fuels and transportation. The population density is high in many settlements areas and its total population is about 694,000 for 8 districts (Karataş, Yumurtalık, Erzin, Dörtyol, Payas, İskenderun, Arsuz, and Samandağ).

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Table 1. Some basic charactering of the fishing port in the area (Density; Ministry's evaluation, Distance: the nearest located settlements)

Properties	Fishing Port			
	Dörtyol	**Iskenderun**	**Çevlik**	**Konacık**
Length of breakwater (m)	1295	785	800	740
Length of berth (m)	370	465	330	270
Protective water area (m^2)	13500	10500	9000	5500
Berth number	90	115	95	60
Number of fishing vessel	200	450	60	45
Number of other ships	4	91	7	5
Density (%)	68	251	43	33
Transport distance (km)	2	0	29	33
Operation of the building	-	+	+	-
Maintenance area	+	+	-	+
Electric	+	+	+	+
Water	+	+	+	+
Operators fishing cooperative	Dörtyol	Iskenderun	Kapısuyu	Konacık Işıklı

Due to the population density, recreational marine fishing activities and yacht tourism are occurring widely in the Bay. Alternatives to sheltering areas are scarce instead of these fishing ports for different vessel sectors such as ship agent boats, the sightseeing boat, service vessels, tour boats and amateur fishing boats for in the many marine activities of the bay.

The present study assessed four fishing ports in Dörtyol, Iskenderun, Çevlik, and Konacık, which serve for the purpose of general use. General information and characteristics of these were provided from the Ministry of Transportation, Communication and Maritime (Table 1).

No data were provided by port authorities and public agencies for risk analysis. Therefore, the current situation and risks in the ports have been primarily determined for those who represent the different stakeholders of the ports. According to the list given below, a preliminary field survey was conducted with selected and contact persons and brief information was given about the subject.

1. Owners of small-scale fishing vessels
2. Owners of fishing ships
3. Recreational angling fishermen
4. Owners of yachts
5. Owners of tourist tour boats
6. Owners of marine agent boats
7. Owners of service ships
8. Fishermen (do not have a ship)
9. Port management (except chief)
10. Port staff
11. Fisheries Engineer from the Ministry of Agriculture and Forestry
12. Personnel of Coast Guard Command
13. Security personnel responsible for public order (Police/Gendarmerie)
14. Personnel of municipal responsible for cleaning
15. Civilian citizens, who have spent time in the port and are not involved in general operation

Four meetings and twelve participants were interviewed for each fishing port and risks were identified and evaluated. These meetings were held to identify and assess the determined the risk of each port. During the meetings, the co-operative chief was not invited for the meeting to be objective to work. Permission requests for public personnel were made at this time. Meetings were held with selected twelve persons from each fishing port. The three meetings were held at the Faculty of Marine Sciences and Technology, Iskenderun Technical University, excepting the meeting for the Çevlik fishing port which is away from the faculty. The meeting for the Çevlik fishing port was held at its own location of the Çevlik fishing port. A presentation was made about the purpose of the research before the meetings. The meetings have three stages:

1. At the first stage of the meeting, risks were identified in three groups of four. Later, members of the group were given the opportunity to check their own risk.
2. For every risk identified, the possible potencies of health, goods, labor and environment losses and frequencies were scored between 1 and 10.
3. The risks identified in the last session of the meetings were determined on the basis of the reason and mean.

There are 4 classes when assessed the potency of health loss including (i) no loss of health, (ii) slight injury, (iii) serious injury, (iv) disability and death. The loss of goods, the loss of occupation, and the environmental damage potency were evaluated in terms of money (Turkish Liras) in five groups. The frequencies were classified as (i) not available, (ii) 6 months, (iii) 1 year, (iv) 1-10 years, and (v) 10-49 years by a time expression for each risk.

The potential and frequency matrices, the losses of health, goods, labor and environment were demonstrated for each port in Figure 1. An evaluation was made according to the value of the potency and frequency by assuming that there is a generated linear line. The one of this linear line consists of three parts in the matrix graph (X-axis the risk potency, Y-axis is the risk frequency): (1) The blue area is lower than 3 for the value of potency and frequency, and it is located under the line, (2) the yellow area is between 3 and 6 for the value of potency and frequency, and it is located on the line, (3) the red area is higher than 6 for the value of potency and frequency, and it is located upper the line.

The causes of risks were examined by risk analysis and these risks were classified as structural, administrative, pollution and public order. A solution was recommended for all risks identified by the participants by considering the assessment of risk analysis.

Table 2. The determination at risks, risk class, risk for frequency and potential loss of health, good, labor and environmental values for Dörtyol Fishing Port (Str: structural, Pol: pollution, Adm: administrative, Sec: security and Env: environmental)

Code	Description	Category	Frequency				Potency			
			Health	Good	Labor	Env.	Health	Good	Labor	Env.
01	Inadequate depth basin	Str.	4.7	5.9	7.0	4.4	1.3	8.0	5.7	5.7
02	Inadequate cleaning	Pol.	1.0	3.3	5.4	7.4	2.5	3.5	4.7	7.3
03	Disputes and problems in boat tying	Adm.	2.9	4.1	4.2	2.5	3.3	5.8	4.9	3.0
04	Public security problem	Sec.	3.5	8.6	6.5	5.4	8.5	9.2	8.4	6.3
05	Income inadequacy for management	Adm.	1.6	3.9	5.7	6.5	4.2	4.5	6.7	7.1
06	Low of west breakwater	Str.	3.5	6.4	4.9	4.7	6.3	6.1	4.6	2.4
07	Swimming attempts	Sec.	4.8	5.1	4.5	4.3	8.4	6.3	5.7	4.0
08	Open to the public entering	Sec.	2.0	5.4	4.5	4.7	3.1	6.1	4.8	4.7
09	Lack of fire extinguishing system	Str.	2.0	2.4	2.5	2.9	5.3	4.6	4.4	4.2
10	Lack of first aid emergency	Adm.	2.5	1.6	1.8	1.4	4.5	2.4	2.1	2.2
11	The inability of the docks	Str.	2.3	4.5	5.7	4.2	5.1	5.5	6.4	4.6
12	The lack of lighting	Sec.	1.5	4.8	5.2	4.3	4.0	6.1	5.7	4.1
13	Lack of the superstructure (the resting place, etc.)	Str.	1.1	1.3	2.5	2.4	2.5	2.4	3.7	2.8
14	Absence of a ship's bilge system	Pol.	1.1	2.4	2.0	6.0	2.9	3.7	4.1	8.4
15	No state office for fishing	Str.	1.0	1.3	3.8	1.2	2.0	2.5	4.3	1.5
16	Illegal angling fishing	Sec.	2.3	3.9	3.9	5.0	4.5	5.4	5.5	6.2
17	No rules for car traffic	Sec.	1.8	3.5	2.6	5.7	5.4	5.8	4.9	6.6
18	Need to WC and shower	Str.	1.8	1.6	4.3	5.5	2.6	2.5	5.0	6.5
19	Fire helicopter closely taking water	Adm.	1.0	1.3	1.0	1.2	1.1	1.5	1.2	1.5
20	No fishing material store	Str.	1.1	4.0	4.5	7.0	2.9	5.6	5.4	7.3
21	The ships in the harbor fast cruise	Sec.	1.5	3.4	2.2	3.8	3.1	4.6	3.7	4.1
22	No management office	Str.	1.0	2.0	5.7	3.7	1.9	3.3	6.4	4.5

Results

The risks associated with each fishing port and their effects were shown with risk frequently groups in Table 2, Table 3, Table 4, and Table 5. While 22 risks were determined for Dörtyol Fishing Port, these risks were defined as ten risks for the structural category, six risks for the security, four risks for the administrative, and two risks for the pollution. The first visible risk for Dörtyol Fishing Port is that the depth of the port is shallow. However, it should be noted that there was no significant ship accident. This fishing port management has imposed the deep process under the more because of the high fish costs. Another structural important risk is the height of the west breakwater, and the height must be increased. The wave height increase may cause undesired impacts on the dock. One of the other structural deficiencies in the fishing ports is the absence of a fire extinguishing system which is vital and essential for the functioning of the ports. The four administrative risk solutions important for Dörtyol Fishing Port were very simple for the port management, but the port manager has been made unauthorized to solve these problems by the law. Security risks were determined as inadequate authority for port management and the lack of security staff due to economic insufficiency. There were two risks associated with pollution including the use of the dock in a sloppy way and the lack of waste systems (Table 2).

Table 3. The determination at risks, risk class, risk for frequency and potential loss of health, good, labor and environmental values for İskenderun Fishing Port (Str: structural, Pol: pollution, Adm: administrative, Sec: security and Env: environmental)

Code	Description	Category	Frequency				Potency			
			Health	Good	Labor	Env.	Health	Good	Labor	Env.
01	Water and electricity not available	Str.	2.5	7.5	8.6	6.7	5.8	6.7	7.9	6.3
02	Public security problems	Sec.	2.7	7.8	4.6	7.2	5.8	7.2	6.2	7.0
03	Insufficient of fire response system	Str.	3.8	6.4	5.7	5.9	7.1	7.1	6.6	6.6
04	Lack of emergency response plan	Sec.	5.0	5.6	7.5	6.5	6.6	6.9	5.7	5.5
05	No dock on the east side	Str.	3.5	5.9	6.2	5.5	6.3	6.5	7.4	6.9
06	Lack of vessels maintenance area	Str.	4.1	6.3	6.4	5.5	6.1	7.3	8.0	6.9
07	Low dock in middle side	Str.	2.4	6.3	6.9	6.7	5.2	7.4	7.5	7.2
08	The lack of main breakwater	Str.	1.2	2.5	3.6	3.3	2.5	2.7	3.8	3.8
09	Income inadequacy for management	Adm.	2.6	5.8	7.4	4.3	5.3	7.5	8.1	6.1
10	Cooperative management method	Adm.	2.6	5.5	7.8	6.7	5.3	6.4	7.5	7.1
11	Bottom wastes from the port	Pol.	2.5	4.6	5.2	7.9	4.1	6.5	5.6	7.7
12	Lack of the superstructure (the resting place, etc.)	Str.	1.5	1.8	2.5	4.0	3.1	3.4	3.9	5.2
13	Lack of park car entry, park and traffic planning	Str.	1.5	3.3	5.3	5.2	4.5	3.9	5.9	5.3
14	Non-payment of fees on a regular basis and adequate for vessels sheltering	Adm.	1.4	3.6	5.3	4.2	2.5	5.5	7.1	5.1
15	Shelter of marine vehicles except fishing	Adm.	2.4	4.0	5.4	4.1	2.7	4.5	5.9	5.1
16	Lack of skilled personnel	Adm.	2.1	5.1	6.1	4.9	3.7	5.8	6.0	4.2
17	No fishing material store	Str.	1.8	5.2	4.5	5.2	3.0	6.9	6.6	5.9
18	Using of the dock for ship maintenance	Str.	1.6	3.3	3.6	4.0	4.3	4.4	4.2	4.9
19	The use of inappropriate system for small ships	Str.	1.8	4.6	4.8	6.5	3.6	4.2	4.9	6.7

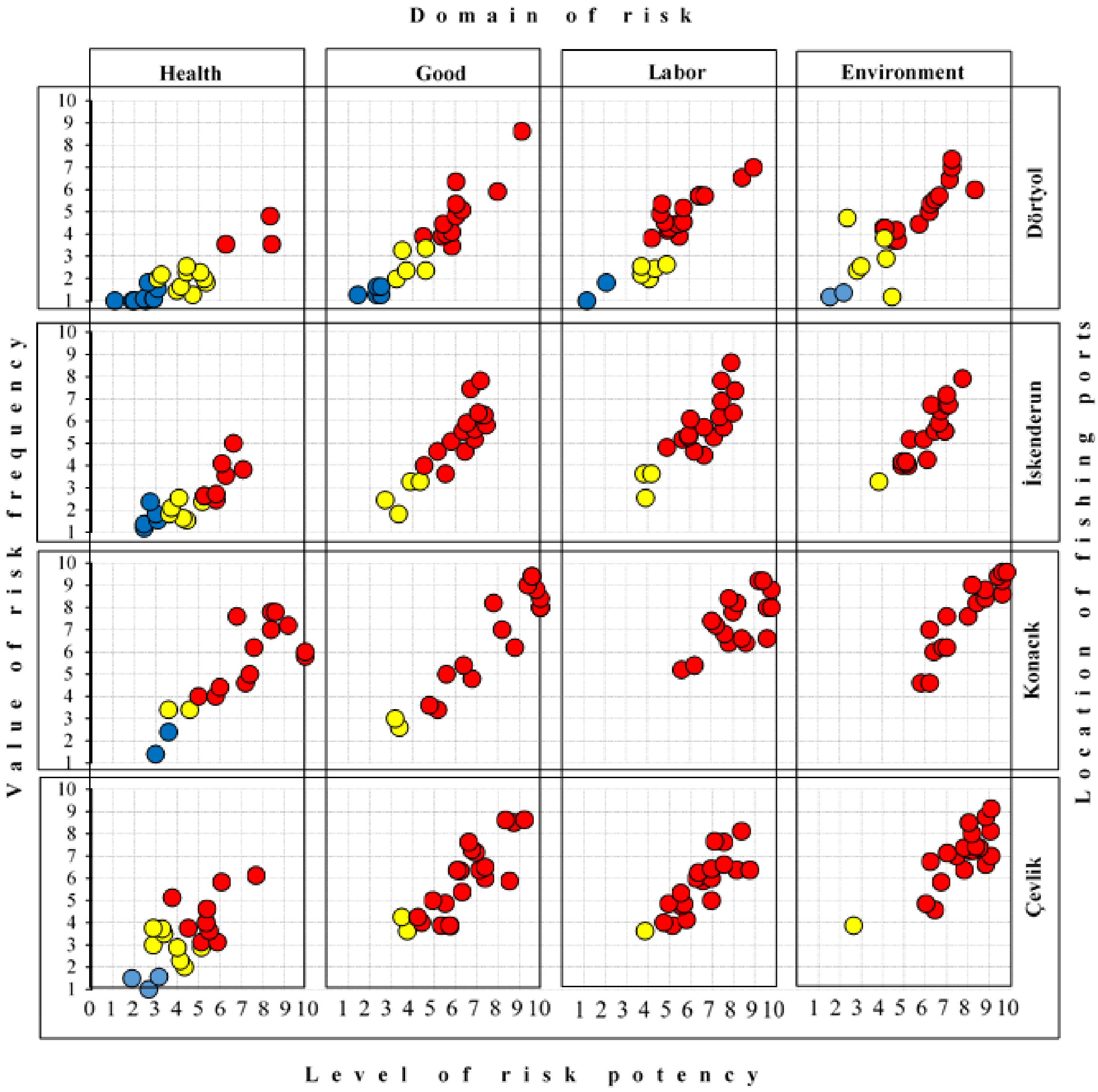

Figure 1. Matrices of the determined risks frequency values and risk potency level with health, good, labor and environmental loses for the four fishing ports in the area (red point: important risk, yellow point; medium risk, blue point: negligible)

There were 19 risk definitions in İskenderun Fishing Port. These risks were classified as eleven risks for the structural category, two risks for the security, six risks for the administrative, and one risk for the pollution. This fishing port has the most intensive fishing, tourism and other maritime activities. In addition, the general public security problem and the irregularities of port-related activities were found to be very high. Electricity and domestic water shortages are often experienced in the port due to the fact that the invoice fees are not paid. All defined administrative risks were related to each other and the main reasons were the low financial income and deauthorization of the port management. Most of the determined structural risks were similar to the Dörtyol fishing port, but there were port-specific risks related to the deficiency of the ship docking areas. The most important problem in terms of pollution is that no ship waste is collected. (Table 3).

While 17 risks were determined for the Konacık Fishing Port, these were defined as seven risks for the structural category, four risks for the security, five risks for the administrative and one risk for the pollution. Konacık fishing port is the most economic insufficiency port, while it has the least structural risk. (Table 4).

Totally 20 risks were identified for Çevlik Fishing Port including nine risks for the structural category, six risks for the security, three risks for the administrative and two risks for the pollution. In general, there were similar risks to the fishing port while there were also regional differences. It is an important structural risk was been absented short outer breakwater of the port, because it was a continuous movement of the sea in the

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port. The insufficient space ship maintenance, the lack of binding vessel dock and the dock consists of the binding systems maintenance were identified as major tree structural risks. In there, this was different from other private fishing port; the risk of not honey a fish sales place was seen as risk. (Table 5).

Increasing risk for all fishing port in the region were requested to potency and frequency matrix. These matrices were given in Figure 1, as each port and the health, goods, labor and environmental losses.

Figure 2 shows the frequency valves of the average potency and risk that arise when the risk ide field at each fishing port are divide into four categories as structural, administrative, security and pollution. This figure shows that as labor losses have the highest average levels arise that they maritime environmental at good losses.

Table 4. The determination at risks, risk class, risk frequency and potential loss of health, good, labor and environmental values for Konacık Fishing Port (Str: structural, Pol: pollution, Adm: administrative, Sec: security and Env: environmental)

Code	Description	Category	Frequency				Potency			
			Health	Good	Labor	Env.	Health	Good	Labor	Env.
01	Public security problem	Sec.	4.6	9.4	7.4	6	7.2	9.6	7	6.4
02	Environmental pollution	Pol.	3.4	4.8	6.8	9.2	4.6	6.8	7.6	9.6
03	The risk created by the floating	Adm.	6.2	6.2	6.4	8.6	7.6	8.8	7.8	9.6
04	Shredding storm breakwater	Str.	7.6	8.2	8.4	7.6	6.8	7.8	7.8	7
05	The lack of electricity and water	Str.	7.8	9	8.8	8.8	8.6	9.4	9.8	8.8
06	The lack of a shower and a WC	Str.	7.8	9	8.8	7	8.6	9.4	9.8	6.2
07	line fishing	Sec.	4.4	5.4	6.6	6.2	6	6.4	8.4	7
08	Lack of first aid intervention	Str.	7	3	7.8	4.6	8.4	3.2	8	5.8
09	Fire response system deficiency	Str.	7.2	8.4	9.2	9.6	9.2	10	9.4	9.8
10	Income inadequacy for management	Adm.	4	5	8.2	6.2	5.8	5.6	8.2	6.8
11	Cooperative management method	Adm.	4	5	8.2	6.2	5.8	5.6	8.2	6.8
12	Lighthouse function insufficient	Sec.	5.8	8	8	8.4	10	10	9.6	8.8
13	The lack of lighting	Sec.	6	8	8	9.6	10	10	9.8	9.6
14	No fishing material store	Str.	4	8.8	6.6	8.2	5	9.8	9.6	8.4
15	Lack of the superstructure (the resting place, etc.)	Str.	2.4	3.4	6.4	7.6	3.6	5.2	8.6	8
16	Non-payment of fees on a regular basis and adequate for vessels sheltering	Adm.	1.4	3.6	5.2	4.6	3	4.8	5.6	6.2
17	No rules follow to individuals	Adm.	5	7	7.2	9	7.4	8.2	7.2	8.2

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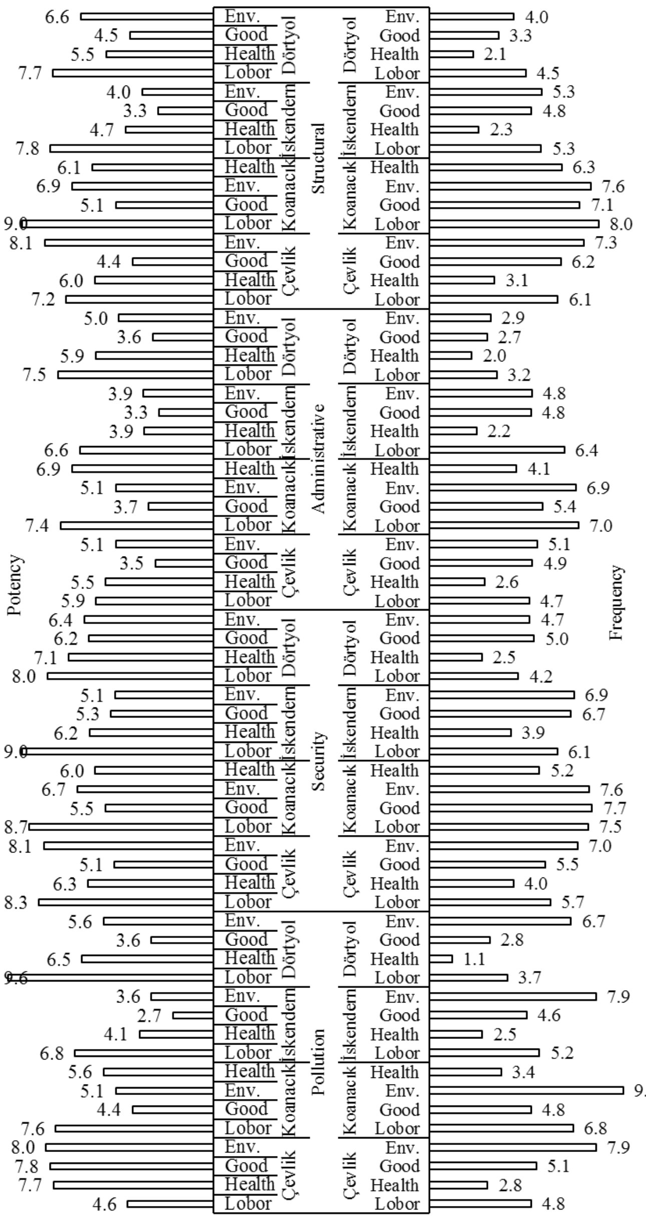

Figure 2. Risk frequency and potency in category in fishing port and domain

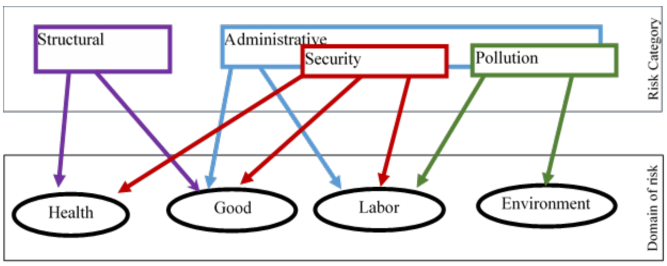

Figure 3. Overview diagram of risk category and domain for the fishing ports

Generally, loss of healthy risk values is lower than average. The categorized results of these values of risk potency and frequency for each fishing port only some points were found statistically difference by multiple analysis of variance (using SPSS 17- MANOVA). When categories of risks and domains of risks were evaluated by SPSS numerically correlations summary schema could have been obtained in Figure 3. This figure illustrated that which risk category is causing losses in the risk.

Discussion

Fishing ports, while providing logistic support for fishing, have not been valued in terms of scientific publication as an important point for realistic fishing control (Flothmann et al., 2010). It is not possible to say that there is a standard application for fishing port construction, business and management in the world (Scheffczyk, 2010). Today there have some clarifications that fishing ports can be turned into tourism support by marinas and others tourism activities due to loss of economic value for fisheries (Kim & Sung, 2016). This and similar expression have been expressed different people in our regional and national arena.

Turkish State makes significant investments for the fishery season in the other maritime sector, there are 385 fishing ports on coast of Türkiye, and this is a negative aspects of sea vehicles as a refuge for the vessels. In addition, these investments are always ongoing for both the improvement of it made and the construction of new fishing ports. The region also carried out a new fifth shelter. Repair of Iskenderun dock and of Çevlik breakwater fishing port were made in recent years and it is planned renovation for Dörtyol fishing port (UDHB, 2022). The ministry of transport communication and the maritime prepared a new fishing put became it did not fit any of the costal stretch, but many serious objections occurred the new circular by different participants (TCRG, 2017). It has estimated that, the risks identified for fishing port in this study will be reduced by this new circular, but the reduce of risk will not provide neither ensuring the satisfaction of the stakeholders nor provide the expected sustainability of fishing port management.

In this study, wide perspectives were formed with the participation of the different status to meeting. In this way, main figures of the four fishing ports' function were reached and it was observed the first time that their roles had been demonstrated as risk to loses of health, goods, labor and environment. As a result of the risk analysis there were a few specific risks for each fishing port, but in the overall, the defined risks were similar, and some were the same. Therefore, in this discussion the reason of the risks identified to assess the differences between fishing port. In this context, all these risks such as structural, administrative, security and pollution were divided into four groups for each fishing ports.

Structural risks, 6, 10, 7, and 9 were defined as Dörtyol, İskenderun, Konacık and Çevlik fishing ports respectively. The

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Table 5. The determination at risks, risk class, risk for frequency and potential loss of health, good, labor and environmental values for Çevlik Fishing Port (Str: structural, Pol: pollution, Adm: administrative, Sec: security and Env: environmental)

Code	Description	Category	Frequency				Potency			
			Health	Good	Labor	Env.	Health	Good	Labor	Env.
01	Ship mooring plan insufficient	Adm.	2.0	6.3	5.3	6.5	4.3	6.2	5.5	6.7
02	Cold storage and exhibition place	Str.	1.0	6.0	4.8	5.8	2.7	7.3	5.7	6.7
03	Public security problems	Sec.	5.8	7.2	7.7	8.0	6.0	7.0	7.2	8.2
04	The outer breakwater short	Str.	2.0	8.5	7.7	8.1	4.3	8.8	7.2	9.0
05	The lack of a shower and a WC	Str.	3.0	4.3	4.8	8.8	2.9	3.5	5.6	9.8
06	Inadequate depth in dock	Str.	5.1	7.6	5.9	7.4	3.8	6.6	6.6	7.8
07	Lack of vessels maintenance area	Str.	3.8	4.9	6.3	6.8	2.9	5.5	6.4	6.3
08	Lack of dock according to the ships number	Str.	3.7	7.3	6.4	7.4	3.3	6.7	7.0	8.3
09	Disputes of vessel sheltering fee	Adm.	3.5	4.3	4.0	3.9	3.4	4.3	4.8	2.6
10	Wastes in bottom	Pol.	4.0	6.5	6.0	8.5	5.3	7.3	6.3	8.0
11	The lack of lighting	Sec.	3.6	8.6	7.6	9.1	5.5	8.3	7.5	9.1
12	Insufficient of fire response system	Str.	3.1	5.9	6.4	7.0	5.9	8.5	8.8	9.1
13	The presence of stray animals	Sec.	3.8	3.9	3.4	6.6	4.5	5.8	5.1	8.8
14	Swimming	Sec.	6.1	3.9	5.0	6.4	7.6	5.4	7.0	7.8
15	Absence of a ship's bilge system	Pol.	1.5	3.6	3.6	7.3	1.9	3.9	3.9	8.0
16	Problem using of water and electricity	Str.	2.9	5.0	6.4	7.4	5.1	5.0	8.1	8.5
17	Illegal angling fishing	Sec.	2.9	5.4	6.6	7.3	4.0	6.3	7.5	8.3
18	Broken bollard in dock	Str.	3.1	6.4	6.0	7.1	5.1	7.1	7.0	7.0
19	Non-payment of fees on a regular basis and adequate for vessels sheltering	Adm.	2.3	4.0	4.9	4.9	4.1	4.4	5.0	6.0
20	Open to the public entering	Sec.	1.6	3.9	4.1	4.6	3.1	5.7	5.9	6.4

common point of structural risks was evaluated by shortcomings in upper structure as lack of operations building, storage for fishing gears, toilet, bathroom, fire-extinguishing system soon. Administrative risk categories are occurred from legal negligence or regional and personal mistakes. In fact, the risk of, security and pollution can be considered as an administrative but more accurately defined because of an excess risk.

The matrix graph shows that the risk area was similar in fishing ports despite of different level. While goods, labor and environment losses risk were very high in all fishing ports, the assessment of the health risks were lower. Nevertheless, different number significant health risks were evaluated for each port. According to the overall assessment, the Dörtyol Fishing Port is showing more favorable condition compares to others. As a result, it can be said that the view point of Dörtyol fishing ports is better than the other fishing port. If we need to dwell on this point, its economic income and expenses can be observed to be more positive than others. This positive case has showed differences in the port administration which uses

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service ships. The members of fishing cooperative who fishing port operator boat have different service for other maritime activities they provide positive income for the fishing port, since this marine sector's is much better to than income especially small-scale fishing. This is good income for the fishing port, it provides a good image compare to the other three ports, but some fisherman who were clamming non-fishing vessels crowded have not to be unhappy with this issue.

In this result of the study, it can be said that, the status of four fishing ports is similar but there are differences in their use, according to regional needs and demands. These differences can give place different results, as listed below.

1. Dörtyol Fishing Port was better than the others in terms of loss of healthy and good.
2. Iskenderun Fishing Port was incorporated serious public security problems for loss of healthy and good.
3. Konacık Fishing Port was a low income to ensuring minimum quality of facility.
4. Çevlik Fishing Port's problems were a common risk for the whole fishing port.

Conclusion

It is clear that the fishing port will not be a regulatory mechanism for the current approach and expense risk management for many people in our country. More important investments for coastal structure areas are made incorrectly only by the operations of the fishing cooperate. Because the operator experts should have at least management elements for sustainability.

In the legal circular for these coastal structures defined fishing port should be need to planned according demands of all maritime activities, and that port operator's, insufficient income should be decreased, which is the biggest problem for the operation of these areas. This approach must be regional or even the port-based instead general management. Moreover, this planning should be included demands of different fishing methods vessel. Because a fishing vessel is changed the activity in the harbor according to fishing method.

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Compliance With Ethical Standards

Authors' Contributions

This study is a part of master thesis of the first author, which was carried out under the supervision of the second author.
ÖA: Study design, Drafting, Writing, Statistical analyses.
AD: Study design, Drafting, Writing, Statistical analyses, Supervision.
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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RESEARCH ARTICLE

In silico analysis and tissue-specific transcription of platyfish (*Xiphophorus maculatus*) catalase gene

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ABSTRACT

The present study focused on conducting in silico analysis and investigating the tissue-specific distribution and expression of the catalase gene in platyfish (*Xiphophorus maculatus*), which can be used as a model organism for studying stress responses in fish. Assay of the steady-state levels of *cat* gene transcripts by real time PCR revealed. The steady-state level of platyfish *cat* transcript was abundant liver (2162.21) compared with the level of *cat* transcript in intestine (1270.94), heart (1241.25), muscle (419.157), brain (46.205), eye (47.57), swimming bladder (28.99), gills (81.18), spleen (95.45), kidney (20.25) ovary (91.16) and testis (113.22). The results suggest that the liver is the major site of *cat* expression in platyfish, with significantly higher expression levels compared to other tissues. In addition, the research involved using bioinformatics tools to analyze the genetic sequence of the catalase gene and predict its structure and function. The results of the study indicated that the *cat* in Platyfish shares a high sequence identity and similarity with its orthologs in other teleost species, including medaka, fugu, and zebrafish. This observation suggests that the *cat* gene is conserved among these fish species, and the gene's function and regulatory mechanisms are likely to be similar. The high conservation of the *cat* gene among teleost fish species highlights the importance of this gene in the antioxidant defense system and its potential role in responding to environmental stressors. Platyfish *cat* gene exhibits a conserved gene structure, as evidenced by its conserved gene synteny with the orthologous *cat/CAT* genes in other teleost fish and humans. Overall, the study provides evidence for the highly conserved gene structure of the *cat* gene in platyfish, which contributes to its functional stability and the maintenance of its critical role in antioxidant defense and stress response mechanisms.

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Introduction

The catalase gene is an essential component of the antioxidant defense system, which helps to protect cells against oxidative stress caused by various environmental stressors (Sies, 2017). Catalase is an enzyme that plays a crucial role in protecting cells from oxidative damage by breaking down hydrogen peroxide into water and oxygen (Chance & Maehly, 1955). Many studies have investigated the expression of catalase genes in fish, which can provide insights into the antioxidant defense mechanisms of fish and how they respond to environmental stressors. Zhao et al. (2019) examined the expression of catalase genes in the liver, muscle, and gill tissues of grass carp (*Ctenopharyngodon idella*) under different environmental stressors, including hypoxia, ammonia, and nitrite exposure. The results showed that catalase gene expression was upregulated in all three tissues under hypoxia, while it was downregulated in the liver and gill tissues under ammonia and nitrite exposure. Bopp et al. (2008) investigated the expression of catalase genes in the liver and kidney tissues of rainbow trout (*Oncorhynchus mykiss*) exposed to copper. The researchers found that catalase gene expression was significantly increased in both tissues after exposure to copper, indicating that the fish were using this antioxidant defense mechanism to combat the oxidative stress caused by the copper exposure. Miryaghefi et al. (2016) found that catalase gene expression was upregulated in both tissues when fish were fed a diet with higher levels of vitamin C, suggesting that vitamin C may enhance the antioxidant defense mechanisms of fish. Fish are frequently exposed to such stressors, making them an excellent model organism for studying stress responses in animals (Balasch & Tort, 2019). Platyfish (*Xiphophorus maculatus*) has been extensively used to study stress responses in fish (Heston, 1982) is an omnivorous freshwater fish live from Northern Mexico to Central and South America (Zaret, 1984) and prefers warm waters, canals and slow-flowing watery trenches, alluvial bases and grassy shores (Arthington, 1989). As known, zebrafish and medaka are the most widely used fish species as a model organism (Iwamatsu, 2004; Howe et al., 2013). Platyfish also has an important place in genetic studies such as these two aquatic organisms (Schartl, 2014). Platyfish has been used as a research model in various fields ranging from genomics and genetics, evolution, ecology, to systematic, since the early 1930s (Kang et al., 2013; Schartl et al., 2013). Platyfish is also in an important point for logistical view of all the other evolutionary models. Its genome sequences are made (Schartl et al., 2013) and possible to reach the sequences in the ENSEMBL genome database (https://www.ensembl.org/Xiphophorus_maculatus/Info/Index).

In recent years, there has been a growing interest in investigating the tissue-specific distribution and expression of the antioxidant enzyme genes in fish to better understand its role in stress responses. In this context, a recent study has conducted in silico analysis and examined the tissue-specific distribution of the *cat* gene in platyfish. The study also employed bioinformatics tools to analyze the genetic sequence of the cat gene in platyfish and predict its structure and function. The study's findings highlight the importance of the cat gene in the antioxidant defense system and its potential role in responding to environmental stressors. Overall, the study provides important insights into the tissue-specific distribution and expression of the *cat* gene in platyfish and its highly conserved gene structure. These findings contribute to a better understanding of the cat gene's role in antioxidant defense and stress response mechanisms in fish and could have implications for developing novel approaches for mitigating the impact of environmental stressors on aquatic organisms. Overall, these studies highlight the importance of catalase gene expression in fish and how it can be influenced by environmental stressors and dietary factors. Further research on catalase gene expression in fish may provide valuable insights into the antioxidant defense mechanisms of fish and how they respond to different environmental conditions.

Material and Methods

Husbandry and Dissection of Platyfish (Xiphophorus maculatus)

The study used a total of 6 platyfish (*X. maculatus*) that were obtained from a commercial supplier in Erzurum, Turkey. The fish, weighing approximately 3.2±0.3 g, were transferred to the Laboratory of Agricultural Biotechnology and fed with commercial fish feed. The water temperature of the aquarium system was maintained at 26±0.4°C. To determine the distribution of tissue-specific expression of the catalase gene, six fish (3 females and 3 males) were used. Liver, gill, testis, ovary, intestine, kidney, stomach, eye, heart, muscle, spleen, and brain tissues were collected from fish. The study followed experimental protocols approved by the Atatürk University Local Ethical Committee for Animal Studies. Before dissection, the fish were anaesthetized with clove oil, and all dissecting instruments and the working bench were sterilized and cleaned

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with a cleaning agent for RNase (RNase ZAP, Invitrogen™). The samples were transferred to nuclease-free tubes containing 1 ml RNAlater (RNAlater™ Stabilization Solution, Invitrogen) and kept at 4°C overnight. The samples were then stored at -80°C until RNA isolation.

RNA Isolation, Reverse Transcriptase (RT) and Real-time PCR (qPCR) Analysis

Samples were removed from RNA later before homogenization with Trizol reagent (Life Technologies) was used for total RNA extraction. Nanodrop 8000 spectrophotometer was used for determine the RNA concentrations and RNA agarose gel-electrophoresis was used for determine the quality of total RNA. RNA was converted to mRNA into cDNA by employing the High-Capacity cDNA Reverse Transcription Kit (Life Technologies) after DNase treatment (DNase I, Amplification Grade, Life Technologies). A Rotor-Gene 6000 thermal cycler system (Qiagen GmbH, Düsseldorf, Germany) and a QuantiTect SYBR Green PCR kit (Qiagen) were used for RT-qPCR analyses to determining transcription of target (*cat*) and reference (ß-actin (*actb*) and elongation factor 1 alpha (*ef1α*)) (number of copies/μL) in different tissues of platyfish. Quantitative PCR was run in the final volume of 20μl by using 10 μL SYBR Green, 5 μL DNAse/RNAse free 2 μL forward primer, 2 μL reverse primer and 1 μL cDNA template. A tube which doesn't include cDNA was used as a negative control for each qPCR reaction. RT-qPCR conditions were initial denaturation (95.0ºC for 15 min), 40 cycles-denaturation (95.0ºC for 20 s), primer annealing [optimum temperature for primers for 30 s] and elongation (72.0ºC for 30 s). To calculate normalized steady-state levels of platyfish *cat* mRNA transcripts in each tissue, copy number of *cat* mRNA transcripts divided to copy number of two reference genes (*actb* and *ef1α*) and constitutively expressed at approximately the same steady-state levels in all tissues and the mean of both values were taken.

Primer Optimization

The forward and reverse primers were designed using NCBI Primer-BLAST for real time qPCR amplification of platyfish *cat* and reference genes (*actb* and *ef1α*) (Table 1). Exon-exon junction model was used for primers designing for avoid PCR amplification of a product from any contaminating hnRNA or genomic DNA (Keşan et al., 2022). Ordered lyophilized primers diluted in TE buffer (10mM Tris, 1mM EDTA and pH 8.0) in such a way that the stock concentration for each primer was100 pmol/μl.

Quantitative Polymerase Chain Reaction

PCR was performed in the final volume of 25μl using 0.4 μM forward and reverse primer as well as 100 ng template cDNA. Platinum PCR Supermix (Invitrogen, Carlsbad, California, USA) was utilized to run a PCR. The annealing temperature was optimized by taking six different temperatures for the target and reference genes. Agarose gel (1%) electrophoresis was performed to confirm the anticipated size of PCR products and the optimum annealing temperature was determined for each primer set by observing thermal gradients.

Identification and Structural Determination of Platyfish Catalase Gene

Sequence identity and similarity among platyfish, medaka, fugu zebrafish, mouse, rat and human *cat* gene was designed using BIOEDIT Program. For this reason, it was collected the amino acid sequences of *cat* in these organisms from NCBI database. Teleost fish exhibits strict evolutionary conservation for gene structure in same gene family. Platyfish *cat* gene sequence was acquired by performing BLAST (http://www.ensembl.org/Multi/Tools/Blast) searches with identical orthologous zebrafish (*Danio rerio*). Ensembl IDs were determined as ENSXMAG00000025437 and ENSDARG00000104702 for catalase gene in platyfish and zebrafish and NCBI cDNA IDs of platyfish and zebrafish were XM_005815279 and NM_130912, respectively.

Phylogenetic Analysis

CLUSTALW (Thompson et al., 1994) at BioEdit software (http://www.mbio.ncsu.edu/bioedit/page2.html) used for sequence alignment of *cat* gene in platyfish (*X. maculatus*). The protein sequence of platyfish Cat was aligned with *Cat/CAT* protein sequences from platyfish, amazon molly, tilapia, fugu, tetraodon, zebrafish, common carp, goldfish, Atlantic salmon, brown trout, chicken, mouse and human. Human LCP2 was used as an external group for phylogenetic tree (Figure 1).

To determine the phylogenetic relationship of the platyfish and its *cat* gene sequence with other fish species, as well as mouse and chicken, a pairwise alignment was performed using the BLOSUM62 matrix to calculate sequence identity and similarity. A maximum-likelihood tree was constructed using the Poisson correction distance model based on amino acid

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Table 1. Sequences of primers used as target and reference genes

Platyfish	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Tm (°C)
cat1	TCTGTGGCTGGGGAGTCTG	GCTGAACAGGAACGACACCT	58.3
ß-actin	ACCCAGATCATGTTTGAGACC	ATGTCACGCACGATTTCCCT	55.8
Elongation factor1	ACGTCAAGATGGAGAGAACTCG	GTGAAGTGAACCCAGAGCGA	56.8

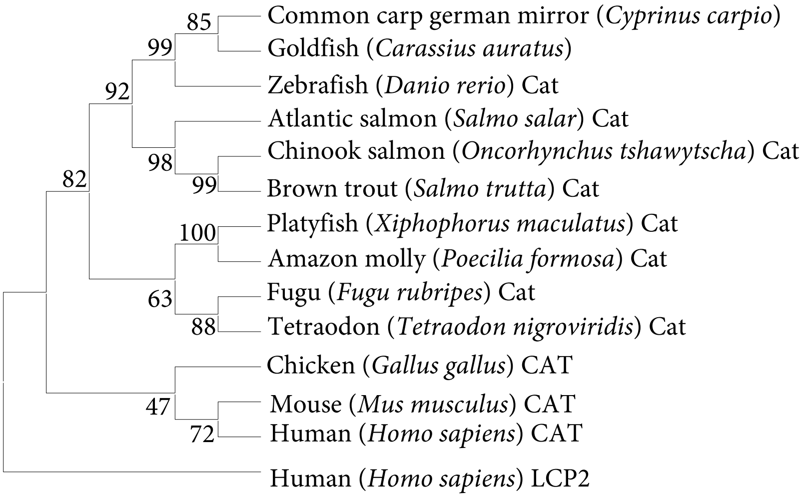

Figure 1. Phylogenetic tree of catalase gene in platyfish

substitution per site, using MEGA11 software. The tree was used to detect the evolutionary relationship of the platyfish with other fish species, mouse, and chicken. To confirm the phylogeny of the cat gene, a bootstrapped neighbor-joining tree was constructed prior to building the maximum-likelihood tree. In addition, the protein sequence of the human lymphocyte cytosolic protein (LCP2) was used as an external group, as done in a previous study (Kell et al., 2018).

Conserved Gene Synteny

To identify co-localized genes and create a conserved gene synteny map for the platyfish *cat* gene and its counterparts in zebrafish and medaka, we manually arranged the gene synteny using the Ensembl database. Specifically, we used the region conceptus selection to select the relevant regions of the genomes and identified the co-localized genes within those regions. By doing so, they were able to generate a conserved gene synteny map that showed the relationship between the *cat* genes of the three species.

Statistical Analysis

In this study, the researchers used SPSS Statistics 17.0 software to perform a one-way analysis of variance (ANOVA) and Duncan's multiple comparison tests. We used these tests to compare the levels of *cat* gene expression platyfish tissues. The experiments were conducted three times, each time in triplicate. The researchers considered a p-value of less than 0.05 to indicate a significant difference between the groups being compared.

RESULTS AND DISCUSSION

Bioinformatics and Computational Analysis of Platyfish Cat Gene

In the bioinformatics section of this study, statistical knowledge was gathered using biological data from Ensembl genomic database, NCBI database, BioEdit software, pairwise alignment of BLOSUM62 matrix program, and MEGA11 program (Tamura et al., 2021). The researchers used the Ensembl genomic database to obtain the longest cDNA sequence, which was used to determine the exon-intron structure of the platyfish *cat* gene. The researchers discovered that these genes have 13 exons and 12 introns, which follow the gt-ag rule. Additionally, the researchers identified putative TATA and CAAT boxes and polyadenylation signal for *cat* gene. Finally, a computational algorithm was tested and evaluated for showing the gene structure (Table 2).

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Table 2. Nucleotide Sequence of platyfish (*Xiphophorus maculatus*) catalase gene

ENSXMAG00000025437	
5'gctataacgttatatatatatatatatatatatatatatatatatatatatatatatata	-300
tatatatatatatatatatatatatatatatataccaaggtgtaggttgtgcaaggaggccc	-240
ctgagacagtccagcacataacagcagggtgcaagatactggcagggaaagcgtacatgg	-180
aacgacataaccaagttgcaggcatagtgtacagaaacatctgtgcagAATATggactgg	-121
aaaccccgagatcaaagtgggaaacacccccaaaggtggcggagaacgccagagctaata	-61
+1	
TCCTGTGGGCCTTCCAGATCCAGACAGACAAAATGGTAATGGCGAACCAACCAGACATTG	60
TCGTAGTGGATAAACAACAGAGGAAAGCCGTTGTGATAGATGTAGCAATACCAAGCGACT	120
GCAACATCAGGAAAAAGGAGCACGAGAAACTAGAGAAATACCAGGGCCTCAGGGAGGAAC	180
TGGAGAGGGCCTGGAAGGTGAAGACCACAGTGGTGCCTGTGGTCATCGGGGCCCTCGGGG	240
CAGTCACCCCCAAACTGGACCAATGGCTACAACAGATCCCAGGAACAACATCAGACATCT	300
CAGTCCAGAAATGTGCAGTCCTTGGCACAGCCAAGATACTGCGCAGAACCCTCAAGCTCC	360
CAGGCCTCTGGTAGAGGACCCGAGCTCAGAGGATAAGAACCACCCGCGGTGGGTGAGAAG	420
GGAATTTTTTTTTTTTTTTATATATATATATATATATATTTTTTTTTATTTTTTTTTTTTTTT	480
GTTTCCTCTTCGTCTTTTGATAAATTTACTCGAAGTAGCTAAAACGTTATACAAGGTCAG	540
CTGCAGCGCTTTTTTTTTTTCTTTTAAAGACAAATAGGTCGCGTTAGTCCTGTCAGCCAAT	600
CAGAAATGAGGGGCGAAGTAGAGGCGGAGTCACGTGGAACAAAGTCCAGGAAGACTTTCT	660
CTGGCATTGAGGTAAACAGATAACGCTTTTCTCCAAGACACCTACCGTGCAGTTAGTGGT	720
CCCGACGCGCTGTTTTGTCGAG**ATGGCGGAGACCAGAGATAAAACTACCGACCAGATGAA**	780
-M--A--E--T--R--D--K--T--T--D--Q--M--K	
GATATGGAAGGAGAACCGAGGCTCCCAGgtttgtacaaatgctgcatttttatcaagggt	840
--I--W--K--E--N--R--G--S--Q-	
tttgtttgctccgtgtccgacttgcaggaattgggtgacaggtcgtttggggtaacaaga	900
gtcgaagcccgtcggttgttcagtctttgctcgttattaacgtgagctaatctcaaacat	960
gctaacacatgttgtttaaatcagcactccggttttgtagtcgttttgttgcagtaatga	1020
gagaggttgctaatgttttgctagctgaaatttaaacaacgccatcaagcgagggctttc	1080
tcctccaagcattttggtcgcgacattaagaagggagctcctaaaatgtctcccgtgggc	1140
taaaattaagttgaaagacgtgaaagtgggtcgtttttattgcacatggtggaacttgag	1200
cctttagttgcataacaagaccacagttcagtttacattagtgcgctaaactgactgaag	1260
tctacctgatcctgcagctgaatgtgagggtttaacacaattcagctctgaaaggattct	1320
tcttattcaatttgccttattgacgcactttatttatcacaatatataaactattcatg	1380
tttatgcaaacaaacacatttcatcgggtaaagtcagtgttattacacttaaaatgggtt	1440
tagttaaaccacatttttcaaaactatcataatatgtgaacaacttagtaagttaatgt	1500
aaaataaagatacatttataaaaaaaacaatgcatttcaaagcacatgatttcttgtgaa	1560
aagtaagaacagagctattgacatgtgtatatatattatgtttttataaactgacatttg	1620
cagggtgttaagaaccagtcaggtcaatttatttccttcgatttacattgtaggttgtga	1680
aaagttttgaccgaaattcatgatatttgatggatgttgtaacttttcaatggttaaaat	1740
aaaggcctgttagttgacatgtgttagtcctttgttttactcaaccacaagatcatattc	1800
gttttcagtataattcaacccttaatttaaaaaaaataacacatatttcagttcaggtg	1860
tttttctattatcttcacatgcatcaaatcaatgttcatgtcacaaacctacacagttt	1920
ttttgtaaaaaaaaaaaaaagtataaatatgcttcctatatcttgacgtctgatccagta	1980
tgttatgagtgtagaaagctaactagctgtgctcatcttgaagacggggctacaccgtat	2040
tcttaaccaaatattagcctaatatttatgttcacactcgcctcctatcatgccggtttt	2100
attggcccattactcttctttttatttcgtgaaggaagtcggacgacgacaaagctgaaa	2160
ctgtcctttcagacaattttggacctttctgtgaggtgtgaataaaaatgtcaggaata	2200
tagttgtttttttttgccctccatagacgggtaaataaacatgctgccttttctgttttc	2280
cttcttgcccaatctaggtctgtgggtgatcaacagactctcgttggattgattaataca	2340
tttagattattagtggtagagctgtagaagaatgtgccacacagacaactgatttaaaaa	2400
agtccacttcttttatgcttttttttttatagttttacctgctctgcttttttaagttcat	2460
attgctgagatattttccagggtgtccacacaaccttaagaagtcttaaattcatatatc	2520
taaaatgaaagactgtctgaaattaattggaaaatgtacaagttggccttagacacgtta	2580
atcacaggtcttaaactttgttctggcaggattattccatgtagttcattgttctcccgg	2640
cgccttttgtgtcgggcaaaatttgactcgctcggaaacatcatcagtagcgtttgaggc	2700
taccgctaactagctgctaataaacactagctagctagctttttttgtgttcatcattgat	2760
aatggcaaatttatcggcagttggctggatgaccttcatcattgccactggctcacttct	2820
gctaccaattcgtacgatgcaatgtgcgttctgttcaaaagaatgaaataaaaatctgga	2880
ttttcttttgtacatttctgttgttacatgtctttaatttcattcataggggtcttaaaa	2940
agtcttaaatttgagttggtgaaaccggcagagatcgtgtttacttctgttgtctttgtt	3000
cagaggaatccaaatcctctcttctcatccaaagaaggttttgacattaaactgaattta	3060
aatgctattcatattgttgtgttaataaacatgtaaagttaggggtgtagttgttagcca	3120

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Table 2. continued

```
gggtaatatgtattaagaatttttcagtcggctttccattgggttactctttgcttgtca      3180
cctcatgagaagcacttttaataagaataagtatgaatggtttgagcccaaacttttaat      3240
atatattttttttaaattcttgaactgcatttggttccagAGACCAGATGTGCTAACGAC      3300
                                        -R--P--D--V--L--T--T
AGGAGGTGGCCATCCCGTTGGGGACAAGCTCAACCTGCAGACTGCGGGGCCGAGGGGCCC      3360
--G--G--G--H--P--V--G--D--K--L--N--L--Q--T--A--G--P--R--G--P
TCTGCTCGTGCAGGATGTGGTCTTCACCGATGAGATGGCCCACTTCGACCGGGAGCGAAT      3420
--L--L--V--Q--D--V--V--F--T--D--E--M--A--H--F--D--R--E--R--I
CCCAGAGAGAGTGGTGCACGCTAAAGGCGCAGgttagctaacggttgtcttacagctcat      3480
--P--E--R--V--V--H--A--K--G--A--
gtgttttggactcaaggtctcatttgctttgataaacaagtttcacgcaataaattcaa      3540
cccctccagacagcaagagttaagtttttattgtagaaactgcttttcgtaaataataat      3600
ttaactttttaccgtacgtgtgtaatttaatttctgagctcttttttgttcaaaggaacgt      3720
tctagaagctttagaacgggacaatttttttccacatccagactttaaacaaacagcaagc      3780
cctcctgaaaagctgctacgttgttctcaatgctgatacgctgaaacattcaacagcatc      3840
gatgtaaaggtgattttgtatttttcttcccatgatgcagGCGCGTTCGGCTACTTTGAG      3900
                                        G--A--F--G--Y--F--E-
GTCACTCACGACATCACCCGCTACTGCAAGGCCAAGGTGTTTGAACATGTTGGGAAAACG      3960
-V--T--H--D--I--T--R--Y--C--K--A--K--V--F--E--H--V--G--K--T-
ACTCCCATCGCTGTCCGGTTCTCCTCTGTGGgtgagagattcactttcatttccaaggaa      4020
-T--P--I--A--V--R--F--S--S--V--
cattctcactctttgtcgtcgtaggaatagctgtgccatgtttgtgcgcaataatttaat      4080
tttacacttgaaaataatttcctccaaagtgatccactgatgtaatttttcatctggag      4140
aaccaaaaaatgtgttgacacgtctaactgtctgtctagcaggtagctacttgctagcta      4200
gccgcagtgttgcaatatgtgtattttttttatttaaccatgatgttcacacgcctctact      4260
cctgcagcacccatattctgttcttttatgatggcagagggcgctgttgtgcaactttta      4320
atatgtggctttgttgcttctgttactgctgtagaaaatataaggtctttgtatgatgaa      4380
gctgcacacaacaaactgaatattataaacgacgattaaaatgcttgtgcgctcagCTGG      4440
                                                        A--G
GGAGTCTGGATCAGCCGACACTGTCCGAGACCCCCGAGGCTTCGCTGTAAAGTTTTACAC      4500
--E--S--G--S--A--D--T--V--R--D--P--R--G--F--A--V--K--F--Y--T
CGAGGAGGGGAACTGGGACCTGACGGGCAACAACACCCCCATCTTCTTCATCAGGGACGC      4560
--E--E--G--N--W--D--L--T--G--N--N--T--P--I--F--F--I--R--D--A
AATGCTTgtaagttggcgtttaaaatttggagttcaaccactttatttgttattttatcg      4620
--M--L-
gtgctgtttgttttctcttcttccgccctgcagTTTCCGTCCTTCATTCATTCCCAGAAG      4680
                                 -F--P--S--F--I--H--S--Q--K-
CGTAACCCTCAAACCCACATGAAGGATCCTGACATGGTGTGGGACTTCTGGAGCCTGAGG      4740
-R--N--P--Q--T--H--M--K--D--P--D--M--V--W--D--F--W--S--L--R-
CCTGAGAGTCTGCATCAGgtagccgcggatattcaacctttagcacttccaggaactaaa      4800
-P--E--S--L--H--Q-
ataaacacatgttctaaaatgatttggtaatggggaacttgcaaaccacagtcgctcccg      4860
ctgatgaacattttttatctgttttatttgtcagtatctactttaacacgctctttttcta      4920
ccttctgtctatgaagacaatctgtttgtcacagattgtctgaaaccctccacacatcca      4980
tgatggagcataaacacgtctagttcctgtttattaagattctcgttagaatcagtagca      5040
tcagcaattcctcctgggtcagattagattaaaaaaagacagatatcatccacaggaaa      5100
tcatattataaatgaggtactaaaatgtaaatggtgatttaaggattggtttactttgcc      5160
ttataaagggctgcaactattatttatttatttattatttttattaagtttatctgttat      5220
tctgacaaatcaagtaaaaaaattggcatattctgcaacattttcattaaactacttcag      5280
cattttctgtataatattagaattacactcaaagatggacataaacaaataatttcgttt      5340
ctgcttttaaaatagggaataataattttattggctgaaattcaataacagccttttttt      5400
gtgaatttcaacagattgaatataaaaaatgacacttgaggatttttttgttaaaatatag      5460
tggtggttttatcttaaatgcaaaacatgtacatttgtattgttttatctcattgctct      5520
gaatatgttgggtttttcagcatttttactccagttaatgattgattgattactaaatta      5580
gttgatgattaatcagatgaattgtttcagccttagtcttgtatactaagattctctcat      5640
atgtagagcacatacagttaccaaaggatcatatcaaaaggactttactgccatcttgtg      5700
gtcagtgatcaaagctaattttcttactataatcaaatgaagcatctccatttgatttaa      5760
aatccatttgacagcaataacagtgataaaacatattttttttgtgtaaataaactcggg      5820
taaacctttttttttttttcttttccctctcagGTGTCGTTCCTGTTCAGCGACCGTGGC      5880
                                 -V--S--F--L--F--S--D--R--G-
CTGCCTGACGGCCACCGCCACATGAACGGCTACGGTTCCCACACCTTCAAGCTGGTCAAC      5940
```


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Table 2. continued

```
-L--P--D--G--H--R--H--M--N--G--Y--G--S--H--T--F--K--L--V--N-
GCTGACGGCGAATGCATCTACTGCAAGTTCCATTACAAGgtctgtttctgaaactggttt    6000
-A--D--G--E--C--I--Y--C--K--F--H--Y--K-
gttggtttgcagagctctgcgatggatttatttatttatttttctctaaagACTG    6060
                                                   -T--
ATCAAGGAATAAAAAACCTGTCTGTGGAGGAGGCGGAGCGCCTCGCAGCCACCAACCCAG    6120
D--Q--G--I--K--N--L--S--V--E--E--A--E--R--L--A--A--T--N--P--
ATTACAGCATCGGAGACCTGTTCAACGCCATCGCTAACGGCGACTTTCCGTCCTGGACCT    6180
D--Y--S--I--G--D--L--F--N--A--I--A--N--G--D--F--P--S--W--T--
TTTACATCCAAGTCATGACCTTTGACCAGGCGGAGAGGTTCCAGTTCAACCCCTTTGATG    6240
F--Y--I--Q--V--M--T--F--D--Q--A--E--R--F--Q--F--N--P--F--D--
TCACCAAGgtacggagtcccaacaaagtgaaacggctgactatcccgccggtttgaactt    6300
V--T--K-
ttggtaaaaagctaaactctgggttcttcgatgccagcaaagtgttactctagtagagga    6360
gaacctttaatcaaaccgcctccttcaatatctctcttgaatgcattcgtctcatttc    6420
aatctgcaatatctaaatctggggctactttttccaacgctcccagGTGTGGTCGCATAA    6480
                                              -V--W--S--H--K
GGAGTTCCCTCTGATCCCTGTGGGCAAACTGGTCCTCAACCGGAACCCGGTCAACTACTT    6540
--E--F--P--L--I--P--V--G--K--L--V--L--N--R--N--P--V--N--Y--F
CGCAGAGGTGGAGCAGCTGGCCTTTGACCCCAGCAACATGCCTCCAGGCATCGAGGCCAG    6600
--A--E--V--E--Q--L--A--F--D--P--S--N--M--P--P--G--I--E--A--S
CCCCGACAAGATGCTGCAGgtgacgcagtaaaccgaatcagctgagaaaggagtgttgaa    6660
--P--D--K--M--L--Q-
atttaccagaggttaacctcgtccggctcggcgcagGGTCGGCTCTTCTCCTACCCAGAC    6720
                                    -G--R--L--F--S--Y--P--D-
ACGCATCGCCATCGCCTGGGAGCCAACTACCTGCAGATCCCGGTCAACTGTCCCTTCAGG    6780
-T--H--R--H--R--L--G--A--N--Y--L--Q--I--P--V--N--C--P--F--R-
GCCCGCGTGGCCAACTACCAACGAGACGGCCCCATGTGCATGTCTGATAACCAGGgtgag    6840
-A--R--V--A--N--Y--Q--R--D--G--P--M--C--M--S--D--N--Q--
ttcttcacctgctgctcagccttaagtctttaagtcttaaattcatgtctaaaattaagg    6900
ccttaaaagtaatgtgttaatcgcaggtcttaaattttatcttgacacgtctgtttattc    6960
atagattatatatttattttaatttttttgttgtttttttttgctggaaatttctgtccgg    7020
caaaacgttgagggttctgccacccactgctaacataccgtcgctaaataactagctagc    7080
ttttttgcatcctgggtaaatgctcatttatcaccagttggctggacaaagtttgcaag    7140
tttttacggagatataggtattaaatttaattcttaacagtcttattaatacttacattt    7200
gaattggtaaaacatgcagaaataattattaattattaattattattattaatattattg    7260
cacttatgctgtctggttcacgatcagtgaggtcaattaaagctgtaattctacatttg    7320
caaatttgtaacaccttaaaactaaactgtcatcattttataacaaaggtcagtttgctc    7380
ccagaaattgtggcgctaaattttcagggttatgacagagctgtaagaattaaaagtatg    7440
aacaacaagaataagtacaaataaataaagttagtctcttatgtattcacaaatataaaa    7500
aaattatctttggttagtccaaggggaaaaaacaacgtcctactatacatttggtttatg    7560
gacactatgactttaaaatgaccagaattggacatgtttatgtataataaatgtataaat    7620
catatatttacccatgtgattaaaacatgtttgtgttgatgattaatcaactgatgtgaa    7680
tttcttcacaccttcttctgtctttgtcccttctgatgtttatatgctcaatccaatatc    7740
atttttttaaaaatctgcctcttaaactcaacaagttcttgcttcgtacgacctctagG    7800
                                                          G
TGGCGCTCCAAACTACTACCCCAACAGCTTCAGCGCCCCTCAGAACCAGCCTCGCTTCGT    7860
--G--A--P--N--Y--Y--P--N--S--F--S--A--P--Q--N--Q--P--R--F--V
GGAGACCAGGTTCGGCGTTTCGCCCGACGTCGCCCGTTACAACAGCGAAGACGAAGACAA    7920
--E--T--R--F--G--V--S--P--D--V--A--R--Y--N--S--E--D--E--D--N
CGTGACGCAGgtgacgatgacgccgccgcctctaatagcctctctgtggctgtttgccag    7980
--V--T--Q-
tcgcacacaggaagtcgatgttttcagcctgttgaattcaatgtagaaaatggtctaaat    8040
atatccttcgaaggccacaccagtaaaacaataaacagttacgtggaatcactatcagag    8100
tataacagagttgtcatgcatctaaaaatgttggcacatccgccgtccttgtagcttcat    8160
gtacgtttggtaacttgaaactggagcgcaatggaaacttaacaattagaccaaatcagc    8220
ttcttctcatcaacaacgctcactccatctctcatgacgacactttaatgcaaagttgc    8280
attaaaagtgtcattatttaccaaatgacactacgtgacatcagtcatgaacattcataa    8340
agactctttcatgttcatgacgtcatttttacctttaaataaaagtgttaccagaataag    8400
tttcaacccctctgtgaaataaacgaccttcccagtaaggttgctgaggtaagtgcagag    8460
aagtgattggttctgtaggactgtgtttaccgtgtctggttctgaccgggtccgtgtgcg    8520
gccggctgcagGTCCGGGCCTTCTACACCCAGGTGCTGAACGAGGACGAGCGCCAGCGGC    8580
```

Table 2. continued

```
                  -V--R--A--F--Y--T--Q--V--L--N--E--D--E--R--Q--R--
TGTGCCAGAACCTTGCCGGGTTCCTGAAGGGAGCCCAGCTCTTCATCCAGAAACGAATGg      8640
L--C--Q--N--L--A--G--F--L--K--G--A--Q--L--F--I--Q--K--R--M-
tgagttcccgtcagactccatgcggagccaaagatgctttctgtcgtgaagatgagtcac      8700
tgcggagggcatttaaaagaaggaaaaacattgttcctctctttaatccacttcaaccac      8760
agggtgctgctgcatgaaactaaacatgcacaccggcctgctttgtggtaatgtggttgc      8820
tacggaaacaaaacatttttaaacaaggaagaatgatggtgaacagagcccaacgtttaa      8880
agcaacacaatctgtatttttttttttttttttaagttttctgctttcaagttctgtaagg      8940
ctttgactaggacatttaaaatgttattttagtaattcaaccacattgtgcaaaagtgaa      9000
tccaaatctttgacattttaagattcaagctattattccttgtaaatttagataattatg      9060
aagtacagataatgaaatcctgacattcagtgtcaaaaataaatgagattatttacataa      9120
caaccattaaaaaccagcatttttaatgtgtgaatgttgtgtaatgcaccatgtcatggc      9180
ccgagttgaacaccagaacggatctatgaggaagatgaggaacgtcaacaacggacactt      9240
tcaaaacaacttgtgtttcagtttcctctcggctgacagcctccgtgccacgccgcatcg      9300
acgcagttattcatgctaaacaagcctcaaccaatcactgagtgcactgaaataaattct      9360
cacatttctgtttaaaatatcctttttttttgtaatatatactatatgttctaattgtctg      9420
aaatacagaattgtggcttactgtgctccaatatttggtttattttaagtcatgattgtg      9480
aaccttcagatgttgcgcagtttagaagaactccctctgaaagtgggaaccttaaatgac      9540
atttatttttttcttctcagttgttttcctgagcgtctttttctcgctttgtctccaccgg      9600
tgtgactcgccaccgcaaagttaatttttaaacatttgaatgagaaataaaaggtgatgt      9660
catttatcgatgtcacagcagccacatataccgctctggtttcctgttttatggaggaaa      9720
aaaaaacaaattcctgtttgcacgtgttgtaaatctgctgccagagttgtaatttgcgca      9780
acctttgacttttttgctagtctcgcatttttttgtgtgcatcgctttaaaaacaggaaac      9840
ctgaatatagatgcatttattcaatgttcttcctgcagGTGGAAAACCTGAAGGCCGTCC      9900
                                      -V--E--N--L--K--A--V--
ATCCAGACTACGGGAACCGGGTCCAGACCTTACTCAACAAGTACAACGCCGAAGCCAAGA      9960
H--P--D--Y--G--N--R--V--Q--T--L--L--N--K--Y--N--A--E--A--K--
AGgtaaccaaacagttggtttgttatcgcggcgtaaaaaagttcagttaacggaaacctg      10020
K-
ctcgtttcctgatgcgctcatttcccgtctgcgtcccagAACACGAACGTCCGCGTCTAC      10080
                                       -N--T--N--V--R--V--Y-
GGCCGTCCAGGAGCCGCCGCCATCGCCGCGTCCTCCAAGATGTGAtcagcggatcggctc      10140
-G--R--P--G--A--A--A--I--A--A--S--S--K--M--*-
acatgaccaccgactggttccaccagcagctggaccagcctcagcggcctaattactgtc      10200
gccgtagtttgctcgagcttggttttggttcattgcaaacagtcaagccttacttttttta      10260
attaattatcaaaaattaattttattgcattgaatgctgtaaggtaacactgttgagatt      10320
aggctgcagttttattaaattacctttaatacatcgatgccttattactgtttcagttaa      10380
attcagttaaccaacaaaaactgatgaacctttttctctaatgttgtaatactggaagatg      10440
tttgtagcgactcaagcaaagttaattttgaaagaacatctgtttgaaacgatgctgatc      10500
tttgtgcctggctcgtaccgcaacgcaatcgatcgcttggtaaagcttctggttttgctg      10560
aggacattattaacgtctgggttgtttaactcggacttgactgaatcacttactgttgaa      10620
tgttaatatagaaaagtattatcctgttttcaaatAATAAAAtcattcaaaatgtt 3'      10680
```

Note: ** Platyfish (*Xiphophorus maculatus*) catalase (*cat*) gene. The exons of the catalase gene are shown in capital letters and the nucleotide positions are numbered at the end of each line. The starting site of transcription is +1, 5 'upstream sequence, 3' downstream sequence and introns are shown in lower case. The TATA box and the poly adenylation signal (AATAAAA) are shown in upper case letters and painted in yellow. Stop codon (TGA) is specified asterisk. The used forward primer is shown in yellow and the reverse primer is shown in red.

Sequence identity and similarity among platyfish, medaka, fugu, zebrafish, mouse, rat, and human *cat/CAT* gene was given in Table 3. The highest identity and similarity rates for platyfish *cat* gene was determined with its orthologs which are medaka (89 and 96%), fugu (87 and 95%) and zebrafish (85 and 93%). However, the study revealed that platyfish has the same identity and similarity rates with mouse, rat, and human (79% and 87%, respectively). The high sequence identity and similarity rates of platyfish *cat* gene with its orthologs in medaka, fugu, and zebrafish suggest a conservation of the *cat* gene among these teleost species. This finding is consistent with previous studies that have reported a high degree of conservation of the catalase gene in vertebrates (Gotoh, 2012; Pan et al., 2022). The high conservation of catalase gene sequence among different species may be due to the importance of this gene in protecting cells from oxidative stress. It is interesting to note that despite the high sequence identity and similarity rates between platyfish and mammalian *cat/CAT* genes, the response of these genes to

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Table 3. The rate of sequence identity and similarity between platyfish (Pf) *cat* gene and the other vertebrates such as medaka (Me), fugu (Fu), zebrafish (Zf), mouse (Mo), rat (Ra) and human (Hu) *CAT* gene

Pf	Cat	1	MAETRDKTTDQMKIWKENRGSQRPDVLTTGGGHPVGDKLNLQTAGPRGPLLVQDVVFTDE
Me	Cat	1	...N.........T.......RK.......A...I.........................
Fu	Cat	1	..DK...A.....L...S..Y....I........I...........K.............
Zf	Cat	1	..DD.E.S.....L...G............A.V.I.....AM..................
Mo	CAT	1	.SDS..PAS....Q...Q.A............N.I.....IM...S..............
Ra	CAT	1	..DS..PAS....Q...Q.AP.K.........N.I.....IM..................
Hu	CAT	1	..DS..PAS...QH...Q.AA.KA......A.N.......VI.V................
Pf	Cat	61	MAHFDRERIPERVVHAKGAGAFGYFEVTHDITRYCKAKVFEHVGKTTPIAVRFSSVAGES
Me	Cat	61	....I...........T.....
Fu	Cat	61	......................................L...............T.G...
Zf	Cat	61	..................................S.......I...........T....A
Mo	CAT	61	..................................S.......I..R........T.T...
Ra	CAT	61	..................................S.......I..R........T.....
Hu	CAT	61	................................K.S.......I..K........T.....
Pf	Cat	121	GSADTVRDPRGFAVKFYTEEGNWDLTGNNTPIFFIRDAMLFPSFIHSQKRNPQTHMKDPD
Me	Cat	121	..................D............V......L.....V.T.............
Fu	Cat	121	......................................L.......T.............
Zf	Cat	121	..S...............D..................TL................L....
Mo	CAT	121	...................D.....V............I................L....
Ra	CAT	121	...................D.....V.............................L....
Hu	CAT	121	...................D.....V...........PI................L....
Pf	Cat	181	MVWDFWSLRPESLHQVSFLFSDRGLPDGHRHMNGYGSHTFKLVNADGECIYCKFHYKTDQ
Me	Cat	181	...........C................Y..................DRV..........
Fu	Cat	181	..................M.........Y................K...V.....F....
Zf	Cat	181	........................I...Y................Q.QPV........N.
Mo	CAT	181	........................I.......................AV..........
Ra	CAT	181	........C.......T.......I....................N..AV..........
Hu	CAT	181	........................I....................N..AV..........
Pf	Cat	241	GIKNLSVEEAERLAATNPDYSIGDLFNAIANGDFPSWTFYIQVMTFDQAERFQFNPFDVT
Me	Cat	241	...........H..S.....A...........NY............E...K.......L.
Fu	Cat	241	..........G...SA....A...........NY............E...K.H.....L.
Zf	Cat	241	....IP....D.....D.....R..Y......N.............E...NWKW....L.
Mo	CAT	241	.....P.G..G...QED...GLR.........NY............KE..T.P.....L.
Ra	CAT	241	.....P....G...QED...GLR.......S.NY............KE..T.P.....L.
Hu	CAT	241	........D.A..SQED...G.R.......T.KY............N...T.P.....L.
Pf	Cat	301	KVWSHKEFPLIPVGKLVLNRNPVNYFAEVEQLAFDPSNMPPGIEASPDKMLQGRLFSYPD
Me	Cat	301	..............RM............................P...............
Fu	Cat	301	.......Y.......M...........Q...M.H..........P...............
Zf	Cat	301	..............RF............................P...............
Mo	CAT	301	...P..DY...........K...........M............P...........A...
Ra	CAT	301	...P..DY..............A........M............P...........A...
Hu	CAT	301	...P..DY.......................I........................A...
Pf	Cat	361	THRHRLGANYLQIPVNCPFRARVANYQRDGPMCMSDNQGGAPNYYPNSFSAPQNQPRFVE
Me	Cat	361	....................T.............F.................ET..Q...
Fu	Cat	361	..................Y.................................EI..QC..
Zf	Cat	361	............L.....Y.T.............H.................DV....L.
Mo	CAT	361	.......P..........Y...............H.................EQ.RSAL.
Ra	CAT	361	.......P..........Y...............H.................EQ.GSAL.
Hu	CAT	361	.......P...H......Y...............Q..............G..EQ..SAL.

cc BY

Table 3. continued

				Identity (%)	Similarity (%)
Pf	Cat	421	TRFGVSPDVARYNSEDEDNVTQVRAFYTQVLNEDERQRLCQNLAGFLKGAQLFIQKRMVE		
Me	Cat	421	SK.Q..........A........CT.F.K.............M..S..E...........		
Fu	Cat	421	SK.K.Y........S.........T...E...DE......E.F..S..............		
Zf	Cat	421	SKCK..........A.D.......T.F......A..E.....M..H.............Q		
Mo	CAT	421	HSVQCAV..K.F..AN........T...K....E..K...E.I..H..D.......KA.K		
Ra	CAT	421	HHSQC.A..K.F..AN........T...K....E..K...E.I.NH..D......RKA.K		
Hu	CAT	421	HSIQY.GE.R.F.TAND..........VN....EQ.K...E.I..H..D..I....KA.K		
Pf	Cat	481	NLKAVHPDYGNRVQTLLNKYNAEAKKNTNVRVYGRPGAAAIAASSKM-	100	100
Me	Cat	481	.................D....Q...SAS.H..N....S........-	89	96
Fu	Cat	481	....I....AS...IF.D...E..E..AH....T....S.V......-	87	95
Zf	Cat	481	..M...S.......A..D.H...G....-.H..S.G..S.V..A...-	85	93
Mo	CAT	481	.FTD......A.I.A..D.....KP..A-IHT.TQA.SHMA.KGKANL	79	87
Ra	CAT	481	.FTD......A...A..DQ..SQKP..A-IHT.VQA.SHIA.KGKANL	79	87
Hu	CAT	481	.FTE......SHI.A..D.....KP..A-IHTFVQS.SHLA.REKANL	79	87

cold stress may be different. This could be due to differences in the regulatory mechanisms controlling the expression of the catalase gene in different species, as well as differences in the physiological responses of fish and mammals to cold stress. Further studies are needed to investigate the functional implications of the observed sequence conservation of the catalase gene in different species. Overall, the results of this study suggest that platyfish can be a useful model organism for studying the effects of cold stress on the expression and activity of the catalase gene. The high sequence identity and similarity rates of the platyfish catalase gene with its orthologs in other teleost species and mammals provide a basis for comparative studies aimed at understanding the evolutionary and functional aspects of this important gene.

Phylogenetic Analysis

It was used identity and similarity data to construct a phylogenetic tree (Figure 1). The analysis revealed a strong clustering of the *cat* gene in platyfish and its orthologs, indicating a close evolutionary relationship. The tree also showed the phylogenetic relationships between the *cat* sequence in platyfish and *cat* sequences from other vertebrates. We used the Maximum Likelihood method (Felsenstein, 1981) to generate the tree. Accession numbers of the sequences used for phylogenetic tree are the following: Platyfish (Cat: XP_005815336, Amazon molly Cat XP_007546846, Fugu Cat ENSTRUT00000041456, Tetraodon Cat ENSTNIT00000008553, Zebrafish Cat: NP_570987, Atlantic salmon Cat:106564824, Chinook salmon Cat: 112218377, Brown trout Cat: 115197208, Common carp German mirror Cat: 30068, Goldfish Cat: 113066695, Chicken CAT: NP_001026386, Mouse CAT: NP_033934, Human CAT: NP_001743.1, Human LCP2: NP_005556.

The phylogenetic analysis conducted in this study revealed a close evolutionary relationship between the *cat* gene in platyfish and its orthologs in other teleost species, particularly in the amazon molly. The high degree of sequence identity and similarity among the *cat/CAT* genes in different species suggests that these genes have been conserved throughout evolution, indicating their importance in cellular metabolism and protection against oxidative stress. The results of the phylogenetic analysis are also in agreement with previous studies that have suggested a close relationship between platyfish and other teleost species such as medaka, fugu, and zebrafish based on their genome sequences (Schartl et al., 2013). The close phylogenetic relationship between these species suggests that they may share similar physiological and molecular responses to environmental stressors such as cold temperature. Overall, the findings of this study provide new insights into the evolution and function of the *cat* gene in platyfish and other teleost species. Further research is needed to better understand the role of this gene in cellular metabolism and stress responses in fish.

Molecular Studies

Catalase *(cat)* transcription levels of the different tissues of platyfish were determined by RT-qPCR. The highest level of *cat* gene transcription in platyfish was found in liver (2162.21). Transcription of the intestine (1270.94) and heart (1241.25) tissues were found significantly lower than liver although transcription was not significantly different from each other. However intestine (1270.94) and heart (1241.25) tissues

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transcriptions were found higher than the all other tissues (muscle (419.157), brain (46.205), eye (47.57), swim bladder (28.99), gill (81.18), spleen (95.45), kidney (20.25), ovarium (91.16), testis (113.22). Although there was no difference in the levels of *cat* gene transcripts between male and female platyfish tissues, it was observed that *cat* gene was highly expressed in liver, intestine and heart tissues (Figure 2).

Conserved Gene Synteny

We observed that the *cat* gene of platyfish showed conserved gene synteny with the orthologous *cat/CAT* genes of other teleost fishes and humans (Figure 3). Specifically, we found that the syntenic genes of the platyfish *cat* gene, located on chromosome 2, had conserved gene synteny with the *cat* genes of zebrafish (located on chromosome 25) and medaka (located on chromosome 6). This suggests that the *cat* gene in platyfish has a highly conserved gene structure. It was also investigated whether the *cat* gene was duplicated in other teleost fish, but did not detect any duplicate copies in the Ensembl database, except for pike (*Esox lucius*) and some cichlid fish (*Zebra mbuna, Maylandia zebra, Neolamprologus brichardi, Astatotilapia calliptera*). These findings suggest that

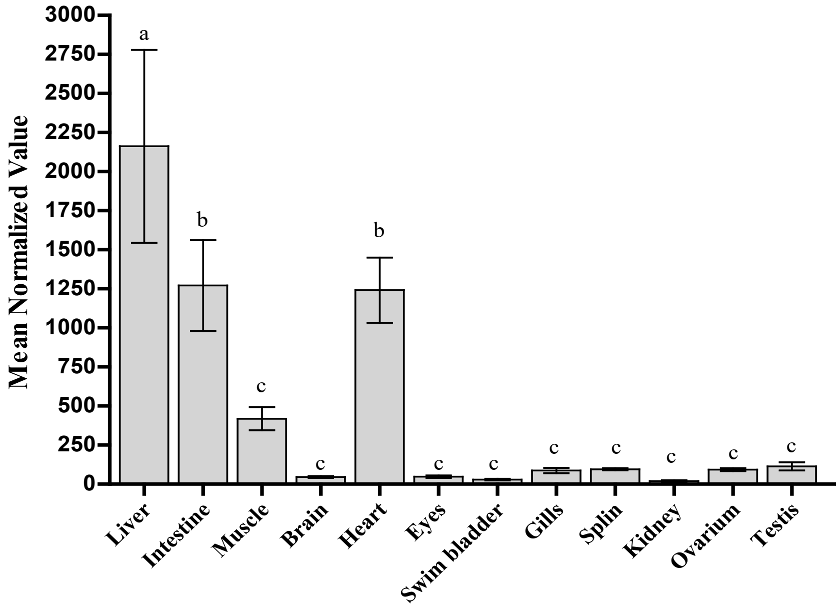

Figure 2. Tissue-specific distribution of catalase transcripts in platyfish

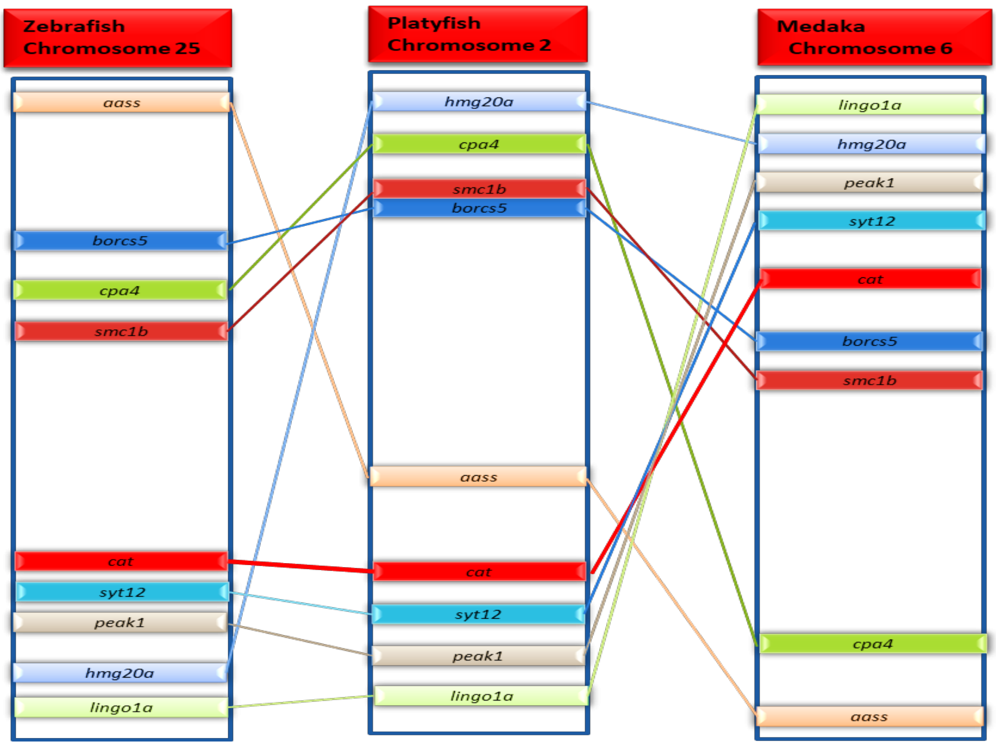

Figure 3. Conserved gene synteny of catalase gene in platyfish (*Xiphophorus maculatus*)

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the loss of a copy of the duplicated *cat* gene is a common occurrence in teleost fish following the teleost-specific whole genome duplication. Overall, these results demonstrate the highly conserved gene structure of the *cat* gene in platyfish. The fact that no duplicate copies of the *cat* gene were detected in the Ensembl database for most teleost fish species, except for a few exceptional cases, suggests that the loss of one copy of the duplicated gene is a common occurrence following the teleost-specific whole genome duplication. This may be due to the fact that duplicate genes can sometimes be non-functional or even detrimental to the organism, leading to their loss over time. There are numerous studies that have investigated the evolution and function of gene duplication events in various organisms, including teleost fish. For example, a study explored the evolution of gene families in teleost fish following the whole-genome duplication event, and found that gene loss and subfunctionalization were common outcomes of this process (Volff, 2005). Another study investigated the evolution of the cytochrome P450 gene family in teleost fish, and found evidence for multiple gene duplication events followed by gene loss and functional diversification (Gesto et al., 2018). These and other studies highlight the importance of understanding the evolutionary dynamics of gene duplication events and their impact on gene function and diversity. Overall, the researchers' findings have important implications for our understanding of the evolution and function of the *cat* gene and other genes in teleost fish. They also underscore the importance of considering gene synteny and duplication events when studying the evolution and function of genes and genomes. By considering gene synteny and other genomic features, researchers can gain insights into the mechanisms and outcomes of gene duplication events in various organisms.

Conclusion

The identification and characterization of stress genes, such as the *cat* in platyfish, can provide important genetic markers for improving stress tolerance in aquaculture and serve as a model for studying stress response in other vertebrates, including humans. In summary, the study found that the cat gene was highly expressed in the liver, intestine, and heart tissues of platyfish, with the liver showing the highest level of expression.

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This manuscript is produced from Esra Can Çapan's master thesis.

Compliance With Ethical Standards

Authors' Contributions

This manuscript is produced from Esra Can Çapan's master thesis.
ECÇ: Literature review, Drafting, Writing, Laboratory experiments
GA: Data analysis and management
MB: Conceptualization, Drafting, Writing, Review, Editing, Supervision
All authors have reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

This study was conducted in 2019, did not receive support from any project and did not require ethics committee approval.

Data Availability Statements

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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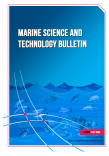

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RESEARCH ARTICLE

The effects on target, by-catch and discard catch of using multifilament and monofilament with selvedge (guarding net) on the trammel nets in the Black Sea coastal fisheries

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ABSTRACT

This study was carried out in 13 fishing operations in the inner harbor area of Sinop, from September through November of 2004. Trammel nets were used, which has 32 mm mesh size and target fish species of the fishing gear is red mullet (*Mullus barbatus*) in the study. Three net groups were used in the experiments, without selvedge (A0) for the control net, multifilament with selvedge (A1) and monofilament with selvedge (A2) respectively. 65.3% Osteichthyes fish (696 individuals), 16.8% Mollusca (179 individuals), 16.4% was Arthropoda (175 individuals) and 1.5% was Chondrichthyes fish (16 individuals) of catch obtained from the operations were form. A total 124 individuals as the target species (red mullet), 398 individuals as bycatch species, and 544 individuals as discarded species were captured sea trials. Catch ratio of A0, A1 and A2 nets were determined 51.88%, 21.58% and 26.55% respectively. 48.38% of the target species, 30.40% of the bycatches and 57.53% of the discarded catch were caught by the A0 net. 25.81% of the target species, 45.23% of the bycatches, and 18.57% of the discard catch were caught with A1 net. 25.81% of the target species, 24.37% of the bycatch and 24.37% of the discarded catch were caught by the A2 net. The results showed that the use of selvedge (guarding net) on trammel nets in the Black Sea coastal fisheries caused a slight decrease in target fish catch, but significantly decreased the amount of discarded catch.

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Introduction

The prevention of bycatch and discarded species in active and passive fishing gear remains up-to-date all over the world (Alverson et al., 1994; Clucas, 1997; Kelleher, 2005; Kenelly, 2007; FAO, 2011, 2019). Set nets are one of the most common fishing gears used in fishing since it is easy use and produce such as gillnets and trammel nets (Karlsen & Bjarnason, 1987; Sainsbury, 1996; Purbayanto et al., 2008). The target species of gillnets, which are used extensively for fishing in Black Sea are red mullet and whiting, which have a very high commercial value (Özdemir & Erdem, 2006; Aydın et al., 2006; Aksu, 2006; Erdem et al., 2019, 2020). The fishing areas are not homogeneous and host many different species. Studies have shown that using gillnets and trammel nets in fishery has caused the decline in many fish populations and the near extinction of a few fish species (Syrja & Valkeaja, 2010). This negative effect is not only limited to fish, but also covers many other marine species and poses a great threat to coastal ecosystems (Regular et al., 2013). Gillnets have low selectivity and high mortality rates for bycatch (Saila, 1983; Alverson et al., 1994; Pascoe, 1997). By ensuring species and size selectivity of fishing gear, other species in the region can be prevented from being harmed by fishing (Erdem, 2004).

Other species have been caught as non-target species in gill nets that equipped for catching target species (Aydın et al., 2015). Even though some of these species are thrown back the sea as discarded, many species are considered as bycatch and create significant economic benefits (Özdemir et al., 2005). The most significant discards are the crabs, due to the damage to gillnet and the caught products (Aydın et al., 2015; Kasapoğlu & Düzgüneş, 2017).

These species can cut the nets and the net may be damaged during their extraction as well. As a result, the discards catch has negative effect on fishery, as it creates damage on other catch products and also increase the time and effort on the cleaning, repair and maintenance of the net (Aksu, 2006; Özdemir & Erdem, 2007).

In addition to the listed side effects, it is important for today's fisheries managements, that are sensitive to the environment and consider the ecosystem as a whole, to prevent the destruction of these discard species, that are the food of other commercially important species, even though they cannot be valued as commercial themselves. While it is not possible to completely prevent the catching of non-targeted species, some measures can be taken to minimize the side effects of fisheries on fish stocks. The measures to be taken in this regard are examined in different categories by different scientists. These can be classified as technical, administrative and economic measures (Pascoe, 1997); measures based on technology and training; measures based on fishing gear and legal regulations (Saila, 1983; Alverson et al., 1994; Godoy et al, 2003; Aksu, 2006; Erdem et al., 2020). By using selvedge (guarding net), norsel ropes and fabrics on the lead side of nets, changing the colour, material or hanging ratio of the net, it has been seen in studies related to species selectivity to prevent the catching of unwanted species, that the catching of discards can actually be reduced (Godoy et al., 2003; Gökçe, 2004; Aksu, 2006; Favaro, 2013; Özdemir et al., 2017; Eryaşar et al., 2021).

The aim of the study is the effect on the amount of target catch and bycatch has been analysed when selvedge application and different net materials being used on trammel nets in small scale fisheries in Sinop coast of Black Sea.

Material and Methods

The study was carried out with trammel nets at depths between 10 and 35 m in Sinop inner harbour region in September-October-November 2004. Sinop region is important fisheries centre of the Black Sea. In addition to trawler and purse seine fishing, coastal fishing also has an important place in the region. Sinop region is an important upwelling area and an important transit point especially for migrating fish (Figure 1). Although bonito fishing attracts attention in the coastal fisheries in the region, especially turbot, red mullet and whiting are widely caught.

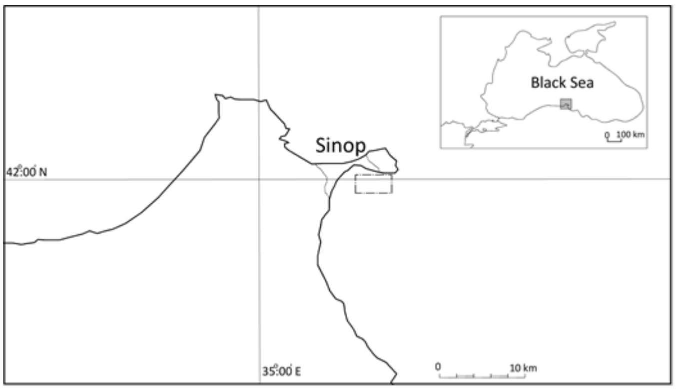

Figure 1. Nautical chart of the study area (dotted line)

The inner nets which used in the study was made of PA material monofilament rope with 32 mm mesh opening, a height of 66 mesh, a length of 200 m, a rope thickness of Ø 0.12 mm. The outer nets are made of PA material with a mesh opening of 220 mm, a depth of 6.5 mesh, a length of 150 m, using PA material fishing line (monofilament) trammel nets with a rope thickness of 210D/6. The outer net hanging ratio

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(E) was 0.67 and the outer/inner net ratio was applied as 2/3 (Figure 2).

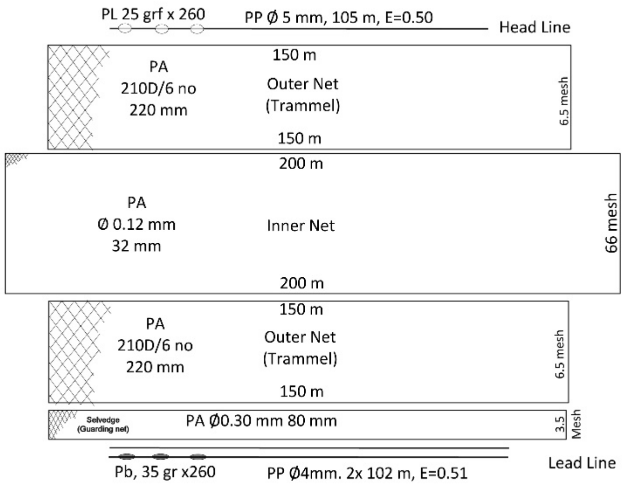

Figure 2. Technical features of the trammel nets used in the study

Figure 3. Used with multifilament and monofilament selvedge nets

Experimental (A1 and A2) nets were added selvedge. A1 nets have selvedge with multifilament material and A2 nets have selvedge with monofilament (Figure 3).

Three nets (A0, A1 and A2) randomly prepared for this purpose were added to each other to form a set net and total 13 fishing operations were carried out. The nets are placed on the deck in such a way that the head and lead line do not interfere with each other in order to facilitate their launch into the sea. Ropes (2 meters) were attached to the head and lead line at both ends of the nets. These ropes are attached to the ropes to which buoys and anchors are attached. The nets were laid into the sea parallel to the shore in the sun set time and they were collected out of sea in the dawn time. All data were brought to laboratories to be crated and classified according to species. Here, evaluations were made by separating the target species, discarded and by-catch. The data of the fished species proportional to the length of time that each net (100 meter) stays in the sea (about 12 hours) were recorded as CPUE values.

CPUE values were calculated using Eq. 1 and Eq. 2 (Gulland, 1983; Erkoyuncu, 1995).

$$CPUE = \frac{n}{Soak\ time\ (12h) \times Net\ length\ (m)} \qquad (1)$$

$$CPUE = \frac{Weight\ (g)}{Soak\ time\ (12h) \times Net\ length\ (m)} \qquad (2)$$

where n is the number of individuals.

The very small ones, the ones that do not have economic value, or the ones that are damaged during the fishing are called discarded. Whether the difference between the nets used depending on the amount of prey is significant or not was determined by the Chi-Square test using the Microsoft Excel and MiniTab 13.0 package program.

Results

In 13 fishing operations, a total of 1066 (42962 g) individuals from 25 species belonging to 4 different groups were caught. The distribution of the species according to the number of individuals have been identified as; 696 Osteichthyes, 179 Mollusca, 175 Arthropoda and 16 Chondrichthyes. 14 species from the group of Osteichthyes, mainly horse mackerel, red mullet, whiting and picarel were caught. Although red mullet is the target species, the most caught fish is horse mackerel. The most of the bycatch species were the *Rapana venosa*. The distribution of other species by groups is given in the Table 1.

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Distribution of Total Catch by Nets of Different Type

A total 553 individuals (51.88%) were caught with the A0 (control net), 230 (21.58%) with the A1 (multifilament with selvedge) and 283 (26.55%) with the A2 (monofilament with selvedge) during the sea trials. The CPUE value calculated as 0.46, 0.19 and 0.24 individual/km, respectively. The highest catch efficiency (19182 g) was obtained with A0 net. Catch amount of the other nets (A1 and A2) were determined 9872 and 13908.11 g, respectively. The CPUE value calculated as 15.99, 8.23 and 11.59 g/km respectively. Also, Osteichthyes, Mollusca, Arthropoda and Chondrichthyes were captured by A0, A1 and A2 nets 322, 171, 203; 132, 26, 21; 97, 30, 48 and 2, 3, 11 individuals, respectively (Table 2).

While most of the Osteichthyes, molluscs and arthropods caught were caught with the A0 net, only Chondrichthyes were caught more with the A2 net. When the ratios of the caught species according to the net type analysed, Osteichthyes with a rate of 58.23% were caught A0 net, the rate of the Osteichthyes were reached higher in the A2 net and A1 net 71.73% and 74.35%, respectively.

Table 1. Distribution of captured species with all nets in the study

Groups	Species	n	%
Osteichthyes	Horse mackerel (*Trachurus mediterraneus* Steindachner, 1868)	163	15.29
	Red mullet (*Mullus barbatus ponticus* Essipov, 1927)	124	11.63
	Whiting (*Merlangius merlangus euxinus* Nordman, 1940)	102	9.57
	Picarel (*Spicara flexuosa* Linnaeus, 1758)	116	10.88
	Anchovy (*Engraulis encrasicolus* Linnaeus, 1758)	11	1.03
	Bluefish (*Pomatomus saltatrix* Linnaeus, 1766)	3	0.28
	Turbot (*Scophthalmus maximus* Linnaeus, 1758)	1	0.09
	Tub gurnard (*Chelidonichthys lucerna* Linnaeus, 1758)	2	0.19
	Scorpion (*Scorpaena porcus* Linnaeus, 1758)	47	4.41
	Stargazer (*Uranoscobus scaber* Linnaeus, 1758)	39	3.66
	Ling (*Gaidropsarus mediterraneus* Linnaeus, 1758)	58	5.44
	Labrus (*Labrus viridis*)	16	1.50
	Goby fish (*Gobius* spp.)	7	0.66
	Greater weever (*Trahinus draco* Linnaeus, 1758)	7	0.66
Chondrichthyes	Spiny dogfish (*Squalus acanthias* Linnaeus, 1758)	12	1.13
	Thornback ray (*Raja clavata* Linnaeus, 1758)	3	0.28
	Common stingray (*Dasyatis pastinaca* Linnaeus, 1758)	1	0.09
Arthropods	Warty crab (*Eriphia verrucosa* Forskål, 1775)	8	0.75
	Swimming crab (*Liocarcinus depurator* Linnaeus, 1758)	163	15.29
	Baltic prawn (*Palaemon adspersus* Rathke, 1837)	4	0.38
Mollusca	Rapa whelk (*Rapana venosa* Valenciennes, 1846)	166	15.57
	Others (*Gibbula* sp.)	13	1.22
Total	**25 Species**	**1066**	**100**

Table 2. Distribution of total catch in the samples

Groups	**Osteichthyes**		**Mollusca**		**Arthropod**		**Chondrichthyes**		**Total**			
Net Type	***n***	***%***	***n***	***%***	***n***	***%***	***n***	***%***	***n***	***%***	***W***	***%***
A0	322	58.23	132	23.87	97	17.54	2	0.36	553	51.88	19182	44.7
A1	171	74.35	26	11.30	30	13.04	3	1.30	230	21.57	9872	22.9
A2	203	71.73	21	7.42	48	16.96	11	3.88	283	26.55	13908	32.4
Total	696	65.29	179	16.79	175	16.42	16	1.50	1066	100	42962	100

Table 3 Catch composition of nets used in the study

Net Type	***Species***	**A0**		**A1**		**A2**	
Groups		***n***	**%**	***n***	**%**	***n***	**%**
Osteichthyes	Horse mackerel	59	10.67	42	18.26	62	21.91
	Red mullet	60	10.85	32	13.91	32	11.31
	Whiting	66	11.93	13	5.65	23	8.13
	Picarel	44	7.96	39	16.96	33	11.66
	Anchovy	8	1.45	2	0.87	1	0.35
	Turbot	1	0.18	0	0	0	0
	Bluefish	0	0	1	0.43	2	0.71
	Tub gurnard	2	0.36	0	0	0	0
	Scorpion	30	5.42	9	3.91	7	2.47
	Stargazer	19	3.44	13	5.65	19	6.71
	Ling	26	4.70	13	5.65	10	3.53
	Labrus	1	0.18	5	2.17	2	0.71
	Goby fish	3	0.54	2	0.87	4	1.41
	Greater weever	3	0.54	0	0	7	2.47
Chondrichthyes	Spiny dogfish	1	0.18	3	1.30	8	2.83
	Thornback ray	1	0.18	0	0	2	0.71
	Common stingray	0	0	0	0	1	0.35
Arthropods	Warty crab	5	0.90	27	11.74	44	15.55
	Blue-leg swimcrab	92	16.64	1	0.43	2	0.71
	Baltic prawn	0	0	2	0.87	2	0.71
Mollusca	Rapa whelk	123	22.24	26	11.30	17	6.01
	Gibbula	9	1.63	0	0	4	1.41
TOTAL		**553**	**100**	**230**	**100**	**283**	**100**

Table 4. Catch amount distribution in the nets of captured groups in the fishing operations

Fishing Operations	**Osteichthyes**			**Molluscs**			**Arthropods**		
	A0	***A1***	***A2***	***A0***	***A1***	***A2***	***A0***	***A1***	***A2***
1	4	11	14	28	2	0	1	1	2
2	6	4	3	7	0	1	0	1	1
3	46	30	30	12	1	0	1	0	3
4	28	5	4	6	3	1	4	0	1
5	36	26	18	31	10	6	6	2	6
6	50	4	6	2	0	0	8	2	2
7	20	11	21	0	0	0	2	0	1
8	14	13	28	12	1	5	3	1	9
9	2	2	2	0	0	0	2	0	0
10	7	7	10	13	2	2	3	0	1
11	63	23	39	16	2	3	52	11	13
12	11	6	8	3	1	0	4	1	1
13	35	29	20	2	4	3	11	11	8
Total (n)	**322**	**171**	**203**	132	26	21	97	30	48
Average	**24.8**	**13.2**	**15.6**	**10.2**	**2.0**	**1.6**	**7.5**	**2.31**	**3.69**

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Among the 322 Osteichthyes caught with A0 net, whiting, red mullet, horse mackerel and picarel took the first four places with the number of 66, 60, 59 and 44, respectively. The ratio of these species in the total prey caught with A0 net was calculated as 11.93%, 10.85%, 10.67% and 7.96%, respectively. *R. venosa* (123 individuals) and blue-leg swimming crab (92 individuals) were the most caught species from Mollusca and Arthropoda. However, few fish were captured from Chondrichthyes group. Captured fish number were 1 thornback ray and 1 spiny dogfish (Table 3).

Comparison of Catch Amounts in the Fishing Operations of Nets

A total of 696 individuals Osteichthyes fish were caught by the A0, A1 and A2 nets in the 13 fishing operations 322, 171 and 203 individuals, respectively. The mean was 24.77±5.51 individuals/km with A0 net, 13.15±2.84 individuals/km with A1 net, and 15.62±3.23 individuals/km with A2 net. The CPUE value for 13 operations is calculated as 0.27, 0.14 and 0.17 individuals/km, respectively. As a result of the Chi-Square test, the difference between the Osteichthyes fish catches among the nets was found to be statistically significant ($P<0.001$). During the study, 179 individuals from the molluscs were caught, of them with A0, A1 and A2 nets 132, 26 and 21, respectively. The average number of Mollusca caught per operation was calculated as 10.15±2.80 in A0 net, 2.00±0.75 in A1 net, 1.62±0.57 in A2 net and 13.77±3.70 in total. The mollusc CPUE value calculated as 0.11, 0.02 and 0.017 individuals/km, respectively. As a result of the Chi-Square test, the difference between the amount of molluscs caught with each net type was found to be statistically significant ($P<0.05$). It was seen that the difference between the mollusc catches in selvedge nets was insignificant, and the use of selvedge prevented the net from catching *R. venosa*. In addition, the difference between mollusc catches with selvedge nets and non-selvedge nets was found to be statistically significant ($P<0.05$). A total of 175 individuals from the arthropod group were caught, 171 individuals of which were crabs and 4 individuals of them were shrimps. The average number of individuals was 13.46±5.65 individuals/km. The number of caught arthropods is 97 individuals with A0 net, 30 individuals with A1 net and 48 individuals with A2 net. The arthropods CPUE value calculated as 0.11, 0.02 and 0.017 individuals/km, respectively. One of the most important data of the study and the most important discard species for gillnets is arthropod species. According to the nets, the average arthropod catch amount was calculated as 7.46±3.81 with A0 net, 2.31±1.09 with A1 net and 3.69±1.12 with A2 net. As a result of the Chi-Square test, the difference between the amount of arthropod prey caught with each net type was found to be statistically significant ($P<0.01$). The outcome has demonstrated importance of using selvedge and the effect of selvedge material on the arthropod catch amount (Table 4).

Comparison of Different Net Types of the Target Fish Species

A total 124 red mullet, target species were caught, of which 60 individuals were caught with A0 net, 32 individuals with A1 net and 32 individuals with A1 net. Of the 3208.81 g red mullet fish caught, 1610.46 g were caught with A0, 808.52 g with A1 and 789.83 g with A2 net. The average of 9.54±1.15 red mullet fish were caught per operation and the averages according to the nets were calculated as 4.62±1.89 in A0 net, 2.46±2.27 in A1 net and 2.46±2.07 in A2 net (Table 5). The red mullet CPUE value calculated as 0.05, 0.03, 0.03 individuals/km and 1.34, 0.67 and 0.66 g/km, respectively. As a result of the Chi-Square test, the difference between the amounts of the target species caught per each net and the operation was found to be statistically significant ($P<0.01$).

Table 5. Distribution of red mullet by nets

Fishing Operations	A0	A1	A2	Total
1	0	3	1	4
2	1	0	0	1
3	6	2	1	9
4	16	0	0	16
5	21	19	14	54
6	0	0	0	0
7	0	0	0	0
8	0	0	1	1
9	0	0	0	0
10	0	0	0	0
11	8	2	6	16
12	3	2	1	6
13	5	4	8	17
Total (n)	**60**	**32**	**32**	**124**
Average	**4.62**	**2.46**	**2.46**	**9.5**

A total of 398 fish individuals were caught from 7 species constituting the bycatch and 45.23% (n=180) of them were caught by A0, 24.37% (n=121) by A1 and 30.4% (n=97) by A2 net. The fish species most captured by the nets were horse mackerel, picarel and whiting Considering the distribution of

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Table 6. Distribution of non-target commercial fish species caught by nets

Fish Species		Nets						Total
		A0		***A1***		***A2***		
H. mackarel	**n**	59	36.2%	42	25.77%	62	38.04%	163
	W (g)	1195.2	34.45%	1041.8	30.03%	1232.4	35.52%	3469.4
Whiting	**n**	66	64.71%	13	12.75%	23	22.55%	102
	W (g)	2340.5	65.08%	411.6	11.45%	844.3	23.48%	3596.4
Picarel	**n**	44	37.93%	39	33.62%	33	28.45%	116
	W (g)	1393.3	39.12%	1182.2	33.20%	985.8	27.68%	3561.3

bycatch species, which are caught commercially, according to the nets; 36.2% of horse mackerel were caught with A0, 25.77% with A1 and 38.04% with A2 net. Bycatch rate distribution in the A1 and A2 nets of the other two fish species were showed in Table 6.

Table 7. Distribution of discard catch caught by nets

Fishing Operations	A0	A1	A2	Total
1	31	5	13	49
2	8	4	4	16
3	34	9	13	56
4	22	8	5	35
5	50	18	16	84
6	14	3	5	23
7	4	3	1	8
8	15	4	18	37
9	2	1	0	3
10	20	4	11	35
11	81	19	24	124
12	11	3	3	17
13	21	20	17	58
Total (n)	313	101	130	544
Average	**24.08**	**7.77**	**10.00**	**41.85**

As a result, the average amount of by-catch, consisting of 398 individuals caught, per operation was found to be 30.62±7.77. According to different types of net, caught 169, 94 and 118 individuals, respectively and the averages were calculated as 13.85±4.27 in A0 net, 7.46±2.11 in A1 net and 9.31±2.88 in A2 net. The bycatch CPUE value calculated as 0.14, 0.08, 0.098 individuals/km, respectively. As a result of the Chi-Square test, the difference between the average catches caught per net and per operation was significant ($P<0.001$).

Among the discarded species, 30 scorpion fish individuals were caught in A0 net, 9 individuals in A1 net and 8 individuals in A2 net. 26, 13 and 19 ling individuals were caught with A0, A1 and A2 nets, respectively. 19, 13 and 7 stargazer individuals were caught with A0, A1 and A2 nets, respectively. 97, 28 and 46 crab individuals were caught with A0, A1 and A2 nets, respectively. *R. venosa* were caught 132, 26 and 21 individuals with A0, A1 and A2 nets, respectively. When the species are considered individually or collectively, it is seen that the unwanted species are caught more with the A0 net. This result was supported by the Chi-Square test. The average of 544 individuals discarded fish caught per operation was 41.85±9.29. Of this amount, 24.08±5.98 individuals were caught with A0, 7.77±1.87 individuals with A1 and 10.00±2.09 individuals with A2 net (Table 7). The discard CPUE value calculated as 0.26, 0.08, 0.11 individuals/km, respectively. As a result of the Chi-Square test, the difference between each net and the average discard catch amounts per operation was found to be statistically significant ($P<0.05$).

Discussion

In this study, the effect of selvedge and selvedge material used in the trammel nets for red mullet fishing has been tried to be revealed. For the study, a set of nets were created with non-selvedge (commercially used) net (A0) as well as experimental nets with multifilament selvedge (A1) and monofilament selvedge (A2).

A total of 1066 individuals were caught, of which 124 were target species, 398 bycatch and 544 discarded species. Together with the target species of red mullet, bycatch species represented 49% of the total catch (Horse mackarel, whiting, picarel, anchovy, turbot, bluefish) and discarded species (tub gurnard, scorpion, stargazer, ling, labrus, goby fish, greater weever, spiny dogfish, thornback ray, common stingray, warty crab, blue leg swimcrab, Baltic prawn, rapa whelk, gibbula) represented 51%. The discarded species can be the target species in other fishing methods. Those of the target species that cannot be evaluated economically and those that are damaged during

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catching are also discarded. It was recorded that the amount of Osteichthyes fish caught with the nets using selvedge was lower than the nets without selvedge. The reason why more fish are caught in the nets without selvedge may be due to the material properties of the gillnets used in the study.

The difference observed in terms of the number of molluscs catch between nets was found to be statistically significant (P<0.05). It was determined that this difference was caused by the use of selvedge, and the effect of selvedge being multifilament or monofilament had been insignificant on catching molluscs which are damaging the nets. This leads to the conclusion that the use of selvedge prevents *R. venosa* from being caught as discarded. This outcome proved that the expected result had been obtained from the use of selvedge in the nets and that selvedge prevented the crabs from climbing into the net.

Gökçe (2004) stated that the catch obtained in his study with selvedge nets equipped with different hanging ratios and nets without selvedge, consisted of arthropods, molluscs, Osteichthyes fish and Chondrichthyes fish groups, and reported that there were significant reductions in the number of both groups and species with the use of selvedge. The study showed that the use of selvedge in shrimp gillnets is effective in reducing bycatch, and that the height of the selvedge is an important criterion in reducing bycatch. The results obtained from different studies conducted in Turkish seas show the diversity of species in fishing with gillnets. Ayaz (2003) caught 392 individuals belonging to 26 species in his study with gillnets in the Aegean Sea. Fish made up 76% of the total catch, crustaceans 24% and arthropods 0.02%.

A total of 124 red mullet fish individuals were caught, of which 60 were caught with A0 net, 32 with A1 net and 32 with A1 net. Averages were calculated as 4.62±1.89 individuals in A0 net, 2.46±2.271 individuals in A1 net and 2.46±2.07 individuals in A2 net. The red mullet CPUE value calculated as 0.05, 0.03, 0.03 individuals/km and 1.34, 0.67 and 0.66 g/km, respectively. It was seen that the difference between the nets in terms of catch amount of red mullet was statistically significant (P<0.05). This outcome revealed that the use of selvedge in the net decreased the amount of target species caught. It was seen that the amount of bycatch decreased with the use of selvedge in the nets. According to the results, it was determined that the difference between the nets in terms of discard amount was statistically significant (P<0.05).

As a result, the average amount of by-catch, consisting of 398 individuals caught, per operation was found to be 30.62±7.77. According to different types of net, caught 169, 94 and 118 individuals, respectively and the averages were calculated as 13.85±4.27 in A0 net, 7.46±2.11 in A1 net and 9.31±2.88 in A2 net. The bycatch CPUE value calculated as 0.14, 0.08, 0.098 individuals/km, respectively. As a result of the Chi-Square test, the difference between the average catches caught per net and per operation was significant (P<0.001).

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If a general assessment regarding the species is made, considering intended outcome of the fishery and its economical evaluation, the amount of discards decreases significantly with the use of selvedges. However, there is a decrease in the amount of prey of commercial species. Although these results are interpreted as negative in terms of fisheries, considering the disadvantages such as the damage caused by undesirable species to the nets and the catch product, the cost of fishing, the time spent during fishing, as well as the unnecessary removal of discarded species, which is caught undesirably and unnecessary and has devastating effects on the ecosystem, all in all it can be assessed that a significant gain has been achieved, not a loss. For example, in cases where crabs, which are the most important discarded species, are caught in the net in large quantities, the fishing gear can be damaged so much that it cannot be used after a few catching operations. Considering the difficulty and economic cost of repairing a net set, it is thought that all fishermen will prefer to catch less discards, even if the amount of the commercial species decreases. In addition, the commercial species caught in the fishing tool are eaten by the crabs and lose their economic value. Crabs and other predatory species harm commercial species at any rate. However, the use of selvedge reduces this rate considerably. All in all, discards reduce the economic return of the products caught without selvedge net and brought it to the level when using selvedge nets. For these reasons, it can be determined by looking at the results of the research that the use of selvedge is very important in the bottom trammel nets. However, while using selvedge, factors such as the number of mesh, mesh size, hanging ratio, material made from and rope thickness should be determined well, and consequently, it should be tried to increase the productivity of fishery. In similar studies (Gökçe, 2004; Metin et al., 2009; Özdemir & Erdem, 2019), it has been reported that nets using selvedge catch less undesired prey of shrimp, crabs, mantis shrimp (*Squilla mantis*, L.), and whelk species than nets without selvedge, and that the height of the selvedge used is an important criterion for reducing bycatch. Likewise, Godoy et al. (2003) and Kara et al. (1991) reported that the norsel ropes and selvedges net attached to the lead line of the net reduced the catching of bycatch species. Eryaşar et al. (2021) aims to prevent non-target species from climbing into the net with the tarpaulin they have equipped on the lead line of the trammel net and they tried to get the target species to ascend from the ground and got trapped in the net, as a result, they reported that there was a decrease in the amount of discards as well as target species.

While it is not possible to completely prevent the catching of non-targeted species, some measures can be taken to minimize the effects of fisheries on stocks. The measures to be taken in this regard are examined in different categories by different researchers. These are classified as technical, administrative and economic measures (Pascoe, 1997), measures based on technology and education, legal regulations (Saila, 1983) or measures based on fishing gear, and measures based on legal regulations (Alverson et al., 1994). In the light of the results of these and other researches and generally accepted scientific facts, it is evident that how important the reduction of bycatch is, for more efficient and environmentally friendly fisheries. Although different methods are used for different fishing gears, it is a fact that various modifications should be made in fishing gears in order to reduce the amount of bycatch, not to endanger the future of the stocks and to keep the fishery under control (Kınacıgil et al., 1999). With this research, it was revealed that the use of selvedge in gillnets reduces the amount of bycatch. For the nets used in the fishing of different species, varying applications should be determined to reduce the undesired catch, and additional techniques other than selvedge should definitely be tried.

Conclusion

In conclusion, improvements in a fisheries bycatch profile can be accomplished by fishing less, by managing and making use of non-target species caught in fishing gear, or by improving the selectivity of fishing gear (Kelleher, 2005; Hall et al., 2007; Favaro, 2013). Reducing the catch of non-target and unwanted species has many advantages such as reducing the fishing pressure on the species, ensuring sustainable fisheries, and preventing time, labour and fuel losses (Brewer et al., 1998).

The findings obtained as a result of this study give us the opportunity to say that the cost of fishing and the amount of catch of unwanted creatures can be reduced without sacrificing the actual product by using selvedge in the trammel nets used in red mullet fishing. Therefore, the necessity of using selvedge on the trammel nets and gillnets used on costal fisheries have been understood from the perspective of sustainable fishing and environmental awareness.

Compliance With Ethical Standards

Authors' Contributions

HA: Preparing the experimental gillnets, Field sampling, Data collection, Data analysis, Drafting

YE: Data collection, Drawing of technical plans of the gill nets, Reviewing, Editing

SÖ: Reviewing, Editing

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SHORT COMMUNICATION

Misidentification of three mullet species under family Mugilidae due to differential pigmentation pattern

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ABSTRACT

The mullet species under the family Mugilidae, considered to be a commercially important teleost, are mostly found in coastal waters throughout the world, distributed in tropical, subtropical and temperate areas. In terms of taxonomic study, biogeography and distribution pattern of mullets remain unclear due to difficulty in separating the species based on morphological characters. Thus, there is a need to study the taxonomy of Mugilidae. Further, phylogeny of family Mugilidae also exceptionally obscure at inter and intraspecific levels challenges exist in species under the family. The present study, has brought a new observation in form of temporary black dots (patrial pigmentation abnormality), especially in three species of Mugilidae were observed. Sometimes these pigmentation pattern can lead to misidentification or identification as different species. Further, DNA Barcoding (COI gene) and morpho-meristic analysis performed to resolve the ambiguity in the species identification, confirmed these species as *Mugil cephalus*, *Planiliza* sp., *Osteomugil perusii*. Present study will help to avoid the misidentification of species, which will assist biologists and managers for acquiring more information their distribution and life history pattern.

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Introduction

Mugilidae commonly known as mullets are most common teleost fishes in the coastal waters, especially in estuaries of the tropical, subtropical and temperate zones of the world (Thomson, 1996; Murgia et al., 2002; Rahman et al., 2013). Family Mugilidae, which was classified under order Perciformes (Menezes et al., 2015), is now placed under the order Mugiliformes (Froese & Pauly, 2022). Hence, this group needs thorough study and revision. The previous taxonomic revision has included varied number of valid genera and species between 14 and 20 genera and 62 valid species (Thomson, 1997); 72 species under 17 genera (Nelson, 2006); 81 species under 18 genera (Zubia et al., 2015; Hassanien et al., 2020); 78 species under 26 genera (Froese & Pauly, 2022). Family Mugilidae relies upon high proportion of monotypic genera which reflect upon general difficulty in classifying mullet species on the basis of few diagnostics or synapomorphic characters. Most of the species are reported under genera *Liza*, *Mugil* and *Valamugil*. Among them, *Liza* and *Mugil* are comprised of 40% of the species richness (Eschmeyer & Fricke, 2011). Morphological characters have been used by fishery biologists to measure discreteness and find out relationship among various taxonomic categories. Mullets are considered to be one of the most difficult taxonomic groups, hence there are conflicts regarding taxonomy of mullets at the generic as well as the species level (Krishnan, 2018). These groups are having highly conservative morphology and therefore, identifying them using only morphometric characters, has proven to be a tedious and complex task. First thorough taxonomic revision on Mugilidae (Schutz, 1946) was based on the mouth anatomy, especially lips and teeth, as supporting evidence. The taxonomy of adult and fry of mullets relies upon external morphology mainly (mouth, teeth, scale, lips, preorbital bones, jaws), meristic count and internal organs (intestine, stomach, pyloric caeca, pharyngo-branchial organs), pigmentation and melanophore pattern (Thompson, 1997; Krishnan, 2018).

DNA Barcoding, using COI gene, has been used for delineation as well as identification of species. In addition to species diagnosis using internal and external characters, in the present study, peculiarity of temporary pigmentation (ecotype) has been observed for three species (*Osteomugil perusii*, *Mugil cephalus* and *Planiliza* sp.). Though some literatures have cited about the pigmentation in fry stages (Minos et al., 2002) and body surface pigmentation (You-jun et al., 2014), there is no clear statement about the pigmentation pattern. Thus, the present study was designed not only to prevent the misidentification of species in the future research, particularly for this groups but also confirms different ecotypes rather than considering them as different species. The results of the present study will have significant contribution towards database with DNA Barcode and an awareness of existing ecotypes in natural environment rather avoid considering them as different species.

Material and Methods

Fishes under family Mugilidae were collected from coast of Eastern Arabian Sea Region (18°18.837 N, 73°45.750 E) (Figure 1), Maharashtra, during November 2021 to March 2022. A total 10 fishes were collected from local fishermen operating gillnet and dolnet, a type of Set bag net (traditional net) which is, 25 ft long and operated at a depth of 7-8 ft in Bhayander estuary. Specimens were brought to the laboratory under fresh condition and identified up to species level by using fish identification key (Luther, 1973; Rahman et al., 2013; Froese & Pauly, 2022; Bhatt & Mankodi, 2023). Tissue samples of the specimens were preserved in absolute alcohol, while whole fish specimens were preserved in formalin. The meristic (7) and morphometric (19) characters were measured with digital callipers to the nearest 0.1 mm precision and recorded in Microsoft® Excel 2019. The data was analysed using software PAST (version 4.30).

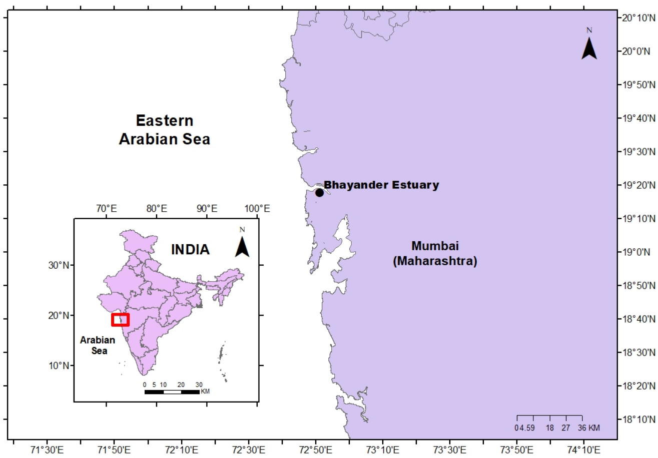

Figure 1. Black dot showing study area (Bhayander Estuary) Eastern Arabian Sea, Maharashtra, India

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a)

b)

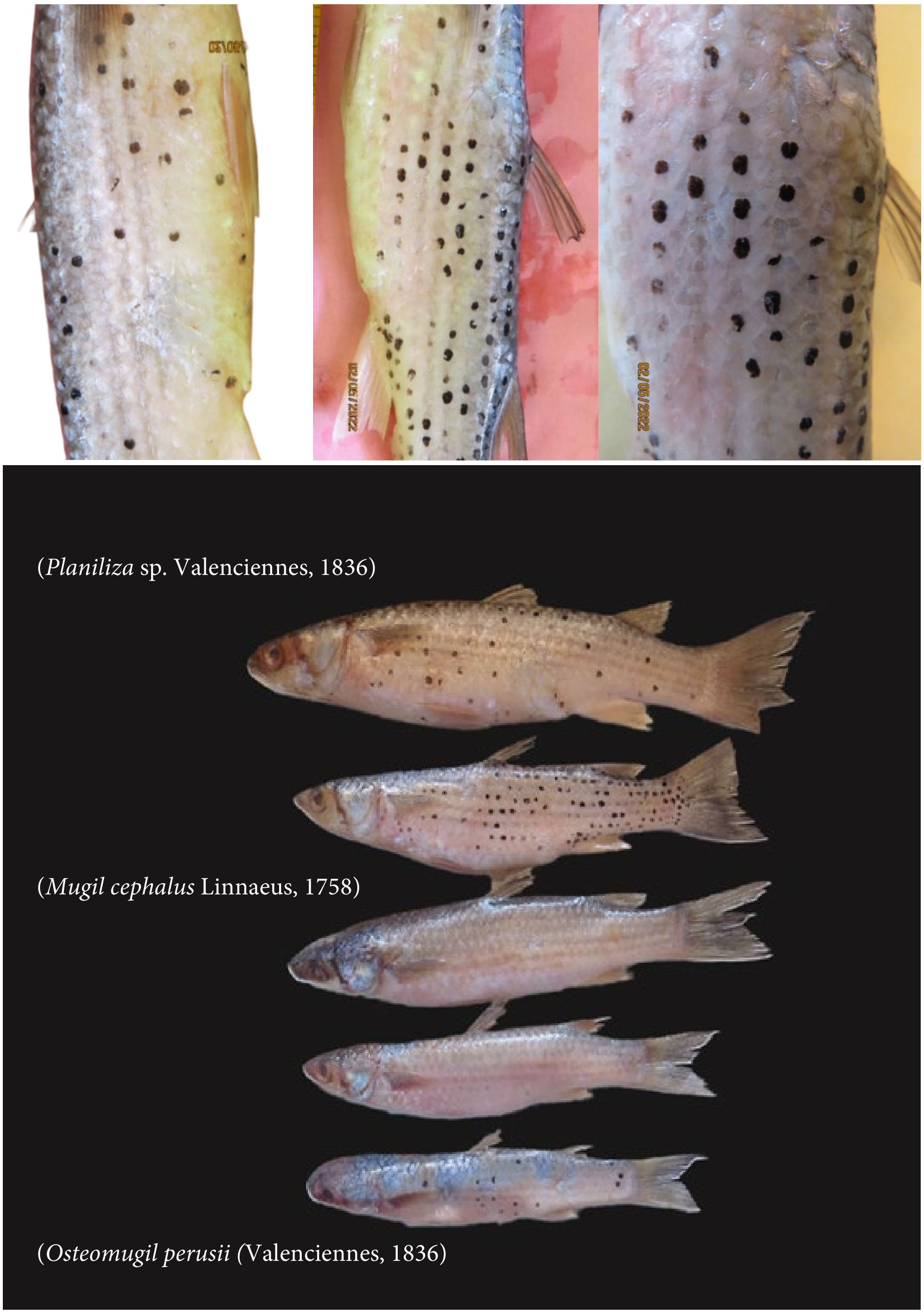

Figure 2. a) Spotted specimens with different pigmentation pattern. b) Observed specimens under 3 different species (Top to bottom- *Planiliza* sp. Valenciennes, 1836); (*Mugil cephalus* Linnaeus, 1758; (*Osteomugil perusii* (Valenciennes, 1836)

DNA Extraction, COI Gene Amplification and Sequencing

DNA was extracted by following Phenol chloroform method (Sambrook & Russell, 2005). Purity of concentration of DNA was checked by nano drop. Further, template DNA was been subjected to PCR amplification of specific region (600 bp) by using primers (F1R1; F2R2) (Ward et al., 2005). Thermal cycling was performed in BIORAD PTC-200, with the following conditions: 95°C for 300s, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 60 s, and a final extension at 72°C for 10 min. PCR products were purified with Qiagen Gel Extraction kit and Promega PCR purification kit following manufacturer's guidelines and sequenced in both directions using a Genetic Analyzer (Massey University Genome Sequencing Service). The purified samples were sent for sequencing to Eurofins Genomics. The Forward and backward sequences were analysed by using MEGA-X software.

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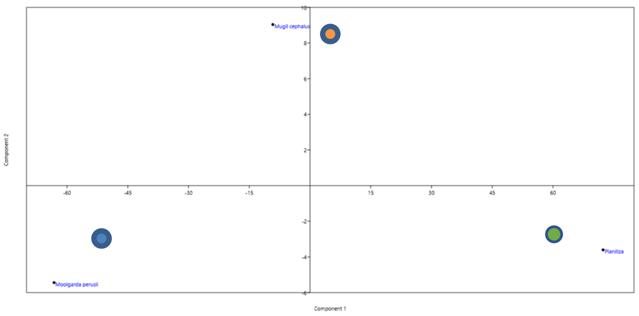

Figure 3. Principal component analysis for *Mugil cephalus* , *Osteomugil* (*Moolgarda*) *perusii* , *Planiliza* sp.)

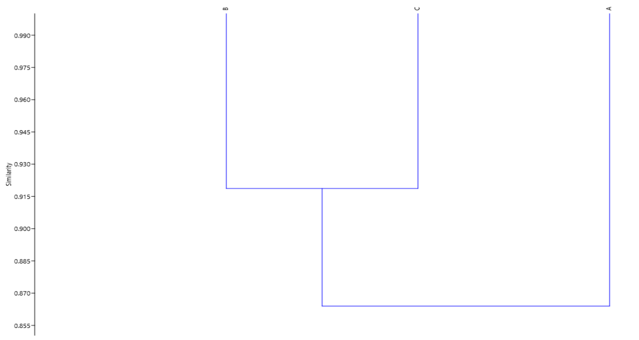

Figure 4. Cluster diagram showing Bray-Curtis similarity Index; (B- *Mugil cephalus*, A-*Planiliza* sp. and C- *Osteomugil (Moolgarda) perusii*

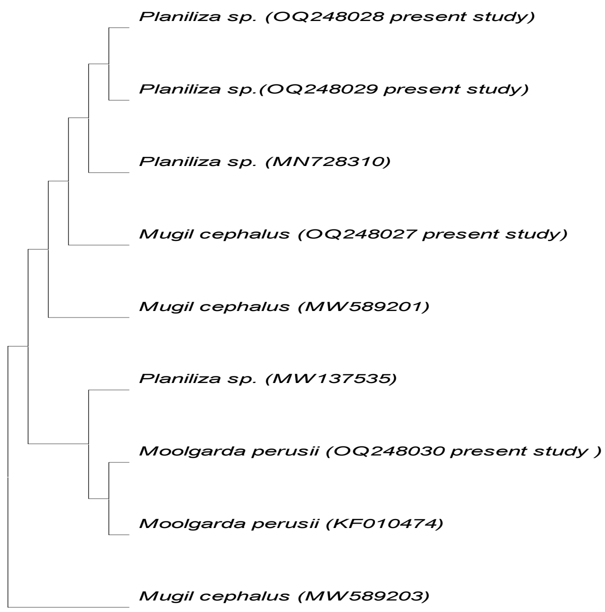

Figure 5. Maximum Likelihood phylogenetic tree for the generated sequence for *Mugil cephalus*, *Planiliza* sp. and *Osteomugil* (*Moolgarda*) *perusii*

Results

The 10 pigmented specimens were collected and identified under 3 different species (Figure 2) under 3 different genera as *Mugil cephalus, Osteomugil perusii* and *Planiliza* sp. based on morpho-meristic traits (Table 1) and molecular barcoding. The highest proportions among these morphometric variables were obtained (Table 1) for eye diameter and pre-orbital length in % HL and inter dorsal space, pre-anal length, and body depth in % SL in case of *Mugil cephalus.* While in *Planiliza* sp., the proportion of caudal peduncle depth in SL was also high. The three species have been clearly differentiated based on morphometric variables through principal component analysis (Figure 3). Further, to understand the Bray-Curtis similarity index based on the morphometric measurements cluster analysis has been performed (UPGMA Method) (Figure 4a, Figure 4b). The least value of similarity index was observed between *Planiliza* sp. and *Osteomugil perusii* (0.82) in comparison with other values among other species (Table 2). Initially, the black dots covering whole fish body, appeared to be natural body colour. But after a long storage in freezer the dots were found to be diminishing. Further, the three identified species were confirmed through molecular barcoding. The maximum likelihood tree has been constructed (Figure 5) by aligning sequences for *Mugil cephalus, Osteomugil* (*Moolgarda*) *perusii* and *Planiliza* sp., by using generated sequences in this present study and downloaded from NCBI.

Discussion

In this present study, characteristics of Mugil *cephalus*, *Osteomugil perusii* (*Moolgarda perusii*) (Rahman et al., 2013) and *Planiliza* sp. were examined and compared with the published records (Table 3). There were insignificant variations in meristic characters in between the specimens (pelvic fin-1 spine, 5 ray, 1st dorsal fin-4 spine, 2nd dorsal fin-8 ray, anal fin spine-2, anal fin ray-9, pre-dorsal scale-18), pectoral fin ray-15 except for *Moolgarda perusii* (pectoral fin ray-14). In addition to this, ambiguity in identification of this group of species has found due to overlapping of characters and pigmentation over the body surface. Melanophore pigmentation was also reported from head of different stages of the fry of 5 grey mullet species (Teleostei: Mugilidae) *Mugil cephalus*, *Liza aurata*, *Liza ramada*, *Chelon labrosus*, *Liza saliens* (Minos et al., 2002) without any taxonomic concern.

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Table 1. Morphometric and meristic parameters of species of *Mugil cephalus*, *Osteomugil perusii and Planiliza* sp. expressed as a percentage of standard length and head length

Parameters	***Mugil* Species**		
	Mugil cephalus **SL – 22.1 cm**	***Osteomugil perusii*** **SL – 18.9 cm**	***Planiliza* sp.** **SL – 27.2 cm**
Characters (% of HL)			
Pr-OL	19.22%	20.86%	18.61%
ED	25.51%	23.48%	21.25%
Po-OL	58.27%	55.24%	56.79%
Characters (% of SL)			
HL	24.04%	23.22%	24.03%
PPtL	24.91%	25.35%	25.01%
PPvL	37.17%	36.51%	37.54%
PVL	13.34%	14.55%	16.05%
PAL	71.0%	68.76%	69.63%
AL	15.44%	14.54%	15.91%
PtL	17.32%	16.86%	16.04%
PDL1	48.69%	50.36%	50.05%
PDL2	73.94%	75.33%	76.38%
DFL1	15.49%	16.22%	14.26%
DFL2	15.57%	16.14%	15.92%
DFB1	6.19%	8.43%	10.02%
DFB2	8.62%	8.63%	9.44%
BD	26.53%	24.40%	22.96%
CPD	11.32%	12.45%	16.83%
IDS	22.04%	19.34%	17.69%

Table 2. Values of similarity in between *Planiliza* sp., *Mugil cephalus*, *Osteomugil perusii*

Species	***Planiliza* sp.**	***Mugil cephalus***	***Osteomugil perusii***
Planiliza sp.			
Mugil cephalus	0.9026		
Osteomugil perusii	0.8253	0.9187	

Table 3. Comparison of morph-meristic characters of *Mugil cephalus*, *Osteomugil perusii and Planiliza* sp. reported by different authors

Parameters	**Species**					
	Mugil cephalus		***Osteomugil perusii***			***Planiliza* sp.**
Author	*Present Study (n=2)*	*Zubia et al. (2015)*	*Present Study (n=1)*	*Rahman et al. (2013)*	*Aaron et al. (2018)*	*Present Study (n=1)*
PrOL	8.32	-	7.46	-	-	9.66
ED	11.04	12.5±0.24	8.4	14.8±0.25	5.71±2.6	11.03
PoOL	25.22	-	19.76	12.4±0.28	21.18±4.1	29.48
HL	43.28	51.44±1.36	35.77	33.8±0.19	34.46±4.7	51.91
PPtL	44.84	-	39.04	-	38.64±4.6	54.03
IDS	39.68	-	29.78	-	-	38.22
PVL	24.02	-	22.41	-	16.00±1.1	34.66
PAL	128.34	-	105.89	-	103.48±10.5	150.4
AL	27.8	35.5±0.25	22.39	-	-	34.37
PtL	31.18	-	25.97	-	33.03±2.4	34.64
BD	47.75	-	37.58	-	37.48±3.0	49.6
CPD	20.38	25.4±0.59	19.17	-	-	36.35

Note: **PrOL** = Pre-Orbital Length, **ED**= Eye Diameter, **PoOL**=Post-Orbital Length, **HL**= Head Length, **PPtL**= Pre-Pectoral Length, **IDS**= Inter Dorsal Space, **PVL**=Pelvic fin Length, **PAL**=Pre-Anal Length, **AL**=Anal Fin Length, **PtL**= Pectoral fin Length, BD=Body Depth, **CPD**=Caudal Peduncle Dept

The genetic variation or ecological factors or interactions between them may also be responsible for morphological differentiation (Krishnan, 2018) and sometimes variation in pigmentation pattern (Jawad et al., 2022). Genetic variations and reproductive isolation of species may result in local adaptation. That is expressed in the morphology, physiology, behaviour and other traits of life (Pakkasmaa & Piironen, 2001; Santos et al., 2016; Hassanien et al., 2020). Pigmentation pattern in animals is noted as a highly variable phenotypes in both inter and intra-specific level, thus suggest to study both genetics of species diversification and adaptation (Mills & Patterson, 2009; Wittkopp & Beldade, 2009; Hubbard et al., 2010). Earlier studies have revealed several reasons for pigmentation such as protective measure from UV ray (Kumar et al., 2023) and water pollution (Bolker & Hill, 2000; Carnikián et al., 2006; Bukola et al., 2015; Jawad et al., 2022). Literatures have also cited pigmentation pattern as a kind of abnormalities categorized under either patrial or hyperpigmentation (Jawad et al., 2022), The pigmentation observed in the present study can be named as under patrial category of pigmentation. Even though morphological traits are the primary requirement for species identification, the molecular traits are confirmatory results. Morphological characteristics, classical taxonomy has made significant contributions in species classification, however, due to morphological plasticity, cryptic species and traditional taxonomy cannot accurately distinguish all species, particularly for similar and closely related species (Pigliucci, 2005; Wang et al., 2020). Therefore, new methods of supporting species identification with classical taxonomy methods are needed. Tautz et al. (2002) first suggested DNA sequencing, namely, DNA taxonomy as the main platform for biological classification. The generated sequences using COI gene has been submitted in NCBI-Gene bank repository (*Mugil cephalus*: OQ248027; *Planiliza* sp.: OQ248028, OQ248029; *Osteomugil* (*Moolgarda*) *perusii*: OQ24803). The information on the differentiation of taxonomic units based on molecular evidence authenticating its phylogenetic status was confirmed by constructing maximum likelihood tree (Figure 5). Further, the sequences deposited in NCBI without confirming morphometry, allows taxonomical complications which need to be verified (Silpa et al., 2021; Behera et al., 2022). This part of information is crucial in establishing a genetic database which will assist in the conservation and efficient management towards fisheries resources in Indian waters.

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Compliance With Ethical Standards

Authors' Contributions

AB, BB & AKJ: Manuscript design
AB: Data collection
AB, SB: Drafting, Writing
AB, APK & AKJ: Data analysis and management
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

The specimens were stored in the museum repository in ICAR-CIFE, Mumbai.

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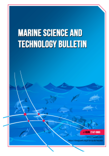

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RESEARCH ARTICLE

Investigation of taxonomic relationship and effect of seasonal temperature changes based on protein profiles of fishes from Beyşehir, Suğla lakes and Dam Apa

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ABSTRACT

Besides traditional methods based on morphological characters, electrophoretic methods such as SDS-PAGE are preferred by taxonomists to make the right decision in the species identification process. In addition, the effect of environmental factors, such as pH, salinity, heat, and temperature on protein profiles are essential in various studies. In this study, we aimed to determine the degree of relationship in some fish species, such as *Squalius lepidus*, *Cyprinus carpio*, *Carassius gibelio*, *Pseudophoxinus anatolicus*, *Tinca tinca*, *Alburnus orontis*, *Scardinius erythrophthalmus*, *Capoeta capoeta*, *Vimba vimba*, *Sander lucioperca* living in Beyşehir, Suğla lakes and Apa Dam by SDS-PAGE method, and to examine seasonal differences by evaluating the effect of hot/cold water on protein profiles in fish. Although there were common major protein bands in all fish species studied, the presence of species-specific minor protein bands led to the separation of the species. The same fish species distributed in different lakes and dams were different both in minor bands, and changes in protein profiles were observed consequently on the same fish species synthesizing different proteins in different seasons. The data obtained from this study can contribute to systematic classification studies of fish.

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Introduction

The family Cyprinidae, represented by 200 genera and 2010 species, constitutes the largest family among freshwater fish (Nelson, 1994; He et al., 2008). Although Cyprinidae members are not found in South America, Australia, and Antarctica, they include many fish with cultural and economic importance (He et al., 2008). A total of 236 species and subspecies belonging to 26 families are found and it constitutes approximately 8% of all fish species in the inland waters of Türkiye (Kuru, 2004).

Mostly in taxonomic studies, the species identification process is based on morphological and anatomical characteristics (Theophilus & Rao, 1998; Yılmaz et al., 2005). Some morphological features may subsequently change as a result of environmental conditions (Fowler, 1970; Ganai et al., 2014). During the definitive identification of a species, classical morphological characters can be misleading due to the existence of these changes over time (Menon, 1989; Ganai et al., 2014). For this reason, comparisons based on morphological characters are not sufficient for taxonomists to make the right decision for determining the species (Hua et al., 2019; Şalcıoğlu et al., 2020). Proteins are used as genetic markers that play an important role in determining taxonomic relationships (Crick, 1963; Nirenberg et al., 1963; Ochoa, 1963; Ganai et al., 2014). In previous systematic studies on fish species, successful identifications were made by electrophoresis of serum proteins, and these studies brought a new perspective to taxonomic evolution (Theophilus & Rao, 1998, Yilmaz et al., 2007).

The aim of this study to evaluate the degree of relationship of some fish species distributed in Beyşehir, Suğla lakes and Apa Dam according to their total protein profiles by using the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method and to determine the effects of seasonal changes on the protein profiles of fish.

Material and Methods

Table 1 indicates the different fish species obtained from Beyşehir, Suğla lakes and Apa Dam, their localities and the seasonal period in which they were obtained.

Protein Isolation

The protocol proposed by Hoffman & Penny (1973) was used, partially modified, for protein isolation from muscle tissue of fish. Five grams of muscle was taken from each fish and thoroughly crushed in a mortar. The shredded muscles were transferred to the falcon tube and 10 ml of distilled water was added. After vortexing, the samples were kept at +4°C overnight. The falcons were thoroughly vortexed the next day and centrifuged for 25 min at 5000 rpm at +4°C in a refrigerated centrifuge (Hettich Universal, Zentrifugen). The supernatant was transferred to a new falcon tube and stored at -20°C until use.

SDS-PAGE Electrophoresis

The SDS-PAGE method was carried out by modifying the method of Laemmli (1970). Before electrophoresis, protein lysate and sample buffer (0.125 M Tris-HCl, 4% SDS, 20% Glycerol, 10%, Mercaptoethanol 2%) were mixed in a 1:1 ratio and boiled in a water bath for five min. The separating gel was a 10% polyacrylamide gel in 1.2 M Tris–HCl (pH 8.8) and 0.3% SDS. The staking gel contained 3% acrylamide in 0.25 mol/l Tris–HCl (pH 6.8) and 0.2% SDS. The electrode buffer contained 0.025 mol/l Tris–HCl, 0.192 M glycine and 0.15% SDS at pH 8.16. The protein standard (Fermantas SM 0431) was used as the molecular weight standard. Running of the proteins was performed using the Biometra vertical electrophoresis system (Biometra, Göttingen) with the size of 120×110×1 mm. The samples were run in electrophoresis at 36 mA until bromophenol blue, which was used as an indicator, reached the end of the running gel. When the electrophoresis process was completed, the gels were removed from the glass plates and placed in staining cuvettes containing the staining solution (50% methanol, 10% Acetic acid, 0.1% Coomassie Brilliant Blue G-250 M, water). After staining, the gels were washed with a washing solution (5% methanol, 7% acetic acid, water). Finally, images of all gels were taken using a gel imaging system (Vilber Lourmat, France).

Data Analysis

Scoring was done according to the absence (0) and presence (1) of protein bands. Similarities were calculated with the Bio1D++ computer program according to Nei's genetic similarity (Nei, 1978). To construct a dendrogram with the UPGMA (unweighted pair-group method and arithmetic averages) method, the degree of relationship and protein differences in summer/winter months were evaluated by cluster analysis.

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Table 1. Names and localities of the fish species used for protein analysis

Order	Family	Genus	Species	Sample Number	Localities	Seasonal Change
Cypriniformes	Cyprinidae	*Cyprinus*	*Cyprinus carpio*	1,2	Beyşehir lake	Winter
				3,4	Apa Dam	Winter
				5,6	Apa Dam	Summer
		Carassius	*Carassius gibelio*	7	Beyşehir lake	Winter
				8,9	Suğla lake	Winter
				10,11	Apa Dam	Winter
				12	Apa Dam	Summer
		Alburnus	*Alburnus akili*	23, 24	Beyşehir lake	Winter
		Vimba	*Vimba vimba*	34	Suğla lake	Winter
		Scardinius	*Scardinius erythrophthalmus*	30	Beyşehir lake	Winter
				31, 32	Beyşehir lake	Summer
		Pseudophoxinus	*Pseudophoxinus anatolicus*	17,18	Beyşehir lake	Winter
				19,20	Beyşehir lake	Summer
				21, 22	Suğla lake	Winter
		Squalius	*Squalius lepidus*	25	Beyşehir lake	Winter
				26, 27	Apa Dam	Winter
				28,29	Apa Dam	Summer
		Gobio	*Gobio microlepidotus*	43	Beyşehir lake	Winter
		Tinca	*Tinca tinca*	13, 14, 15	Beyşehir lake	Summer
				16	Suğla lake	Winter
		Capoeta	*Capoeta capoeta*	33	Suğla lake	Winter
	Cobitidae	*Cobitis*	*Cobitis bilseli*	42	Beyşehir lake	Winter
Mugiliformes	Atherinidae	*Atherina*	*Atherina boyeri*	44	Beyşehir lake	Winter
Perciformes	Percidae	*Sander*	*Sander lucioperca*	35, 36	Beyşehir lake	Winter
				37, 38	Suğla lake	Winter
				39, 40	Apa Dam	Winter
				41	Apa Dam	Summer

Results

Total proteins belonging to fish species used in this study were isolated and SDS-PAGE electropherograms were taken. (Figure 1). A total of 96 polypeptide bands, ranging in size from 18.4 kDa to 116 kDa, were observed in thirteen different fish species.

Cluster analysis was performed using the UPGMA method with the Bio1D++ computer program. According to the dendrogram obtained (Figure 2), fish species were divided into two main groups with 38% similarity. While *Cyprinus carpio*, *Pseudophoxinus anatolicus*, *Squalius lepidus*, *Alburnus orontis*, *Scardinius erythrophthalmus*, *Carassius gibelio*, *Tinca tinca*, *Capoeta capoeta*, *Vimba vimba*, *Gobio microlepidotus*, *Sander lucioperca*, *Atherina boyeri* were in the first main branch, *Cobitis bilseli* species were in the second main branch. Fish species belonging to Cyprinidae and Cobitidae families in Cypriniformes order were clustered and divided into two branches. But members of the Mugiliformes and Perciformes clustered and separated within the first main branch. In other words, while protein profiles distinguish two families belonging to Cypriniformes order with 38% similarity, *A. boyeri* (Mugiliformes) and *S. lucioperca* (Perciformes) species belonging to different orders and are placed in Cyprinidae family of Cypriniformes.

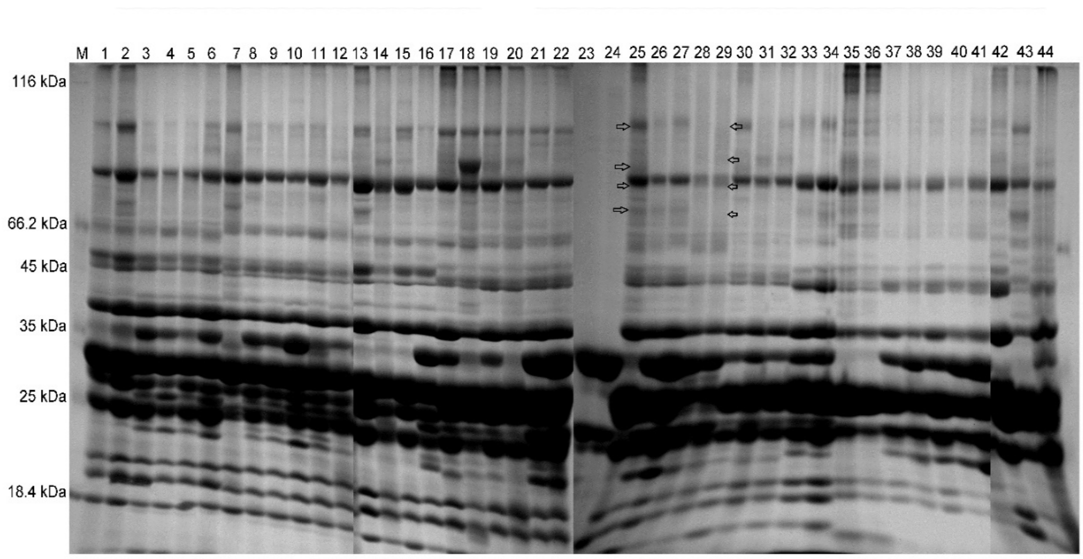

Figure 1. SDS-PAGE protein profiles of the fish species

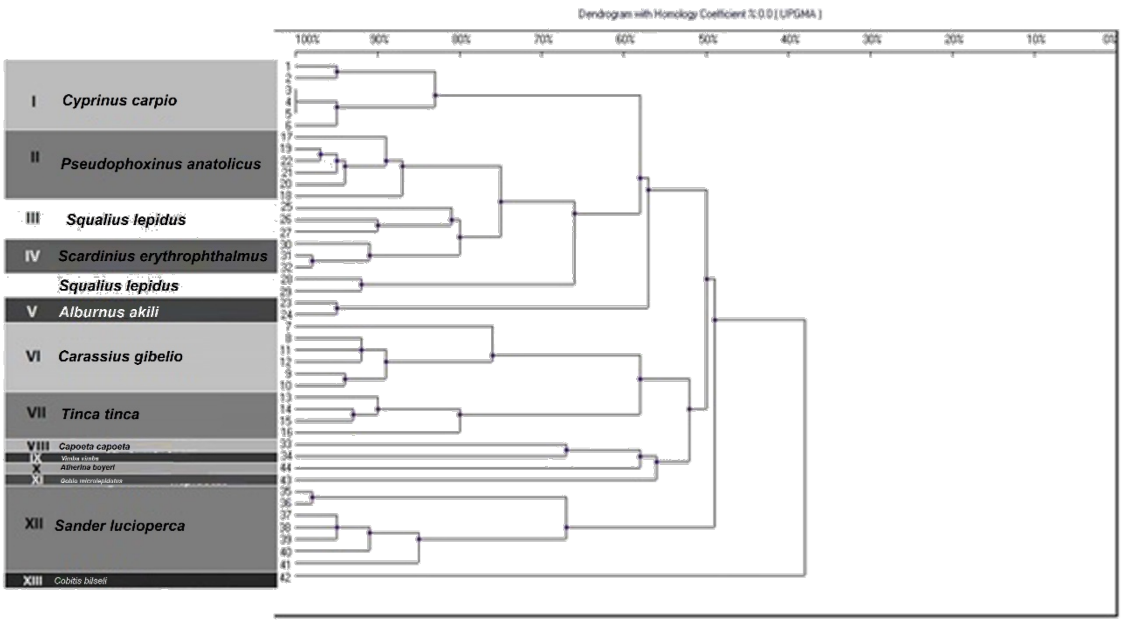

Figure 2. UPGMA dendrogram showing relationship between fish species based on protein profiles

In the first group, different populations (obtained from Beyşehir Lake in winter season, Suğla Lake in Winter, from Apa Dam in Winter and Summer) of *S. lucioperca* (pikeperch) were clustered with a similarity rate of 48%. *C. carpio*, *P. anatolicus*, *S. lepidus*, *S. erythrophthalmus*, and *A. akili* were included in one clade, with 50% similarity, while the other clade consisted of *C. gibelio*, *T. tinca*, *C. capoeta*, *V. vimba*, *A. boyeri* and *G. microlepidotus*. In the first clade, *P. anatolicus*, *S. lepidus* and *S. erythrophthalmus* species had closely related each other than carp. Contrary to expectations, *C. gibelio*, *C. capoeta*, *V. vimba*, *A. boyeri*, and *G. microlepidotus* were in the second clade, 50% away from the grass carp and located closer to the *T. tinca*.

When different fish species and populations of the same species were evaluated in total, 13 small groups were formed in the dendrogram (Figure 2). The same fish species collected from different regions were included in the same group. The fact that the species in these different populations in each group are 10-20% distant from each other can be attributed to the seasonal changes and their collection from different geographical regions.

Based on the UPGMA dendrogram similarity levels results, while the *S. lepidus* species (25, 26, 27) were quite similar to each other (81-89%), the *S. lepidus* 28 and 29 were found to be less similar than the others at the rate of 67.5%. This is due to differences in protein profiles as a result of seasonal changes (as indicated in Figure 1 different bands are indicated by arrows).

When we evaluate the seasonal changes within the species, the synthesis of some proteins increased while others decreased.

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The most obvious change was observed in *S. lepidus* species. Other types have minor changes.

Discussion

Many researchers have successfully differentiated fish species using serum protein profiles via SDS-PAGE, isoelectric focusing and two-dimensional electrophoresis methods. In this study, we evaluated protein identification SDS-page method to understand taxonomy of Cyprinid and other Order fishes. The most important finding for this study is that species-specific minor protein bands provide good differentiation between species. In addition, another important finding is that the minor band profile changes even in the same fish species depending on seasonal changes. In the electropherogram obtained from some studies revealed that protein bands are characteristic to the species (Yılmaz et al., 2005; Berrini et al., 2006; Ganai et al., 2014). Yılmaz et al. (2000) examined the serum proteins of 66 *Capoeta trutta* and 92 *Capoeta umbla* by SDS-PAGE method. It has been stated that there are taxonomically significant differences between the serum proteins of these two fish species. They reported that these differences were due to differentiation of the genes which synthesizes different proteins.

On the other hand, Yilmaz et al. (2007) evaluated the differentiation of *S. lepidus, Acanthobrama marmid*, and *Chondrostoma regium* fish species from Karakaya (Malatya) Dam Lake according to serum protein profiles obtained as a result of Native-PAGE and SDS-PAGE. In this study, they observed 13 protein bands from *S. lepidus* and 11 protein bands from the *A. marmid* and *C. regium*. In addition to the 80.3 kD protein band observed in the electrophoretograms obtained from the Native-PAGE method, the protein bands of the three fish species were observed to be 21.7 kD in the results obtained from the SDS-PAGE method, where the molecular weights of the protein bands were different. In addition, although the protein bands obtained mostly have similar molecular weights, those with 63.4, 52.3 and 49.5 kD weights were observed only in *S. lepidus* and *C. regium* species.

In DNA-based phylogenetic studies, there are some studies do not agree with the degree of relationship obtained as a result of the protein profiles in this study. For example, in a phylogenetic tree based on mitochondrial genome analysis, *T. tinca*, and *C. carpio* were separately located in distant clades, consistent with our results. However, contrary to our results, *Alburnus alburnus* was found to be closer to *T. tinca* and much farther from *C. carpio* (Imoto et al., 2013). Consistent with our results, in a phylogenetic tree based on cyt *b* gene, *Alburnus escherichii* and *Squalius lepidus* were closely located in a clade, while *C. carpio* and *C. capoeta* were located in the same clade, although not very closely (Durand et al., 2002).

While *C. carpio* and *Carassius auratus* species are quite close to each other, *A. alburnus, T. tinca* and *Gobio gobio* have taken place separately in different clades farther away from them (Tang et al., 2010, 2011). Similarly, in another phylogenetic analysis based on mitochondrial 16S rRNA, contrary to the results of our study, it was shown that *C. carpio* and *Carassius carassius* species, and *Leuciscus* genus and *Gobio* genus are closely related each other (Li et al., 2008). Similar to current result, Imoto et al. (2013) found the *Cobitis striata* to be the most distant species from other Cyprinidae family members.

It is known that environmental conditions lead to changes in the amount and number of proteins, which are the expression products of the gene. It has been stated that Native-PAGE and SDS-PAGE methods are useful in separating the proteins of fish samples whose structure changes as a result of exposure to high pressure (Etienne et al., 2001). Muhammad et al. (2018) analyzed the liver proteins of three different fish species using the SDS-PAGE technique and the similarities and differences between the species were determined. In addition, according to the results of their studies, they suggested that the SDS-PAGE method could be used to examine the toxicological aspects of the species (Muhammad et al., 2018). Another group of researchers were able to distinguish processed fish samples using different staining methods of SDS-PAGE (Martinez et al., 2001). Tokur & Kandemir (2008) analyzed the effect of different thawing methods of frozen *Oncorhynchus mykiss* and *Sardina pilchardus* on protein quality by SDS-PAGE and the differences were determined between two species.

Ihuţ et al. (2020) evaluated the seasonal changes in blood biochemical parameters of *Hucho hucho*, and it was observed that some blood parameters increased significantly in the spring and decreased in the summer. On the other hand, Abolfathi et al. (2022) found the presence of proteins with molecular weights ranging from 7-224 kDa in a study in which the seasonal changes in skin-epidermal structure and mucosal immunity parameters of the skin of *O. mykiss* were examined by SDS-PAGE method. They also reported that there were noticeable differences between the number and size of protein bands in seasonal changes. In the study, they reported that small proteins with a molecular weight of less than 35 kDa were found in high proportion in the late summer and spring, while

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proteins with a larger molecular weight (> 35 kDa) were clearly observed in the winter season. Similarly, in our study, the synthesis of some proteins increased in the winter season, while others decreased in the summer season (for example: Figure 1 indicated by the arrow). This change was most prominently observed in *S. lepidus* species.

Conclusion

Although the electrophoretic results of the proteins are incompatible with DNA-based methods, in taxonomic studies, it allows successful differentiation at the level of species and higher characters, as well as the determination of seasonal changes in protein number and amount.

The presence of species-specific protein bands in this study led to the determination of the degree of relatedness. In addition, it was determined that protein profiles changed according to seasonal changes in the same fish species. It was observed that this change was quite evident in the protein profile of *S. lepidus*. In further studies, specific protein determination and quantification by methods such as Westernblot or typing by methods such as peptide mapping can be performed.

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Compliance With Ethical Standards

Authors' Contributions

EA: Study design, Writing
EGM: Writing, Data analysis and management
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

Data Availability Statements

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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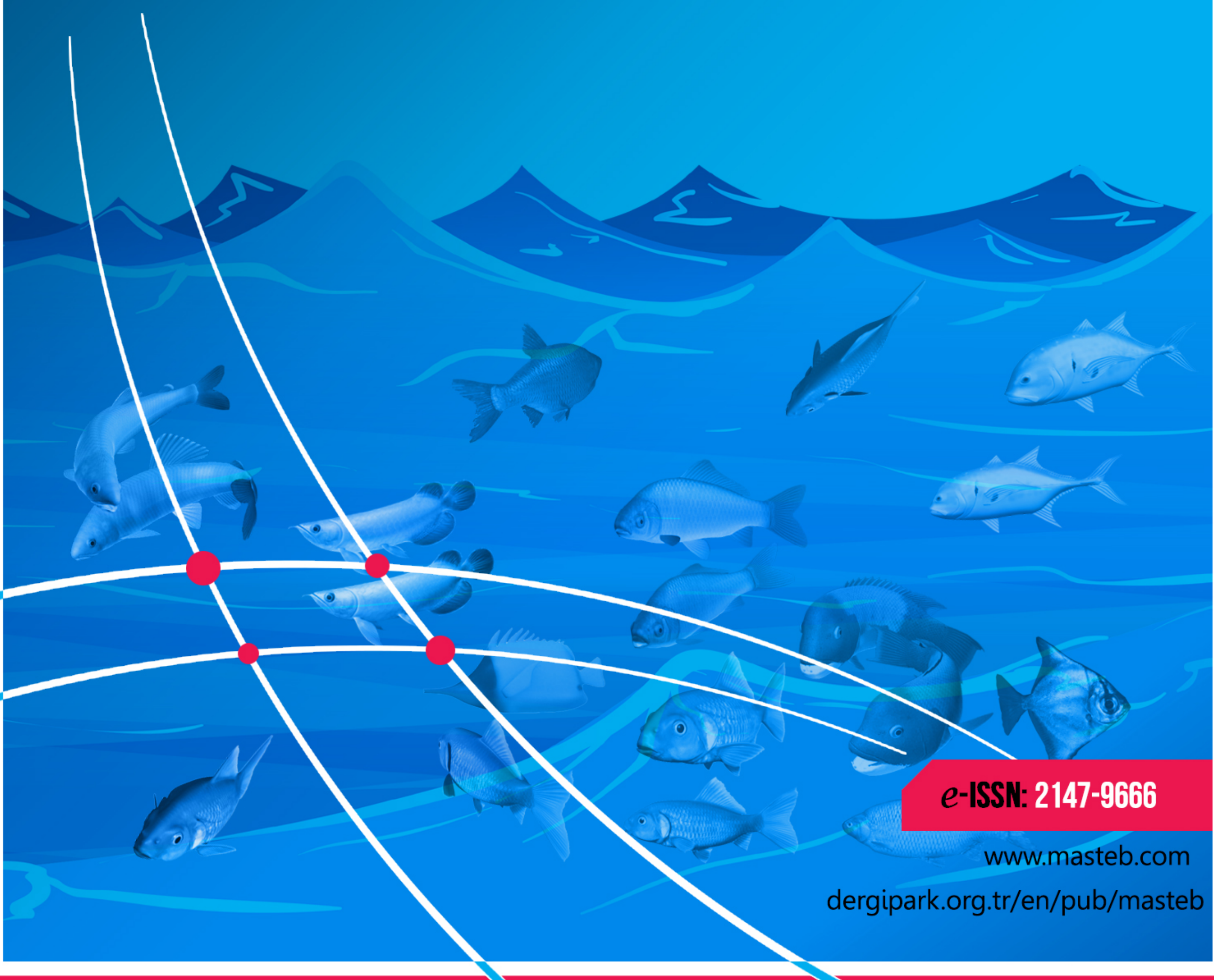

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