MARINE SCIENCE AND TECHNOLOGY BULLETIN

Volume 11 - Issue 4 - YEAR 2022

e-ISSN: 2147-9666

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MARINE SCIENCE AND TECHNOLOGY BULLETIN

2022 • VOLUME: 11 • ISSUE: 4

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Mar. Sci. Tech. Bull. (2022) 11(4): 384-396
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1159477

Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Tracking liquefied natural gas fuelled ship's emissions via formaldehyde deposition in marine boundary layer

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ARTICLE INFO

Article History:
Received: 08.08.2022
Received in revised form: 19.09.2022
Accepted: 07.10.2022
Available online: 22.12.2022

Keywords:
Aerosol Effects
Air Pollution
Formaldehyde
Greenhouse Gas Emissions
Satellite Imagery Sensors
Shipping Corridors
Maritime Transportation

ABSTRACT

One of the reasons that anthropogenic greenhouse gas emissions estimation is imprecise is the uncertainty of aerosol impacts on cloud properties. Maritime transportation is slowly changing fuel preferences. With the policy framework changing regulations, the shipping business is going in a direction that emits less sulfur dioxide and black carbon, which are the compounds that cause linear cloud formations known as ship tracks. Aside from their effects on the total radiative forcing of a transportation mean, this phenomenon enables the detection of ships via satellite imagery sensors. The rapidly increasing trend of shifting propulsion of maritime transportation from conventional heavy fuel oil and distillate marine fuels to liquefied natural gas causes enormous hikes in methane emissions. Therefore, oxidation of the volatile organic compound in the marine boundary layer by the hydroxyl radical in the troposphere makes significant deposition of formaldehyde which causes human effects, ecosystem damage, and climate impact. The primary triggering substance among the compounds in the ship plume is methane. This paper discusses methods to assess near real time tracking of anomalies and the deposition of the short lived substance in different seasons in one of the main occurring areas, shipping corridors. The study also employs anomaly map analysis for June and December 2010 and 2020. Several global tracking methods are available with satellites, monitoring experiments, and other satellite tracking tools. Apart from a few areas the results are not indicative since the formaldehyde formations caused by LNG fueled ships are not widespread enough alongside with overall LNG fueled fleet. On the other hand, the analysis and method are promising for the follow-up of the emissions in the future.

Please cite this paper as follows:

Çalışkan, U. Y., & Zincir, B. (2022). Tracking liquefied natural gas fuelled ship's emissions via formaldehyde deposition in marine boundary layer. *Marine Science and Technology Bulletin*, *11*(4), 384-396. https://doi.org/10.33714/masteb.1159477

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Introduction

There are around 450 compounds in a diesel marine fuel exhaust (Deniz & Durmuşoğlu, 2008). In the recent inventory study of International Maritime Organization (IMO, 2021), in addition to the six initial gases acknowledged under the United Nations Framework Convention on Climate Change process as GHG (carbon dioxide [CO_2], methane [CH_4], nitrous oxide [N_2O], hydrofluorocarbons [HFCs], perfluorocarbons [PFCs] and sulfur hexafluoride [SF_6]), relevant substances to climate change considered as nitrogen oxides (NO_x), nonmethane volatile organic compounds (NMVOCs), carbon monoxide (CO), particulate matter (PM), sulfur oxides (SO_x), and black carbon (BC).

IMO (2018) planned the reduction of shipborne Greenhouse Gas (GHG) emissions with new means. These strategies range from improving the existing energy efficiency framework to employing alternative or renewable fuels. One of the measures in the short term was undertaking additional GHG emission studies. A recent anthropogenic emissions inventory study of IMO (2021) shows that CH_4 emission rates increased more than 2.5 times in 2018 compared to 2012. In the same period, CO_2, NO_x, SO_x, and fine particulate matter ($PM_{2,5}$) also increased by 5.6%, 1.2%, 5.5%, and 3.6%, respectively. To prevent air pollution, IMO has reduced the fuel sulfur limit to 0.1% with the IMO global sulfur cap 2020 regulation within the emission control zones (Bilgili, 2021). Subsequently, Heavy Fuel Oil (HFO) with high sulfur and black carbon concentration is prohibited on the arctic sea routes that the use of which has intensified in the last decade due to melting elements in its cryosphere (in force after 2024) (IMO, 2022a). HFO fuel is the most preferred fuel with 70% in shipping. IMO left these controls to port and flag states and provided three possibilities as solution elements; the use of very low sulfur fuel oil or marine distillate fuels with a lower sulfur content (1), the use of alternative fuels such as Liquefied Natural Gas (LNG), liquefied petroleum gas, biodiesel, biofuel, methanol, ethanol, hydrogen fuel, or hybrid use (2), or the use of a filtration attachment on vessels, closed or open loop (scrubber) (3), (United Nations Conference on Trade and Development [UNCTAD], 2021a).

While most operators chose to equip their ships with scrubber systems, a clear shift to alternative fuels has begun. World LNG fleet by fuel type as of 1st of January 2021 indicates there are 621 LNG ships. More than half of this statistic uses LNG with Very Low Sulfur Intermediate Fuel Oil (VLS IFO). Although the aggregated number of LNG fueled ships do not represent even 1% (0.61%) size wise their deadweight (dwt) represents 1.84% of total dwt of the world merchandise fleet, indicating the cargo volume importance (Authors curation based on UNCTAD, 2021a). According to the statistics obtained from UNCTAD (2021b) data, LNG ships constitute 8.3% of the liner ships arriving at Turkish ports in 2020. Calling ships are 60% younger than the general average, with an average age of 10, and 336% larger than the closest type, container ships, compared to the average size of the ships.

According to previous studies in the literature (Lauer et al., 2007; Fuglestvedt et al., 2009; Eyring et al., 2010), shipping had negative radiative forcing values, but a recent one (Jin et al., 2018a) claims the gap in between climate neutral and cooling effect is closing. The main reasons that these negative values are reaching equilibrium are related to sulfur content, methane and ethanol based fuel choices, and obligations (Lauer et al., 2007; Lindstad et al., 2015; Jensen et al., 2016; Jin et al., 2018a; Sofiev et al., 2018; Kontovas, 2020; Pavlenko et al., 2020; Peng et al., 2020; Bilgili, 2021). Most control policies on substances target non-CO_2 emissions that have tangled linear and nonlinear relationships with fuel usage. In addition, some of the controls on pollutants have uncertain effects on global climate warming (Myhre et al., 2013). One of the examples of this is the Twomey effect which describes how anthropogenic pollution may help to reflect more significant amounts of solar radiation via enhancing the albedo of clouds (Twomey, 1974). Increasing Cloud Condensation Nuclei (CCN) can escalate the cloud droplet number concentration (N_d), thus, increasing reflectivity (Christiensen et al., 2022). In addition, reduced precipitation affected by maritime emissions can cause smaller cloud droplets that enhance moisture in the atmosphere and eventually enhance cloudiness (the so called lifetime effect) (Albrecht, 1989). This phenomenon creates cloud clearing and ship tracks.

The amounts of main polluters released into the atmosphere by the LNG and diesel fuel combustion processes are similar in the tank to propeller cases. From the perspective of life cycle assessment, the quantity doubles for LNG. At the same angle, different emission metrics that calculate emissions in their effective time window show at least a twofold impact against LNG fuel. Average Global Warming Potential 20-year pulse comparison on energy based approach with diesel, LNG fuel scores two times more impactful emissions (Shine et al., 2005; Pavlenko et al., 2020; Peng et al., 2020; Argonne National Laboratory, 2021). LNG fuel's main output is methane emissions which are directly correlated with tropospheric ozone emissions. Most LNG fuelled ships use low pressure dual fuel technology with fossil fuels, which is the least efficient in terms of methane slip. Furthermore, control measures are linear (Pavlenko et al., 2020; IMO, 2021; Christiensen et al., 2022). Another inefficiency is the CO emissions, with almost 25 times more emissions due to the dual fuel technology. In other spark ignited otto cycle versions, this inefficiency is not visible, indicating the low technological readiness level (Pavlenko et al., 2020; IMO, 2021). In Table 1, CO and NO_x emission factors are eye catching. The highest correlations among substances released after LNG combustion are between CH_4 and CO and HCHO (Miller et al., 2020).

Table 1. Emission factors of polluters after the combustion of common marine fuels in the tank to propeller fuel based case (mg emissions/ g fuel), Authors curation based on IMO (2021), missing values of OC is procured from Corbin et al. (2020)

	LSHFO (1% S)	MDO (0.1% S)	LNG
CO_2	3114	3206	2750
N_2O	0.38	0.36	0.41
CH_4	0.11	0.12	2.73
NO_x	29.32	30.83	35.53
SO_x	19.60	1.40	0.03
BC	0.35	0.18	0.03
OC	0.30	0.31	0.0007
CO	6.09	0.55	14.21
VOC	5.94	6.25	5.46
PM	9.65	1.96	0.13

Climatic and Air Quality Interactions: Two opposing indirect effects of NO_x emissions complicate radiative forcing calculations. On the one hand, emissions of NOx inclining O_3 enhancement and, on the other hand, cause CH_4 reductions. Because of the lower altitudes of maritime transportation, CH_4 destruction outweighs the O_3 enhancement compared to land based sources (Myhre et al., 2013). Although the NO_x effect varies in the different time scales and emission metrics, the consensus is on the cooling effect for the emissions (Shine et al., 2005). SO_2 emissions do not have a dispute on them. Most known metrics agree on the cooling effect. The main issues of these two main compounds that differentiate maritime transportation from other transportation are the effects on air quality, regional photochemical oxidants, acidification, and eutrophication (Kontovas, 2020). The imperfect combustion process also causes high CO emissions. Air quality effects of PMs out of ship exhaust include increased human mortality and morbidity, primarily via cardiovascular and respiratory diseases (Brandt et al., 2013).

Aerosol Cloud Interactions: SO_x and NO_x emissions via atmospheric deposition and aerosol nitrate formations are causing a disturbance over terrestrial habitat biodiversity (European Environment Agency [EEA], 2021). These chemical interactions affect precipitation patterns emissions (Shine et al., 2015). The essential aerosols that interact with clouds in order are Sulfate (SO_4) which is the oxidated form of SO_2, BC, Organic Carbon (OC), and nitrate (NO_3). The signal from shipping decays rapidly due to the substance's short lives. Sensitivity studies indicate that three fourth of all direct and indirect aerosol effects can be associated with fuel's sulfur content, whereas BC and PM only contribute 0.4–1.4% and 0.1–1.1%, respectively (Lauer et al., 2007). The direct aerosol effect by scattering and absorbing the solar and thermal radiation from shipping is small (Lauer et al., 2009). The indirect impact is changing cloud properties via aerosol cloud interactions that are most uncertain, but values to refer to aerosol cooling are possibly overestimated (Lauer et al., 2007). Cloud clearing is strongly correlated to SO_x emissions. In order to reach the state of CCN, an aerosol goes through nucleation, condensation, and coagulation, if it is not diluted in the atmosphere. Particles of aerosol act as CCN, enhancing N_d when they enter the cloud. Systematic studies of ship tracks show varying influences on Liquid Water Path (LWP) due to the different atmospheric backgrounds depending on the cloud's pollution level. Significant increases occur under clean conditions and decreases under more polluted conditions (Gryspeerdt et al., 2019). Despite this, inconsistency throughout the studies on the increases in LWP due to aerosol perturbations is argued (Christiensen et al., 2022). When interacting with a cloud, BC emissions, one of the aerosols, can reduce the entrainment when it resides above the cloud and burn off the cloud when it resides in the cloud layer (Johnson et al., 2004). The in-situ measurements indicate that shipping emissions differ vastly within Sulfur Emission Control Areas (SECA) and open seas. Emission factors of SO_4 decrease by 94%. It is also predicted that SECA can decline total CCN by 80%. Seemingly, BC and organic compounds emission factors are not responding to the fuel's sulfur content (Yu et al., 2020).

Shipping emission studies using satellite imagery are mainly formed around ship tracks (Beirle et al., 2004; Peters et al., 2011; Topic et al., 2021). Tied to the given background on the subject, declined CCN indicates fewer cloud formations due to the fuel regulations hence the disappearance of ship tracks (Yuan et al., 2022). This paper discusses the changing use of satellite imagery on maritime transportation emissions. Due to the nature of shipping emissions, previous studies mainly keep the focal point in their studies as sulfur and black carbon originated aerosol effect which causes ship tracks. Although these effects are still visible, the main compound is more restricted (Christiensen et al., 2022). The changing fuel preferences via policies directs shipowners to alternative fuels such as LNG, which causes unexpected atmospheric effects. Few studies concentrated on formaldehyde (HCHO) emissions caused by maritime transportation and their deposition on the Marine Boundary Layer (MBL) in shipping corridors. On top of that, this issue is not associated with rising CH_4 emissions due to the reshaping of bunkering. The paper attempts to investigate means of studying HCHO emissions arising from ship plume via satellite imagery. Various near real time tracking satellites are mentioned, which indicates their availability. An anomaly study has been included via Ozone Monitoring Instrument (OMI) on AURA satellite with the help of Panoply software, comparing June and December of 2010 and 2020. Results are not on a satisfactory level to track emissions of HCHO effectively due to the proliferation of LNG not exceeding the threshold of being one of the main fuel preferences as a bunker.

Materials and Methods

Source Identification

The main causing reason for HCHO in the atmosphere comes primarily from methane oxidation by the hydroxyl radical (OH) in the troposphere. A decade ago, a study observing HCHO emissions on the shipping routes concluded whether or not increased methane degradation due to enhanced OH concentrations derived from emissions of maritime transportation can cause increased levels of atmospheric deposition. This study's results also indicate that the degradation of emitted nonmethane hydrocarbons will not likely explain the increased levels of HCHO values (Marbach et al., 2009). Three possible sources of HCHO are discussed for the source identification of ship plumes in an indirect in situ measurement study: (1) primary HCHO emission from ships, (2) secondary HCHO production in the atmosphere from nonmethane VOCs emitted from ships, and (3) atmospheric process of oxidation of methane. It is found that the atmospheric chemical process of methane oxidation via enhanced levels of OH radicals is the headmost reason for the higher rates of HCHO by 91% (Song et al., 2010). Two studies' explanation of the phenomenon of the OH levels resulting in HCHO along with acidic substances, nitric acid (HNO_3) and sulfuric acid (H_2SO_4), was the oxidation of NO_2 and SO_2 (Kim et al., 2009; Song et al., 2010). Photochemical HCHO production can be done via two atmospheric reactions in Eq (1) and Eq (2).

$$CH_4 + OH + O_2 \rightarrow CH_3O_2 + H_2O \quad (1)$$

$$CH_4 + O(^1D) + O_2 \rightarrow CH_3O_2 + OH \ \& \ CH_4 + O(^1D) \rightarrow HCHO + H_2 \quad (2)$$

Due to $O(^1D)$ radicals often reacting with the abundant N_2 and O_2 molecules (quenching reactions) and then H_2O molecules in the MBL, compounds in the second equation can be denoted as an adjunct (Song et al., 2010). Field observations indicate vessels took part in the elevation of O_3 and or OH levels in the MBL (Burkert et al., 2001; Davis et al., 2001; Kim et al., 2009). HCHO emissions are causing air pollution, high GHG effects, radiative forcing, coastal acidification, coral bleaching, and eye, nose, and throat irritations. Also, they are carcinogens. A recent study observed HCHO concentrations in a corridor from Sri Lanka to Indonesia. Satellite data shows that emissions are mainly on the MBL from the observations of clear and cloudy situations. The emissions are in the location of the shipping corridor and vary according to the seasonal shift of the corridor. The data complies with the chemistry transport model (Gopikrishnan & Kuttippurath, 2021).

Tools of Satellite Observation for Formaldehyde

The spectral resolution of the Global Ozone Monitoring Experiment (GOME) is used to enable the tracking of tropospheric ozone precursors; NO_2 and HCHO (Burrows et al., 1999). To obtain better resolutions and near infrared observing capability on CO, CO_2, and CH_4 observations, SCIAMACHY can be utilized (Bovensmann et al., 1999). The inclusion of the record of ultraviolet imagery covering more detailed and high quality came with Ozone Monitoring (Callies et al., 2000; Levelt et al., 2006). According to Tropospheric Emission Monitoring Internet Service (TEMIS, 2022), which was used to be part of the European Space Agency (ESA) H_2CO (chemical formula of formaldehyde) data products from ERS 2 GOME and SCIAMACHY level 1 data (indicating levels further from raw data at full instrument resolution [0 to 4]) can be produced from ESA at the German Aerospace Centre. Level 2 and level 3 H_2CO developments from ERS 2 GOME and SCIAMACHY should be taken from ESA through the TEMIS. GOME 2 H_2CO operational product developed by EUMETSAT can be used for German Aerospace Centre products as well. For Ozone Monitoring Instrument (OMI) data products, level 1 data can be procured from the National Aeronautics and Space Administration (NASA) ozone watch program's tool NASA/KNMI, and level 2 and level 3 OMI H_2CO developments are supported as part of the Sentinel 5 precursor TROPOMI level 2 project. Also following data products gives near real time data recording: OMI on AURA, SCIAMACHY on ENVISAT, GOME 2 on METOP A, and METOP B. Their further specifications are shown in Table 2. Selective visualizations of data products from the database of TEMIS are given in Figure 1.

Table 2. Instruments of satellite to measure tropospheric HCHO column density (Jin et al., 2018b)

Instrument	Platform	Period	Nadir Resolution (km^2)	Overpass time (local time)	Global coverage (days)
GOME	ERS-2	1995– 2003	320 × 40	10:30 AM	3
SCIAMACHY	ENVISAT	2002 to present	60 × 30	10:00 AM	6
OMI	AURA	2004 to present	24 × 13	1:45 PM	1
GOME-2	MetOp	2006 to present	80 × 40	9:30 AM	1
TROPOMI	Sentinel-5	2017 to present	7 × 3.5	1:30 PM	1

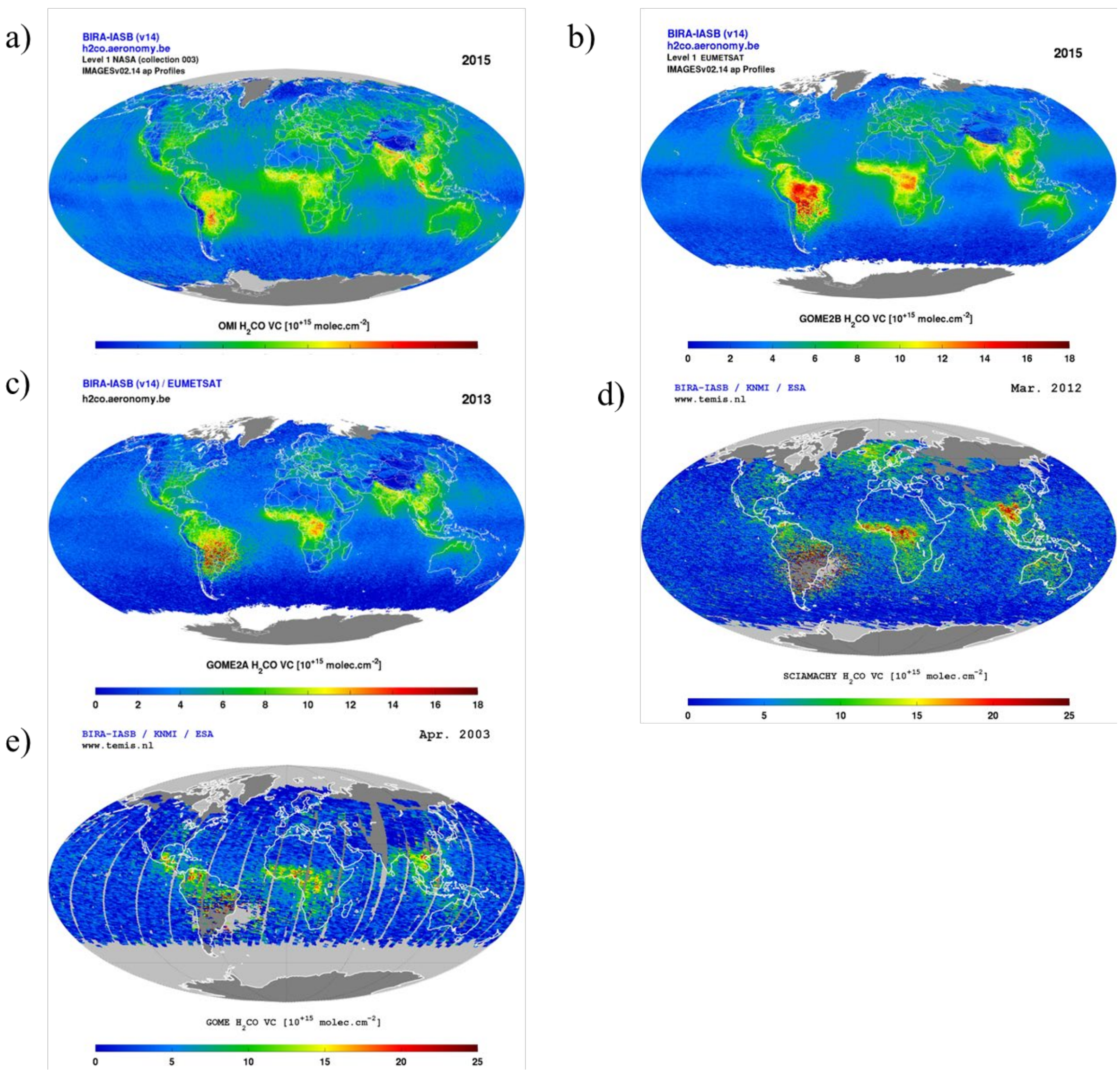

Figure 1. Tropospheric H_2CO columns visualizations from different instruments (TEMIS, 2022) a) yearly mean of 2015 from OMI on AURA. b) yearly mean of 2015 from GOME 2 on METOP B. c) yearly mean of 2013 from GOME 2 on METOP A. d) monthly mean of March 2012 from SCIAMACHY on ENVISAT. e) monthly mean of April 2003 from GOME on ERS-2.

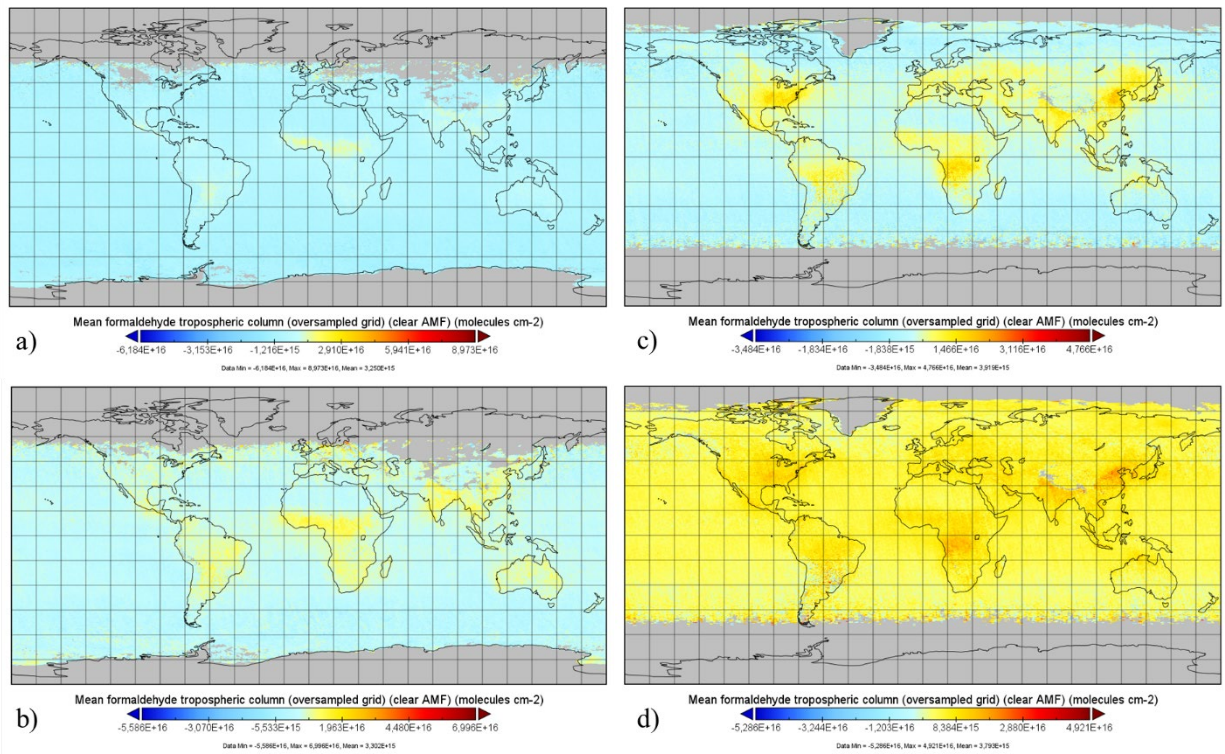

Figure 2. Tropospheric H_2CO columns visualizations of OMI on Panoply; a) 2010 December b) 2020 December c) 2010 June d) 2020 June.

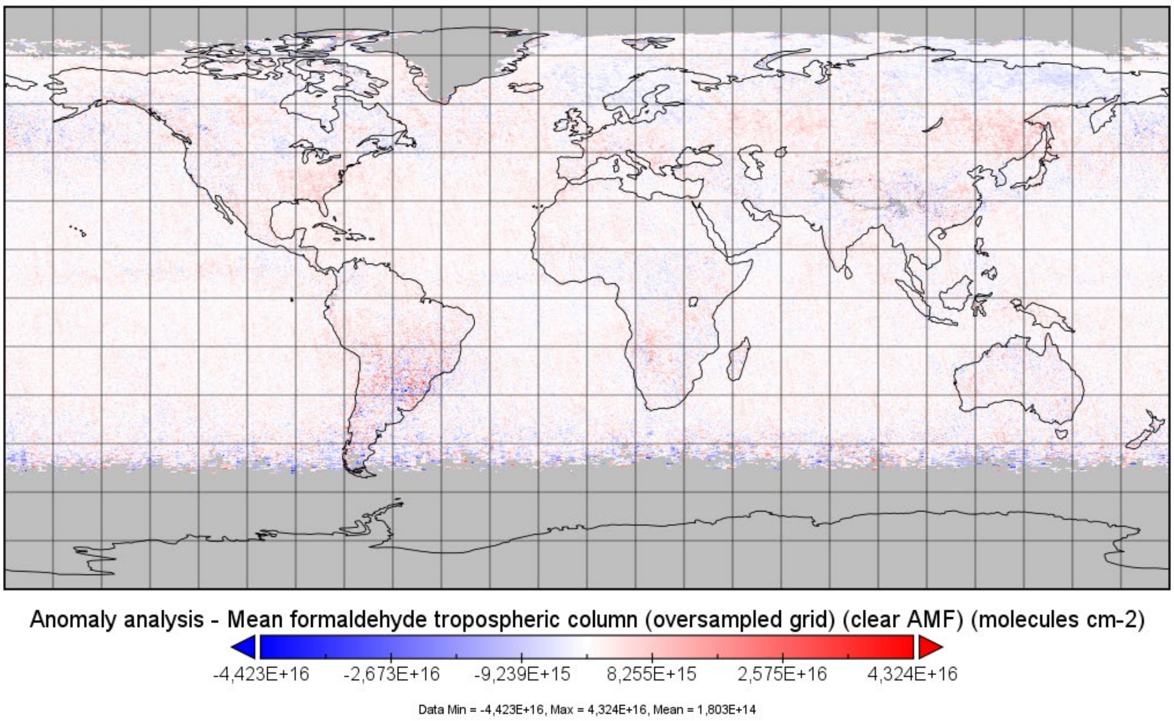

Figure 3. Anomaly map of OMI's global tropospheric H_2CO columns retrievals for June 2010 compared to 2020

Anomaly Analysis

OMI has a cross-track field of view of 115°, a swath of 2600 km, and pixel size between 26 × 135 km² at the swath edges. The OMI HCHO gridding algorithm filters out pixels affected by row anomalies (González Abad et al., 2015). Zhu et al. (2016) provided detailed instructions and the validation of the OMI HCHO instrument. Quality Assurance for Essential Climate Variables (QA4ECV) project provides the dataset for HCHO tropospheric column data from OMI (De Smedt et al., 2017). Level 3 clear Air Mass Factor (AMF) interpolated figures of tropospheric HCHO column retrievals from OMI for the periods of 2010 and 2020 December and 2010 and 2020 June have been given in Figure 2. No interpolation or extrapolations are carried out for filling the missing values. Interpolation mentioned here implies one pixel to other transitions, which are used to obtain better visualization.

The periods represent the recent changes in the maritime transportation policy framework (e.g., Initial GHG Strategy, 2018; IMO, 2020). The first action can be denoted as adopting the Energy Efficiency Design Index in 2011. The constant improvements in the framework favored alternative marine fuels, including LNG (IMO, 2022b). The favoritism can be attributed to the Marginal Abatement Cost Curve (MACC) analysis included in feasibility studies for potential regulations (IMO, 2010). The recent one included in the 4th GHG Study envisions 64.08% of total CO_2 reduction to be contributed by using alternative fuels, followed by speed reduction with 7.54% (IMO, 2021). The difference of summer and winter creates a significant effect on tropospheric formaldehyde column retrievals. Therefore, both times are considered.

Results and Discussion

June

As much as the global warming effects are in place, the impact of HCHO attributed only by shipping is not easily distinguishable. Still, an apparent deterioration can be spoken of. Apart from the equatorial, subequatorial, and tropic zones, visible worsening can be seen in the Sea of Okhotsk, East Coast of United States, Sea of Japan, Bay of Biscay, Arctic Sea Routes, Strait of Gibraltar, Suez Canal, and British Columbia. Figure 3 shows the anomaly of HCHO emissions for 2010 and 2020.

The prominent mentioned geographic locations correspondingly hold major trade flows in shipping. In line with the works of Gryspeerdt et al. (2019) and Yu et al. (2020), Canada's busiest shipping flow Port of Vancouver is where the emissions are visible, but the emissions do not lessen in the following SECA. This implies that even though sulfur regulations are in place, shipping originated HCHO emissions did not show extraordinary fluctuations. The analysis is in line with the elaborated work of Gopikrishnan & Kuttippurath (2021), hence implying the same trade routes. In Figure 4, Europe is focused on.

LNG fueled service vessels are mainly used in European countries. In addition, emissions are regulated with emission control areas, implying HCHO emissions will be more distinguishable. Therefore, the Baltic and the North Sea are the areas of importance for further studies in tracking shipping HCHO emissions. In the Black Sea trade flow, the Gallipoli Peninsula and Strait of Kerch have similar significance for being shipping corridors in the area. There is a visible track of emissions near the entrance of the Suez Canal. The most visible emissions of HCHO for shipping occur west of the Bay of Biscay and the Strait of Gibraltar, where the busiest shipping routes are. Another shipping route is in the Adriatic Sea, which ends mostly in Port of Trieste, where emissions of HCHO are depicted in Europe centered tropospheric HCHO anomaly map.

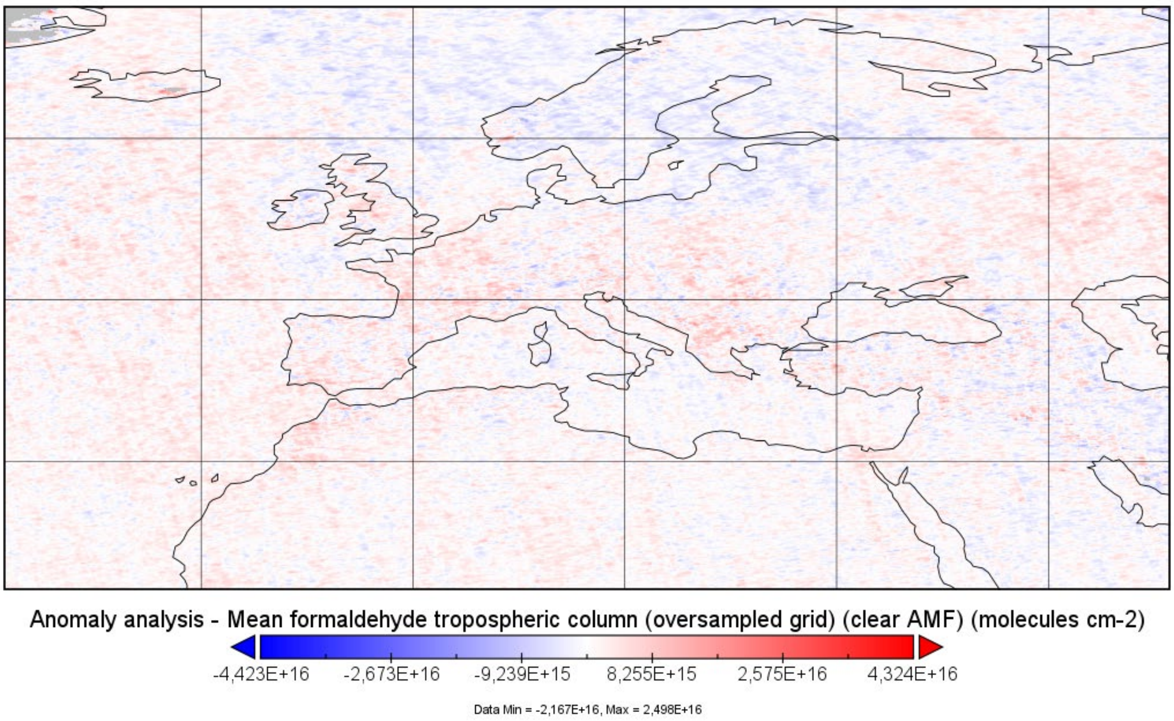

Figure 4. Anomaly map of OMI's tropospheric H_2CO columns retrievals for June 2010 compared to 2020 centered on Europe

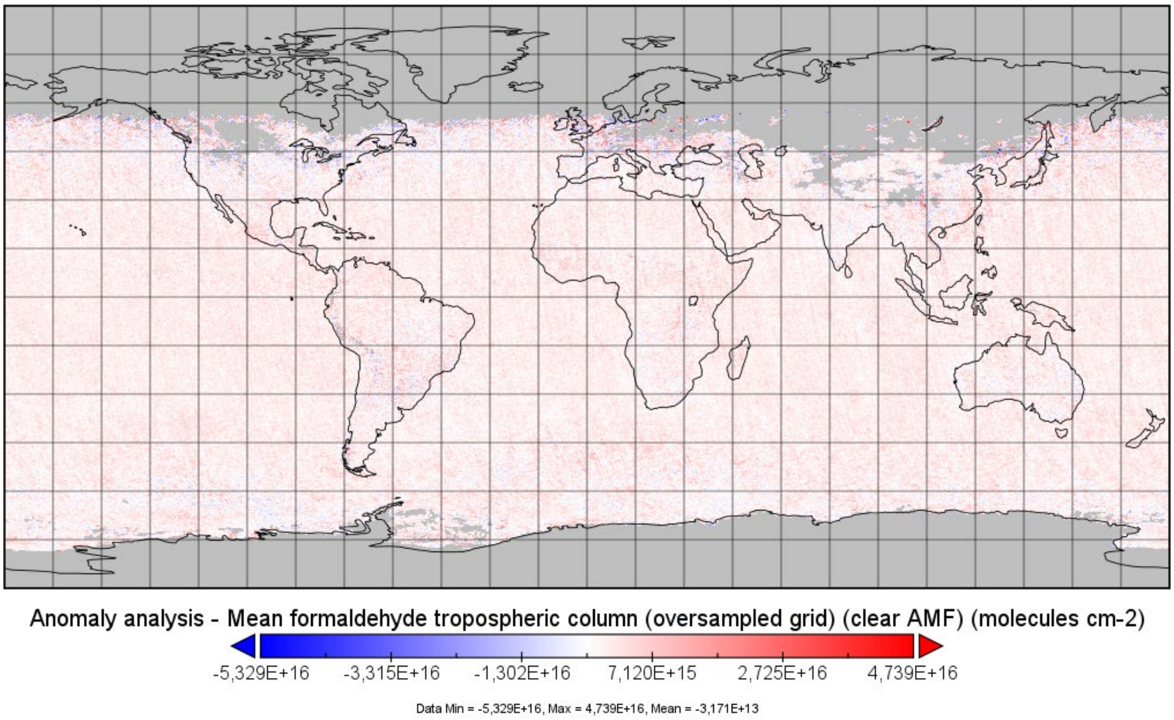

Figure 5. Anomaly map of OMI's tropospheric H_2CO columns retrievals for the months of December 2010 compared to 2020

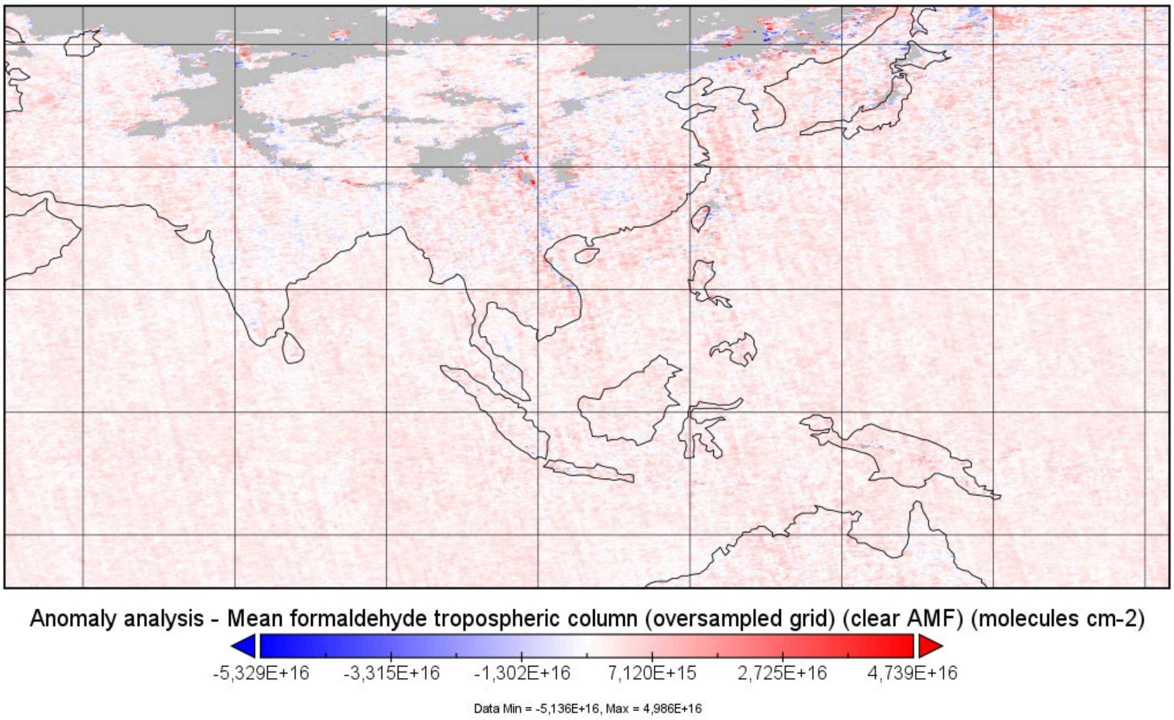

Figure 6. Anomaly map of OMI's tropospheric H_2CO columns retrievals for the months of December 2010 compared to 2020 centered on Asia

December

Exclusion from the heat effect shows that dispersion of HCHO emissions is proportionate to overall global warming. The leading LNG exporters are Qatar, the United States of America, and Australia (Filimonova et al., 2022). According to the International Gas Union (IGU, 2022) report, the most significant global LNG trade flow is in Intra Asia pacific trade. The leading importers are China and Japan, and their biggest exporter is Australia. Australia is followed by Qatar, the United States, and Russia. LNG trade flow includes many re exports. The import of the trade flow consists of small groups that reach out to the whole world. In Figure 5, an equally distributed increase in the overall trend of HCHO emissions can be seen.

Fig. 6 shows the Intra Asia HCHO emissions. The tracks of anomaly can be traced in between above mentioned Australia to Japan and China trade flow. Subsequently Strait of Malacca, sub continent India, and Strait of Hormuz are polluted areas. The Sea of Japan is another central point of anomalies in this period of HCHO emissions.

Overall, tropospheric emissions of HCHO are not strongly visible, which was an expected outcome considering the fuel proliferation is around 1%. In a study by Det Norske Veritas Germanischer Lloyd (DNV GL, 2018), it is said that LNG will be the most dominant fuel type among marine fuels, with a share of between 40% and 80% in 2050 forecasts. Also, Gopikrishnan & Kuttippurath (2021) validated the retrievals of the deposition of HCHO in the shipping corridors with decade long daily emissions. Between anomaly maps, there is a common trend of increased HCHO emissions. Also, main shipping corridors share many similar traceable emissions of HCHO, for instance, the Suez Canal and the Sea of Japan.

Conclusion

This paper discusses the evaluation of changing satellite tracking of maritime transport emissions due to the allocation of emissions. In the older anthropogenic emission trends, satellite tracking was relatively easy to execute due to the formation of cloud clearing and still discussed enhancing LWP. The highly visible Twomey effect enabled scientists to study the impact of sulfur and other aerosols' interactions with the clouds, which scientists even called opportunistic experiments (Christiensen et al., 2022). The transition to the disputable less air polluting fuels allocates emissions, thus, changing the interactions in the tropospheric atmosphere. Formaldehyde, mainly caused by the methane deposition interaction with hydroxyl radical in the MBL, can be attributed to multifold damage on human effects, ecosystem damage, and climate impact compared to dominant sulfur based (2.7% S) fuel emissions. In addition, the increasing volatility of global temperature changes will form more occasional heat waves that will catalyze the deposition of formaldehyde in more significant accumulation in the marine environment and the atmosphere.

This paper suggests that the tracking of formaldehyde via satellite imagery and calculations of anomalies in annual periods will show a clearer picture of the tentative impact of the future with LNG fuel as the transition to alternative marine fuels with greener aspects. OMI on AURA, GOME 2 on METOP A and B, and SCIAMACHY on ENVISAT are identified as near real time possible data sources of formaldehyde to keep track of LNG emissions in shipping corridors. In prior studies, OMI on the AURA satellite has been used to assess the impact created by formaldehyde over the Indian Ocean and the busiest shipping routes. The paper also applied two anomaly analyses for June and December 2010 and 2020. Although the results are not specific since LNG fuel and its emissions are not widespread yet, it shows that the applicability of these prospective analyses and the impact of formaldehyde can be followed like ship tracks.

Further suggestions for studies should include employing different satellite tracking methods with more frequent time windows. Volatile organic compounds and cloud interaction effects can be clarified more. Finally, in situ measurements will be explicitly revealing as well as the satellite tracking on this specific subject.

Acknowledgements

The authors would like to express their gratitude to the respondents for their valuable inputs and contributions.

Compliance With Ethical Standards

Authors' Contributions

UYÇ wrote the original draft of the manuscript. BZ reviewed, edited, and gave supervision. 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.

Funding

The research presented in the manuscript did not receive any external funding.

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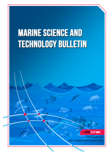
Mar. Sci. Tech. Bull. (2022) 11(4): 397-415
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1162688



Marine Science and Technology Bulletin

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REVIEW ARTICLE

Ballast water problem: Current status and expected challenges

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ARTICLE INFO

Article History:
Received: 16.08.2022
Received in revised form: 15.09.2022
Accepted: 07.10.2022
Available online: 22.12.2022

Keywords:
Ballast water management
Ballast water treatment
Invasive species

ABSTRACT

Transporting non-native species in ballast tanks has been a major challenge over the years. The number of surviving species in the host environment is quite small compared to those of all introduced. However, even a single species can cause great harm to the environment, economy, and public health. Ballast water treatment issues are difficult and complex as the performance of the treatment is highly affected by the variable characteristics of the seawater. In addition, targeted organisms are in a wide spectrum. The International Convention on the Control and Management of Ship Ballast Water and Sediments requires ships to manage ballast water with a Type Approved System in compliance with the Ballast water discharge standard defined in the Convention. The Ballast Water Management Systems Approval (G8) Guide was revised in 2016 and accepted as the BWMS Code (Ballast Water Management Systems Approval Code) as the mandatory regime in 2018. According to the implementation schedule of this mandatory approval regime, the ballast water management system installed on or after 28 October 2020 must be type-approved according to the IMO's revised G8 requirements. Several systems use different methods with their limitations. However, the ballast water problem does not seem to end only with the installation of the systems on ships. Although substantial international progress has been made in ballast water management (both technically and regulatory), there are still several issues regarding effectiveness, compliance monitoring, and the environment.

Please cite this paper as follows:

Bilgin Güney, C. (2022). Ballast water problem: Current status and expected challenges. *Marine Science and Technology Bulletin, 11*(4), 397-415. https://doi.org/10.33714/masteb.1162688

ABBREVIATIONS

BW	Ballast Water
BWMS Code	Ballast Water Management Systems Approval Code
BWM Convention	International Convention for the Control and Management of Ship's Ballast Water and Sediments
BWTS	Ballast water treatment system (the same as BWMS)
BWMS	Ballast water management system (the same as BWTS)
D-1	Regulation D-1, Ballast Water Exchange Standard
D-2	Regulation D-2, Ballast Water Performance Standard
G8	Guidelines for Approval of Ballast Water Management Systems
IMO	International Maritime Organization
MEPC	Marine Environment Protection Committee
USCG	US Coast Guard

Introduction

Ballast is a term that describes any solid or liquid placed on a ship to provide safe navigational conditions by increasing draft, changing trim, regulating stability, or keeping stress loads within acceptable limits. With the use of steel-hulled ships, water began to be used as ballast. However, as a result, ships not only transfer commercial products and people but transfer

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around 12 billion tons of ballast water among biogeographic regions annually (Bax et al., 2003).

Mostly, the ballasting procedure occurs at the ports when the ships discharge their load. As they discharge their load, ships need to compensate for this lost weight. In addition to this, ships also need to compensate for weight loss caused by fuel consumption, freshwater consumption, etc. along the voyage. To compensate for the weight loss, water from the surrounding is pumped into the ballast tank. During the ballasting process, anything small enough to pass through the ballasting system, including the organisms, is also taken into tanks. These organisms are then translocated with the ballast water and discharged to regions where they did not exist before. Due to human activities, many nonindigenous species enter new environments all over the world, but invasive species are among the most important human-induced threats to the oceans (Gollasch, 2006), and ballast waters have the most important share among all vectors. (Lavoie et al., 1999; Olenin et al., 2000; Steve Raaymakers, 2002; Occhipinti-Ambrogi & Savini, 2003).

The transportation of non-indigenous species in ballast tanks has attracted the scientific world's attention since the 1970s (Medcof, 1975; Carlton, 1979). The detection of the Black Sea-origin zebra mussel in the Great Lakes region of America (Hebert et al., 1989)., the poisonous seaweed species originating from Japan in Australia (Hallegraeff & Bolch, 1991), and the carnivorous honeycomb jellyfish from the eastern coast of America in the Black Sea (Berdnikov et al., 1999) raised also the attention of the governments and the public.

Ballast water management is a multifaceted issue where international rules, ship-related technical solutions, and environmental factors coexist. Due to the dimensions of the problem global action was required to solve it. The International Maritime Organization (IMO) (the United Nations' specialized agency responsible for the safety and security of shipping and the prevention of marine and atmospheric pollution by ships) adopted the International Convention for the Control and Management of Ship's Ballast Water and Sediments in 2004 and the Convention is in force since September 8, 2017. The convention is also known as Ballast Water Management Convention (BWM Convention for short). Currently, there are 91 contracting parties (representing 92.23% of world merchant tonnage) to the BWM Convention (the actual numbers can be followed from IMO's official website). All vessels registered under the BWM Convention Contracting Parties that receive and use ballast water during international voyages are obliged to comply with the Convention rules.

On the other hand, instead of being a party to the convention, the United States of America has developed its own Legislation (i.e., Final Rule). The 'Final Rule' is enforced by the US Coast Guard (USCG) and the Environmental Protection Agency. 'Final Rule' became effective in June 2012 and applies to all vessels discharging in U.S. waters that take on ballast water outside the U.S. and Canadian Exclusive Economic Zone.

The Consequences of Transport of Ballast Water Organisms

There are numerous examples of introductions of nonindigenous species to new regions with ballast waters all over the world. The European Environment Agency reports that 346 ballast-related non-indigenous species were introduced into European seas between 1949 and 2021 (European Environment Agency, 2021). The number of species transported to the Baltic Sea with ballast waters was reported to be 105 as of 2005 (Leppäkoski & Gollasch, 2006), in the Laurentian Great Lakes (located on the Canada-US border) 43 non-native animals and protists were established between 1959 and 2003 and the introduction of 67% of these species was again related to ballast water (Grigorovich et al., 2003).

The number of surviving species in the host environment is quite small compared to the total number of introduced species. However, even a single species can cause serious damage to the receiving ecosystem. The settlement of organisms in a new environment depends on certain factors. The most important of these include the absence or scarcity of natural enemies, the organism's ability to spread widely, and the existence of suitable and empty ecological niches (Cirik & Akçalı, 2002). The survival of these species in the new environment is largely determined by differences in physical and chemical properties between donor and recipient sites; the greater these differences, the less likely the survival of living organisms, but never zero (National Research Council, 1996). Their invasion success, on the other hand, depends on the inoculation density of the organisms and their ability to survive and reproduce (Hess-Erga et al., 2019). If all conditions are favorable in the new area, survived species may become invasive, and significant changes occur in the ecology: these species struggle with local species for habitat and food; they use local species as a food source; they can live as parasites on native species; they may cause hybridization of local species; they change the habitat; they may change environmental conditions such as water clarity and hydraulic regime, chemical regime; they change the food web in the ecosystem and displace native species, causing a reduction in natural biodiversity (Nichols, 2001; Raaymakers, 2002). There are numerous examples of the introduction of invasive species by ballast water, some of which have devastating effects.

The introduction of zebra mussels (*Dreissena polymorpha*) into North America is one of the most severe examples due to its rate of spread and continuing ecological and economic consequences. They were first identified in North America in 1988 (Hebert et al., 1989). The studies on the physiological ecology of North American zebra mussels suggest that they probably originated from the northern shore of the Black Sea and reached to Great Lakes region by ballast water (McMahon, 1996). Just a few years after they were detected, they had spread to many of the inland waterways. (Roberts, 1990) and by 2022, zebra mussels are reported to be found in 31 states in the USA (Benson et al., 2022). Their biological features (such as high fertility, pelagic larval stage, and bysso-pelagic drifting ability of juveniles) and human activities such as commercial shipping, fishing, and boating were the main reasons for their rapid spread (Griffiths et al., 1991). Their ability to attach to hard surfaces with byssal threads with around 1-2 Newtons and to form extremely large colonies (up to 700,000 individuals/m^2) makes zebra mussels a major threat (Dölle & Kurzmann, 2020).

They colonize water supply pipes of many structures such as public water supply plants, industrial facilities, hydroelectric and nuclear power plants, etc. (Roberts, 1990). Monitoring and control of zebra mussels cost an average of US$30 million per year in the Great Lakes area of the United States during the mid-1990s (Burtle, 2014) and the economic losses of US and Canadian water users in the Great Lakes region between 2000 and 2010 are estimated to be 5 billion dollars (Glomski, 2015).

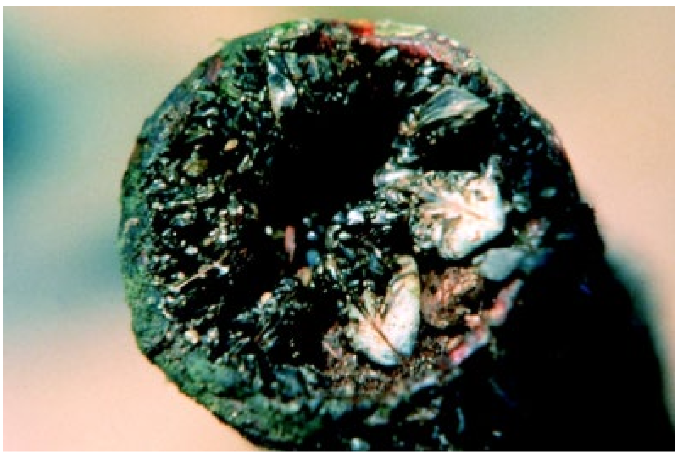

Figure 1. Water intake pipe clogged with adult zebra mussels (de Kozlowski et al., 2002)

Mnemiopsis leidyi, with its devastating impact on the fisheries in the Black Sea and Azov Seas, is another notorious example of the invasive species introduced with ballast water. *M. leidyi* is a north American comb jelly, introduced to the Black Sea in the early 1980s by ballast water from ships, probably coming from the northwest Atlantic coastal region; and in 20 years spread into the Sea of Azov, Sea of Marmara, the Aegean Sea, and lately the Caspian Sea (Shiganova et al., 2001). Through the years, the density of this species in the Black Sea increased up to 1 kg of biomass per m^2 (Raaymakers, 2002). The success of *M. leidyi* was related to the lack of its predators, the ability for competing with pre-existing gelatinous consumers of zooplankton (such as *Aurelia aurita*), and the predation on eggs and larvae of zooplankton-eating fish (Shiganova et al., 2001). The sharp decline in pelagic fish stocks (especially anchovy stocks) in the Black Sea during this period is largely explained by the mass occurrence of the *M. leidyi* (Kideys, 1994), and as a result, the arrival of this species in this region has had a major impact on the fisheries in the Black Sea and Azov Seas; and The Black Sea coast of Türkiye has been the region most affected by this species (Knowler, 2005).

Over the decades, ballast waters and sediments are also associated with the transfer of phytoplankton that will cause harmful algae blooms (HABs) (Olenin et al., 2000; Butrón et al., 2011; Hallegraeff, 2015a; H. Wu et al., 2017; Lin et al., 2021). In his extensive article, Hallegraeff (2015a) states that in the studies on ships' ballast waters for many years, almost all known harmful algae bloom species have been documented in the viable form (Hallegraeff, 2015a). Harmful microalgae species, which have different structures and degrees of toxicity, directly affect fish and shellfish and cause many diseases such as skin allergies, respiratory disorders, and digestive system disorders in people who consume them. Tourism can be damaged due to aesthetic losses such as foaming on the sea due to discoloration caused by some algae explosions and bad odors (van den Berghl, 2002).

In addition to invasive species, it has been determined that many pathogens, including enterobacteria, *Vibrio* spp., and *Escherichia coli*, which threaten human health, can be transported to different regions in ballast tanks (McCarthy & Khambaty, 1994; Ruiz et al., 2000; Takahashi et al., 2008; Altug et al., 2012; Wu et al., 2017; Lv et al., 2018b).

Shipboard Management of Ballast Water According to The IMO BWM Convention

In the BWM Convention, ballast water management is defined as *'any mechanical, physical, chemical, and biological processes, either singularly or in combination, to remove, render harmless, or avoid the uptake or discharge of Harmful Aquatic Organisms and Pathogens within Ballast Water and Sediments'* (IMO, 2004). Ballast water management on the ship includes all the applications made on the ship for the above-mentioned purpose. 'Control and Management Requirements for Ships' are specified in Part B of the Annex to the Convention. The requirements can be summarized in three items:

1. All ships should have a *Ballast Water Management Plan* approved by the Administration
2. All ships should have a *Ballast Water Record Book*
3. Fulfill ballast water management requirements for ships

The 'ballast water management requirements for ships' stated in the last item are carried out by performing *ballast water exchange* on ships or using a *ballast water treatment system.* The Convention requires ships to manage their ballast water, with a method in compliance with *The Standards for Ballast Water Management* which are defined in the D section of the Annex of the Convention:

Regulation D-1, Ballast Water Exchange Standard is an interim measure that requires ships to exchange their coastal ballast water with open seawater with an efficiency of at least 95 percent volumetric exchange. The ballast water exchange should be conducted 200 nautical miles from the nearest land and in waters with a depth of at least 200 m (MEPC, 2017b).

The main idea behind this method is that large numbers of coastal organisms taken up with ballast water have a low chance of survival when discharged into the open sea, and a small number of offshore organisms taken up during the exchange cannot survive after being released into coastal areas due to the physical and chemical differences between the donor and recipient regions. There are three acceptable ballast water exchange methods such as sequential method, flow-through method, and dilution method.

The sequential method is applied by emptying the existing coastal water in the ballast tanks in the open sea and filling the tanks with open seawater again (MEPC, 2017b). With this method, at least 95% of the ballast water by volume must be exchanged.

The flow-through method is carried out by overflowing the ballast water taken into the ballast tanks from the overflow outlets on the deck or by using different devices (MEPC, 2017b). To achieve the desired 95% exchange standard, it is necessary to pump water up to three times the volume of the tanks and allow it to overflow from the tanks.

In the dilution method, replacing the existing ballast water in the ballast tanks is accomplished by discharging the same amount of water from the bottom at the same rate as the water is taken from the top (MEPC, 2017b). To achieve a 95% volume change with this method, three times the volume of the tanks should be filled from the top and discharged from the bottom of the tank.

Regulation D-2, Ballast Water Performance Standard (IMO, 2004) is the ultimate standard to achieve. This standard defines the maximum permissible concentration of viable organisms and specified indicator microbes harmful to human health in the discharge (Table 1). To achieve this standard ships should be installed with ballast water treatment systems (BWTSs) which are Type Approved according to *Regulation D-3 Approval requirements for Ballast Water Management systems.* (IMO, 2004):

Table 1. Organism limits per volume of ballast water (Campara et al., 2019)

Organism Size Indicator Microbes	**IMO D-2 Regulation BW Performance Standard**	**USCG Regulation BW Discharge Standard**
Size ≥50 µm in min dimension	<10 viable organisms/m^3 of BW	<10 living organisms/m^3 of BW
10 ≤ Size < 50 µm in min dimension	<10 viable organisms/mL of BW	<10 living organisms/mL of BW
Toxicogenic *Vibrio cholera* (O1 and O139)	<1 cfu /100 mL, or <1 cfu/g (wet weight) zooplankton samples	<1 cfu/100 mL
Escherichia coli	<250 cfu/100 mL	<250 cfu/100 mL
Intestinal enterococci	<100 cfu/100 mL	<100 cfu/100 mL

Note: µm: micrometer / cfu: colony forming unit / mL: mililiter / m^3: cubic meter

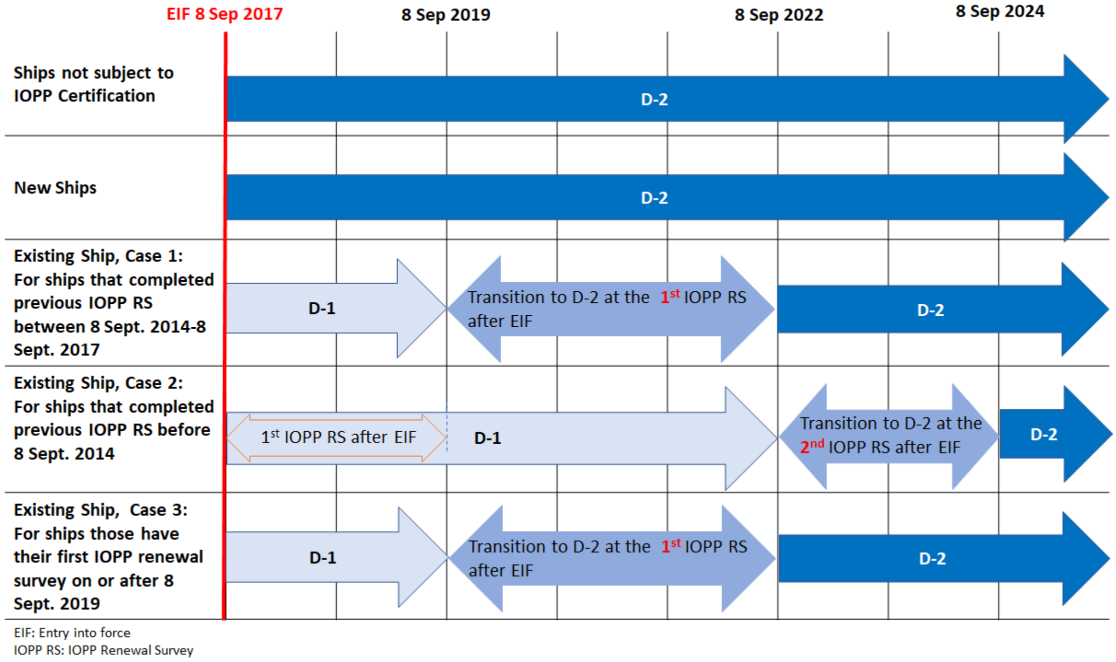

Figure 2. Schedule for compliance with the BWM Convention

As the BWM Convention is in force, the ships registered by the contracting Parties are required to be installed with a ballast water treatment system within a schedule related to their IOPP renewal survey[1] (MEPC, 2017a) and by 8 September 2024, those ships all shall be installed with BWTSs (Figure 2). In Figure 2, '*New Ships*' refer to ships whose keel is laid on or after 8 September 2017, and *'Existing Ships'* refer to ships whose keel is laid before 8 September 2017.

The numerical values of IMO D-2 standards are similar to those identified by the USCG (Table 1). However, there are some differences in the methodologies that they accept for detecting organisms within the size class '10 ≤ size < 50 µm in minimum dimension' and also in their approval processes.

For the uniform application of the BWM Convention, 14 sets of Guidelines were developed by the IMO Member States from July 2005 to October 2008. Some of these Guidelines have been revised since their initial adoption. However, *Guidelines for Approval of Ballast Water Management Systems(G8)* adopted by the Marine Environment Protection Committee (MEPC) is of quite different importance among all guidelines as it is the main component for the implementation of the BWM Convention. These guidelines were also called G8 for short. All ships need to be installed with a system that has a Type Approval Certificate granted following G8. If a ballast water treatment system makes use of an active substance, a second approval process is included in the above-mentioned approval process. The active substance is defined as '*a substance or organism, including a virus or a fungus, that has a general or specific action on or against Harmful Aquatic Organisms and Pathogens* in the BWM Convention (IMO, 2004). With this additional process system's suitability for safety, human health, and the environment is evaluated. This additional process should be following the *Procedure for approval of ballast water management systems that make use of active substances (G9)*, which is called G9 for short.

The Revision of Guidelines for Approval of Ballast Water Management Systems(G8)

Even though the first G8 has provided an important and detailed description of the tests that must be completed and the procedures that must be followed to grant IMO Type Approval to a ballast water management system (BWMS), some gray areas have been identified over the years. Recognizing the differences in the approval processes of the systems based on the applications of the administrations, the IMO Marine Environment Protection Committee determined that there are some uncertainties in the G8 guideline regarding the approval processes and these uncertainties may affect the system reliability. In addition, shipowners have experienced that despite investing millions of US dollars to purchase and install a BWTS approved according to this manual, it does not always perform as expected when installed and operated on board their

[1] The IOPP renewal survey refers to the renewal survey associated with the IOPP Certificate (International Oil Pollution Prevention Certificate) required under MARPOL73/78 Annex I

ships. In summary, the revision of the G8 guidelines became inevitable and was in part promoted by shipowner organizations such as the International Chamber of Shipping (ICS).

As a result, the Marine Environment Protection Committee of IMO adopted the *2016 Guidelines for Approval of Ballast Water Management Systems (G8)* (MEPC, 2016). This revised G8 was made mandatory for the approval of ballast water management systems at the Committee's 72nd Session in April 2018 and was adopted as the *BWMS Code (Code for Approval of Ballast Water Management Systems)* as a mandatory regime (MEPC, 2018). This was an evolutionary change for the Type Approval process of the systems because the old G8 was only a guideline, not a regulatory requirement. Also, the 2016 Guidelines include more prescriptive explanations and stricter requirements for the required type approval tests compared to the old G8.

One of the most significant revisions regards testing facilities. In the old G8, there were no requirements regarding the independence of the laboratory of the manufacturer (MEPC, 2008). However, the 2016 Guidelines and the BWMS Code stipulate that test facilities must be independent; this means that laboratories cannot be affiliated in any way with the manufacturer, vendor, or supplier of any ballast water management system.

Another important issue that was not specified in the old G8 was the temperature. The revised G8 and BWMS Code requires BWMS performance should be checked through a ballast water temperature range of 0°C to 40°C (2°C to 40°C for freshwater) and a mid-range temperature of 10°C to 20°C should be the subject of an assessment verified by the Administration.

The 2016 Guidelines and the BWM Code are more stringent on the requirements for land-based and shipboard testing. For example, land-based tests consist of at least five consecutive successful test cycles in each salinity, and for ship testing, the BWMS should be set up to allow the ship to be used in all ballast operations during the 6-month test period and at least three consecutive valid tests.

Another important revision is about the requirement of the System Design Limitations. According to the 2016 Guidelines and also the BWMS Code manufacturer should identify the System Design Limitations, this limitation should be validated during testing, and indicated on the Type Approval Certificate. System Design Limitations should be established for all known parameters to which the design of the BWMS is sensitive and that are important to the operation of the BWMS. There was no requirement in the old G8 regarding the System Design Limitations

Ballast Water Treatment Systems

Ballast water treatment is more difficult and complex compared to wastewater or drinking water treatment. The physical and chemical properties of ballast waters differ significantly depending on the region where the ballast operation is carried out. Each of these properties can cause different effects on the method to be used. In addition, the targeted organisms vary depending on the region where the ballast operation is carried out, and they range from benthic organisms living at the bottom to pelagic organisms in the water phase, from crustaceans to jellyfish, from viruses to fish. Moreover, the technical and operational conditions of ships are also among the limiting factors in the development of ballast water systems.

There is no single method that sufficiently eliminates all ballast organisms. Hence, combined systems in which more than one method is used together for ballast water treatment have been developed and presented to the market.

Many of the systems have two stages. In the first stage (pre-treatment), Particles and large organisms in the ballast water are removed from the ballast water by mechanical methods, making the ballast water ready for the next treatment stage. In the treatment stage, several methods can be used. By employing more than one method in the systems, it is aimed to expand the range of targeted organisms while increasing the flexibility of the BWTS with combined systems consisting of several steps.

Treatment Technologies Used in The System

Mechanical treatment methods

Screen filters, membrane filters, and disc filters are widely used in ballast water treatment. Filtering mesh size is very important in terms of efficacy (Bailey et al., 2022). Although there are systems with different filtration capacities in the market, the major concern is clogging problems (Matheickal et al., 2003). However, in many filter systems, this problem is overcome by backwashing, and the cleaning of the filters can be ensured by short-term automatic backwashing by making use of sensors that determine the pressure difference (McCluskey et al., 2005; Dobroski et al., 2007).

Hydrocyclones are another option for pre-treatment. In hydrocyclones, the fluid enters the system tangentially and the cylindrical chamber ensures that the flow is spiral; the rotational flow is realized in this way and the centrifugal force on the fluid ensures that the high-density solid particles are pushed toward the separator wall and thrown out (McCluskey & Holdø, 2009). Its effect on organisms is not as efficient as filters (Parsons & Harkins, 2002; Waite et al., 2003) and may vary depending on the density and shape of the particles and organisms contained in the ballast water (Zhou et al., 2005).

Physical treatment methods

Every organism has an optimal temperature range in which it can live. Exceeding this range can lead to the death of many organisms. The applicability of heat treatment depends on the supply of energy that can raise the temperature of the ballast water to the required temperature during the voyage and keep it at this temperature for the time required for treatment. As the temperature increases, the time required for treatment decreases (Oemcke & Van Leeuwen, 2005; Quilez-Badia et al., 2008). On the other hand, Quilez-Badia et al. (2008) suggest that the required treatment can be achieved by prolonging the exposure time at lower temperatures. Several studies have been conducted to provide the energy from the main engine, auxiliary machinery, and other heat sources on the ship and alternative systems that can be applied for different ship types have been proposed (Rigby et al., 1999; Mountfort et al., 2003; Quilez-Badia et al., 2008; Balaji et al., 2014).

Ultraviolet (UV) radiation provides treatment by disrupting the chemical bonds in DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) molecules and cell proteins of organisms (Hijnen et al., 2006; Hess-Erga et al., 2008) This method is highly effective on microorganisms however its efficiency is directly related to the clarity of the water(Hijnen et al., 2006; Azar Daryany et al., 2008; Hess-Erga et al., 2008), therefore, it is suggested to combine UV with an efficient pre-treatment in the systems. UV is utilized in most of the BWTS in combination with a filter. The ballast water management systems are generally tested for the IMO Type approval in warmer seasons when plankton concentrations are high; however, Casas-Monroy et al. (2018) demonstrated that when combined with a filter, UV-C irradiation is also effective across a range of low temperatures (18°C, 12°C, and 2°C) on organisms from two size classes (≥ 10 to < 50 µm and ≥50 µm) (Casas-Monroy et al., 2018). On the other hand, higher life forms and crustacean organisms are highly resistant to UV-based treatment and therefore UV-based treatment systems do not have sufficient effect on such organisms. To overcome the current limitations, the UV method can be used in conjunction with advanced oxidation techniques based on oxidizing radicals formed by photolysis in a significant proportion of BWTSs (Wu et al., 2011a; Wu et al., 2011b; Zhang et al., 2014). Also, the DNA damage of organisms can be repaired through different mechanisms, and among these mechanisms photo repair is the most important, therefore lower UV doses may be sufficient if the water is treated at the intake and left in dark ballast tanks; higher UV doses may be more efficient in the absence of post-dark treatment (Olsen et al., 2016; Romero-Martínez et al., 2021).

The purpose of ultrasound (US) technology is to create acoustic cavitation with high-frequency vibrations created in the liquid and to benefit from the disinfectant effect of the physical and chemical processes that occur during this period. When the microscopic gas bubbles formed during cavitation burst, very high local heat is released, and it also causes the formation of disinfectants such as hydroxyl radicals and hydrogen peroxide (Sassi et al., 2002; Viitasalo et al., 2005). The effect of this technology depends on the size of the organism, results can be obtained with lower energy in a shorter time in large organisms compared to small organisms (Gavand et al., 2007; Holm et al., 2008). The cavitation produced, depends on the frequency, power density, duration of action, and the properties of the water. On the other hand, high-intensity ultrasound energy is required to provide the desired standard in microbiological disinfection in large-scale waters (Joyce et al., 2003). This system is more suitable for low ballast capacity and low flow rates due to the higher cost per ballast water (Mesbahi, 2004). However, hydrodynamic cavitation, which is not currently involved in ballast water treatment, has significant potential in ballast water treatment due to lower energy consumption and operating costs (Cvetković et al., 2015).

Chemical injection

The use of chemical methods in ballast water treatment has become an important field of study as a result of the inclusion of microbial organisms in the scope of IMO's ballast water discharge standards, and the investigation of the effects of various chemical substances, including commercial products, on some target species has accelerated.

Chemicals to be used in ballast water treatment can generally be examined under two major groups oxidizing biocides and non-oxidizing biocides. Oxidizing-type biocides, which work by destroying the cell membrane and other organic structures, are also used in wastewater treatment (Dobroski et al., 2007; Kazumi, 2007). Chlorine, chlorine dioxide, bromine, hydrogen peroxide, peracetic acid, and ozone are among the leading biocides. Peraclean® Ocean, a commercially available liquid form product for ballast water treatment, is a rapid oxidizer. Its active ingredients are peracetic acid (PAA) and hydrogen peroxide (H_2O_2) (Yves de Lafontaine et al., 2008; R Fuchs & de Wilde, 2003; Rainer Fuchs et al., 2001) and it is effective on bacteria spores, yeasts, molds, protozoa, algae, and viruses between pH 5 and 9 (Montemezzani et al., 2015). Peraclean® Ocean was able to rapidly eliminate organisms in the water column of the ballast tank under different environmental conditions during a test with real ballast conditions on board; however, treated water needs to be managed appropriately to minimize potential environmental impacts due to toxicity levels (De Lafontaine et al., 2009). In addition, corrosion is an important point to be considered in the use of biocide in ballast water disinfection as it increases with the increase of the oxidizing potential due to biocides (Kornmueller, 2007).

Non-oxidizing biocides are being developed as an alternative to oxidizing biocides due to the corrosion problem. This group of biocides provides inactivation of organisms by destroying the reproductive and nervous systems of organisms or by interfering with their metabolic functions (Kazumi, 2007). In a laboratory study of 18 low molecular weight quinones (common building blocks of many biological molecules, e.g., vitamin K1), four of them (juglone, plumbagin, menadione, and naphthazarin) were found to be effective on most planktonic organisms even concentrations below 1.0 mg/l (Wright et al., 2007). The commercial product marketed under the brand name SeaKleen® (with menadione as the active ingredient) was tested in various studies. It is effective on cladocerans and rotifers, rather than green microalgae (Montemezzani et al., 2015). SeaKleen® was also found to be effective on resting eggs of different taxa (Raikow et al., 2006) however insufficient as a biocide on bacteria (Gregg & Hallegraeff, 2007). In addition, the slow degradation of SeaKleen® after the time required for disinfection was underlined (Raikow et al., 2006; Gregg & Hallegraeff, 2007).

Electrochemical systems

The chemical injection may not be appropriate for all ships due to several reasons such as the limited available space for storage, supply problems in the ports, and safety risks to the crew. On the other hand, *in situ* production of various disinfectants such as chlorine (Cl_2) gas and hypochlorous acid (HOCl) by applying electrochemical processes constitutes an important alternative for ballast water treatment. Two distinct methods employ electrochemical (el-chem) technology.

In the first method, the electrochemical reactor functions based on the electrolysis of NaCl present in seawater to produce chlorine species such as hypochlorite and hypochlorous acid or sodium hypochlorite. (Matousek et al., 2006; Bilgin Güney & Yonsel, 2013). In this system a portion of the main ballast stream (so-called side-flow) is passed through the electrolysis cells to produce disinfectant that is rich in chlorine species, then this produced disinfectant is injected into the ballast stream (Cha et al., 2015; Petersen et al., 2019; Joo et al., 2022). The second method is direct electrolysis, where the whole ballast water (so-called full-flow) passes through the electrochemical reactor (Tsolaki et al., 2010; Nanayakkara et al., 2011; Lacasa et al., 2013; Moreno-Andrés et al., 2018). During this process, a low concentration of disinfectant is also produced. The disinfection is mainly based on the lethal effect of the electrical field, in addition to this produced disinfectant is also utilized. Although no active substance is added to the seawater in both methods, active substances are released during electrolysis.

The efficiency of electrochemical systems is affected by the salinity and temperature of seawater, as well as the pollutants and their concentrations (Bilgin Güney & Yonsel, 2013; Yonsel et al., 2014). Therefore, the electrochemical systems to be used in ballast water treatment should be optimized according to the properties of seawater that are subject to treatment. It should also be taken into account that electrolysis products can accelerate corrosion in ballast tanks (Kim & Jang, 2009).

Ballast Water Treatment Systems Approved Compliance with the 2016 Guidelines (G8) or the BWMS Code

According to the implementation schedule of the mandatory approval regime, BWTS installed on or after 28 October 2020 must be type-approved according to the IMO revised G8 requirements. However, if BWTS was installed before 28 October 2020, the existing type approval remains valid.

The BWTSs which received IMO Type approval by the 2016 Guidelines (G8) or the BWMS Code (resolution MEPC.279(70) or MEPC.300(72)) are given with their treatment components in Table 2. The names of the approved system are gathered from IMO's official website (IMO, 2021), and the information on the treatment technologies of the individual systems is found through an internet search.

According to IMO's website, there are 47 IMO Type Approvals granted by the 2016 Guidelines (G8) and the BWMS Code. When that list was studied, it is noted that PureBallast and Echlor Systems have been Type Approved two times, once by 2016 Guidelines (G8) and once under the BWMS Code. So, these brands have been listed in Table 2 only once each. Among the brands in Table 2, 'Envirocleanse inTank™' systems (8th and 11th rows) have changed the name to 'inTank BWTS™' and have once granted IMO Type Approval under the new name. And lastly, Trojan Marinex BWT listed in the 39th row has exited the market due to business reasons as a result of regulatory delays and challenging market dynamics. However, there are ships already equipped with the Trojan Marinex Ballast Water Treatment (BWT) system.

Table 2. BWTSs approved by the 2016 Guidelines (G8) or the BWMS Code

	Name of the ballast water management system	Pre-treatment	Treatment
1	Ecochlor® Ballast Water Management System	Filter	Chemical injection (ClO_2)
2	GloEn-Patrol 2.0	Filter	UV
3	BalClor® Ballast Water Management System	Filter	El-chem (side stream)
4	BSKY™ Ballast Water Management System	Hydrocyclone and US	UV
5	CompactClean ballast water management system	Filter	UV
6	OceanGuard® Ballast Water Management System	Filter	AEOP and US
7	HiBallast™ Ballast Water Management System	Filter	El-chem (side stream)
8	Envirocleanse inTank™ Electro chlorination Ballast Water Treatment System	Not employed	El-chem (side stream)
9	Evolution Mini, Evolution BWMS	Filter	UV
10	ERMA FIRST BWTS, model FIT 75-3000	Filter	El-chem (full stream)
11	Envirocleanse inTank™ Bulk Chemical Ballast Water Treatment System	Not employed	Chemical injection (NaOCl)
12	BLUE OCEAN SHIELD BWMS	Filter	UV
13	Bawat BWMS Mk2	Not employed	Heat (pasteurization)
14	PureBallast 3.2 and PureBallast 3.2 Compact Flex ballast water management system	Filter	UV
15	Oceansaver Ballast Water Treatment System MKIIB	Filter	Electrodialysis (side stream)
16	Hyde GUARDIAN -US BWTS	Filter	UV
17	ECS HYCHLOR™ BWMS	Filter	El-chem (side stream)
18	Miura BWMS	Filter	UV
19	LeesGreen® Ballast Water Management System (LeesGreen® BWMS)	Filter	UV
20	Wärtsilä Aquarius UV BWMS	Filter	UV
21	MICROFADE II BWMS	Filter	Chemical injection (TRO)
22	Seascape BWMS	Filter	UV (US for lamp cleaning)
23	NiBallast™ ballast water management system (NiBallast™ BWMS)	Filter	Microfiltration 10 μm, Nitrogen generator
24	Wärtsilä Aquarius EC BWMS	Filter	El-chem (side stream)
25	Cyeco Ballast Water Management System	Filter	UV
26	KBAL BWMS	Pressure vacuum reactor	UV
27	oneTank	Not employed	Chemical injection (TRO)
28	Semb-Eco BWMS	Filter	UV
29	Electro-Cleen™ System (ECS)	Not employed	El-chem (full stream)
30	Purimar™	Filter	El-chem (side stream)
31	EcoGuardian™ BWMS	Filter	El-chem (side stream)
32	TLC-BWM	Filter	UV
33	SKF BlueSonic BWMS	Filter	UV (US for lamp cleaning)
34	Optimarin Ballast System (OBS) and Optimarin Ballast System Ex (OBSEx)	Filter	UV
35	ATPS-BLUEsys BWMS	Not employed	El-chem (full stream)
36	inTank BWTS	Not employed	El-chem/chemical (NaClO)
37	BALPURE® Ballast Water Management System	Filter	El-chem (side stream)
38	Trojan Marinex BWT™	Filter	UV
39	NGT BWMS	Filter	UV
40	JFE BallastAce®	Filter	Chemical injection (NaClO)
41	PACT marine Ballast Water Management System (Pact marine BWMS)	Filter	UV
42	KURITA BWMS	Not employed	Chemical (NaClO)
43	BIO-SEA® BWTS	Filter	UV
44	SeaCURE® BWMS and SeaCURE Models SC-F-500 to SC-F-6000	Filter	El-chem (side stream)
45	Atlantium Purestream™ 100/200/300/500/900/1200/1500	Filter	UV

Examining Table 2, out of 45 brands 37 have a pre-treatment stage. The most employed pre-treatment technique (35 systems) is filtering. Most of these systems can filter organisms >40 µm. Pressure vacuum reactor and hydro-cyclone (coupled with the ultrasound) were used in only one system each. Systems without a pre-treatment stage are mostly chemical or electrochemical systems. Only one uses pasteurization with heat treatment.

Considering the treatment stage, it is seen that 24 brands rely on physical methods, that is, they do not use active substances. UV technology is the most used technology not only among the physical systems but also among all the systems in the list and it is used in 22 systems. One system uses microfiltration (organisms >10 µm) coupled with Nitrogen as inert gas and one system uses heat treatment for pasteurization.

21 systems make use of active substances either through direct injection or onboard generation. The most widely used among these systems is electrochemical technology with 13 systems. 9 of the electrochemical systems use the electro-chlorination method (i.e., side flow process) to produce disinfectant by processing a small amount of ballast water, which is then injected into the entire ballast water. Among these, the inTank system is based on chemical injection and recirculation of the ballast water. However, the chemical can either be generated from seawater onboard or optionally liquid bulk is dosed. The last 3 of the electrochemical systems treat all ballast water directly through the electrochemical process (i.e., full flow process). Totally 6 systems use direct chemical injection (other than the inTank system), one uses electrodialysis and one uses Advanced Electrocatalysis Oxidation Process coupled with ultrasound.

It should be noted that the type approval processes of systems using active substances also require a second approval process that will be conducted following the G9 guideline. Thus, the Type Approval of these systems takes longer than those that do not use active substances, and this process is costlier for system manufacturers.

While selecting a system, along with the environmental acceptability, ships' specifications, operational characteristics, system limitations, safety, installation, and operational cost also must be evaluated (Satir, 2014; Ren, 2018).

Expected Challenges

One of the main issues raised is about *Regulation D-2, Ballast Water Performance Standard,* as there is no general limitation for most bacteria and the majority of other microorganisms with dimensions less than 10 µm in the D-2 Standard. Both IMO Convention and US Coast guard regulations have limitations based on some indicator organisms regarding the human health for this size class. However, these indicator microorganisms are rarely detected in even untreated ballast water samples (Doblin & Dobbs, 2006; Lv et al., 2018a; Petersen et al., 2019) while there is a high abundance of diverse microbial communities hosted by ballast tanks including pathogens and viruses (Altug et al., 2012; Lv et al., 2017; Hwang et al., 2018). Hence, standards based on indicator organisms may not be sufficient when evaluated in terms of human health, the aquaculture industry, and food security, (Cohen & Dobbs, 2015; Drillet, 2016).

The second issue is about the methodology that will be used for assessing compliance with Regulation D-2. The fact that limiting standards address viable/live in ballast water makes it very difficult to study organisms smaller than 10 µm and currently available methods for examining microorganisms do not fully meet all the main criteria for BWM in terms of accuracy, feasibility, and reliability (Bailey et al., 2022; Hess-Erga et al., 2019). The methodology issue also applies to the size class of organisms ≥10 to <50 µm, although not as much for organisms smaller than 10 µm (Casas-Monroy et al., 2022). Casas-Monroy et al. (2022) tested indicative analysis devices against microscopy for size class of organisms ≥10 to <50 µm. However, the results of indicative devices had a weak correlation with microscopy based on numeric estimates, and uncertainty for abundances below and close to the D-2 standard was higher.

In addition to methodological problems, recolonization is another challenge to consider. The disinfection may increase the biological availability of organic matter and even after successful disinfection, recolonization may occur (Hess-Erga et al., 2019). Type-approval test results, where the concentration of microorganisms in treated discharges exceeds those in untreated by up to three orders of magnitude, suggest that onboard treatment systems could turn ballast tanks into 'bacterial incubators' (Cohen & Dobbs, 2015). Also, as the majority of the approved BWMSs are based on disinfection with UV, the repair mechanism against UV-induced damage (Tosa & Hirata, 1999; Jungfer et al., 2007; Guo et al., 2009) needs to be considered.

The available information on the effects, advantages, and disadvantages of BWTS systems is mostly obtained from laboratory-scale studies. Due to the complex nature of ballast water treatment, the methods need to be extensively tested under realistic conditions (Hess-Erga et al., 2019). The study for evaluation of ballast water management systems on operational ships gives us important insight into the future of the ballast water problem (Bailey et al., 2022). In that study, Bailey et. al. (2022) evaluated ballast water samples from 29 different ships calling on the Canadian Ports. The results showed that 48% of these samples exceed the standards for organisms ≥ 50 µm in minimum dimension. Bailey et. al. (2022) discusses multiple reasons extensively. Among these, Bailey et. al. (2022) suggests the incorrect installation of the systems and the inadequacies to be experienced during the operation and maintenance of the installed systems could also lead to exceeding the ballast water discharge limit, as operational issues were reported in 10% the tests where the discharge limits were exceeded. Briski et. al. (2015) carried out experiments on 3 individual ships having different types of treatment systems. They tested the efficacy of the management strategies as 'ballast water treatment alone' and 'ballast water treatment plus ballast water exchange'. They observed combining with ballast water exchange had a significant additional effect on reducing the plankton (Briski et al., 2015).

Also, the ballast sediment can pose implications due to its biotic and abiotic properties. The majority of the approved ballast water treatment systems have primary treatment. They are expected to reduce the amount of sediment to be accumulated to a degree, but they may sufficiently eliminate the accumulation. Because the ballast tank sediment particles are mostly in clay (2 µm or less) and silt (2–63 µm) form (Maglić et al., 2016) which are smaller than the treatment limits of the major pre-treatment systems having 40-50 µm mesh size. In addition, some of the approved systems do not include a pre-treatment stage). Bailey et al. (2022) detected fine sediment in one-third of the samples collected from 29 BWMS-built ships (some without a pre-treatment stage); this suggests that even if vessels are set up with BWMSs, sediment can accumulate at the bottom of the tank. The bottom sediment can host a variety of organisms and some of these organisms are extremely resistant with the capability of germination when conditions are favorable (Johengen et al., 2005; Hallegraeff, 2015b; Bilgin Güney et al., 2016; Lv et al., 2018b; Shang et al., 2019; Dong et al., 2021; Tang et al., 2022). Accumulated sediment should be handled cautiously (Maglić et al., 2019). There are a few studies on reducing sediment accumulation and/or facilitating sediment removal (Yuan et al., 2017; Bilgin Güney et al., 2020; Pereira et al., 2021; Bilgin Güney, 2022); these suggested systems were effective to some extent in laboratory scale.

A significant number of ballast water treatment systems use active substances for disinfection. Disinfection by-products (DBPs) that will be released after disinfection vary not only depending on the chemical used but also on other available substances, factors such as pH, and temperature (Werschkun et al., 2014; Moreno-Andrés & Peperzak, 2019; Zhu et al., 2020). Despite the neutralization phase of systems, disinfectant by-products remain an important issue to be controlled and monitored, especially in receiving environments (Jang & Cha, 2020; Kurniawan et al., 2022) and spreading areas. (Maas et al., 2019) as some of them may reach levels that can pose risk to aquatic organisms (David et al., 2018)

Discussion and Conclusion

Ballast water management has become a major environmental challenge for the IMO and the global shipping industry. The BWM Convention was developed as the result of decades of rigorous work and additional 12 years were needed to meet its entry into force requirements. However, efforts continue to eliminate uncertainties and ensure more efficient implementation. Among these efforts, the revision of Guidelines for Approval of Ballast Water Management Systems(G8) in 2016 and adopting it as a BWM Code was an evolutionary action. With the revisions, the two most important international regimes for ballast water management, the IMO BWM Convention and the U.S Coastguard Final Rule, have been more harmonized, which will relieve system manufacturers and shipowners.

From June 2008, when the first Type Approval was given, until 2018, a total of 78 Type Approvals were given. Some of them are already tested against BWM Code and some of them are on the queue. Yet, there are tens of thousands of ships that are already installed systems granted approvals in compliance with the old G8. As the shipowners fulfill their responsibilities on time, their approvals are valid. That means they are not required to take new action for compliance again. The consequences of the problem areas identified in the old G8 will only become clear with time.

Moreover, there is no available system that sufficiently eradicates the whole ballast organisms. The repair mechanism and regrowth of the organisms are still an issue for future translocation. It is seen that even if the ships are installed with ballast water management systems the discharge standards can be exceeded. Although ballast water exchange is a temporary requirement of the BWM Convention, it can be considered an integral component of a ballast water management strategy to support ballast water treatment systems, as it reduces the number of organisms to be disinfected in ballast tanks.

In addition to system-related reasons, inadequacies to be experienced onboard the ship during the operation maintenance of the system could also result in limit exceeding. This shows that comprehensive training of the crew is of great importance. Trainings should be well planned taking into account the educational and professional history of the crew responsible for operating and maintaining the system on board. Training documents and manuals should be clear and concise.

On the other hand, there are still considerations on the compliance testing methodologies both for organisms less than 10 μm and organisms in the size class of ≥10 to <50 μm and smaller. Available methods for testing organisms smaller than 50 μm for compliance assessment do not fully meet all the key criteria for BWM in terms of accuracy, feasibility, and reliability.

In addition, one of the main issues raised is that there are no general regulations for organisms less than 10 μm. Only three indicator organisms regarding human health, are subject to control, many others are ignored. Public health risks from the introduction of human pathogens other than these three organisms via ballast water may persist so more comprehensive limitations can be needed regarding human health.

Technologies based on chemical treatment are used in a significant part of ballast water treatment systems. Before the ballast water is discharged, disinfection chemicals and by-products are reduced to an acceptable level by the neutralization stage of the treatment system. However, even if they are present in low concentrations in ballast water, there will be a high total inflow of disinfection by-products as there will be continuous and high volumes of ballast water discharge, especially to large international ports. Therefore, disinfection byproduct concentration in seawater and sediment should be monitored in these ports and the spreading areas of the discharged waters.

Accumulated sediment is another concern as it can host a variety of organisms in different life forms. Although the pre-treatment stage of some ballast water treatment systems reduces sediment amount, they cannot completely prevent sediment intake. It is important to minimize the sediment intake with practical applications and precautions that can be taken on the ship.

In conclusion, although great progress has been made toward solving the ballast water problem, there are still many issues that need to be considered by the different stakeholders of ballast water management and due to the nature of the problem, the considerations are not limited to those reviewed in this study. Applications and technologies that will be alternatives to ballast water treatment systems such as the use of potable ballast water, permanent ballast, and port-based treatment options should be investigated. In addition, ship design alternatives should be developed instead of traditional methods to build the ships of the future. For this purpose, the concepts proposed to completely eliminate or reduce the need for ballast water (i.e., *ballastfree* ship, *zero-ballast* ship, *minimal ballast* ship) can be revisited, or new concepts can be developed by changing the existing perspective on conventional shipbuilding approaches.

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.

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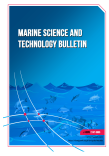
Mar. Sci. Tech. Bull. (2022) 11(4): 416-434
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1166310



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RESEARCH ARTICLE

A research on determining the senior level managerial competencies for container ports in Türkiye

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ARTICLE INFO

Article History:
Received: 24.08.2022
Received in revised form: 28.09.2022
Accepted: 07.10.2022
Available online: 22.12.2022

Keywords:
Container ports
Delphi method
Managerial competencies
Senior managers
Structured interview

ABSTRACT

As essential and significant interfaces in the maritime transportation chain, ports operate in a dynamic and competitive environment. Port managers' competencies are critical intellectual capital for ports to react to the demands of this changing environment. This research aims to identify the critical managerial competencies of container port senior managers in strategically managing the container port in a dynamic and competitive environment. The study was carried out in two steps, and structured interview and Delphi methods were used. As a result, 26 critical managerial competencies for senior managers of container ports are determined in this study, including technical and operational, human and social, cognitive and conceptual competencies. The outcomes of this study are expected to contribute to the relevant literature and provide useful insights to practitioners in designing and implementing human resource development strategies in port management.

Please cite this paper as follows:

Baran Kasapoğlu, E., & Ayan, M. S. (2022). A research on determining the senior level managerial competencies for container ports in Türkiye. *Marine Science and Technology Bulletin*, *11*(4), 416-434. https://doi.org/10.33714/masteb.1166310

Introduction

The earlier study of McClelland (1973) originated the idea of competence, which was further popularized by Boyatzis (1982) and Spencer & Spencer studies (1993). Competencies are "the underlying characteristics of a person that lead to or cause effective and outstanding performance," according to Boyatzis (1982). In addition, it was defined by Spencer & Spencer (1993) as "an underlying characteristic of an individual that is casually related to criterion-referenced effective and/or superior performance in a job or situation". Even though competencies have been described from various viewpoints, three key aspects are common: appreciable performance, the quality of the consequence of the individual's performance, and the fundamental characteristics of an individual (Hoffman, 1999).

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Organizations are altering their management practices promptly as the business environment is becoming more complicated, unpredictable, and risky. For managerial tasks to be accomplished successfully, new requirements emerge. In the current global environment, managers who can successfully manage people and physical assets in the twenty-first century are essential for enterprises (Nekoranec, 2013). Intangible resources such as human capital play a pivotal role in port performance in managing the difficulties of the contemporary era. Consequently, port managers need to have the essential skills, expertise, and talents to be efficient and successful in their managerial role, as well as be completely competent to overcome the difficulties that ports experience in their highly competitive environment (Thai et al., 2015).

As senior managers are critical decision makers and important intellectual capital in port management, it was considered important to investigate their competencies. Yet, despite the importance of managerial competencies and extensive literature on managerial competencies, research on the competencies of port managers is very limited. After a detailed literature review, it has been determined that there is a research gap in Türkiye related to managerial competencies in the port sector. To the best of the authors' knowledge, no study has been reached in the literature regarding the senior-level managerial competencies of container ports. Thus, this study aimed to contribute to the literature by conducting research on the critical competencies for senior managers of Turkish container ports. Therefore, the main research questions of this study are: (i) Which managerial competencies are critical for senior managers in container ports? (ii) What are senior managers' critical technical, human, and conceptual competencies in container ports? The study is structured as follows: the literature on general managerial competencies and managerial competencies in ports are reviewed, and the content analysis of the competencies is presented in the next section. The material and methods section details the research method consisting of two steps. Then the results section presents the findings from the interviews and the Delphi method. The results are compared with the previous studies in the conclusion section, the study's limitations are expressed, and further research directions are proposed.

Literature Review

While the competencies are discussed from the organizational perspective by some scholars (Prahalad and Hamel, 1990), other researchers (Boyatzis, 1982; Burgoyne, 1989; Collin, 1989) examined employees' competencies. Core competencies of employees are sometimes referred to as personal competencies. For example, the "managerial competencies" are widely used to describe the competencies of effective managers (Abraham et al., 2001). A description of managerial competency that emphasizes the significance of abilities, expertise, and personal attributes was proposed by Quinn et al. (1996). They stated that competency term means both the ability to behave properly and the existence of knowledge (Wadongo et al. 2011).

Katz (1955) stated that the three managerial competencies are necessary for successful management: technical, human, and conceptual. Additionally, other various classifications of managerial competencies have been suggested in the literature. For instance, affective, perceptual, symbolic, and behavioral competencies were the categories proposed by Kolb et al. (1986). A total of 21 competencies were categorized as consummate and threshold competencies in conceptual research by Boyatzis (1982). According to Mintzberg (1990), there are ten fundamental roles of managers, which are grouped as interpersonal, informational, and decisional. Competencies are divided into two groups by Spencer & Spencer (1993): visible competencies (knowledge and skill) and hidden competencies (self-concept, trait, motive). Eight managerial roles, including 24 managerial competencies, were proposed by Quinn et al. (1996). Cameron (1997's Management Development Questionnaire grouped 20 competencies into five categories: "managing change," "planning and organizing," "interpersonal skills," "result orientation," and "leadership." Kurz & Bartram (2002) defined the "Big Eight" competency factors, which are leading & deciding, supporting & co-operating, interacting & presenting, analyzing & interpreting, creating & conceptualizing, organizing & executing, adapting & coping, enterprising & performing. The Holistic Competence Model, developed by Le Deist and Winterton in 2005, includes cognitive, functional, social, and meta-competence. Viitala (2005) proposed a hierarchical model of management competencies including six clusters, namely technical, business, knowledge management, leadership, social and intrapersonal competencies. Chong (2008) used the Managerial Assessment of Proficiency instrument, which has four groups (administrative, communication, supervisory, and cognitive) and a total of 12 competencies. The managerial competencies scale elements were divided into five categories (focus, leadership, management skills, purpose and action, and human resources) by Çetinkaya & Özutku (2010, 2012). Agnihotri & Misra (2022) proposed a framework for managerial competency development and classified managerial skills as intrapersonal, interpersonal, business and leadership.

Reichel (1996) investigated the aspects and features of a competent manager in Israel, and the findings emphasized behavioral skills and features. The findings also pointed out that efficiency, adaptability to various circumstances, and interpersonal skills were among the most important. Professional knowledge and problem-identification skill competencies have the lowest value. Abraham et al. (2001) examined the critical competencies to managerial success. Their survey research from various industries revealed that 6 out of 23 competencies are the most critical ones. These are leadership skills, customer focus, result orientation, problem solver, communication skills, and teamwork. Sangka et al. (2019) prioritized managerial competencies of the Indonesian third-party logistics (3PL) sector with the AHP method. They proposed a conceptual framework including 15 competencies under four categories: "management," "logistics," "business," and "information and communication" technologies. While the logistics competency category was identified as most important, the management and business competency categories were perceived as moderately important. Although the competencies from the information and communication technology category were perceived as the least important, they will have a significant role in the future. Bondarenko et al. (2021) provided an investigation of how the managerial competencies has changed as a result of the transformative shifts in the global environment. Analyses are included the forecasts and changes in the fundamental managerial skills for the year 2025 and intercultural and digital competencies are categorised as theoretically new skills needed by a contemporary manager.

Shet & Pereira (2021) focused on identifying the managerial competencies required for an Industry 4.0 environment to succeed and outlined 14 managerial competencies as being essential. Sukalova (2022)'s research is aimed to identify managers' competencies in the context of management diversity throughout the globalization era. And it is stated global thinking, worldwide experience, and strong technical and strategic abilities are often required of managers working in a global context.

Some of the research in the literature examined competencies according to managerial levels. For instance, Labbaf et al. (1996) conducted research about senior managers in the Iranian steel industry and categorized the managerial skills as "analytical and self-related," "people-related," and "task-related." Their findings demonstrated that the improved effectiveness of the senior manager is based on people and analytical & self-related skills rather than task-related skills. Siu (1998) conducted a survey study investigating middle managers' competencies in the Hong Kong hotel sector. A total of eleven competencies were evaluated according to their perceived importance. Communication, customer concern, leadership, planning, and team-building competencies were perceived top 5 crucial competencies for the hotel sector in Hong Kong. Qiao & Wang (2009) used focus group, critical incidence interview, and survey methods to identify managerial competencies necessary for middle managers in China. Team building, communication, coordination, execution, and continual learning were identified as critical competencies for successful middle managers. Furthermore, their research supported those middle managers need a different set of abilities compared to senior managers. For instance, vision and strategic thinking competencies were mentioned less frequently as crucial. Fang et al. (2010) detected a competency model of middle managers in the Taiwanese healthcare sector with the AHP method. In order of importance, the competency groups were determined as personality, managing, planning, professional, and interpersonal.

Mbokazi et al. (2004) investigated the differences in competency importance among hierarchical managerial levels. Their findings showed that senior-level managers perceive interpersonal competence as more critical than middle managers, and first-line managers perceived operational competence as more crucial than middle managers. However, no differences have been found in the perceptions of managers at different levels about the leadership, analytical, and business awareness competencies. Çetinkaya & Özutku (2012) conducted empirical research in the Turkish Automotive sector and evaluated managerial competencies for all management levels. The most important managerial competencies for senior management include reliability, regular, planned and quality work, success orientation, self-confidence, teamwork and collaboration, being an investigator and innovative, customer focus, assisting staff development, good interpersonal relationship skill, and decisiveness. While the managerial competencies considered most important for middle-level management are the same as those considered important for senior-level management, time management competency was emphasized.

Table 1 lays out the many managerial competencies that have been gathered from previous research and are displayed

Table 1. Content analysis of managerial competencies in the literature

Author/s	Effective Communication	Analytical Thinking	Developing others/employees	Teamwork and Cooperation	Managing team / Team Leadership	Achievement & Result Orientation	Planning	Decision making	Managing time and stress	Adaptability and Flexibility	Conflict management	Goal orientation / Goal setting	Information Management	Initiative / Taking initiative	Problem Identification and solving	Interpersonal skills / Relationship	Creative thinking / Imaginative	Customer focus/ orientation	Leadership	Motivation / Motivating others	Organizing	Delegation / Empowerment	Self-Confidence	Expertise / Specialized knowledge	Risk taking and management	Strategic awareness / orientation / thinking	Learning orientation	Negotiating ability	Change management / Managing change	Impact and Influence	Innovation	Listening	Use of power & Authority	Business awareness / sense	Controlling	Having a Vision	Presentation ability	Productivity and efficiency orientation	Reliability Confidential / Integrity	Resilience and Stamina	Self- management	Quality focus/ orientation	Accountability / Responsibility	Commercial awareness/ concern	Ethical concern	Persuasiveness	Sensitivity	Conceptualization / Conceptual thinking	Empathy with people	Self-control	Cultural awareness	Counseling & coaching	Performance Appraising	Proactivity	Self-development	Understanding self & others	Discipline	Fairness	Loyalty	Objectivity	Self-assessment	Judgment
Boyatzis (1982)	✓		✓		✓					✓						✓							✓	✓									✓				✓	✓		✓								✓		✓				✓						✓	✓	
Parry (1992)		✓	✓				✓		✓		✓	✓	✓		✓						✓	✓			✓							✓																				✓	✓				✓					
Spencer & Spencer (1993)		✓	✓	✓	✓	✓							✓	✓		✓							✓	✓						✓																		✓														
Quinn et al. (1996)	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓			✓			✓	✓	✓						✓	✓				✓		✓		✓	✓																		✓						
Cameron (1997)	✓	✓	✓	✓		✓	✓	✓		✓				✓		✓		✓		✓					✓		✓				✓		✓	✓						✓		✓					✓															
McClelland (1998)		✓	✓		✓	✓				✓			✓	✓		✓							✓							✓				✓														✓														
Siu (1998)	✓			✓	✓	✓	✓	✓										✓	✓	✓																		✓						✓																		
Robertson et al. (1999)	✓						✓			✓					✓		✓		✓	✓	✓			✓		✓					✓									✓		✓		✓		✓	✓															
McCredie & Shackleton (2000)						✓				✓				✓		✓							✓									✓			✓				✓	✓																						
Abraham et al. (2001)	✓		✓	✓		✓			✓	✓		✓			✓	✓	✓	✓	✓					✓	✓									✓					✓			✓																				
Anderson & Pulich (2002)	✓			✓	✓		✓	✓			✓	✓							✓		✓	✓													✓																											
Agut et al. (2003)	✓				✓	✓		✓	✓	✓	✓	✓		✓	✓	✓					✓		✓								✓	✓				✓		✓				✓	✓	✓	✓					✓										✓		
Mbokazi et al. (2004)	✓	✓	✓	✓				✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓		✓				✓	✓			✓	✓				✓					✓		✓	✓				✓				✓							✓
Chong (2008)		✓	✓				✓		✓		✓	✓	✓		✓						✓	✓			✓							✓																				✓	✓				✓					
Qiao & Wang (2009)	✓		✓		✓						✓				✓	✓				✓				✓			✓																✓																			
Wickramasinghe & De Zoyza (2009)	✓			✓		✓	✓		✓	✓	✓	✓					✓	✓				✓		✓	✓	✓	✓	✓	✓			✓				✓				✓		✓		✓	✓				✓			✓										
Fang et al. (2010)		✓	✓	✓			✓			✓				✓		✓		✓	✓		✓			✓							✓										✓																					
Bakanauskienė & Martinkienė (2011)	✓	✓				✓			✓		✓		✓					✓					✓				✓					✓									✓		✓		✓	✓				✓					✓	✓		✓	✓			
Wadongo et al. (2011)	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓			✓			✓	✓	✓						✓	✓				✓		✓		✓	✓																		✓						
Çetinkaya & Özutku (2010, 2012)		✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓			✓			✓	✓	✓		✓		✓	✓			✓									✓	✓												✓	
Martina et al. (2012)	✓	✓						✓	✓			✓					✓		✓				✓				✓	✓									✓		✓				✓											✓					✓			

Table 1 (continued)

Author/s	Effective Communication	Analytical Thinking	Developing others/employees	Teamwork and Cooperation	Managing team / Team Leadership	Achievement & Result Orientation	Planning	Decision making	Managing time and stress	Adaptability and Flexibility	Conflict management	Goal orientation / Goal setting	Information Management	Initiative / Taking initiative	Problem Identification and solving	Interpersonal skills / Relationship	Creative thinking / Imaginative	Customer focus/ orientation	Leadership	Motivation / Motivating others	Organizing	Delegation / Empowerment	Self-Confidence	Expertise / Specialized knowledge	Risk taking and management	Strategic awareness / orientation / thinking	Learning orientation	Negotiating ability	Change management / Managing change	Impact and Influence	Innovation	Listening	Use of power & Authority	Business awareness / sense	Controlling	Having a Vision	Presentation ability	Productivity and efficiency orientation	Reliability Confidential / Integrity	Resilience and Stamina	Self- management	Quality focus/ orientation	Accountability / Responsibility	Commercial awareness/ concern	Ethical concern	Persuasiveness	Sensitivity	Conceptualization / Conceptual thinking	Empathy with people	Self-control	Cultural awareness	Counseling & coaching	Performance Appraising	Proactivity	Self-development	Understanding self & others	Discipline	Fairness	Loyalty	Objectivity	Self-assessment	Judgment
Mohd Shamsudin, & Chuttipattana (2012)	✓			✓							✓		✓						✓																	✓																										
Arditi et al. (2013)	✓	✓	✓	✓		✓	✓	✓		✓				✓		✓		✓		✓					✓		✓				✓		✓	✓						✓		✓					✓															
Bhardwaj & Punia (2013)	✓			✓		✓		✓									✓								✓	✓										✓					✓													✓								
Bucur (2013)				✓	✓	✓													✓							✓	✓			✓																																
Chong (2013)	✓	✓	✓			✓	✓	✓	✓	✓			✓	✓	✓		✓			✓	✓	✓			✓	✓		✓		✓		✓		✓			✓		✓	✓	✓					✓	✓						✓									✓
Shang & Yu (2013)	✓	✓	✓		✓		✓	✓	✓		✓	✓	✓	✓			✓			✓	✓	✓						✓	✓				✓		✓	✓	✓	✓																		✓						
Hawi et al. (2015)					✓			✓							✓			✓								✓																																				
Paulienė (2017)			✓	✓	✓	✓								✓	✓								✓							✓											✓								✓	✓												
Veliu & Manhari (2017)	✓	✓		✓		✓	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓				✓	✓		✓	✓	✓	✓	✓			✓							✓		✓		✓		✓	✓			✓		✓				✓			✓				
Sangka et al. (2019)	✓	✓		✓									✓						✓					✓				✓	✓																✓						✓											

as a result of conducting content analysis. Hardworking, safety-conscious, foreign language, independence, tenacity, detail consciousness, energy, process-oriented, ambition, spontaneity, action orientation, and concern for excellence are additional competencies that cannot be listed in the table.

There has been limited research on the port manager and employee competencies (see Table 2). Ahn & McLeanN (2008) conducted an expert interview and categorized 16 competencies under six clusters: policy, system management, service, product development and promotion, information, and globalization. A 65-item competency framework was constructed by Thai (2012), Thai & Lirn (2012), and Thai et al. (2015). Tezcan & Kuleyin (2019a, 2019b) examined the operational managers' competencies in relation to the environmental sustainability practices of container ports. They categorized competencies as technical, job, knowledge management, leadership, and social. As is seen, except for the foreign researchers proposing a general competency list in the port and logistics sectors, and researchers conducting a specific study on operations managers in Türkiye, the field of research on managerial competencies in the port sector is quite limited. The literature review has shown that there is no study on the competencies of senior managers who are decision-makers in the strategic management processes of container ports in Türkiye. Therefore, with this study, it is thought that it will be beneficial for the literature to reveal the critical competencies of senior managers in container ports in Türkiye.

Table 2. Studies related to competencies of port employees and port managers

Author/s	Sample	Method	Findings (Competencies)
Ahn & McLeanN (2008)	Experts from the port and logistics sectors (Busan, Korea)	Expert interviews	Six competency groups 16 sub-competencies <u>**Competency Groups:**</u> Policy, System Management, Service, Product Development and Promotion, Information, Globalization
Thai (2012)	Port employees (Singapore and Vietnam)	In-depth interviews & survey	Port employees at the supervisory level and above should have a wide range of skills in three key areas: **business**, **port and logistics affairs**, and **management.** The most crucial ones are port related.
Thai & Lirn (2012) Thai et al. (2015)	Port executives in Vietnam and Taiwan (2012) Port executives in Vietnam and Korea (2015)	Survey	**Three key groups of competencies:** – Port and logistics affairs-related competencies – Business-related competencies – Management competencies Total 65 critical competencies were categorized.
Tezcan & Kuleyin (2019a)	3 Academicians 5 senior port managers	Structured Interview	– **Technical:** Emergency practices, Basic vocational knowledge, Cargo knowledge, Cargo stowage, Regulations procedures – **Job:** Field knowledge/ Expertise, Business understanding, Port and operation planning, Customer-oriented, Organization, Planning, Management skill – **Knowledge Management:** Analytical thinking, Problem-solving – **Leadership:** Action-oriented, Target-oriented, Decision making, Motivation, Coaching, Teamwork ability and management, Delegating – **Social:** Sensitivity
Tezcan & Kuleyin (2019b)	13 academicians	Delphi Technique	**15 competencies have received consensus.** (*Safety Management, Security Management, Emergency Practices, Decision Making, Regulations / Procedures, Problem Solving, Open-Minded, Analytical Thinking, Action-Oriented, Target-Oriented, Management Skill and Basic Vocational Knowledge, Field Knowledge/Expertise, Delegating*)

Material and Methods

In order to address the research questions of this study, the structured interview and Delphi methods were used (Figure 1). A prepared standardized question list is required for the structured interview procedure. This study's interview form is divided into three sections. The interview form's first section includes a list of general managerial competencies. A list of managerial competencies for the port business was included in the second section. For senior managers at container ports, the competencies were asked to be rated in accordance with their importance (5-point Likert Scale). The last section of the interview form included open-ended questions focused on Katz's (1955) three managerial competency groups.

The structured interview research sample includes respondents who occupied managerial positions in various departments at container ports in Türkiye. Interview questions were answered by e-mail or online question form in accordance with participants' preferences. 18 managers from nine distinct container ports participated in the study and responded to the interview questions. The profile information of the participants is presented in Table 3.

Participants of interviews were chosen from various departments at container ports. Seven participants hold master's degrees, two hold doctoral degrees, and nine participants hold bachelor's degrees. The participants' total experience in the port industry ranges from 1 year to 20 years.

Table 3. Profile information of participants in structured interviews

Age	**N**	**Managerial Experience in Port Sector (Year)**	**N**
Younger than 35	1	1-5 years	3
35-39 years old	5	6-10 years	9
40-44 years old	8	11-15 years	6
45-49 years old	3		
Older than 49	1		
Total Experience in Port Sector (Year)	**N**	**Total Work Experience (Year)**	**N**
1-5 years	1	7-11 years	2
6-10 years	6	12-16 years	5
11-15 years	9	17-21 years	6
16-20 years	2	22-26 years	4
		32 years	1
Departments	**N**	**Titles**	**N**
Commercial & Budget and Reporting	4	General Manager	1
Digital Products	1	Commercial Director	1
General Management	1	Budget and Business Controller Manager	1
Human Resources	3	Business Process Manager	1
HR and Corporate Communication	1		
Operation and Planning	5	HR Director & Manager	3
		HR and Corporate Communication Manager	1
Marketing and Sales	1	Digital Products Manager	1
Support Services & Technical	2	Technical Manager	1
		Sales & Marketing Director	2
		Sales Manager	1
		Support Services Manager	1
		Operation Director & Manager	4

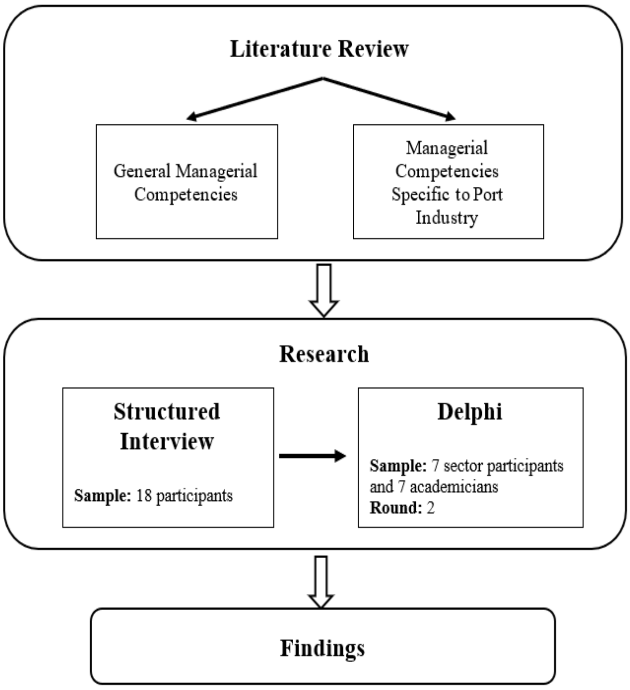

Figure 1. The framework of the study

In order to reach an expert consensus on the critical managerial competencies of senior-level port managers, Delphi research was completed in the second step of the study. The Delphi method is a repetitive procedure that uses a variety of data collecting and analysis approaches combined with feedback to gather anonymous expert opinions. The original Delphi approach was created in the 1950s by Norman Dalkey of the RAND Corporation (Skulmoski et al., 2007). Delphi is a judgment-gathering approach that seeks to overcome the limitations of focusing on a single expert, a one-time group average, or a roundtable conversation. It attempts to establish the most reliable group consensus through a series of rigorous surveys with controlled feedback (Clayton, 1997).

An expert is someone who has the knowledge and expertise required to participate in Delphi research (Clayton, 1997). Although there is no definite rule about the level of knowledge and expertise needed from the Delphi members, it is recommended that the participants of the Delphi study should have i) expertise of and background to the topics being investigated, ii) Capability and desire to participate, iii) adequate time to take participation, iv) strong communication abilities (Skulmoski et al., 2007). Therefore, all these criteria were considered while determining the sample of Delphi research. Furthermore, because the judgmental selection of experts is critical to the reliability of the data gathered throughout the Delphi process, the purposive sampling method (as suggested by Hasson et al., 2000; Skulmoski et al., 2007; McPherson et al., 2018) was used in this study. Based on the purposive sampling method, this study tries to reach managers and academicians with knowledge and experience about port businesses.

There are various opinions on the number of participant sizes necessary for the Delphi method in the literature, such as 15-30 experts for a homogeneous sample and 5-10 experts for a heterogeneous sample (Clayton, 1997), 10- 20 experts (Şahin, 2001), 10–18 experts (Okoli & Pawlowski, 2004), 10-15 experts in the homogeneous group (Skulmoski et al., 2007), 10-50, but no fewer than 7 experts (Hon et al. 2011). Accordingly, in this study, Delphi's first round was completed with 14 participants, 7 of whom were managers from the sector and 7 were academicians (Table 4). Table 5 describes the profile information of the participants in the Delphi research.

The Delphi procedure typically starts with open-ended questions in the first round. However, if the preliminary information about the investigated issue is accessible and useable, a modified Delphi process can be used (Hsu & Sandford, 2007). In addition, open-ended questions in round one can be eliminated if the round two survey questionnaire can be created based on the literature study and interviews. Therefore, it was decided to skip the exploratory first round of the Delphi, as the literature on competencies was reviewed, and preliminary interviews were conducted. As mentioned by Karataş Çetin (2012), in Delphi studies, most of the changes of opinion take place in the first two rounds (Dodge & Clark, 1977), and sending the questionnaires more than twice, especially in Classical Delphi, does not increase the contribution to study too much (Ford, 1975; Mitchell & McGoldrick, 1994). Accordingly, it seemed appropriate to conduct a two-round Delphi study considering that prolonging the Delphi process may reduce the interest and participation of Delphi members. As a result, the competencies evaluated by structured interview participants as being highly important (mean value 4.5 and above) were included in the first round Delphi questionnaire form.

Table 4. Details of Delphi research

Criteria	Choice
Aim of the research	Evaluation
Rounds	Two sequential sets of rounds
Sample	7 sector participants and 7 academicians
Implementation	Face-to-face and E-mail
Anonymity of group	Full

Note: Source: Derived from Day & Bobeva (2005).

Table 5. Profile information of participants in Delphi research

ACADEMIC PARTICIPANTS				
Age	**Title**	**Faculty**	**Total Academic Experience**	**Total Experience in Sector**
46	Prof. Dr.	Maritime Faculty	20 years	2 years sector 13 years consultancy
46	Asst. Prof. Dr.	Naval Architecture and Maritime Faculty	9 years	15 years
41	Asst. Prof. Dr.	Maritime Faculty	20 years	3 years (part-time)
39	Asst. Prof. Dr.	Marine Science and Technology Faculty	11 years	5 years (officer)
32	Asst. Prof. Dr.	Naval Architecture and Maritime Faculty	5 years	1.5 years
33	Dr.	Maritime Faculty	10 years	9 months
33	Dr.	Maritime Faculty	9.5 years	6 months
SECTOR PARTICIPANTS				
Age	**Education**	**Department / Position**	**Managerial Experience in Port Sector**	**Total Experience in Port Sector**
56	Master's degree	Top Management / General Manager	20 years	22 years
46	High-school graduate	Operation / Manager	19 years	25 years
41	Master's degree	Customer Services / Manager	10 years	20 years
42	Bachelor's degree	Finance / Manager	10 years	18 years
40	Master's degree	Trade / Commercial Manager	8 years	8 years
43	Bachelor's degree	Finance / CFO (Chief Financial Officer)	8 years	8 years
34	Bachelor's degree	Trade / Marketing Manager	4.5 years	8. 5 years

Results

The findings obtained from the structured interview and Delphi research are presented in this section.

Structured Interviews

A total of 84 competencies, including 65 general managing abilities and 19 port-specific managerial competencies, are identified from the literature. The competencies were assessed according to their level of importance for container port senior managers. In order to identify the most critical managerial competencies, it was decided to choose competencies with a mean value of 4.5 and above, which are rated as very important. A total of 36 competency criteria have been revealed to have a mean value of 4.5 or above. Table 6 displays the managerial competencies' mean values.

Following Katz's (1955) three fundamental managerial skill dimensions, open-ended questions were asked in the interview's last section. The answers given to the open-ended questions show similarities with high-value competencies determined in the previous section. Most repeated responses include analytical thinking leadership, effective communication, strategic orientation, problem solving, sector knowledge, customer orientation, port operations, and planning. Different from these competencies, competition management and competitive analysis are frequently mentioned in open-ended questions. Therefore "competitive analysis and competition management" competency is included in the Delphi research form. In addition, before the

Table 6. Mean values of managerial competencies

Managerial Competencies	Mean	Managerial Competencies	Mean
Planning	4.88	Creative thinking	4.39
Port operations and operational planning	4.88	Understanding others	4.38
Analytical thinking	4.83	Accountability	4.38
Port financial management	4.83	Learning orientation	4.38
Port strategic planning	4.83	Performance appraising	4.38
Decision making	4.78	Flexibility	4.38
Organizing	4.77	Judgment	4.33
Problem identification and solving	4.77	Self-assessment	4.33
Development of port service quality	4.77	Port quality management	4.33
Port customer relations and management	4.77	Self-management	4.33
Effective Communication	4.72	Responsibility	4.33
Port marketing management	4.72	Ethical concern	4.30
Customer orientation	4.72	Quality orientation	4.30
Strategic orientation and thinking	4.72	Fairness	4.28
Commercial awareness/concern	4.70	Goal orientation	4.27
Managing change	4.66	Conceptual thinking /Conceptualization	4.27
Reliability and Integrity	4.66	Negotiating ability	4.27
Port tariffs and pricing	4.66	Self-control	4.27
Achievement and Result orientation	4.61	Delegating / Empowerment	4.27
Developing others/employees	4.61	Safety and security at the port	4.27
Leadership	4.61	Port policy development and evaluation	4.22
Having a Vision	4.61	Information and operations about cargo types	4.22
Port management information system	4.61	Port Equipment – operational, maintenance, and repair	4.22
Port performance and efficiency management	4.61	Discipline	4.22
Understanding and using port information systems and technologies	4.61	Empathy	4.22
Global industry analysis	4.61	Objectivity	4.22
Teamwork and Cooperation	4.60	Business orientation/awareness	4.17
Managing time / Time management	4.56	Customs and regulatory procedures	4.11
Effective Listening	4.55	Emergency management	4.11
Port roles and functions	4.55	Self-confidence	4.11
Interpersonal relationship skills	4.50	Controlling	4.06
Motivation and motivating	4.50	Impact and Influence	4.00
Self-development	4.50	Persuasiveness	4.00
Risk-taking and management	4.50	Resilience	3.94
Team Leadership /Managing team	4.50	Sensitivity	3.94
Expertise / Specialized knowledge	4.50	Proactivity and Entrepreneurship	3.94
Initiative	4.44	Counseling and coaching	3.83
Productivity and efficiency orientation	4.44	Conflict management	3.72
Innovation	4.44	Presentation ability	3.67
Adaptability	4.40	Cultural awareness	3.66
Managing stress / Stress management	4.40	Authority/power	3.39
Information Management	4.39	Loyalty	3.39

Delphi process, the competencies were reviewed and simplified. Because communication is a two-way action and listening plays an integral part in communicating, "effective listening" competency is unified under "effective communication" competency. Similarly, it is thought that team leadership and team management are covered within the broader meaningful competencies of "leadership" and "teamwork". To prevent the recurrence of similarly meaningful competencies, they were unified in the Delphi stage.

Delphi Research

The APMO (Average Percent of Majority Opinion) technique (Equation 1) was used in this study as a consensus criterion. In maritime literature, the use of the APMO formula is supported by Saldanha & Gary (2002), Özer & Tuna (2002), Yılmaz & Cerit (2005), Karataş Çetin & Cerit (2010), Emiroglu et al. (2014), Ayaz & Çetin (2018), Göçer et al. (2019).

The APMO cut-off percentage rate for the first round of the Delphi survey is 98.1%, as calculated by the responses of 14 participants. As a result of the evaluation, a total of 476 responses are given by the Delphi participants, including 421 "agree", 46 "disagree", and nine "no comment" statements (Table 7). Since the participants are experienced and knowledgeable about the port business and have an opinion on the evaluated competencies, quite a few evaluations were made as "no comment". This situation caused the APMO value to be quite high.

A statement was deemed to have achieved consensus when it attained 70% or above, according to Brett & Roe (2010). Low consensus rates were defined as those between 70 and 79%, medium consensus rates as those between 80 and 89%, and high consensus rates as those between 90 and 100%. Therefore, 12 competencies are entered into the subsequent round. Their consensus rates are between 61.5% and 85.7%. Although 8 of these statements have a low and medium consensus rate, they entered round three because their consensus rates are lower than the high consensus rate (90%) and APMO rate (98.1%).

A total of 17 competencies have achieved the full consensus with 100%, four competencies have reached a high consensus with the 92.9%, and one competency has reached a high consensus with the 92.3%. Five competencies have a consensus rate lower than APMO but higher than 90%, so they have a high consensus rate. Hence, these five competencies haven't been included in the Delphi study's second round. The findings of the first round of the Delphi research are displayed in Table 8.

Table 7. Summary of Delphi's first round of results

Response	Values
Total Agreements	421
Total Disagreements	46
Total Answers	476
APMO Rate	98.1%
Competencies with Low Consensus Levels - (70–79%)	5
Competencies with Medium Consensus Levels - (80-89%)	3
Competencies with High Consensus Levels - (90-100%)	22
Competencies less than 70% Consensus	4
Competencies to be included in Delphi Round 2	12

The second round Delphi form was prepared using 12 competencies that were not agreed upon by the participants in the first round. The second round of Delphi received responses from 12 participants. For the second round of Delphi, the APMO cut-off percentage rate was found to be 96.5% (Table 9).

According to the result of the second round (see Table 10), four competencies have received a high consensus. Eight competencies could not reach a consensus in the second round. As a result, 26 of the 34 competencies reached consensus by the experts. These competencies were categorized as "technical & operational", "human & social", and "conceptual & cognitive" by the participants (see Table 11). Marketing management was perceived as both technical and conceptual competency.

$$\text{APMO} = \frac{\text{Majority Agreements} + \text{Majority Disagreements}}{\sum \text{Opinions Expressed}} \qquad 98.1 = \frac{421+46}{476} \tag{1}$$

$$\text{APMO} = \frac{\text{Majority Agreements} + \text{Majority Disagreements}}{\sum \text{Opinions Expressed}} \qquad 96.5 = \frac{103+36}{144} \tag{2}$$

Table 8. First-round results of the Delphi research

Managerial Competencies	Consensus	No Consensus*
Analytical thinking	100 % agreed	
Achievement and Result orientation		85.7 % (agree)14.3 % (disagree) ***(Second Round)***
Developing others/employees	100 % agreed	
Managing change	100 % agreed	
Effective communication	100 % agreed	
Reliability and integrity	100 % agreed	
Decision making	Agreed with 92.9 %	
Interpersonal relationship skills		85.7 % (agree) 14.3 % (disagree) ***(Second Round)***
Leadership	Agreed with 92.9 %	
Motivation	100 % agreed	
Customer orientation	100 % agreed	
Organizing	100 % agreed	
Self-development	100 % agreed	
Planning	Agreed with 92.9 %	
Problem identification and solving	100 % agreed	
Risk-taking and management	100 % agreed	
Strategic orientation and thinking	100 % agreed	
Teamwork and cooperation	100 % agreed	
Commercial awareness/concern	100 % agreed	
Expertise / Specialized knowledge	100 % agreed	
Having a vision	100 % agreed	
Managing time / Time management	Agreed with 92.9 %	
Port roles and functions		70.9 % (agree) 23.1 % (disagree) ***(Second Round)***
Financial management		69.2 % (agree) 30.8 % (disagree) ***(Second Round)***
Port tariffs and pricing		64.3 % (agree) 35.7 % (disagree) ***(Second Round)***
Marketing management		78.6 % (agree) 21.4 % (disagree) ***(Second Round)***
Port operations and operational planning		71.4 % (agree) 28.6 % (disagree) ***(Second Round)***
Development of port service quality		78.6 % (agree) 21.4 % (disagree) ***(Second Round)***
Port strategic planning	100 % agreed	
Management information system		61.5 % (agree) 38.5 % (disagree) ***(Second Round)***
Port performance and efficiency management		78.6 % (agree)21.4 % (disagree) ***Second Round)***
Understanding and using port information systems and technologies		58.3 % (agree) 41.7 % (disagree) ***(Second Round)***
Global industry analysis	Agreed with 92.3 %	
Competitive analysis / Competition Management		85.7 % (agree) 14.3 % (disagree) ***(Second Round)***

* Consensus rates that are lower than the high consensus rate (90%) and APMO rate (98.1%)

Table 9. Summary of Delphi's second round of results

Response	Values
Total Agreements	103
Total Disagreements	36
Total Answers	144
APMO Rate	96.5%
Competencies with Low Consensus Levels - (70–79%)	1
Competencies with Medium Consensus Levels - (80-89%)	1
Competencies with High Consensus Levels - (90-100%)	4
Competencies less than 70% Consensus	6

Discussion

The main aim of this study is to determine the managerial competencies critical for senior managers in container ports. For this purpose, a two-step study method was designed. In the first step of the study, interviews were conducted, and the majority of the competencies identified through the literature were assessed to be important (over 4.0 mean value). However, the competencies with the high mean value (over 4.5) were determined as the most important ones. Among the general managerial competencies presented to the expert opinion, "planning", "analytical thinking", "organizing", "problem identification and solving", "decision making", "effective communication", "customer orientation," and "strategic orientation" were the competencies considered to be of the utmost importance for senior port managers. "Port operations", "port financial management", "port strategic planning", "port service quality," and "port customer relations" are the sector-specific competencies that are perceived most important for senior managers in the interview part. However, some of these competencies haven't reached expert consensus in the second step of the study. The reason may be due to differing opinions between academic and sector experts. The competencies that are reached consensus in this study such as team working (Siu, 1998; Abraham et al., 2001; Qiao & Wang, 2009, Çetinkaya & Özutku, 2012), customer focus (Siu, 1998; Abraham et al., 2001, Çetinkaya & Özutku, 2012), communication skills (Siu, 1998; Abraham et al., 2001; Qiao && Wang, 2009), leadership skills (Siu, 1998; Abraham et al., 2001), problem-solving (Abraham et al., 2001), planning, decision making, commercial concern (Siu, 1998), developing employees and interpersonal relationship skill (Çetinkaya & Özutku, 2012) has some similarities with empirical studies from different sectors. In addition, some of the competencies determined in this study for senior-level managers are the same as those determined for operation managers concerning the sustainability performance of container ports by Tezcan & Kuleyin (2019b). These are decision-making, problem-solving, analytical thinking, and expertise. There are also some different findings with the literature. For instance, unlike the study of Reichel (1996), professional knowledge and problem-identification competencies were found essential for the port senior managers. Siu (1998), Abraham et al. (2001), and Çetinkaya & Özutku (2012) revealed that the result and success orientation is one of the critical competencies to managerial success. However, this competency has not reached a consensus in our study.

Table 10. Second-round results of the Delphi research

Managerial Competencies	Consensus	No Consensus*
Achievement and Result orientation		75.0 % (agree) 25.0 % (disagree)
Interpersonal relationship skills	Agreed with 91.7 %	
Port roles and functions		81.8 % (agree) 18.2% (disagree)
Financial management		63.6 % (agree)36.4 % (disagree)
Port tariffs and pricing		54.5 % (agree) 45.5 % (disagree)
Marketing management	Agreed with 90.9 %	
Port operations and operational planning		63.6 % (agree) 36.4 % (disagree)
Development of port service quality		66.7 % (agree) 33.3 % (disagree)
Management information system		66.7 % (agree) 33.3 % (disagree)
Port performance and efficiency management	Agreed with 91.7 %	
Understanding and using port information systems and technologies		50.0 % (agree) 50.0 % (disagree)
Competitive analysis / Competition Management	Agreed with 91.7 %	

Note: * Consensus rates that are lower than the high consensus rate (90%) and APMO rate (96.5)

Table 11. Categorization of managerial competencies by participants

	Technical & Operational	Human & Social	Conceptual & Cognitive
CONSENSUS	• Expertise / Specialized knowledge • Port performance and efficiency management • Planning • Marketing management	• Reliability and integrity • Interpersonal relationship skills • Teamwork and cooperation • Effective communication • Leadership • Motivation • Self-development • Developing others/employees • Organizing • Customer orientation and relations	• Global industry analysis • Competitive analysis / Competition Management • Analytical thinking • Strategic orientation and thinking • Commercial awareness/concern • Problem identification and solving • Managing change • Decision making • Risk-taking and management • Port strategic planning • Managing time / Time management • Having a vision • Marketing management
NON-CONSENSUS	• Port operations and operational planning • Understanding and using port information systems and technologies • Management information system • Development of port service quality • Port tariffs and pricing • Port roles and functions • Financial management		• Achievement and Result orientation

In the Delphi method, the majority of the competencies that have reached consensus are gathered under *conceptual* and *human* categories. All human-related competencies have reached a consensus. It shows the importance of these competencies for senior managers are supported by expert opinions for the port sector. Out of the conceptual competencies, only achievement and results orientation did not reach expert consensus. Experts' comments include that success or result-oriented managers are mostly not interested in the process, but they should focus on the whole process rather than just success and outcome. It is also mentioned that the understanding that "the ends justify the means" is not true. Although the technical competencies have been evaluated as very important in the interview findings of the study, few of the technical competencies have reached consensus by experts in the Delphi method. For most of the technical competencies, it is expressed that it is sufficient for senior managers to have a general idea, not technically and in detail. Department managers who are experts in the subject can fulfill their duties by having these competencies. For instance, expert opinions show that port operations and operational planning are too detailed for senior managers, they can be aware of important developments in the port field, but the senior manager does not need to have competence in field planning. These findings have similarities with the common understanding in the management literature and empirical studies. It is generally accepted in the management literature that as managerial levels increase, technical competencies would be given less importance than conceptual and interpersonal skills. Technical competency is regarded as being crucial for the organization's lower-level managers. Similarly, Labbaf et al. (1996) argued that people-related, analytical and self-related skills rate more critical than task-related skills for senior managers. Mbokazi et al. (2004) found out interpersonal competencies are more critical for senior-level managers, and first-line managers perceived operational competence as more critical.

Conclusion

In conclusion, this study demonstrated expert consensus on 26 competencies critical to strategic management in a dynamic and competitive environment for senior managers in container ports. The findings of this study can be taken into consideration by container port management in designing their human resource development policy. The identified competencies can provide useful insight to practitioners in the selection of new managers, designing promotion and career development programs, organizing training and development activities, and evaluating manager performance. The maritime training and education institutes or universities may benefit from the findings of this study to train and educate future manager candidates in the sector. In addition, container ports managers can consider these competencies when assessing their own competencies and development needs. This study has some limitations regarding research scope; first, the evaluation of competencies includes the opinions of experts who agreed to participate in the research in Türkiye. Second, the competencies were investigated for only senior-level managers. Also, the competency requirements have been evaluated only for container ports, and other port types are not included. For further research, the research scope can be expanded by including the middle and first-line managers, and a comparison can be made between them. Also, other studies can be conducted in other cargo-type ports such as liquid bulk, dry bulk, general cargo, passenger, and ro-ro. Similarities and differences in the competencies that are critical in other cargo-type ports can be revealed. In addition, as a comparative study, managerial competency requirements in public and private ports can be examined in future studies.

Acknowledgements

This paper was prepared within the scope of the doctoral thesis being carried out under the supervision of Asst. Prof. Dr. M. Serdar Ayan. The findings of interview part of the research were presented at 12th International Scientific Research Congress.

Compliance With Ethical Standards

Authors' Contributions

EBK: Manuscript design, Drafting, Writing
MSA: Manuscript design
Both authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

Ethical approval received for structured interview from Dokuz Eylül University, Social and Human Sciences Research and Publication Ethics Committee, 28.04.2021, E-57485104-300.99-49747. Ethical approval received for Delphi research from Dokuz Eylül University, Social and Human Sciences Research and Publication Ethics Committee, 24.11.202, E-57485104-300-144856.

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Mar. Sci. Tech. Bull. (2022) 11(4): 435-442
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1170665

Marine Science and Technology Bulletin

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https://prensip.gen.tr/

RESEARCH ARTICLE

A new maximum length for the grey triggerfish, *Balistes capriscus* Gmelin, 1789 for the Mediterranean Sea and first confirmed record in the Çanakkale Strait (Turkish Strait System)

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ARTICLE INFO

Article History:
Received: 07.09.2022
Received in revised form: 28.10.2022
Accepted: 30.10.2022
Available online: 22.12.2022

Keywords:
Maximum length
First record
Balistes capriscus
Grey triggerfish
Çanakkale Strait

ABSTRACT

A single specimen of the grey triggerfish, *Balistes capriscus* Gmelin, 1789, was caught by a speargun at 18 m water depth in the vicinity of the Çanakkale Strait in the Turkish Straits System, between the Aegean Sea and the Marmara Sea. Its total length was 57.8 cm and weight was 2270 g. The given size is the maximum observed length for the grey triggerfish, *B. capriscus,* in the Mediterranean Sea. In addition, this paper also documented the first confirmed record of the grey triggerfish, *B. capriscus* in the Çanakkale Strait (the Turkish Straits System).

Please cite this paper as follows:

Kale, S., & Tan, E. (2022). A new maximum length for the grey triggerfish, *Balistes capriscus* Gmelin, 1789 for the Mediterranean Sea and first confirmed record in the Çanakkale Strait (Turkish Strait System). *Marine Science and Technology Bulletin*, *11*(4), 435-442. https://doi.org/10.33714/masteb.1170665

Introduction

The grey triggerfish, *Balistes capriscus* Gmelin, 1789, distributes around reefs and mainly over rocky bottoms (Dance & Wells, 2018). The body form is laterally flattened to let manoeuvring in shallow rocky areas or wrecks that it inhabits (Reeds, 2008). *B. capriscus* is a carnivorous fish species and does not leave its habitats (Tortonese, 1986). The species is mainly a demersal fish that feeds predominantly on benthic invertebrates such as marine molluscs and crustaceans (Ofori-Danson, 1981). The species also feeds on zooplankton like amphipods and copepods (Aggrey-Fynn, 2007). Dance & Wells

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(2018) claimed that grey triggerfish was more dependent on the reef structure for foraging opportunities. The authors also indicated that *Balistes capriscus* mostly consumed pelagic gastropods and reef-associated prey. It is an oviparous species and the sexes are separate (Tourenne et al., 2020). The spawning time of the species was indicated as the period between April and June for the Mediterranean Sea by Akşıray (1987). Tourenne et al. (2020) documented that the triggerfish can change colour during reproduction, and the head turns white, the dark bands are more contrasted with the rest of the body which clears. The spatial distribution range of the species covers both sides of the Atlantic coasts from Nova Scotia to Argentina in the western parts of the Atlantic and from Norway to South Africa in the eastern parts of the Atlantic, the Mediterranean Sea (Figure 1). Aggrey-Fynn (2007) noted that *B. capriscus* has a very wide bathymetric distribution in Ghanaian coastal waters. It is assessed as "Vulnerable" and is not considered to be threatened by IUCN (Liu et al., 2015). The maximum reported total length (TL) of the grey triggerfish is 61.7 cm in the Gulf of Mexico (Jefferson et al., 2019).

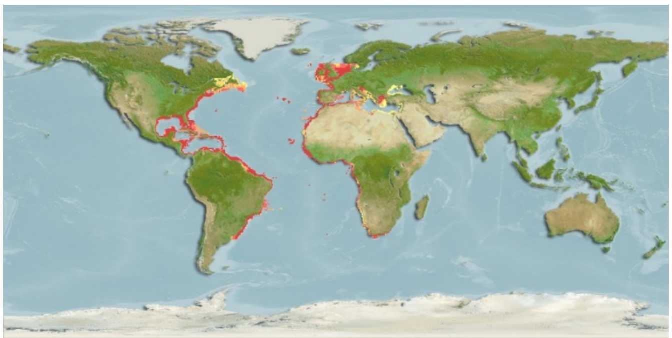

Figure 1. Distribution range of *Balistes capriscus* (Aquamaps, 2020) (Distribution range colours indicate degree of suitability of habitat which can be interpreted as probabilities of occurrence. Relative probabilities of occurrence ranges from high (red) to low (yellow))

The maximum length and maximum age are important theoretical parameters in fisheries science (Acarli et al., 2018). Dulčić & Soldo (2005) noted that the measurements of maximum length and maximum age have been commonly used directly or indirectly in most stock assessment models. Therefore, bringing information about maximum sizes up to date is important for fisheries-related sciences. The spatial distribution of the grey triggerfish B. capriscus was documented by several authors in the Greek exclusive economic zone of the Aegean Sea (Maldura, 1938; Konsuloff & Drenski, 1943; Kalogirou, 2009; Kalogirou et al., 2010, 2012). On the other hand, several authors previously reported the occurrence of the grey triggerfish in different locations of the Turkish continental shelf including the coasts of Gökçeada Island (Ulutürk, 1987), İskenderun Bay (İşmen et al., 2004), Edremit Bay (Meriç et al., 2007), Aegean Sea (Mater et al., 2009; Cerim et al., 2021), Saros Bay (Cengiz & Paruğ, 2020). However, there is no document reporting the occurrence of the species in the Çanakkale Strait or Turkish Straits System. The aim of this paper is to provide new data on the first record of the grey triggerfish in the Çanakkale Strait and the maximum observed length for the Mediterranean Sea.

Material and Methods

A specimen of the grey triggerfish, *B. capriscus*, was caught by a speargun off the coasts of Kumkale in the Çanakkale Strait (Figure 2). The specimen was captured on 6 October 2020 during the daytime at 18 m water depth. Fish species was classified according to Mater et al. (2009) by considering taxonomic determinations. The fish was stored under frozen conditions (at -18°C) and covered with ice for 5 hours to let the morphometric measurement successfully. The specimen was measured to the nearest millimetre and weighted to the nearest gram according to the manual for the measurement of fish proposed by FAO (Holden & Raitt, 1974). Unfortunately, the specimen could not be catalogued or deposited as the fisherman did not allow us to catalogue the specimen in the museum. The habitat structure was covered by reefs and sea grass. Figure 3 illustrates the habitat structure and the environment when the specimen has been caught by a speargun.

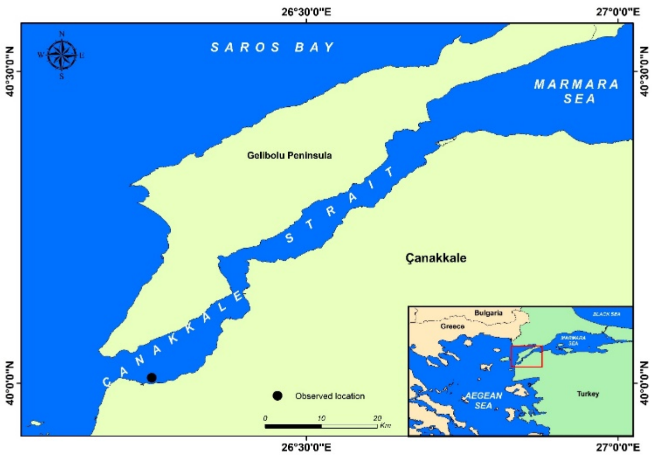

Figure 2. The observed location of the grey triggerfish *Balistes capriscus*

Results

This paper provides the first confirmed record of the grey triggerfish in the Çanakkale Strait. The total length of the specimen was measured as 57.8 cm (Figure 4) and the total weight (TW) was calculated as 2270 g. Morphometric measurements considered for the species were given in Table 1.

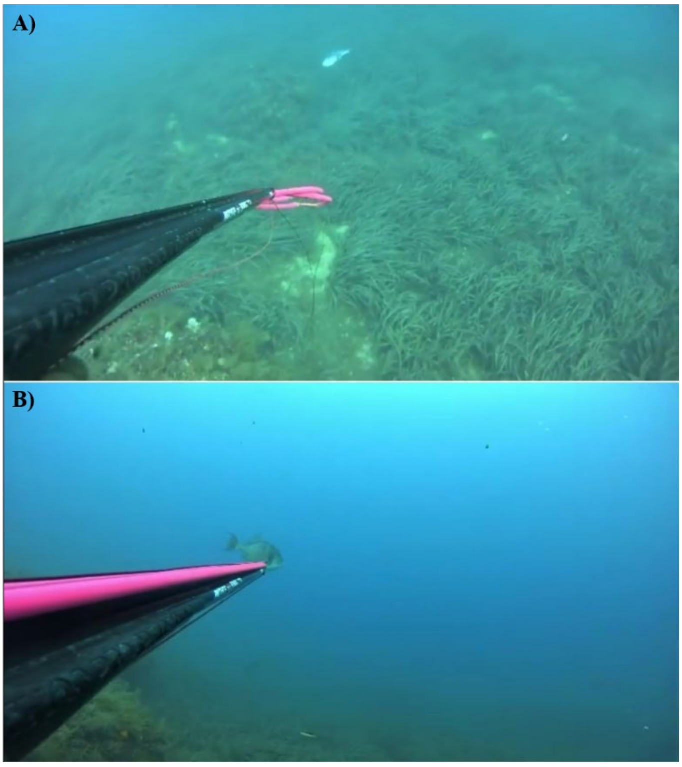

Figure 3. The habitat structure (A) and the environment (B) when the grey triggerfish *Balistes capriscus* caught by a speargun in the Çanakkale Strait

The total length measured in the present paper is the maximum length for the grey triggerfish in the Mediterranean Sea whereas the global maximum total length for the species was noted as 61.7 cm by Jefferson et al. (2019). The present paper provides recent data and significant contribution to our knowledge of the reported maximum size and the first record of *B. capriscus* in the Çanakkale Strait.

Figure 4. *Balistes capriscus*. The grey triggerfish (TL: 57.8 cm, TW: 2270 g) caught on 6 October 2020 in the Çanakkale Strait

Discussion

The maximum length of the species was previously reported as 61.7 cm (FL) by Jefferson et al. (2019) in the Gulf of Mexico. On the other hand, several maximum length reports have also been documented for different locations by several authors (Table 2). Harmelin-Vivien & Quéro (1990) noted that the maximum total length was 60.0 cm in the eastern tropical Atlantic coasts. Kelly-Stormer et al. (2017) documented that the maximum fork length was 57.8 cm for the Atlantic Coast of the South-eastern USA. In the Mediterranean Sea, Dulčić & Soldo (2005) reported the maximum total length as 52.50 cm for the Adriatic Sea. Kacem et al. (2015) reported the maximum fork length as 42.7 cm for Tunisian coasts. İşmen et al. (2004) noted that the maximum total length was 25.5 cm for İskenderun Bay. Cerim et al. (2021) recently documented the maximum total length as 53.5 cm in the Gökova Bay, southeastern Aegean Sea while Cengiz & Paruğ (2020) reported the occurrence of the species in the Saros Bay, northern Aegean Sea. The authors noted that the total length of the specimen was 36.4 cm. The present paper reports the maximum length not only for the Çanakkale Strait (Turkish Straits system, Aegean Sea) but also for the Mediterranean Sea. Moreover, this paper also documented the first confirmed record of the grey triggerfish, *B. capriscus* in the Çanakkale Strait.

Table 1. Morphometric measurements of *Balistes capriscus* caught on 6 October 2020 in the Çanakkale Strait

Morphometric Measurements	Values (cm)
Total length	57.8
Fork length	48.2
Standard length	42.6
Pre dorsal length	16.7
Pre anal length	26.8
Pre ventral length	20.2
First dorsal fin length	6.4
Second dorsal fin length	15.8
Anal fin length	14.1
Pectoral fin length	4.2
Girth	47.7
Head length	19.5
Ocular diameter	1.8
Pre orbital length	4.2
Post orbital length	1.4

Table 2. The comparison of the maximum lengths recorded in different areas for *Balistes capriscus* (TL refers to total length. FL refers to fork length)

Area	Author(s)	Length (cm)	Length type	Depth (m)	Fishing gear
Gulf of Mexico, Atlantic	Jefferson et al. (2019)	**61.7**	FL	20-40	Vertical longline, Hook-and-line, Trawl, Plankton purse seine, Pole-spear
Eastern Atlantic	Harmelin-Vivien & Quéro, (1990)	60.0	TL	UNK	UNK
Gulf of Mexico, Atlantic	Ingram (2001)	58.3	FL	21-32	Tagging trip
Southeastern USA, Atlantic	Kelly-Stormer et al. (2017)	57.8	FL	14-92	Chevron traps
Southeastern USA, Atlantic	Burton et al. (2015)	56.7	FL	UNK	Fishing line
Gökova Bay, Aegean Sea, Mediterranean	Cerim et al. (2021)	53.5	TL	15	Speargun
Adriatic Sea, Mediterranean	Dulčić & Soldo (2005)	52.5	TL	50	Bottom trawl
Western Gulf of Guinea, Atlantic	Aggrey-Fynn (2009)	52.0	TL	22-60	Bottom trawl, Pelagic trawl
South Carolina, Atlantic	Shervette et al. (2021)	52.0	FL	UNK	Conventional vertical hook-and-line gear
Gulf of Gabès, Mediterranean	Kacem et al. (2015)	42.7	FL	UNK	Pelagic trawl net
Southeastern Brazilian Coast, Atlantic	Bernardes (2002)	41.0	FL	UNK	Bottom trawl
Gulf of Mexico, Atlantic	Dance et al. (2018)	38.2	FL	13-32	Vertical longline, Trap
Saros Bay, Mediterranean	Cengiz & Paruğ (2020)	36.4	TL	30	Fishing line
Coasts of Ghana, Atlantic	Ofori-Danson (1981)	35.0	FL	UNK	Bottom trawl
İskenderun Bay, Mediterranean	İşmen et al. (2004)	25.5	TL	50	Bottom trawl
Çanakkale Strait, Mediterranean	**Present Paper**	**57.8**	**TL**	**18**	**Speargun**

Note: UNK indicates that there is no information on this data.

The observation of uncommon growth in total length could be associated with variations in morphologic characteristics (Bauer, 1961) or an outcome of hereditary factors (Borges, 2001). Fish populations exposed to high fishing pressure response to the fishing pressure by reproducing at smaller sizes and ages (Helfman et al., 2009). Individuals not exposed to high fishing pressure might extend the largest weight and length (Acarli et al., 2018). It seems that there is no potential high fishing pressure for this species in the Çanakkale Strait at the moment. Nevertheless, fisheries activities should be regulated to prevent the over-exploitation of the species and to ensure the fish stocks' sustainability. On the other hand, several factors may possibly affect the growth of the individuals such as environmental conditions (temperature, salinity, dissolved oxygen, light, pollutants, current speed, food availability) and biological competitions (pray-predator relationships, interspecific or intraspecific interactions, genetics) (Helfman et al., 2009).

As fisheries of some fish species have become exhausted and more closely controlled, previously untargeted species such as the *B. capriscus* have become more frequently harvested and progressively valuable (Kelly, 2014). Tourenne et al. (2020) pointed out that its workforce has grown significantly in 30 years and this was attributed to the warming of the waters. Increase in seawater temperature might have significant effects on the spatial distribution of fish species. Correspondingly, several researchers noted that climate change will affect the spatial and bathymetric distribution of fish species (Kale, 2019;

Kale & Acarli, 2019). Kacem et al. (2015) indicated that the spatial distribution of *B. capriscus* has expanded to the north over the last decade due to global warming and climate change. Cengiz & Paruğ (2020) reported the northernmost observation of the grey triggerfish in the Mediterranean Sea by pointing out the observation in Saros Bay. The present paper provides the recent further expansion of the grey triggerfish in the Mediterranean Sea. Çanakkale Strait has different environmental conditions (such as higher current speeds, two-layered current flows, maritime transportation activities, and human interventions) compared to Saros Bay. On the other hand, Saros Bay is closed to fishing activities by the local government, and therefore, it provides a protected area and lower fishing pressure on fish species. Therefore, the grey triggerfish may establish a new population in Saros Bay in the near future. On the other hand, the Çanakkale Strait is a part of the Turkish Straits System and a transition zone from the Mediterranean Sea to the Black Sea. Since the present paper has reported the first occurrence *of B. capriscus* in the Çanakkale Strait, the species may possibly migrate to the Black Sea through the straits system and it may also establish a new population there in the near future.

Kacem et al. (2015) reported that the catch amounts and commercial value of this species have increased due to its greatly appreciated flesh meat by local consumers in Tunisia. The same case might happen in the local fisheries in the Çanakkale Strait. Currently, *B. capriscus* is a non-targeted species for commercial fishing activities in Türkiye. However, it can be targeted if the catch amounts and commercial value increase. Therefore, although this paper documents the first occurrence of the species in the Çanakkale Strait, nevertheless, the population structure and dynamics of the species in the Çanakkale Strait should be continuously investigated in future studies. In addition, further investigations should be carried out to understand the prey-predator relationships between interspecies and intra-species in the Çanakkale Strait.

Citizen science and local ecological knowledge have demonstrated to be extremely important in discovering the occurrence and further distribution of rare or alien species in the Mediterranean Sea (Rizgalla et al., 2016; Crocetta et al., 2017; Rizgalla & Crocetta, 2020; Tiralongo et al., 2020). The present study appreciates the contributions of citizen science and local ecological knowledge in exploring the spatial distribution of rare species in the Çanakkale Strait.

Conclusion

This paper provides a new maximum length for the grey triggerfish, *Balistes capriscus* Gmelin, 1789, for the Mediterranean Sea from the Çanakkale Strait (Turkish Strait System, Aegean Sea). On the other hand, there is no paper reporting the occurrence of the species in the Turkish Straits System. Therefore, the present paper is also the first document that reports the first confirmed record of the grey triggerfish *B. capriscus* in the Çanakkale Strait.

Acknowledgements

We would like to thank the recreational spear fisherman Ozan Önen (Çanakkale, Türkiye) for bringing the specimen to our consideration, for having allowed using his underwater photographs, and for sharing his information.

Compliance With Ethical Standards

Authors' Contributions

SK: Manuscript design, Writing, Editing
ET: Manuscript design
Both authors read and approved the final manuscript.

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.

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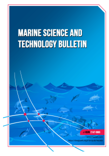
Mar. Sci. Tech. Bull. (2022) 11(4): 443-453
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1175251



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RESEARCH ARTICLE

Determination of shelf life during cold storage of fish fingers coated addition of goji berry

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ARTICLE INFO

Article History:
Received: 07.09.2022
Received in revised form: 28.10.2022
Accepted: 30.10.2022
Available online: 22.12.2022

Keywords:
Luciobarbus esocinus
Goji berry
Cold storage
Shelf life
Fish finger

ABSTRACT

The present study was aimed to evaluate shelf life of Pike Barbell (*Luciobarbus esocinus* Heckel, 1843) experimental samples of fish finger, that ordinarily packed and stored at 4±1°C. Together with the control group, three experimental groups fish finger were obtained with the addition of the goji berry extract into this content by 1% and 2%. According to the findings obtained in this study, it was concluded that fish fingers with goji berry addition had a positive effect on chemical, microbiological and sensory qualities and this study could also establish a basis for many scientific studies with the application of herbal extracts used in experimental samples on various fish products in more different concentration.

Please cite this paper as follows:

Gurel Inanli, A., & Muhammed Amin, B. L. (2022). Determination of shelf life during cold storage of fish fingers coated addition of goji berry. *Marine Science and Technology Bulletin*, *11*(4), 443-453. https://doi.org/10.33714/masteb.1175251

Introduction

Demand for seafood has consistently increased during recent years with fish protein being the major animal protein consumed in many parts of the world. To assure the quality of raw material used for processing, fish has to be treated carefully before and after harvest. Often fish and shellfish undergo some type of handling or primary processing (washing, gutting, filleting, shucking, etc.), before the main processing occurs, to assure their quality and safety, as well as to produce new, convenient and added-value products (e.g., packed fish fillets instead of unpacked, whole ungutted fish). Processing of seafood mainly inhibits and/or inactivates bacteria and enzymes which results in shelf-life extension and also assures food safety. While the main role of processing is preservation, processing not only extends shelf life but also creates a new range of products (Boziaris, 2014).

The literature on fishery fast foods is quite new. Concerns regarding the safety of synthetic food additives and the toxicity of such chemicals have lately been on a rise. The essential ingredients of coating materials largely consist of flour and water (Vareltzis et al., 1997).

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People are tended to produce ready-to-serve foods since women who do not have the opportunity to cook at home are tended to ready-to-serve foods with the increasing number of working women today, working people have short time for lunch; places such as schools, hospitals, cafeterias and restaurants want to serve in a short time. This service is carried out in a short while with developments in world food technology. Ready-to-serve foods are the products which are made of food by processing either directly or heated and consumed by themselves or with some nutrients, having a certain shelf life and on which favourable processing techniques and methods are applied (Gökoğlu, 1994).

It is known that microbial and chemical reactions lead to the quality deterioration of fish. Fish fingers are made from fish slices as battered and breaded foods. They are usually stored and marketed as frozen (Sarma et al., 2000).

Extracts are antiseptic, antioxidant, digestive stimulant antimicrobial and enzymatic effects as natural products used in food preservation. Plant extracts are a very large part of the GRAS list Goji berry (*Lycium barbarum*) or wolfberry in the world known as wolfberry but in our country, little-known Wolf is one of the highest nutritional value rape fruits. This is a very powerful antioxidant fruit has been used for 2000 years in the medical field in China. Goji berry high antioxidant activity of β-carotene and phenolic components have been found to have originated from. Other natural antioxidants present in Goji berries are tannin, lignin, and flavonoids (Lee et al., 2002; Kammerer et al., 2007).

In this study, it was aimed to determine the chemical, microbiological and sensory changes during the storage of Pike barbell (*Luciobarbus esocinus* Heckel, 1843) fish fingers coated addition of goji berry at 4°C and to investigate the shelf life of this products.

Material and Methods

Materials

In this study, Pike barbell (*Luciobarbus esocinus*) which are caught from Keban Dam Lake was used. Fresh fishes were brought to the laboratory in cold and fish slices were obtained and aseptic conditions.

Goji berry extract was obtained from a commercial company (Xi'a Xin Sheng Bio-chem Co., Ltd, China) to add to coverings prepared in the study. This extract used is for food purpose, and has ISO 22000, GMP, FDA and halal certificates. The study was carried out as two repeats and two parallel in the research.

Preparation of Experimental Samples

Fish slices prepared were kept on the fridge for 1 hour in brine of ratio as 1.5%. Liquid and solid coverings were prepared to cover the fish slices.

Briefly, egg white (75%), carbonate (3%), breadcrumbs (15%), wheat starch (5%), salt (1%) and sugar (1%) were prepared by mixing for liquid coating. Additionally, onion powder (2%), garlic powder (2%), red pepper (1%) crumbs bread (40%), corn flour (30%) and wheat flour (25%) were mixed as solid covering.

Firstly, salted fish slices were covered with liquid, and then covered with simple solid covering (control), solid covering with additional 1% goji berry extract (1% GB) and solid covering with an additional 2% goji berry extract (2% GB). Thus, were created 3 groups.

All of the fish fingers were fried for 1 min in oil at 180 C°. In this way the samples would be pre-cooked. After that each treatment was packaged in styrofoam packages and was stored at +4°C before the analyses on 0^{th}, 3^{rd}, 6^{th}, 9^{th}, 12^{nd}, 15^{th} and 18^{th}days.

The analyzes would be carried out in three stages, before fish processing, after processing and under storage conditions. In order to determine the shelf life of experimental fish finger, chemical, microbiological and sensory analyzes were performed with 3 replications every three days. Nutritional composition was determined before processing fish meat and after making fish finger. In addition, in the treatments, pH, Thiobarbituric Acid Numbers (TBARs), Total Volatile Basic Nitrogen (TVB-N) in certain days of the storage and sensory analyzes were performed.

Analytical Methods

Proximate composition

The moisture content of experimental samples was measured according to the official method 950.46. Crude protein of samples was determined according to the micro Kjeldahl method (method 928.08). Soxhlet method was used (method 960.39) to determine lipid content. Ash was determined according to method (920.153). The pH values of samples were measured with a pH meter (Thermo Scientific Orion 3-Star). The sample was homogenized in distilled water in the ratio 1: of 10 (wt/vol), and the measurement was done with a pH meter (AOAC, 2002a, 2002b, 2002c, 2002d, 2002e).

Total volatile basic nitrogen

The TVB-N content was determined according to the method stated by Antonacopoulos (1973). Volatile bases were kept in the H_3BO_3 solution of 3% with the distillation after that MgO was minced to the sample. The distillate was collected in a flask containing a 3% (w/v) aqueous solution of boric acid and a mixed indicator produced by dissolving 0.1g of methyl red and 0.05g of methylene blue to 100ml of ethanol. The boric acid solution turned green when the distilled TVB-N made it alkaline. Finally, the boric acid solution was titrated with a 0.01 mol/L HCl solution until it turned pink. The quantity of TVB-N in mg/100 g sample was then calculated.

Determination of thiobarbituric acid number

The method stated by Tarladgis et al. (1960) was applied. Absorbance of red colour at 538 nm given by malondialdehydes formed by oil oxidation, with thiobarbitruic acid at glacial acetic acid environment was read on spectrophotometer. Malondialdehyde value was calculated by multiplying the read absorbance value with 7.8 factor.

Microbiological analyses

For microbiological analyzes of samples were weighed as 10 g in the special bag, and it was homogenized in the stomacher (Stomacher 400) for 60 s by adding 90 ml from the sterile 0.1% peptoned water on it for the microbiological analyses. Thereby, dilution of samples of 10^{-1} (1/10) were prepared. Other decimal dilutions were made by means of using the same diluting agent from this dilution e.g., up to 10^{-6} (Harrigan 1998).

Plate Count Agar (PCA Merck1.05463) medium was used for total viable counts (TVC) and psychrotrophic bacterial counts (PTC) in samples. Colonies consisted after cultured plates were incubated for 72 hours at 30±1 C° were counted TVC. PTC were evaluated after incubated for 7 days at ±5 C°. Plates using Potato Dextrose Agar (PDA Merck1.10130) medium, of which pH was reduced to 3.5 by adding 10% tartaric acid (Merck1.00802) for the count of yeast-mold (YMC) in the samples, was evaluated after 5 days incubation at 21±1°C (ICMSF 1986; Harrigan 1998).

Sensory evaluation

Sensory characteristics of the fish fingers were evaluated by 10 panelists in terms color, appearance, odor, flavor, texture and overall acceptability. Scores were measured as "like extremely" = 5 to "dislike extremely" = 1, and tests were conducted storage days of 0th, 3rd, 6th, 9th, 12nd and 15th (Kurtcan & Gönül, 1987).

Statistical analyses

In this study, results during the production of samples fish finger which were prepared experimentally were reported statistical analysis. The statistical analysis was run using SPSS® version 22.0 software computer package statistical program (SPSS, Chicago, IL, USA). Kruskal-Wallis test was applied to determine whether there is a meaning difference between the groups or not, and the difference between the groups was determined with Duncan test.

Result and Discussion

Quality of Raw Fish

Proximate composition, chemical and microbiological quality of *Luciobarbus esocinus* used in the study is presented in Table 1.

Table 1. The properties of fish meat used in the study

Parameters	Values
Moisture (%)	70.35±1.85
Crude Protein (%)	15.66±0.83
Crude Fat (%)	11.68±1.66
Ash (%)	0.93±0.17
pH	6.26±0.35
TVB-N (mg/100 g)	7.59±0.58
TBA (mg MDA/kg)	0.90 ±0.22
TVC (log cfu/g)	1.88±0.01
PTC (log cfu/g)	2.69±0.30
YMC (log cfu/g)	1.87±0.18

Proximate Composition

The results of proximate composition analysis belonging to simple fish fingers (control) and fish finger samples with the addition of goji berry extract 1.0% and 2.0% are determined in this study.

As a result of the analyses, 63.80% moisture, 15.74% protein, 14.82% fat and 3.01% ash were calculated in control. In other samples with the addition of 1% goji berry extract, 61.96% moisture, 16.39% protein, 15.73% fat, 3.42% ash were determined in the samples of 1%GB, 61.99% moisture, 16.76% protein, 16.32 % fat and 2.92% ash were found in the samples of 2% GB.

When the data obtained as results of the study were analyzed statistically, this difference between the groups was found significant in terms of moisture, protein, fat and ash amounts determined in control fish finger samples and the samples prepared with the addition of extract ($p<0.05$).

Tokur et al. (2006) respectively found the water, protein, fat and ash amounts in carp sticks as follows: 68.50%, 15.50%, 6.00% and 2.20%. In their study, Gülyavuz & Timur (1991) obtained very similar amounts as a result of their investigation on the nutrient components of carp: 15.92% protein, 9.50% fat, 1.13% ash and 73.02% moisture.

In a different study, water, protein, fat, ash and carbohydrate amounts were investigated in sardine sticks (52.04%, 14.39%, 16.16%, 2.61% and 14.80%), in the whiting sticks (63.01%, 6.71%, 15.98%, 3.33% and 10.97%) and the sander sticks (69.73%, 4.28%, 15.75%, 2.75% and 7.49%) (Çaklı et al., 2005). In Yanar & Fenercioglu's (1999) studies, the amounts of 16.67% crude protein, 8.45% lipid, 73.04% moisture and 1.18% ash were found in carp meat, and the amounts of 15.34 crude protein, 6.98 lipid, 75.89 moisture and 1.09 crude ash were discovered in minced meat as percentage. In different study fish fingers were produced from *Carassius gibelio* and evaluated through nutritional parameters. The moisture, crude fat, crude protein and crude ash contents of fish fingers were determined as 56.543 ± 0.113, 10.507 ± 0.116, 15.577 ± 0.382 and 2.027 ± 0.133, respectively (Izci, 2010).

Chemical Changes

The chemical changes of samples fish fingers prepared during storage are displayed in Table 2.

Table 2. Physico/chemical deterioration of samples fish fingers

Parameters	Groups	Storage Days						
		0	**3**	**6**	**9**	**12**	**15**	**18**
pH	**Control**	6.19±0.06^{aA}	6.13±0.01^{aA}	6.47±0.12^{aA}	5.93±0.42^{aA}	5.13±0.18^{bB}	**N.A.**	**N.A**
	1%GB	6.09±0.11^{aA}	6.26±0.03^{aA}	6.35±0.34^{aA}	6.31±0.22^{aA}	6.30±0.23^{aA}	5.86±0.36^{aA}	5.82±0.05^{aA}
	2%GB	6.24±0.04^{abA}	6.54±0.62^{aA}	5.89±0.35^{abA}	6.48±0.35^{aA}	6.46±0.10^{abA}	5.92±0.09^{abA}	5.62±0.05^{bA}
TVB-N mg/100g	**Control**	8.11±0.13^{dA}	12.92±1.19^{cA}	21.26±0.74^{bA}	24.01±2.24^{bA}	29.19±1.32^{aA}	**N.A.**	**N.A.**
	1%GB	5.99±1.40^{dAB}	6.04±1.12^{dB}	8.99±0.78^{dB}	14.02±1.22^{cB}	18.37±1.59^{bB}	22.49±1.84^{aA}	26.06±2.76^{aA}
	2%GB	3.76±0.51^{dB}	4.16±0.23^{dB}	5.78±0.59^{dC}	9.75±0.46^{cB}	13.28±2.80^{bB}	15.94±0.5^{abB}	16.94±0.54^{aB}
TBARs mgMDA/kg	**Control**	1.26±0.08^{dAB}	2.80±0.12^{cA}	3.14±0.11^{cA}	4.31±0.15^{bA}	5.45±0.64^{aA}	**N.A.**	**N.A.**
	1%GB	1.58±0.08^{cA}	2.31±0.33^{bA}	2.53±0.17^{bB}	3.74±0.12^{aA}	3.76±0.34^{aB}	4.12±0.15^{aA}	4.21±0.16^{aA}
	2%GB	1.09±0.16^{cB}	2.07±0.35^{bA}	2.05±0.10^{bC}	2.35±0.25^{bB}	2.87±0.17^{aB}	3.22±0.04^{aB}	3.14±0.11^{aB}

Note: $^{a, b, c}$ shows the statistical differences between the storage days of the same group. $^{A, B, C}$ indicate the statistical differences between the groups in the same storage period.

Table 3. Microbiological growth in samples of fish fingers log cfu/g.

Parameters	Groups	Storage Days						
		0	**3**	**6**	**9**	**12**	**15**	**18**
TVC	**Control**	2.10±0.06^{aA}	3.16±0.51^{bA}	5.47±0.34^{cA}	5.94±0.43^{cA}	6.40±0.62^{cA}	**N.A.**	**N.A.**
	1%GB	2.09±0.15^{aA}	2.74±0.57^{bA}	3.56±0.47^{cB}	4.27±0.14^{dB}	4.88±0.85^{dAB}	5.85±0.14^{eA}	6.71±0.12^{fA}
	2%GB	2.26±0.02^{aA}	2.45±0.03^{bA}	2.80±0.14^{cB}	3.30±0.42^{cB}	4.27±0.26^{dB}	4.50±0.58^{dA}	4.19±0.15^{dB}
PTC	**Control**	2.13±0.18^{aA}	3.53±0.14^{bA}	6.38±0.60^{cA}	6.27±0.11^{cA}	6.60±0.49^{cA}	**N.A.**	**N.A.**
	1%GB	2.83±0.57^{aA}	2.73±0.67^{aA}	4.33±0.21^{bB}	4.83±0.66^{bAB}	5.59±0.09^{cB}	6.41±0.05^{dA}	8.07±0.16^{eA}
	2%GB	3.16±0.16^{aA}	3.12±0.07^{aA}	3.34±0.19^{aB}	3.59±0.80^{aB}	4.59±0.17^{bC}	4.94±0.66^{bB}	5.64±1.19^{bB}
YMC	**Control**	2.54±0.54^{aA}	3.05±0.14^{aA}	3.39±0.12^{bA}	3.89±0.18^{cA}	5.51±0.33^{dA}	**N.A.**	**N.A.**
	1%GB	2.35±0.21^{aA}	2.64±0.48^{bA}	2.69±0.41^{bAB}	3.01±0.28^{cAB}	3.57±0.60^{cB}	3.92±0.78^{cA}	6.28±0.27^{dA}
	2%GB	1.68±0.13^{aA}	2.67±0.32^{bA}	1.77±0.26^{aB}	2.18±0.31^{cC}	3.33±0.16^{dB}	3.58±0.11^{eA}	4.58±0.59^{fB}

Note: $^{a, b, c}$ shows the statistical differences between the storage days of the same group. $^{A, B, C}$ indicate the statistical differences between the groups in the same storage period.

pH Values

The changes in pH give information about quality of fish products. The average pH of freshness fish muscle is 7.0 (Kyrana et al., 1997). However, postmortem pH can vary from 6.0 to 7.1 depending on some factors such as species, season, and gender (Church, 1998; Simeonidou et al., 1998).

pH values of the control fish finger and the samples with the addition of goji berry extract in certain proportions were determined during the storage at 4°C. These determined values are presented in Table 1.

When the data obtained as a result of the study were analyzed statistically, it was found that the difference of the pH values between the groups end of storage was significant in the simple samples and the samples with the addition of extract ($p<0.05$).

It was reported that pH value increased from 6.25 to 6.48 after 6 days of storage in Sardine meatballs (Kılınç et al., 2008). Similarly, the pH value of anchovy meatballs increased from 6.33 to 6.56 after 10 days of storage. In another study, it was reported that the pH value of anchovy meatballs increased throughout the storage (Turhan et al., 2001). In general, the pH value of fresh fish is stated as 6.0-6.5, consumability limit value as 6.8-7 (Gülyavuz & Ünlüsayın, 1999). Öksüztepe et al. (2010) expressed researchers that the value was found between 6.12 and 6.49 in meatballs of rainbow trout stored at 4±1°C when the effect of sodium lactate addition on the meatballs of Rainbow trout (*Oncorhynchus mykiss* W.) was investigated. In a study conducted by Varlık et al. (2000) in order to determine the shelf life of the marinated fish meatball, the initial pH value of the meatball marinades stored in cold was 4.19, that is, it firstly increased. Then, it decreased depending on the storage duration. In the study on making meatballs from remaining fillets of sander and tench, the pH values of sander and tench meatballs at +4°C were initially determined as 6.16±0.02 and 6.93±0.42, respectively. During the study, the values first decreased and then increased; they were then discovered as 9.12±0.88 and 6.78±0.09 on the 14th day of the study (Ünlüsayın et al., 2002). The pH value of 6.279 was determined in fish fingers obtained from *Carassius gibelio* by Izci (2010). At the end of the storage, the pH values in all the meatball groups were observed between 4.92±0.00-5.56±0.01. In the study investigating the effect of sodium lactate and thymol on meatballs made from mirrored carp, the pH value which was 6.69 on the 0th day dropped to 6.58 on the 6th day. Again, in the other groups with sodium lactate and thymol addition, pH which was initially in the range of 6.58-6.70 decreased in general during the storage (Erol & Ilhak, 2015). The pH values obtained in our study show similarities and differences with the findings in the aforementioned studies. The reasons behind the differences can be attributed to the applied solution proportions, fish species, storage temperature and the extracts used.

TVB-N value

The TVB-N value is used as an indicator of spoilage in aquaculture products. This value increases as a result of the activities of endogenous enzymes and bacteria causing spoilage (Kyrana et al., 1997). In other words, Huss (1995) reports the amount of TVB-N contained in the newly caught fresh fish as 5-20 mg/100g and the acceptable freshness limit value as 30-40 mg/100g. The concentration of TVB-N in freshly caught fish is typically between 5 and 20 mg TVB-N/100 g flesh, whereas levels of 30–35 mg/100 g flesh are generally regarded as the limit of acceptability for iced stored cold-water fish (Connell, 1995).

The high initial content of TVB-N may be attributed to the high level of NPN present in the flesh of gilthead sea bream. Breakdown of low molecular weight nitrogenous compounds occurs under the conditions of analysis, releasing volatile base nitrogen (Perez-Villarreal & Howgate, 1987; Whittle et al., 1990).

When the data (Table 1) obtained in this research were analyzed statistically, in generally the difference between the simple samples and the samples with the addition of goji berry in terms of the TVB-N amount during the storage was found to be significant ($p<0.05$). In addition, the difference between the days of analysis in terms of the amount of TVB-N determined during the storage of the samples was also significant ($p<0.05$).

When the findings were examined, the mean TVB-N value of the control fish finger was discovered as 8.11±0.13mg/100g on the first day. In other fish finger samples prepared with the addition of goji berry, the mean TVB-N value was found as 5.99±1.40 for group 1% GB and 3.76±0.50 mg/100g for group 2% GB. These values increased during the storage time of the samples.

In a study, the TVB-N value, which was found as 11.0 in the 6th month of the storage of the sander meatballs at -18°C, was obtained as 12.0 in fish meatballs with rice (Yanar & Fenercioglu, 1999). In the study conducted on investigating some quality parameters of meatballs made from raw and boiled fish meat, the TVB-N value of meatball samples made from raw anchovy increased depending on the storage duration. 39.33 mg/100 g was obtained on the 18th day of the storage in the 1st experiment, and 36.03 mg/100 g was discovered on the 15th day of the storage in the 2nd experiment, which means both values exceeded the limit (Akkuş et al., 2004). In the studies executed on making meatballs from remaining Sander and tench fillets, Ünlüsayın et al. (2002) initially found the TVB-N values in sander and tench meatballs as 11.4±1.10 mg/100 g and 11.2±1.14 mg/100 g at +4±1°C, respectively. On the 14th day of the study, 39.6±1.90 mg/100 g and 36.2±1.57 mg/100 g were obtained, respectively. In the study of Varlık et al. (2000), the TVB-N value was initially determined as 10.70 mg/100g in marinated meatballs, which is a sign of fish spoilage, and it was 10.45 mg/100g at the end of the storage. In Erol & Ilhak's (2015) study, the TVB-N value was discovered as 16.27 on the 0th day and 22.87 at the end of the storage in the control group. Again, in the meatball groups with different contents, this value was determined as 15.83-17.83 on the first day and 20.28-23.08 on the last day of the storage. In the study of Öksüztepe et al. (2010), the TVB-N value increased from 8.16 mg/100g to 22.56 mg/100g in the control group. In the other meatball groups, it changed as 7.13-23.65 mg/100g. When the TVB-N values are examined during the storage in our study, there is an increase in all the groups depending on time, and the TVB-N amounts can be said to increase later in meatball groups with extract addition compared to group A. The increases in the TVB-N value were determined to be significant as the storage time passed ($p<0.05$), and the change in the TVB-N values between the groups was found statistically significant ($p<0.01$). When all the groups were compared in terms of TVB-N values during storage days, significant differences were discovered ($p<0.05$). Izci et al. (2011) determined total volatile basic nitrogen (TVB-N) as 6.737±0.012, 19.583±0.087 mg/100 g in fish fingers during frozen storage in their study. Emir Coban (2013) determined the effect of ginger oil (0.5% and 1%) on chemical and sensorial quality and production of fingers from *Sarda sarda*. In this study the amount of TVB-N in fish finger 7.76 mg/100 g was determined. When compared to different studies, these values obtained depending on the storage are similar.

Thiobarbituric Acid (TBARs) Value

When the findings (Table 1) were examined, they were found to increase during the storage. When the data obtained as a result of the study were analyzed statistically, it was found that the difference in the TBARs amounts of the control samples and the samples with extract addition between the groups and days during the storage was significant ($p<0.05$).

The amount of TBARs is an indicator of lipid oxidation and it is another method used to determine the freshness of fish and fish products. The amount of thiobarbituric acid (TBA) may vary depending on fish species, hunting places and methods, storage conditions. The TBA value, which emerges as a result of fat oxidation in aquaculture products and is the index of bitterness, is considered as "good quality" between 1−3 mg MDA/kg, "medium quality" between 3−5 mg MDA/kg, "low quality" between 5–8 mg MDA/kg, and the products exceeding 8 mg MDA/kg is classified as "inconsumable" (Sinnhuber & Yu, 1958; Varlık et al., 2007). In the study conducted on the making fish meatballs from carp meat, the TBA values of the product ranged from 0.8 to 2.2 (mg MDA/kg) at -18±1°C during the storage (Yanar & Fenercioğlu, 1999). In the study conducted on making meatballs from remaining fillets of sander and tench fillets, the TBA values of sander and tench meatballs were initially determined at 4±1°C as 1.71±0.87 mg MDA/kg and 1.41±0.6 mg MDA/kg, respectively. On the 14th day of the study, 9.12±0.88 mg MDA/kg and 8.56±1.23 mg MDA/kg were obtained respectively (Ünlüsayın et al., 2002). Izci et al. (2011) determined total volatile basic nitrogen (TBA) as 0.293±0.013 mgMDA/g in fish fingers during frozen storage in their study. In another study, Can (2012) found the value as 0.79 mg MDA/1000g on the first day and 4.8 mg/100g on the last day in the control group within the storage days, but it was determined as 0.74-0.79 mg/kg on the first day and 1.74 mg MDA/kg-1.79 mg MDA/kg on the last day of the storage in other meatball samples. In our study, increases in TBARs values were determined in all the groups as the storage time passed ($p<0.05$) and significant differences were also observed in all the groups when they were compared within the days of storage ($p<0.05$). In different study was examined the effect of ginger oil (0.5% and 1%) and value TBA in fish finger was found as 0.69 mg MDA/kg (Emir Coban, 2013). In our study, the TBARs values determined during the storage were assessed and found similar to the aforementioned studies. In another study, the value was 0.08 during the storage in the control group, but it varied between 0.06- 0.10 in other carp meatball groups (Erol & Ilhak, 2015). In addition, the TBARs number determined in our study during preservation were lower in goji berry extract groups than in other groups. Its reason can be expressed as the antioxidant effect caused by the components in the extract used. The reason behind the similarities and differences in our findings can be attributed to the applied solutions and their proportions, fish species, storage temperature and the extracts used.

Microbiological Changes

In general, the muscle of a newly caught healthy fish is sterile. Microorganisms are normally found in the skin, gills and intestines of the fish. Depending on the operations, temperature and duration applied after the fish are caught, microorganisms can move to muscle from gills, skin and intestines. As a result, the quality of the product deteriorates depending on the type of the microorganism, so consumers may be exposed to infection or toxicity. Therefore, the number and type of microorganisms in the muscle of the fish are important for health and storage (Gram & Huss, 1996, 2000).

Total viable count (TVC), psychrotrophic bacterial count (PTC), and yeast-mold count (YMC) of the control fish finger and the samples with the addition of goji berry extract in certain proportions were determined during the storage at 4°C. These determined values are presented in Table 3.

Total Viable Count (TVC)

The activity of microorganisms is one of the main factors causing fish spoilage. The total viable counts (TVC) as a traditional and helpful indicator are used to assess the freshness of different kinds of aquatic products. Meanwhile, most of countries have established standards, guidelines, and specifications of fish freshness evaluation based on TVC index with diverse storage conditions of temperature, time, and atmosphere. This indicator is useful for accurate detection of the degree of fish freshness and for predicting the remaining shelf life of fish (Cheng et al., 2015). TVC are taken the 7 log cfu/g as an upper acceptable level for precooked breaded fish (ICMSF, 1986).

In the study TVC of group 2% GB were enumerated as 2.26±0.02 log cfu/g on the first day of the storage, it reached 4.19±0.15 log cfu/g on the 18th day.

An increase was observed in the TVC depending on spoilage throughout the storage. When the data obtained as a result of the study were analyzed statistically, it was determined that the intergroup difference between all the experimental groups in terms of aerobic mesophilic bacteria counts was statistically significant ($p<0.05$).

TVC was determined in our study to exceed the acceptable limit on the 12th day in group control and on the 15th day in groups 1%GB and 2%GB, which were stored at 4°C. In the study conducted on making meatballs from remaining pike perch and tench fillets, TVC of pike perch and tench meatballs were initially determined as 4.0×10^4 cfu/g and 4.2×10^4 cfu/g at +4±1°C, respectively; afterwards, they increased and appeared respectively as 1.2×10^7 cfu/g and 1.4×10^7 cfu/g on the 14th day of the study (Ünlüsayın et al., 2002). Erol & Ilhak (2015) discovered in a study on meatballs that the TVC generally increased during the storage. In the study conducted on the shelf life of anchovy meatballs stored in cold, TVC increased by the storage duration and was determined as 4 log cfu/g (1.0×10^4 cfu/g) at the beginning of storage, reaching 7.76 log cfu/g (5.8×10^7 cfu/g) on the 10th day of the storage (Turhan et al., 2001). In the study investigating some quality parameters of the meatballs made from raw and boiled fish meat, TVC of the meatballs made from raw anchovy increased depending on the storage duration and it was found as 4.8±0.007 log cfu/g in the 1st experiment and 4.6±0.021 log cfu/g in the 2nd experiment at the beginning of the storage, exceeding the limit value by reaching 7.4±0.014 log cfu/g in the 1st experiment and 7.3±0.002 log cfu/g in the 2nd experiment on the 12th day of the storage (Akkuş et al., 2004). In the study where Öksüztepe et al. (2010) investigated the effect on the fresh rainbow trout (*Oncorhynchus mykiss* W.) meatballs prepared with sodium lactate addition, the following levels were reached: 8.83 log cfu/g on the 8th day of the storage in the control group, 8.79 log cfu/g on the 10th day in group A, 8.90 log cfu/g on the 12th day in group B and 8.80 log cfu/g on the 16th day in group C. It can be expressed that these findings are in parallel with our findings. In addition, when we look at the total viable counts determined in our study, it can be seen that herbal extracts have antibacterial effect. When the findings are compared, we can attribute the reason behind some similarities and differences to the differences in the applied solutions and their proportions, fish species, storage temperature and the extracts used.

Psychrotrophic Bacterial Count (PTC)

PTC are the most important group of microorganisms responsible for aerobic spoilage of fresh fish stored at low temperatures (Sallam, 2007). In our study, the counts of psychrophilic bacteria increased in all the groups during the storage depending on time. When the data of psychrotrophic bacterial count obtained as a result of the study were analyzed statistically, the difference between the groups in terms of PTC was not significant in control samples and samples with goji berry addition ($p>0.05$). In addition, the differences between PTC within days during the storage were found significant ($p<0.05$).

In a study conducted on the suitability of tench (*Tinca tinca* L., 1758) for making meatballs and the changes in the nutritional properties after processing, the total PTC load values ranged between 3.99±0.11 log cfu/g and 6.91±0.014 cfu/g at +4°C. The PTC increased time-dependently. All the samples remained below the limit value (Çapkın, 2008). In the study investigating some quality parameters of the meatballs made from raw and boiled fish meat, the PTC of the meatballs made from raw anchovy increased depending on the storage duration; it was found as 4.0±0.001 log cfu/g in the 1st experiment and 4.5±0.004 log cfu/g in the 2nd experiment at the beginning of the storage, exceeding the limit value by reaching 7.7±0.002 log cfu/g in the 1st experiment and 7.8±0.014 log cfu/g in the 2nd experiment on the 12th day of the storage (Akkuş et al., 2004). Erol & Ilhak (2015) discovered in a study on meatballs that PTC generally increased during the storage. On the 6th day of the storage, the PTC increased above 7 log cfu/g in the control group and groups containing 0.1% thymol and 1% sodium lactate + 0.1% thymol. In the group containing 1% sodium lactate + 0.25% thymol, almost 7 log cfu/g was reached on the 8th day, 7 log cfu/g was exceeded on the 10th day of the storage in the group containing 1% sodium lactate. Similar results were also obtained in this study. It can be stated that the antibacterial effect of goji berry extract used in our study influenced the results.

Yeast & Mold Count (YMC)

YMC are not found in normal flora in fish. They are generally of earth-origin and transmitted from water as soon as they are caught or tools and materials used after catching the fish. In our study YMC of the control fish finger and the samples with goji berry addition determined by incubation at 21°C. The YMC at the end of the storage were determined in samples stored at 4°C as 6.47±0.37 log cfu/g in group control, 4.96±0.69 log cfu/g in group 1%GB and 4.58±0.59 log cfu/g in group 2% GB. When the data obtained as a result of the study were analyzed statistically, the difference between the groups in terms of yeast-mold count during the storage was found to be significant in the control samples and samples with extract addition ($p<0.05$). Erol & Ilhak (2015) discovered in a study on meatballs prepared from mirrored carp that the yeast-mold counts had increases during the storage. Only in the group containing 1% sodium lactate + 0.25% thymol, the yeast-mold count on the 4th day of the storage was slightly lower than the 0th day. In another study, the YMC of fish meatballs obtained from Rainbow trout was determined as 1.56 log cfu/g in the fillet. This number increased in all the groups, including the control, due to the progress of the storage and it was observed to be at the same levels in the other groups except the group C with 2% sodium lactate addition (Öksüztepe et al., 2010). In terms of the YMC, the effect of storage duration on all groups was found to be statistically significant ($p<0.05$). In our study, when the change in YMC during the storage was examined considering the antimicrobial properties of goji berry, the difference in group B and C with extract addition is seen compared to the control group.

Sensory Evaluation

During the sensory evaluation of the control fish finger samples and samples prepared with the addition of goji berry extract in different proportions, the appreciation of the samples' the color, smell, appearance, taste and texture were evaluated by 10 panelists using the hedonic scale. Throughout the storage, the panelists evaluated the control (without the addition of goji berry extract) fish finger samples and the samples with the addition of goji berry extract, which were made ready for consumption, according to their color, smell, appearance, taste and texture. The data obtained as a result of the panelists scoring were evaluated and the change in the general appreciation levels of the samples during the storage were presented. The sample of group A lost its consumability on the 12th day of the storage and the samples of group B and C with fish finger samples containing extract on the 18th day; samples were not included in the analysis.

Overall Acceptability

Overall acceptability of group control, 1% GB and 2% GB in term of storage days (0th, 3rd, 6th, 9th, 12nd and 15th) are given in Figure 1.

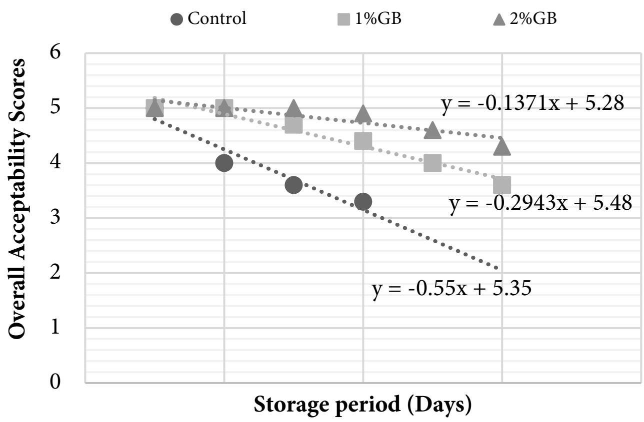

Figure 1. Regression of storage time equation for total acceptability of fish finger samples. Linear regression plot with time

It was found that the effect of groups and duration on the overall acceptability level of the samples' during storage was significant ($p<0.05$).

In terms of the overall acceptability scores in the evaluation between the groups, there was no statistically significant difference between the groups on the 0th day ($p>0.05$) while the difference between the control group and the groups with coating was significant during storage ($p<0.05$).

When the data obtained as a result of the study were analyzed statistically, it was found that the difference between the groups in terms of the general appreciation level of the control group A and group C sample from samples (group B and C) containing goji berry extract was significant ($p<0.05$).

The sensory analysis used when purchasing any food product is the first method, we apply to determine the freshness of the product. It is widely used in determining the quality of foods because it gives fast results. Chemical, microbiological and physical analyses are associated with sensory analyses in determining the shelf life.

Although physical, chemical and microbiological analyses used to determine the freshness level of the fish can significantly evaluate the shelf life, sensory analyses have a much more important place in consumption, because people make use of sensory analyses instead of experiments organized under laboratory conditions when deciding on consuming a food.

As a result of the sensory analyses on the products prepared in the research, a major part of the panelists liked the fish finger samples very much. In terms of the examined fish finger samples, group B and C received higher scores due to some specific physical properties of the goji berry extract.

Conclusion

The results obtained from this study showed that goji berry extract prolongs the shelf life of food. The use of goji berry extract in the food industry will contribute to the shelf life of the products in a positive way. The results show that fish fingers prepared with goji berry addition can contribute to the national economy.

Now, the positive effect of fish consumption on human health is known through scientific studies conducted in various fields. Increasing fish consumption in our country is an important matter for both economy and health. Countries that are aware of balanced nutrition are looking for new products which will satisfy the consumer in sensory terms in order to further enrich the protein sources and can be prepared easily, and they make investments accordingly. Although the consumption of fish is low in our country, the consumption form of fresh fish is limited to a few cooking methods. In this study, fish finger was prepared with mirror carp as a ready-to-use product. As it is understood from the sensory data, the product prepared in the study was highly appreciated by the panelists and the appreciation continued during the storage duration.

With this study, it is possible to express that the shelf life can be extended through natural extracts without the use of chemical additives, which are used to prolong the duration of lasting that can also be defined as the shelf life of the food, and if used unconsciously, threaten the consumer health.

Acknowledgements

This study was summarized from the master's thesis of the second author at Fırat University Graduate School of Natural and Applied Sciences.

Compliance With Ethical Standards

Authors' Contributions

AGI: Designing, draft checking, editing
BLMA: Analyzing, 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.

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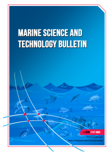
Mar. Sci. Tech. Bull. (2022) 11(4): 454-466
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1185291



Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Recent update on the distribution of alien and neonative fishes in the Aegean Sea

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ARTICLE INFO

Article History:
Received: 06.10.2022
Received in revised form: 08.11.2022
Accepted: 08.11.2022
Available online: 22.12.2022

Keywords:
Gibraltar Strait
Suez Canal
Native species
Non-native species
Bioinvasion

ABSTRACT

The Mediterranean Sea is considered an aquatic biodiversity hotspot that hosts approximately 782 fish species and 72 of them are categorized as endemic. However, non-native species introductions that are of natural or human-mediated origin have posed a major threat to the biodiversity of the Mediterranean Sea which is showing high rates of endemism. Here, we represent historical and recent updated data alien (*Champsodon nudivittis, Nemipterus randalli, Pterois miles, Scarus ghobban, Scomberomorus commerson*), neonative (*Seriola fasciata, Sphoeroides pachygaster*) fishes spreading on the Aegean Sea.

Please cite this paper as follows:

Aydın, İ., Yıldırım, Ö., Topçuoğlu, K., & Yapıcı, S. (2022). Recent update on the distribution of alien and neonative fishes in the Aegean Sea. *Marine Science and Technology Bulletin*, *11*(4), 454-466. https://doi.org/10.33714/masteb.1185291

Introduction

In terms of marine bio-invasion, the Mediterranean Sea is considered a crossroads for non-native marine organisms that introduce via the Gibraltar Strait and the Suez Canal (Rilov & Galil, 2009; Edelist et al., 2012; Zenetos et al., 2012). The Mediterranean Sea hosts approximately 17000 species and more than a third of them are endemic species classified as endangered, threatened, or vulnerable on the IUCN Red List (Coll et al., 2010). Although non-native species diversity displays sharp differences throughout the west-east axis of the Mediterranean Sea, the endemic and endangered taxa of the Mediterranean Sea have faced the invasion of the non-native species (Coll et al., 2010). The rate of influx of non-native

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species classified as alien and neonative into the Mediterranean is affected by several vectors that such as ballast waters, water mass circulations, and increasing sea temperature (Bianchi, 2007). The fact that negative effects of invasive species are not limited to ecosystem services they also affect negatively socio-economic activities forces scientists and public authorities to close monitoring of new introductions/range expansions of non-native species.

Until today, Mediterranean marine biodiversity has gone altered by many thousands of species that have spread beyond their natural ranges because of intentionally or unintentionally human drivers. As well known, the temperature is the primary factor that determines the boundaries of the natural habitats of species, hence changing sea temperature level is the keystone causing to shift in the geographical range of aquatic organisms. This functional factor also affects migration patterns, seasonal activities, species interactions, and abundances of species (Walther et al., 2002). This continuing warming trend has enabled the introduction and expansion of thermophilic non-native species and transformed some of them into invasive. These non-native freshwater and marine species that are introduced directly or indirectly by human activity are an ever-increasing problem in the Mediterranean, such that the number of non-native species in Europe has been reported as 42% (Stranga & Katsanevakis, 2021).

In the present study, we aimed to present the recent distribution data related to alien and neonative fish species that are two sides of the same coin in the Mediterranean marine bio-invasion. We also believe that the results of this study will provide recent data for scientists, stakeholders, and public authorities on several topics such as reacting against alien species, distribution modelling, risk assessment protocols, and update of marine conservation actions.

Material and Methods

Alien Species

Champsodon nudivittis

On 30 March 2022, a single specimen was collected by bottom trawl at a depth of 70 m on a sandy-mud bottom in the Edremit Bay (North Aegean Sea). The identification of the specimen was following the instruction provided by Nemeth (1994). The specimen was deposited in Muğla Sıtkı Koçman University Faculty of Fisheries Museum.

Nemipterus randalli

On 17 July 2020, a specimen was captured from the Edremit Bay (North Aegean Sea), at a depth of 96 m. The identification of the specimen was following the instruction provided by Russell (1990). The specimen was deposited in the fish collection of the Fisheries Faculty, Ege University (ESFM-PIS/2021-01)

Pterois miles

On 05 February 2022, a single specimen was caught by trammel net from Edremit Bay (North Aegean Sea) at a depth of 30 m on rocky bottom. The identification of the specimen was following the instruction provided by Golani & Sonin (1992). The specimen was deposited in Muğla Sıtkı Koçman University Faculty of Fisheries Museum.

Scarus ghobban

On 28 September 2021, a single specimen was caught by fishing pot at a depth of 2 m on a sandy-mud bottom in the Gökova Bay (SE Aegean Sea). The identification of the specimen was following the instruction provided by Goren & Aronov (2002). The specimen was deposited in Muğla Sıtkı Koçman University Faculty of Fisheries Museum.

Scomberomorus commerson

On 03 March 2022, a single specimen was collected by trammel net at the 25 m in the Edremit Bay (North Aegean Sea). The identification of the specimen was following the instruction provided by Collette (2001).

Neonative Species

Seriola fasciata

On 10 October 2021, a single specimen was caught by trammel net at a depth of 30 m on a sandy-mud bottom in the Edremit Bay (North Aegean Sea). The identification of the specimen was following the instruction provided by Smith-Vaniz & Berry (1981). The specimen was deposited in Muğla Sıtkı Koçman University Faculty of Fisheries Museum.

Sphoeroides pachygaster

On 29 July 2020, one specimen was caught by bottom trawl at a depth of 93 m on a sandy and muddy bottom from the Edremit Bay (North Aegean Sea). The identification of the specimen was following the instruction provided by Smith & Heemstra (1986). The specimen was deposited in the fish collection of Ege University, Fisheries Faculty (ESFM-PIS/2021-02).

Results

Alien Species

Champsodon nudivittis

Systematics:
Class: Actinopteri
Family: Champsodontidae Jordan & Snyder, 1902
Genus: *Champsodon* Günther, 1867
Champsodon nudivittis (Ogilby, 1895) (Figure 1a)

Diagnostic characteristics: Compressed body dark brown dorsally. Caudal lobes pale. Scaleless chin covered with melanophore spots. The breast was scaled. The maxilla extended beyond the eye (Nemeth, 1994).

Measurements (mm): Total length 112, standard length 94, pre-anal length 50.2, pre-dorsal length 35.1, pre-pectoral length 29.6, body depth 16.7, caudal peduncle depth 6.4, head length 28.7, eye diameter 4.8.

Distribution: The first Mediterranean record of *C. nudivittis* was given by Çiçek & Bilecenoğlu (2009) from eastern Mediterranean. The following records are in the Levantine-Aegean axis: Greece (Kalogirou & Corsini-Foka, 2012; Kousteni & Christidis, 2019) Israel (Goren et al., 2011), Lebanon (Bariche, 2010, 2011), Syria (Ali et al., 2017), Türkiye (Ergüden & Turan, 2011; Gökoğlu et al., 2011; Dalyan et al., 2012; Gökoğlu & Özvarol, 2013; Filiz et al., 2014; Akyol & Ünal, 2015; Aydın & Akyol, 2015; Kebapçıoğlu & Dereli, 2016; Yapıcı et al., 2016; Dalyan et al., 2021). The present study provides additional record for northernmost distribution of *C. nudivittis* in the Aegean Sea (Figure 2).

Nemipterus randalli

Systematics:
Class: Actinopteri
Family: Nemipteridae Regan, 1913
Genus: *Nemipterus* Swainson, 1839
Nemipterus randalli Russell, 1986 (Figure 1b)

Diagnostic characteristics: Mouth terminal; five pairs of small recurved canine teeth on the premaxilla. Body covered with big ctenoid scales. Long and pointed pectoral fins, reaching beyond the level of anal fin origin. Caudal fin with a filamentous extension on the upper lobe. Upper part of the body is pinkish while ventral surface is silvery. Three or four pale yellow stripes on the lateral side. Three or four pale yellow stripes exist on the lateral side. The pectoral base includes a golden blotch. The dorsal fin pales bluish, upper margin edged with red, with yellow striped. The anal fin colour is pale bluish and includes a narrow yellow medial band (Russell, 1990).

Measurements (mm): Total length 187, standard length 153, fork length 167, pre-anal length 91.8, pre-dorsal length 42.8, pre-pectoral length 44.1, body depth 47.8, caudal peduncle depth 14.9, head length 44.3, eye diameter 12.6.

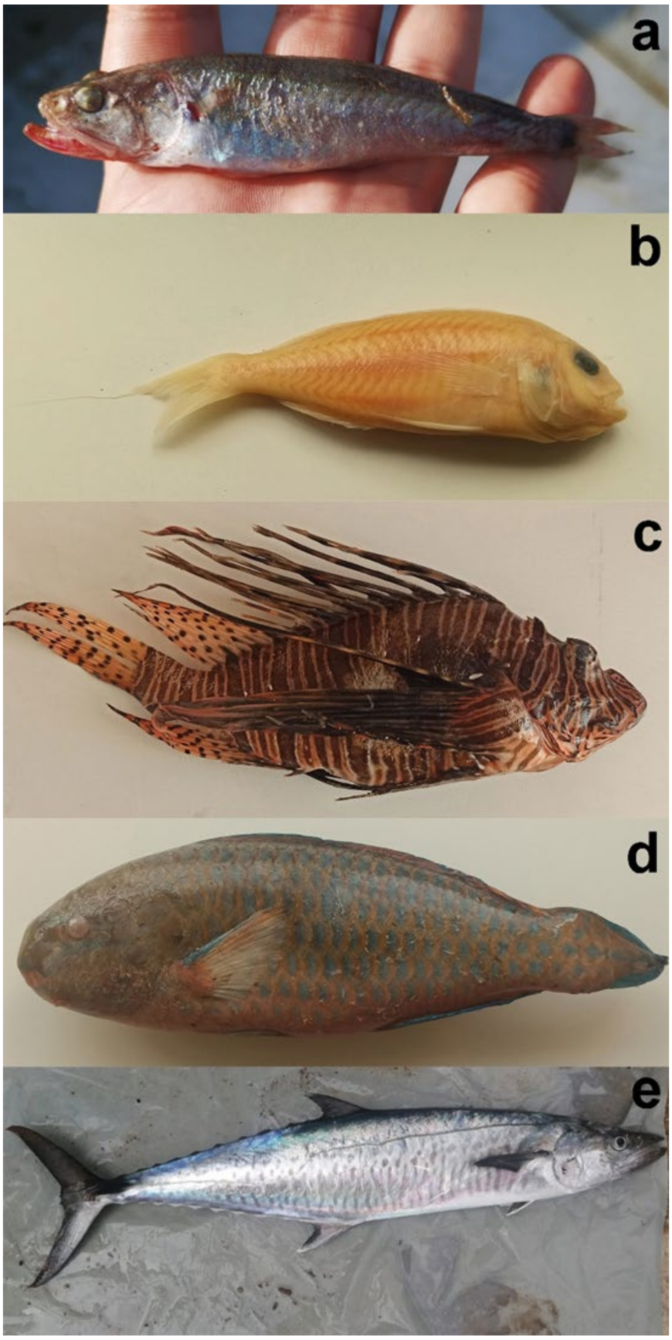

Figure 1. Alien species in the Aegean Sea. A) *Champsodon nudivittis*, B) *Nemipterus randalli*, C) *Pterois miles*, D) *Scarus ghobban*, E) *Scomberomorus commerson*

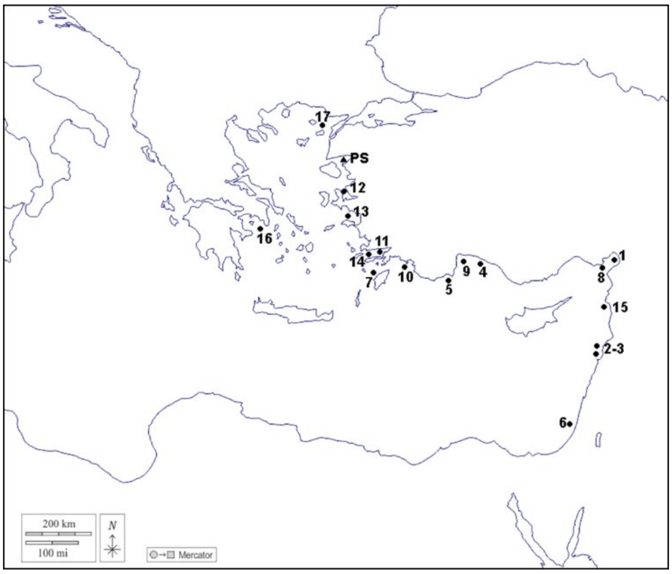

Figure 2. *Champsodon nudivittis* recordings from the Aegean-Levantine Sea up to date: [1]Çiçek & Bilecenoğlu (2009), [2]Bariche (2010), [3]Bariche (2011), [4]Gökoğlu et al. (2011), [5]Erguden & Turan (2011), [6]Goren et al. (2011), [7]Kalogirou & Corsini-Foka (2012), [8]Dalyan et al. (2012), [9]Gökoğlu & Özvarol (2013), [10]Filiz et al. (2014), [11]Akyol & Ünal (2015), [12]Aydın & Akyol (2015), [13]Kebapçıoğlu & Dereli (2016), [14]Yapıcı et al. (2016), [15]Ali et al. (2017), [16]Kousteni & Christidis (2019), [17]Dalyan et al. (2021), [PS]Present study

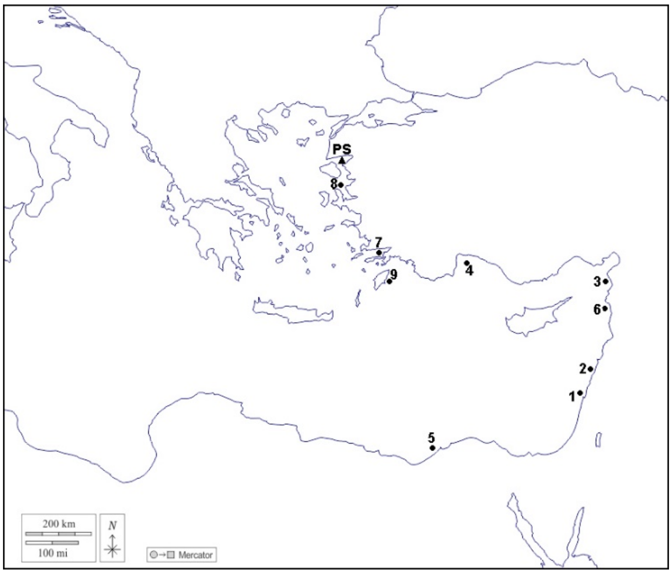

Figure 3. *Nemipterus randalli* recordings from the Aegean-Levantine Sea up to date: [1]Golani & Sonin (2006), [2]Lelli et al. (2008), [3]Bilecenoğlu & Russell (2008), [4]Gökoğlu et al. (2009), [5]Halim & Rizkalla (2011), [6]Ali et al. (2013), [7]Gülşahin & Kara (2013), [8]Aydın & Akyol (2017), [9]Kampouris et al. (2019), [PS]Present study.

Distribution: *N. randalli* was first reported from Israeli coasts by Golani & Sonin (2006) in the Mediterranean. The following records are in the Levantine-Aegean axis: Egypt (Halim & Rizkalla, 2011), Greece (Kampouris et al., 2019), Lebanon (Lelli et al., 2008), Syria (Ali et al., 2013), Türkiye (Bilecenoğlu & Russell, 2008; Gökoğlu et al., 2009; Gülşahin & Kara, 2013; Aydın & Akyol, 2017). The present study provides the northernmost occurrence of *N. randalli* in the Aegean Sea (Figure 3).

Pterois miles

Systematics:

Class: Actinopteri

Family: Scorpaenidae Risso, 1827

Genus: *Pterois* Oken, 1817

Pterois miles (Bennett, 1828) (Fig. 1c)

Diagnostic characteristics: Moderately compressed body is reddish to tan and numerous thin dark bars on body and head vertically; median fins covered with visible small spots. Long and venomous dorsal spines longer than body (Golani & Sonin, 1992).

Measurements (mm): Total length 244, standard length 181, pre-anal length 124, pre-dorsal length 53.1, pre-pectoral length 57.3, body depth 64.1, caudal peduncle depth 20.0, head length 60.4, eye diameter 12.0, snout length 18.0

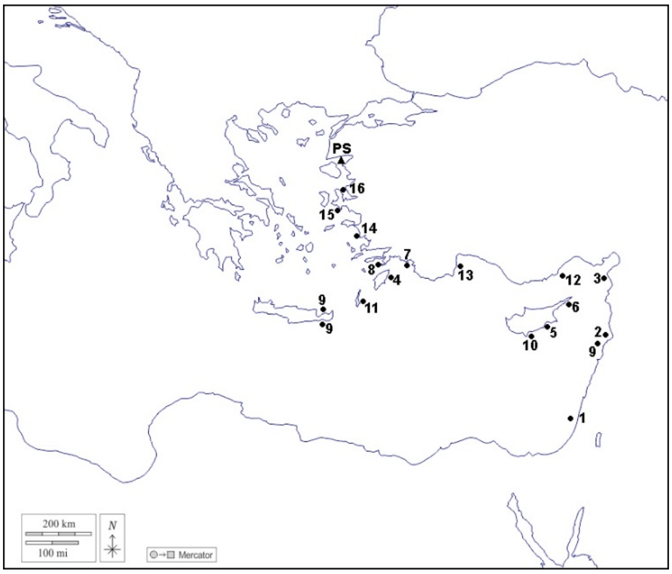

Figure 4. *Pterois miles* recordings from the Aegean-Levantine Sea up to date: [1]Golani & Sonin (1992), [2]Bariche et al. (2013), [3]Turan et al. (2014a), [4]Crocetta et al. (2015), [5]Iglésias & Frotté (2015), [6]Oray et al. (2015), [7]Turan & Öztürk (2015), [8]Bilge et al. (2016), [9]Dailianis et al. (2016), [10]Jimenez et al. (2016), [11]Mytilineou et al. (2016), [12]Yağlıoğlu & Ayaş (2016), [13]Özgür Özbek et al. (2017), [14]Yapıcı (2018), [15]Özgül (2020), [16]Oruç et al. (2022), [PS]Present study

Distribution: *P. miles* was first reported by Golani & Sonin (1992) in the Mediterranean. The following records are: Cyprus

(Iglesias & Frotte, 2015; Oray et al., 2015; Jimenez et al., 2016), Greece (Crocetta et al., 2015; Dailianis et al., 2016; Mytilineou et al., 2016), Lebanon (Bariche et al., 2013), Türkiye (Turan et al., 2014a; Turan & Öztürk, 2015; Bilge et al., 2016; Yağlıoğlu & Ayas 2016; Özgür Özbek et al., 2017; Yapıcı, 2018; Özgül, 2020; Oruç et al., 2022). The present study indicates the northernmost occurrence of *P. miles* in the Aegean Sea (Figure 4).

Scarus ghobban

Systematics:
Class: Actinopteri
Family: Scaridae Rafinesque, 1810
Genus: *Scarus* Forsskål, 1775
Scarus ghobban Forsskål, 1775 (Figure 1d)

Diagnostic characteristics: The body was dorsally brightly salmon pink with green-blue marks and stripes and ventrally was brownish. Scales were turquoise-blue with brown margins. The head was covered by irregular bands of turquoise-blue to brownish colour. The coloration of the lower and upper margins of the pectoral and pelvic fins was turquoise-blue while the middle part included a longitudinal band of reddish-brown (Goren & Aronov, 2002).

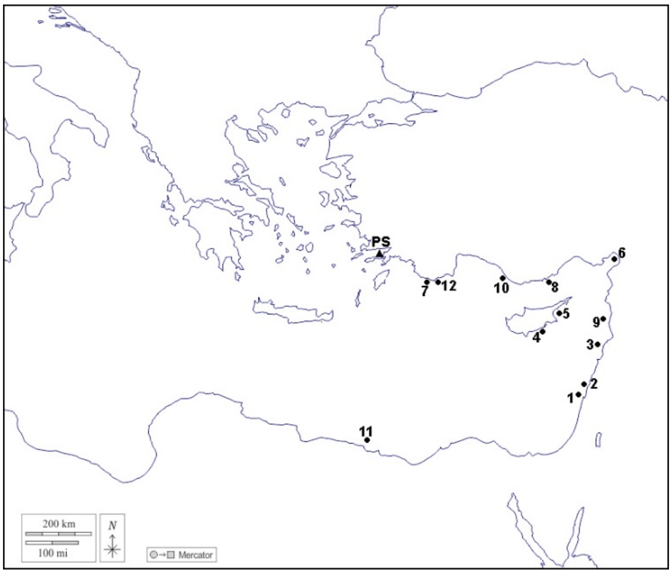

Figure 5. *Scarus ghobban* recordings from the Aegean-Levantine Sea up to date: [1]Goren & Aronov (2002), [2]Golani & Levy (2005), [3]Bariche & Saad (2008), [4]Ioannou et al. (2010), [5]Filiz & Sevingel (2014), [6]Turan et al. (2014b), [7]Karachle et al. (2016), [8]Yağlıoğlu & Ayaş (2016), [9]Soliman et al. (2018), [10]Ergüden et al. (2018), [11]Al Mabruk et al. (2020), [12]Tüney-Kızılkaya & Akyol (2020), [PS]Present study

Measurements (mm): Total length 279, standard length 230, fork length 273, pre-anal length 138.8, pre-dorsal length 75.1, pre-pectoral length 68.1, body depth 83.2, caudal peduncle depth 33.0, head length 70.8, eye diameter 8.3, snout length 24.5.

Distribution: Its first Mediterranean record was given from Israeli coasts by Goren & Aronov (2002). The following records are in the Levantine-Aegean axis: Cyprus (Ioannou et al., 2010; Filiz & Sevingel, 2014), Greece (Karachle et al., 2016), Israel (Golani & Levy, 2005) Lebanon (Bariche & Saad, 2005), Egypt (Al Mabruk et al., 2020), Syria (Soliman et al., 2018), Türkiye (Turan et al., 2014b; Yağlıoğlu & Ayaş, 2016; Ergüden et al., 2018; Tüney-Kızılkaya & Akyol, 2020). The present study provides the northernmost record of *S. ghobban* in the Aegean Sea (Figure 5).

Scomberomorus commerson

Systematics:
Class: Actinopteri
Family: Scombridae Rafinesque, 1815
Genus: *Scomberomorus* Lacepède, 1801
Scomberomorus commerson (Lacepède, 1800) (Figure 1e)

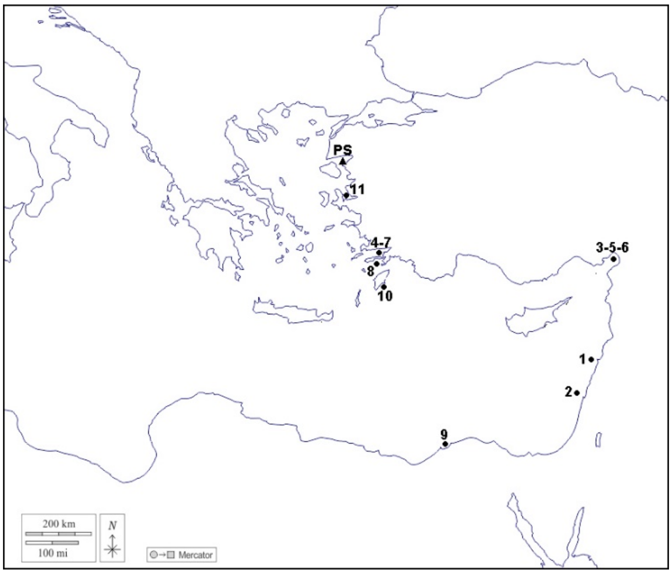

Figure 6. *Scomberomorus commerson* recordings from the Aegean-Levantine Sea up to date: [1]George & Athanassiou (1965), [2]Ben-Tuvia (1971), [3]Gücü et al. (1994), [4]Buhan et al. (1997), [5]Başusta & Erdem (2000), [6]Torcu & Mater (2000), [7]Öğretmen et al. (2005), [8]Öz et al. (2007), [9]Bakhoum (2007), [10]Corsini-Foka & Kalogirou (2008), [11]Akyol & Tosunoğlu (2019), [PS]Present study. (*The first Mediterranean record (Hornell, 1935) could not be shown on the map because the coordinate information could not be reached)

Diagnostic characteristics: Body shape elongated and moderately strongly compressed. The posterior margin of the eye intersects with the upper jaw. Two dorsal fins, the first of which is longer than the second. Finlets originating after the second dorsal fin reach out to the back end of the body. Lateral line abruptly bent downward below end of second dorsal fin. The dorsal side of the body is iridescent blue-grey and covered with distinctive thin-wavy vertical bands (Collette, 2001).

Measurements (mm): Detailed measurements could not be realized since the fisherman despatched a specimen to sell. However, he stated that the specimen was 80 cm in total length and weighed ~ 3 kg.

Distribution: Its first Mediterranean record was given from Palestine by Hornell (1935). The following records are in the Levantine-Aegean axis: Egypt (Bakhoum, 2007), Greece (Corsini-Foka & Kalogirou, 2008), Israel (Ben-Tuvia, 1971), Lebanon (George & Athanassiou, 1965), Türkiye (Gücü et al., 1994; Buhan et al., 1997; Başusta & Erdem, 2000; Torcu & Mater, 2000; Öğretmen et al., 2005; Öz et al., 2007; Akyol & Tosunoğlu, 2019). The present study provides the northernmost record of *S. commerson* in the Aegean Sea (Figure 6).

Neonative species

Seriola fasciata

Systematics:
Class: Actinopteri
Family: Carangidae Rafinesque, 1815
Genus: *Seriola* Cuvier, 1816
Seriola fasciata (Bloch, 1793) (Figure 7a)

Diagnostic characteristics: Body shape is elongated, moderately deep, somewhat compressed, and also covered with small cycloid scales. The body is dark olive dorsally, sides lighter, and belly silvery. Two dorsal fins, first lower than second. A dark band exists between the eye and the anterior of the dorsal fin. Seven dark irregular and broken bands throughout the body; 8th band is small and dark, at the end of the caudal peduncle (Smith-Vaniz & Berry, 1981).

Measurements (mm): Total length 210, standard length 161, fork length 182, pre-anal length 94.5, pre-dorsal length 54.8, pre-pectoral length 49.8, body depth 64.3, caudal peduncle depth 9.5, head length 36.7, eye diameter 10.0, snout length 17.3

Distribution: Its first record in the Mediterranean was reported by Massutí & Stefanescu (1993) from Balearic Island. The following records are in the Levantine-Aegean axis: Greece (Corsini et al., 2006), Israel (Sonin et al., 2009), Syria (Jawad et al., 2015), Türkiye (Kapiris et al., 2014; Doğdu et al., 2019; Yapici & Filiz, 2020; Akyol & Ünal, 2021), Egypt (Stamouli et al., 2017). The present paper provides the northernmost record and of *S. fasciata* for the Aegean Sea (Figure 8).

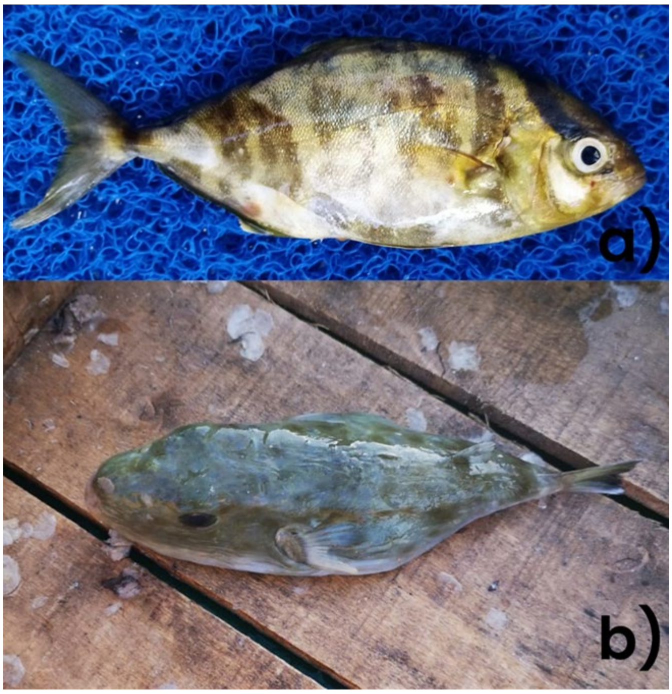

Figure 7. A) *Seriola fasciata* B) *Sphoeroides pachygaster*

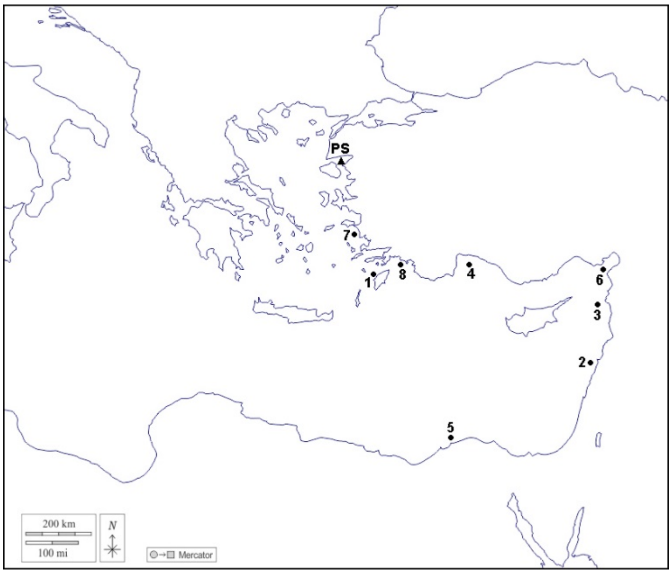

Figure 8. *Seriola fasciata* recordings from the Aegean-Levantine Sea up to date: [1]Corsini et al. (2006), [2]Sonin et al. (2009), [3]Jawad et al. (2015), [4]Kapiris et al. (2014), [5]Stamouli et al. (2017), [6]Doğdu et al. (2019), [7]Yapıcı & Filiz (2020), [8]Akyol & Ünal (2021), [PS]Present study

Sphoeroides pachygaster

Systematics:
Class: Actinopteri
Family: Tetraodontidae Bonaparte, 1831
Genus: *Sphoeroides* Anonymous [Lacepède], 1798
Sphoeroides pachygaster (Müller & Troschel, 1848) (Figure 7b)

Diagnostic characteristics: Body compressed and inflatable. Scaled absent. One lateral line. Skin covered by small spines often present on back and belly. Caudal fin truncated or rounded. Two nostrils on each side of snout. Body of the dorsal surface and the flanks is greyish to olive green with many pale dots and ventral side is whitish (Smith & Heemstra, 1986).

Measurements (mm): Total length 162, standard length 139, pre-anal length 114, pre-dorsal length 106, pre-pectoral length 57.9, body depth 48.2, caudal peduncle depth 20.0, head length 51.3, eye diameter 11.0.

Distribution: The first Mediterranean record of *S. pachygaster* was given by Oliver (1981) from Balearic Island. The following records are in the Levantine-Aegean axis: Cyprus (Katsanevakis et al., 2009; Akbora et al., 2021), Egypt (Farrag et al., 2016), Greece (Zachariou-Mamalinga & Corsini, 1994; Peristeraki et al., 2006; Dailianis et al., 2016), Israel (Golani, 1996), Lebanon (Gerovasileiou et al., 2017), Syria (Abdul Rahman et al., 2014) and Türkiye (Eryılmaz et al., 2003; Eleftheriou et al., 2011; Akyol & Aydın, 2017; Akbora et al., 2021). The present study provides additional record for northern distribution of *S. pachygaster* in the Aegean Sea (Figure 9).

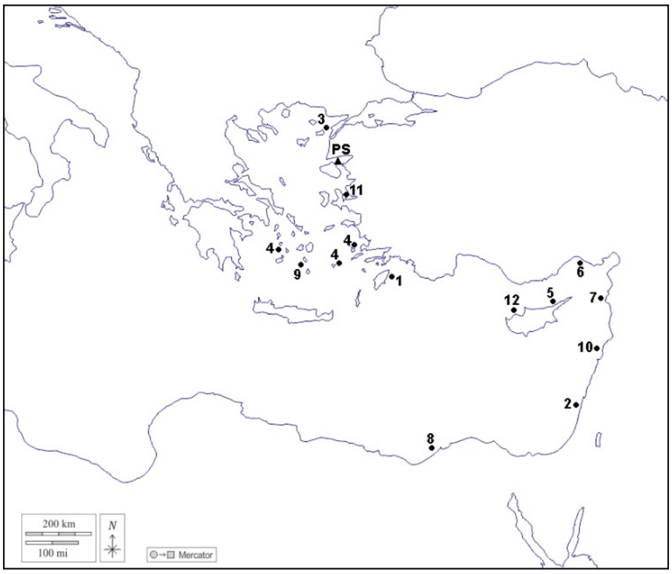

Figure 9. *Sphoeroides pachygaster* recordings from the Aegean-Levantine Sea up to date: [1]Zachariou-Mamalinga & Corsini (1994), [2]Golani (1996), [3]Eryılmaz et al. (2003), [4]Peristeraki et al. (2006), [5]Katsanevakis et al. (2009), [6]Eleftheriou et al. (2011), [7]Abdul Rahman et al. (2014), [8]Farrag et al. (2016), [9]Dailianis et al. (2016), [10]Gerovasileiou et al. (2017), [11]Aydın & Akyol (2017), [12]Akbora et al. (2021), [PS]Present study

Discussion

Invasion processes are controlled by the interaction between the characteristics of the invasive species and the conditions of the receiving environment and so are species-specific. Biodiversity of the Mediterranean that includes high endemism has been remodelled by non-native species introduced from the Gibraltar Strait and Suez Canal (Coll et al., 2010). Even though all non-native Atlantic and Lessepsian species are termed alien species, the invasion processes actually differ from each other. Therefore, range-expanding non-native species were classified into two groups as alien and neonative species (Essl et al., 2019). According to this definition, alien species directly exploit human agency (human-made canals, tunnels) in their range expansions, while neonative species may use human-induced changes of the biophysical environment to expand their ranges, however, do not use a directly human agency, intentional or unintentional. This classification clarifies that alien species (Lessepsian species) are often reported in unexpected areas distant from their native range and neonative (Atlantic species) are usually observed in new areas close to their native range.

There is a marked difference in the diversity of non-native species between the western and the eastern basin in the Mediterranean, however, this difference tends to decrease due to changes in the physicochemical conditions in the Mediterranean Sea. Because remodelling of the sea currents in the Mediterranean has given the opportunity to reduce or eliminate the adaptation phase of non-native species. Considering the historical spread of invasive species in the Mediterranean, the 38th parallel had considered a breaking point for the invasion because non-native species (especially Lessepsian migrants) could not appear, due primarily to the cold-water temperature (Papaconstantinou, 1990). Additionally, most scientists have suggested that even though a taxonomic richness of the alien biota, there is not essentially an expansion of the Lessepsian province above the 38th parallel (Bilecenoğlu, 2016). Sea surface temperature (SST) of the Aegean Sea that involves the 38th parallel mainly depends on the exchange between cold/fresh Black Sea and warm/saline Levantine basin (Poulain et al., 2012).

However, the effects of global warming have altered the borders of existent sea currents in the Mediterranean. In particular, water-mass modifications in the Mediterranean Transient (EMT)) have caused to penetration of a warm-water masses (Levantine Surface Water and Levantine Intermediate Water) into northern colder sectors of the Aegean Sea. Similar changes are also observed in the Atlantic Ocean, which connects with the Mediterranean Sea via the Strait of Gibraltar. Biton (2020) declared that water mass that flows from the Mediterranean Sea into the Atlantic (Mediterranean Overflow Water - MOW) has been caused the eastern Atlantic including the Strait of Gibraltar to be as saltier (~0.36 psu), warmer

(1.8°C), and so changed the characteristics of the Atlantic Meridional Overturning Circulation (AMOC). Effects of transport of warm water mass that Levantine origin to the northern sectors and the equalization trend of temperature and salinity between the eastern Atlantic and the Mediterranean on the introduction and dispersal of subtropical and tropical alien and neonative species are already observed throughout the Mediterranean (Katsanevakis et al., 2020; Dalyan et al., 2021). On the other hand, studies on the effect of environmental factors on the distribution of demersal Lessepsian species have not detected a correlation between temperature and dispersal mechanism. According to the results, only *N. randalli* had a positive correlation with water temperature. However, based on the results of the present study, it could be asserted that *N. randalli* is experiencing the temperature-dependent integration phase of the invasion. Similarly, Poursanidis et al. (2022) simulated the distribution of *P. miles* in the Mediterranean via the Bayesian framework and outputs showed this species would invade and settle whole the Aegean coasts for the 2040-2050 period. The northernmost observation of *P. miles* presented in this study proves the accuracy of the simulation outputs.

The process and success of the invasion in the Mediterranean cannot be completely attributed to the increase in SST, but it is also obvious that the increase in SST shortens the invasion processes and increases the success of the invasion. Because considering the distribution of non-native species in the Mediterranean (except *Fistularia commersonii*), veteran species took longer to settle and spread than newly introduced species. For example, invasion momentum in the Levant-Aegean axis is ~290 km/y^{-1} for *N. randalli* and 15 km/y^{-1} for *Siganus luridus*. In recent years, many thermophilic non-native and native taxa reported on northern Aegean coasts clearly reflect remodelling biodiversity in the Mediterranean (Katsanevakis et al., 2020; Dalyan et al., 2021; Esposito et al., 2021).

Conclusion

Non-native species that are directed by climate change and other human-induced vectors and native species are an integral part of biodiversity. Consequently, monitoring these species will enable scientists and public authorities to understand current/possible changes and their long-term effects on biodiversity.

Acknowledgements

The authors would like to thank captains Hasan BİRAN and Uğur Ali BİRAN of the F/V "DÜLGER" for supporting the survey. We also thank Mr. Yüksel TÜRK for providing some specimens as part of citizen science. Some data in this study were obtained in the project funded by Ege University Scientific Research Projects Coordination (2017/SÜF/005).

Compliance With Ethical Standards

Authors' Contributions

İA: Designed the study, provided the data.
ÖY: Provided the data.
KT: Provided the data.
SY: Designed the study, wrote the first version of manuscript.
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.

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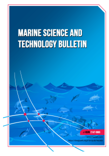
Mar. Sci. Tech. Bull. (2022) 11(4): 467-474
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1184165



Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Antibacterial activity of eucalyptus (*Eucalyptus camaldulensis*) essential oil against fish pathogen bacterium, *Aeromonas caviae*

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ARTICLE INFO

Article History:
Received: 05.10.2022
Received in revised form: 13.11.2022
Accepted: 14.11.2022
Available online: 22.12.2022

Keywords:
Aeromonas caviae
Antibacterial activity
Essential oils
Eucalyptus

ABSTRACT

The intensive use of antibiotics in aquaculture has resulted in increased resistance among fish pathogens, and this situation has led researchers to investigate the antibacterial properties of natural resources. The present study focused on an essential oil isolated from the leaves of *Eucalyptus camaldulensis* as a potential antibacterial that could be used against *Aeromonas caviae.* Eighteen compounds were identified in the essential oil, representing 86.68 % of the total oil. The components were found to be p-cymene (20.09%), β-phellandrene (18.61%), α-phellandrene (7.50%), α-terpineol (6.02%), terpinen-4-ol (5.50%), Crypton (5.36%), spathulenol (4.26%), linalool (3.56%), 1,8-cineole (2.77%), farnesol (2.31%) Cumin aldehyde (2.13%), limonen (2.12%), α-thujene (1.94%), fellendral (1.13%), γ-terpinene (1.10%), sabinene (0.97%), α-pinene (0.68%) and α -terpinen (0.63%). The antibacterial efficiency of essential oils against *Aeromonas caviae* was determined using Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, ranging from 200 µg/ml to 400 µg/ml respectively. Our findings revealed the potential of essential oils isolated from Eucalyptus (*Eucalyptus camaldulensis*) as a natural antibacterial agent that could efficiently contribute to the control of *Aeromonas caviae* infection in fish.

Please cite this paper as follows:

Bektaş, S., & Özdal, M. (2022). Antibacterial activity of eucalyptus (*Eucalyptus camaldulensis*) essential oil against fish pathogen bacterium, *Aeromonas caviae. Marine Science and Technology Bulletin*, *11*(4), 467-474. https://doi.org/10.33714/masteb.1184165

Introduction

Fish diseases, especially those caused by infectious organisms like bacteria which are responsible for the majority of infectious diseases, often result in serious economic losses in aquaculture facilities (Miller & Mitchell, 2009; Scott et al., 2011). The disease poses a major threat to aquaculture in many

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ways, such as losses caused by dead fish, decreased growth and the cost of treatment (Alfred et al., 2020).

Aeromonas spp. can cause diseases in fish with a high mortality rate (Bektas et al., 2007; Martínez-Murcia et al., 2008; Xue et al., 2022). Among the 31 species of *Aeromonas*, *A. hydrophila*, *A. sobria, A. caviae, A. allosaccharophila, A. encheleia* and *A. veronii* are classified as motile aeromonads (Chen et al., 2016; Stratev & Odeyemi, 2017).

Although, motile *Aeromonas* septicaemia (MAS) caused by motile aeromonads mostly associated with *A. hydrophila*. *Aeromonas sobria* and *Aeromonas caviae* are the other two most common species of the genus that cause disease. These opportunistic pathogens cause haemorrhagic septicaemia and ulcerative conditions in a variety of aquatic and terrestrial animals (Austin & Austin, 1993; Baumgartner et al., 2017).

Antimicrobials have a wide range of usage in preventing and treating the disease in intensive fish farming. These intensive applications of antimicrobials can cause the development of antimicrobial-resistant strains (Miller & Harbottle, 2018; Saengsitthisak et al., 2020). Xue et al. (2022) isolated *A. caviae* WH21406 from diseased largemouth bass (*Micropterus salmoides*) and pathogenicity of the strain was confirmed with the histopathological observation and virulent gene analysis. They also reported resistance of the strain against florfenicol, neomycin sulfate, compound sulfamethoxazole, doxycycline and tetracyclines. On the other hand, antibiotic-resistance genes may be passed to humans with food chain and this may complicate the treatment of some diseases in humans (Harikrishnan et al., 2009).

The mentioned side effects associated with synthetic antimicrobials have led scientists to develop alternative substances that can be used in case of a bacterial disease. (Sharma et al., 2012). Being a natural product, use of essential oils (EOs) which have antibacterial activity and low toxicity levels in the prevention and treatment of bacterial disease, makes them beneficial alternative against commonly used antibiotics in aquaculture (Da Cunha et al., 2018: Wińska et al., 2019).

Effective use of EOs in case of a bacterial infection in fish can be a promising strategy for reducing the consumption of antimicrobials in aquaculture (Klūga et al., 2021).

Eucalyptus is widely used in the pulp and paper industry, but its seeds and leaves also have the potential to produce essential oils. Being secondary plant metabolites, EOs have an important role in decreasing bacterial virulence and also weaken the mechanisms of bacterial invasion (Kartiko et al., 2021; Kouki et al., 2022).

Arial parts of *Eucalyptus* species are mostly used for their antibacterial, antifungal, analgesic and anti-inflammatory properties *Eucalyptus* essential oils exhibit high antimicrobial activity against a wide range of bacteria (Ghalem & Mohamed, 2008; Mulyaningsih et.al., 2011; Clavijo-Romero et al., 2019).

The objectives of the present study were to determine the chemical composition of the essential oils obtained from leaves of eucalyptus (*Eucalyptus camaldulensis*) and to determine the antimicrobial activity of this essential oil against fish pathogenic bacteria, *Aeromonas caviae.*

Material and Methods

Plant Materials and Extraction of the Essential Oil

Plant materials were collected from the leaves of Eucalyptus tree, growing wild in Sinop (Türkiye) region. Samples were cleaned and dried at the ambient temperature, protected from direct light.

About 500 g of fresh leaves were crushed and subjected to hydro-distillation using a Clevenger's apparatus. Briefly, crushed eucalyptus leaves were completely immersed in water and heated to boiling. The essential oil (EOs) evaporated together with water vapour passed through the refrigerant and collected into the condensation flask, after liquid phase removed the essential oil was collected in a glass vial (Ghalem & Mohamed, 2008; Mazumder et al., 2020).

Test Organism

The bacterium, *Aeromonas caviae* LipT51 (GenBank ID: MN818567.1), isolated from Ilica Hot Springs (Erzurum, Turkey), was obtained from the Microbiology Laboratory, Department of Biology, Atatürk University, Erzurum, Turkey. The bacterial isolate was identified by both 16S rRNA gene sequencing and classic identification tests (Gurkok & Ozdal, 2021). The bacterial strain was maintained in Nutrient Broth containing 20% glycerol at −86°C throughout the study and used as stock cultures.

GC and GC/MS Analyses

Hewlett Packard system, HP 5973 Mass Selective Detector System and GC–MS 6890 GC system were used in analyses. Agilent HP innowax column (60 m in length, inner diameter of 0.25 mm, film thickness of 0.25 μm) was used. As a carrier gas helium was used. The injection temperature was 250°C and the oven temperature was kept at 60°C for 10 minute and programmed to 220°C at a rate of 4°C/min and kept constant at 220°C for 10 min and programmed to 240°C at a rate of 2°C/min for 40 min. Relative amounts of the characterized components were expressed in percentages and the retention time (RT) was recorded in minutes (Sevindik et al., 2016). GC-MS analyses were conducted in Eskisehir Anadolu University Medicinal Plants, Drugs and Scientific Research Center (AUBİBAM).

Determination Of Antibacterial Concentration

The Minimum Inhibitory Concentration (MIC) of the Eucalyptus oil was measured by Broth Dilution Method (Rath & Priyadarshanee, 2017). Shortly, varying volumes of the oil were mixed with NB (Nutrient Broth containing 0.5% of Tween 20, v/v) to give a concentration of 50-1000 µg/ml by serial dilution method. A total of seven different concentrations used in the study. One hundred microliters of overnight grown culture (5×10^5) were inoculated into the tubes containing various concentrations of eucalyptus oil. The tubes with a volume of 4 ml were incubated with shaking at 37°C for 24 h and the lowest concentration inhibiting bacterial growth (no turbidity) was determined as MIC of the particular oil against the specific test organism. The optical density (OD600) was measured for MIC analysis at two different times: t_0, before incubation, and t_{24}, following incubation for 24 hours. To determine MBC, a 20-µl solution from the final three tubes that did not demonstrate bacterial growth was inoculated on NA plates and incubated overnight at 37°C.

Results and Discussion

The isolate was identified based on 16S rDNA sequence analyses and its morphological and biochemical characteristics. *A. caviae* LipT51 was characterized as a Gram-negative, non-spore forming, rod-shaped, catalase, oxidase, and protease-positive, urease-negative bacterium, forming cream-colored, bright, and round colonies (Gurkok & Ozdal, 2021).

GC-MS analyses of the essential oil of eucalyptus (*Eucalyptus camaldulensis*) revealed p-cymene and β-phellandrene as the major compounds with the highest peaks (Figure 1).

Although, components of essential oils obtained from plants differ relating to many factors such as region, climate, harvesting season, soil, relative humidity, day length and sampling methods (Sartorelli et al., 2007; Heikal, 2017), the major compounds of EO from *Eucalyptus* species were reported as 1,8-cineole (eucalyptol), p-cymene, terpinen-4-ol, β-pinene and γ-terpinene, (Sebei et al., 2015; Sabo & Knezevic, 2019; Almas et al., 2021).

Eighteen compounds were identified in the essential oil representing 86.68% of the total oil. The essential oil composition presented in Table 1.

Similar to our results p-cymene and β-phellandrene were reported as the major component in *Eucalyptus camaldulensis* (Dagne et al., 2000; Grbović et al., 2010). p-Cymene and β-phellandrene are monoterpenes, belong to the terpenes which are the major components of essential oils of various plant species (De Oliveira et al., 2015; Noriega, 2020). To date, various biological activities of these compounds have been reported. (Wang & Liu, 2010; Marchese et al., 2017).

Anastasiou et al. (2019), tested the EOs of twelve Mediterranean plants against certain bacterial fish pathogens like as *Aeromonas*, *Vibrio*, *Edwardsiella* and *Photobacterium* species. They have reported that compounds such as carvacrol, p-cymene and γ-terpinene showed a strong inhibitory activity on growth all of this fish pathogenic bacteria. They were also determined that these EOs had the highest total antioxidant capacity.

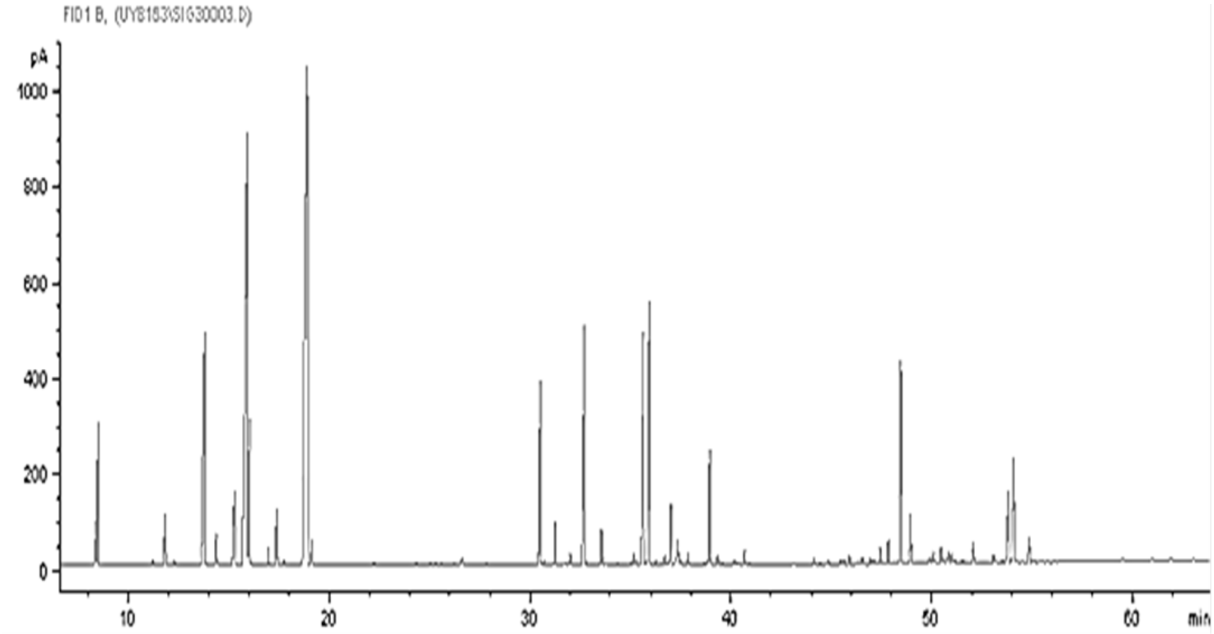

Figure 1. Gas chromatogram of the essential oil from *Eucalyptus camaldulensis*

Table 1. Essential oil composition of *Eucalyptus camaldulensis*

RT (min)	Component	Quantity (%)
8.33	p-cymene	20.09
8.46	β-phellandrene	18.61
11.80	α-phellandrene	7.50
13.78	α-terpineol	6.02
14.35	terpinen-4-ol	5.50
15.25	Crypton	5.36
15.89	Spathulenol	4.26
16.01	linalool	3.56
17.42	1,8-cineole	2.77
18.92	Farnesol	2.31
30.54	Cumin aldehyde	2.13
32.74	limonen	2.12
35.67	α-thujene	1.94
35.98	Fellendral	1.13
37.05	γ-terpinene	1.10
38.98	sabinene	0.97
48.50	α-pinene	0.68
54.12	α-terpinen	0.63
Total		86.68

Note: RT: Retention time

In our study, *Eucalyptus* oil presented antibacterial activity against *Aeromonas caviae*. The antibacterial efficiency of essential oils was detected by MIC and MBC values, which were observed at 200 µg/ml and 400 µg/ml respectively. The comparative MIC and MBC values of the eucalyptus essential oils are presented in Table 2.

Eucalyptus essential oils contain antibacterial active ingredients such as volatile terpenes and aromatic compounds (Barbosa et al., 2016; Gadhoumi et al., 2022). On the other hand, eucalyptus essential oil can cause cell wall and membrane dysfunction. He et al. (2022) determined by SEM that *S. putrefaciens* and *S. aureus* cells treated with eucalyptus oil showed abnormal and rough surface. This can lead to significant loss of intracellular ATP, pH disturbance and intracytoplasmic changes (Sabo & Knezevic, 2019; Limam et al., 2020).

Insuan & Chahomchuen (2020) reported the high antibacterial activity of essential oils of *Eucalyptus citriodora* against *Staphylococcus intermedius* and *Pseudomonas aeruginosa* as determined by the MIC and MBC values, and they suggested that *E. citriodora* oil affected the cell wall and cell membrane.

Bogavac et al. (2019) reported the MIC values of *Eucalyptus globulus* essential oil against *S. aureus* 2, *E. coli* 1, and *P. mirabilis* as 6.2, 12.5 and 25 µL/ml, respectively. The essential oil extracted from the fresh leaves of *E. grandis* showed MIC 0.31, 1.25 and 1.25 mg/ml and MBC 0.63, 2.5 and 1.25 mg/ml against bacteria such as *Klebsiella pneumonia*, *S. aureus* and *Moraxella catarrhalis*, respectively (Sewanu et al., 2015). The minimum inhibitory concentration (MIC) of leaf oil (*E. globulus*) was 0.72 µl/ml for *S. epidermidis*, 0.74 µl/ml for *S. aureus*, 1.5 µl/ml for *P. aeruginosa* and 2.75 µl/ml for *Klebsiella pneumoniae* (Bachheti, 2015).

Knezevic et al. (2016) reported the major components of essential oils obtained from *E. camaldulensis* as spatulenol, cryptone, p-cimene, 1,8-cineole, terpinen-4-ol and β-pinene and they have detected MICs values of essential oils against *Acinetobacter baumannii* wound isolates in a range of 0.5 to 2 µl/ml according to these findings they claimed that the polar terpene compounds and spathulenol are at least partially responsible for the antibacterial activity.

Table 2. Comparative MIC and MBC values of eucalyptus essential oils against fish pathogenic bacteria

Bacteria	*Eucalyptus*	MIC	MBC	References
Aeromonas hydrophila	*E. camaldulensis*	125 µL/mL	500 µL/mL	Debbarma et al., 2013
Lactococcus garvieae	*E. globulus*	31.24 µg/mL	125 µg/mL	Park et al., 2016
Edwardsiella tarda	*E. globulus*	7.81 µg/mL	125 µg/mL	Park et al., 2016
Vibrio harveyi	*E. globulus*	7.81 µg/mL	62.50 µg/mL	Park et al., 2016
Vibrio ichthyoenteri	*E. globulus*	125 µg/mL	250 µg/mL	Park et al., 2016
Photobacterium damselae	*E. globulus*	31.24 µg/mL	125 µg/mL	Park et al., 2016
Citrobacter freundii	*E. globulus*	0.36 µg/mL	0.36 mg/mL	Damjanović-Vratnica et al., 2011
A. caviae	*E. camaldulensis*	200 µg/mL	400 µg/mL	Present study

There are so many studies reporting variable results for MIC and MBC values of essential oils against Aeromonas strains. Chagas et al. (2020) reported MIC values ranging between 1.25 to 10 and MBC values 1.667 to 10 µg/mL of the essential oils against *Aeromonas* spp. isolates. In a study which aimed to evaluate in vitro efficacy of 13 essential oils and 16 compounds against four *Aeromonas salmonicida* subsp. *salmonicida* strains, researchers observed that MIC and MBC values of studied EOs and compounds, varied slightly among the strains (Hayatgheib et al., 2020). Monteiro et al. (2021) determined the antimicrobial activity of *Lippia sidoides*, *Ocimum gratissimum* and *Zingiber officinale*'s essential oils against *Aeromonas* spp. isolates and they have reported MIC and MBC values ranging from 625 to 5,000 µg/mL.

These differences among the studies may be due to many factors such as the extraction method and purity of the essential oil, the difference in the bacterial species studied, the inoculum volume and the difference in the medium used.

Conclusion

Bacteria concerned with fish disease are one of the limiting factors that have caused major production problems in aquaculture facilities. In case of a bacterial infection, antibiotics are the most applied chemicals for inhibiting the pathogen growth and also to control the disease. However intensive and unregulated use of antibiotics has led to antimicrobial resistant fish pathogens. Increased antimicrobial resistance (AMR) is a global concern, because microorganisms, causing disease are becoming resistant to antimicrobials which are used to control them.

Since the antibiotic resistant bacteria limited the use of conventional treatment with antibiotics, has led the researchers to seek alternative natural resources with plant origin that can replace antibiotics. A literature based survey has demonstrated that the use of essential oil extract for the prevention and treatment of infectious diseases, could substitute synthetic antibacterial.

The antimicrobial effects of EOs, mainly p-cymene and β-phellandrene being major components obtained from Eucalyptus (*Eucalyptus camaldulensis*) reported in the present study demonstrate that these components have an antimicrobial activity against one of a fish pathogen bacteria, *Aeromonas caviae.*

Compliance With Ethical Standards

Authors' Contributions

SB: Designed the study, wrote the first draft of the manuscript.
MÖ: Performed laboratory experiments.
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

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Mar. Sci. Tech. Bull. (2022) 11(4): 475-492
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1178141

Marine Science and Technology Bulletin

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https://prensip.gen.tr/

RESEARCH ARTICLE

Sustainability of Karacaören-I Dam Lake rainbow trout cage farming (Türkiye) in terms of cultural energy and carbon footprint expended on compound diet and transportation

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ARTICLE INFO

Article History:
Received: 21.09.2022
Received in revised form: 13.11.2022
Accepted: 16.11.2022
Available online: 22.12.2022

Keywords:
Carbon footprint
Cultural energy
Compound diet
Rainbow trout
Sustainability
Transportation

ABSTRACT

The purpose of this study was to assess the consumed compound diet and juvenile fish, harvested fish, and compound diet transport of Karacaören Dam Lake-I rainbow trout cage farming (KRTC) in terms of cultural energy (CE) and carbon footprint (CF) expended sustainability. Data was collected through face-to-face interviews with the farmers. Cultural energy and carbon footprint were calculated with the data obtained from the literature. The lowest and highest FCRs in KRTC were 0.91 and 1.18, the closest and farthest distances related to transportation were 387 and 427 km for aquafeed factories, 7 and 650 km for hatcheries, and 67 and 450 km for processing factories. Cultural energy and carbon footprint expended on consumed compound diet (CECD-Gcal and Mcal kg^{-1}, and CFCD-tonne CO_2e and kg CO_2e kg^{-1}) and cultural energy and carbon footprint expended on transportation analyzes (CET-Gcal and Mcal kg^{-1}, and CFT-tonne CO_2e and kg CO_2e kg^{-1}) were performed according to the literature of 20-40 g fish stocked in the beginning of November 2020 and 270-500 g harvested until early June 2021 in the basin. In the access of sustainability, the CE (Mcal kg^{-1}) and CF (CO_2e kg^{-1}) expended values in kg of the harvested fish were given. The average values of CE expended of 5 different aquafeed groups used in the basin were 3.65, 3.58, 3.41, 3.25, and 3.55 Mcal kg^{-1}, respectively and the average values of CF expended were 1.05, 1.03, 1.14, 1.40, and 1.10 kg CO_2e kg^{-1}, respectively. The average share of CE and CF in the compound diet was 86.59% and 86.61%, respectively. The KRTC sustainability criterion for compound diet and transportation values was 2.9260 CE:CF. It is recommended to develop a sustainability index of aquaculture systems and species-specific CE and CF expended values.

Please cite this paper as follows:

Diken, G. (2022). Sustainability of Karacaören-I Dam Lake rainbow trout cage farming (Türkiye) in terms of cultural energy and carbon footprint expended on compound diet and transportation. *Marine Science and Technology Bulletin*, *11*(4), 475-492. https://doi.org/10.33714/masteb.1178141

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Introduction

The aquaculture studies of countries with a high share in world food production are a reference in the species-based evaluation of sustainability against global climate change. Freshwater aquaculture meets 77% of the world's edible aquaculture production, excluding aquatic plants. Freshwater aquaculture has provided 80% of the finned finfish production with external feed support since 2000 (Zhang et al., 2022). Türkiye is an innovative country with a strong sectorial direction in intensive finfish aquaculture on a world scale. Türkiye, which is the leading country among European and Mediterranean countries in portioned rainbow trout farming was the world leader until 2012, but after this year it left the world leadership to Iran. In 2019, 22.48% of the world rainbow trout production shares were provided by Iran and 13.43% by Türkiye (FAO, 2022). Rainbow trout farming in Türkiye's inland waters increased regularly from 42,572 tonnes in 2000 to 135,732 tonnes in 2021 (GDFA, 2022).

The length of the coastline is 8,333 km, with an area of 8,903 km² nearly 200 natural lakes, approximately 177,714 km of rivers, 342,377 hectares of dam lakes, 70,000 hectares of lagoon lakes, Türkiye has a total aquaculture production capacity of 25,577,200 hectares (Demir & Sevinç, 2020; Arslan & Oguzhan Yıldız, 2021). Karacaören-I Dam Lake, which is located within the borders of Isparta and Budur provinces of Türkiye's Lakes Region Basin, was built on the Aksu stream for irrigation, flood control, and electricity generation. The dam is 93.00 m high from the stream bed and has a normal water code of 270 m, and the reservoir volume and area at normal water level are 1234 hm³ and 45.50 km², respectively (Becer Ozvarol & İkiz, 2009) (Figure 1).

In terms of the stability of the world and examining the extent of that stability, the true cost of resource consumption and environmental degradation will help us determine the energy value of the production system (Henriksson et al., 2010). In aquaculture, species, feeding habits, and aquaculture systems cause differences in energy use, making it difficult to establish basic rules for determining energy use efficiency (Pelletier et al., 2011). Along with the food systems, the fishery and aquaculture sector is also associated with the source of greenhouse gases that cause global climate change, which is dependent on the energy use of non-renewable fossil fuels (Pelletier et al., 2011; Muir, 2015; Boyd et al., 2019). However, the energy use of aquaculture in the fossil fuel-based global food system is around 1%. As a cross-sectoral approach, the energy efficiency of farmed fish, calculated as energy input per protein-energy output, is better than the production of livestock (Hargreaves et al., 2019). It is important to evaluate the resources and practices of the fisheries sector, to determine the sustainability of energy use and to determine resource dependence (Muir, 2015).

In the agrifood chain, our direct or indirect energy needs and the purpose of using it reveal whether it can meet food security and sustainably support development goals (FAO, 2012). Cultural energy (CE) and carbon footprint (CF) studies in aquaculture can be considered as a concept that offers important approaches to sustainability (Diken et al., 2021, 2022; Diken & Koknaroglu, 2022). The carbon footprint in food production is expressed as the total kg of CO_2*equivalent* (*e*) emitted per kg of an edible product obtained within the scope of all activities. The calculation is based on estimations of the emission amounts for each input during the product life cycle (Lutz, 2021). CE or embodied energy results of aquaculture are fossil-based non-renewable energy values that include calculations of energy values other than solar sources (Kurnia et al., 2019). Agrifood production, which currently relies heavily on fossil fuels, needs energy at every step to meet the growing demand for food. Improving access to energy, using energy more efficiently, and increasing the use of renewable energy sources will be beneficial to energy input and thus increase efficiency (FAO, 2012). A global-scale aquaculture feed factory is turning to low-emission feed production in its feed production planning without sacrificing quality and feed production to contribute to the sustainability of the aquaculture industry (Hatchery Feed & Management, 2021). At the same time, within the scope of reducing the CF values caused by the transport of the produced feed to the farm, private feed facility investments belonging to the farm have also been started (Hatchery International, 2021).

The energy expenditure share of feed in intensive cage farming was 79 and 78% for salmon and grouper/bass, respectively (Flos & Reig, 2017). CE and CF expended consumed compound diet share of Turkish rainbow trout cage farming was close to 80% and 75%, respectively (Diken et al., 2021, 2022). In this study, the status of sustainable aquaculture in Karacaören-I Dam Lake which is one of the important inland aquaculture areas of Türkiye was determined by calculating the CE and CF budget of compound diet, and compound diet and fish transportation of rainbow trout cage farming in Karacaören-I Dam Lake.

Material and Methods

Rainbow Trout Cage Farming Management

This study is based on the data of 22 cage farms rearing rainbow trout in Karacaören-I Dam Lake within the borders of Isparta and Burdur provinces in Türkiye's Mediterranean Region Lakes Region (Figures 1, 2). Data was collected through

Table 1. Karacaören-I Dam Lake rainbow trout cage farming management*

Cage		Juvenil initial		Harvested fish		Diet (kg)									Transportation (km)		
Code	*Technical*	*AW (g)*	*Σ (kg)*	*AW (g)*	*Σ (kg)*	*Code*	*1.9 mm*	*3 mm*	*4 mm*	*4.5 mm*	*5 mm*	*6 mm*	*Σ*	*FCR*	*Aquafeed*	*Hatchery*	*Processing factory*
1	Offshore (8 pieces; ø=20 m, h=15 m)	23	31.050	300	400.950	A		28.000	52.000		108.000	161.188	349.188	1.18	315	7	100.5
						C		3.500	6.500		13.500	20.149	43.649		417		
						E	3.500	6.500		13.500		20.149	43.649		413		
						Σ	3.500	38.000	58.500	13.500	121.500	201.485	349.188				
2	Offshore (20 pieces; ø=12 m, h=6 m, and 15 pieces; ø=16 m, h=7 m)	40	16.560	500	205.000	A		7.000	55.000		68.000	75.000	205.000	1.09	380	7	380 75
3	Octagonal (15 pieces; l=4.6 m, 10.5 h=6 m)	20	2.560	270	33.250	A		2.000	6.000		10.000	14.000	32.000	1.04	380	9	380
4	Offshore	28	5.600	250	49.000	D		5.000	10.000		15.000	20.000	50.000	1.15	427	98	442
5	Square & octagonal (25 pieces; l=5 m, h=4 m & 3 pieces; l=6.4 m, h=6 m)	20	2.400	300	35.000	C		3.000	9.000		10.000	11.000	33.000	1.01	417	45	75
6	Offshore (10 pieces; ø=12 m, h=6 m)	25	4.125	300	48.000	B		4.250	8.500		16.000	19.500	48.250	1.10	315	7	120
7	Offshore (10 pieces; ø=12 m, h=6 m)	25	4.250	300	49.000	B		4.250	8.500		16.500	20.000	49.250	1.10	315	7	120
8	Offshore (12 pieces; ø=12 m, h=7 m)	25	4.000	300	45.000	B		4.000	8.000		15.000	18.000	45.000	1.10	315	7	120
9	Offshore (1 piece; ø=20 m, h=9 m)	23	4.510	253	49.092	A		3.200	7.200		15.200	20.600	46.200	1.18	315	7	100.5
						C		400	900		1.900	2.575	5.775		417		
						E	400	900		1.900		2.575	5.775		413		
						Σ	400	4.500	8.100	1.900	17.100	25.750	57.750				
10	Square & octagonal (10 pieces; l=5 m, h=4 m & 3 pieces; l=6.4 m, h=6 m)	20	1.700	300	25.050	C		2.000	6.500		7.000	8.000	23.500	1.01	417	45	75
11	Octagonal (4 pieces; l=6.4 m, h=6 m)	20	2.000	260	30.000	B		2.000	6.000		10.000	14.500	32.500	1.16	315	7	120
12	Offshore (24 pieces; ø=16 m, h=5 m)	25	10.000	320	120.000	E	40.000	60.000			20.000		120.000	1.09	408	230	72

Table 1 (continued)

Cage		Juvenil Initial		Harvested Fish		Diet (kg)									Transportation (km)		
Code	*Technical*	*AW (g)*	*Σ (kg)*	*AW (g)*	*Σ (kg)*	*Code*	*1.9 mm*	*3 mm*	*4 mm*	*4.5 mm*	*5 mm*	*6 mm*	*Σ*	*FCR*	*Aquafeed*	*Hatchery*	*Processing factory*
13	Offshore (25 pieces; ø=20 m, h=7 or 9 m)	20	30.000	350	500.000	D		20.000	120.000		200.000	160.000	500.000	1.06	401	40	67
14	Offshore (14 pieces; ø=20 m, h=9 m and 10 pieces; ø=24 m, h=9 m & 5 pieces; ø=30 m, h=9 m)	23	31.050	300	400.950	A		28.000	52.000		108.000	161.188	349.188		315		
						C		3.500	6.500		13.500	20.149	43.649	1.18	417	7	100.5
						E	3.500	6.500		13.500		20.149	43.649		413		
						Σ	3.500	38.000	58.500	13.500	121.500	201.485	436.485				
15	Offshore (20 pieces; ø=20 m, h=7 or 9 m)	20	36.000	330	450.000	D		20.000	80.000		150.000	200.000	450.000	1.09	401	40	67
16	Offshore (12 pieces; ø=16 m, h=6 m)	35	35.000	400	346.000	D		55.000	100.000		150.000	22.000	327.000	1.05	402	650	69
17	Offshore & octagonal (16 pieces; ø=16 m, h=6 m & 7 pieces; l=6.4 m, h=7 m)	20	12.000	330	170.000	D		5.000	20.000		100.000	45.000	170.000	1.08	401	40	450
18	Offshore (9 pieces; ø=12 m, h=5 m, and 5 pieces; ø=16 m, h=7 m)	27	9.600	300	75.388	A		2.400	38.000		24.100		64.500	0.98	391	58	166
19	Offshore (4 pieces; ø=16 m, h=6 m)	35	14.000	400	95.000	D		14.000	25.000		40.000	6.000	85.000	1.05	402	650	69
20	Offshore (5 pieces; ø=16 m, h=7)	20	2.000	400	25.000	D		4.000	8.000		6.000	7.000	25.000	1.09	401	40	68
21	Offshore (5 pieces; ø=16 m, h=7)	20	2.000	400	25.000	D		4.000	8.000		6.000	7.000	25.000	1.09	402	41	68
22	Octagonal (5 pieces; l=6.4 m, h=4 or 5 m)	20	2.000	258	25.000	B		1.000	5.000		5.000	10.000	21.000	0.91	315	7	76
TOTAL & Average			262.405		3,201.680		47.400	299.400	646.600	28.900	1,128.700	1,085.720	3,236.720	1.08	387.0	93.1	150.3

Note: *Total annual project capacities were 3,840 tonnes (Anonymous, 2021).

face-to-face interviews with the farmers. Rainbow trout juveniles stocked in Karacaören-I Dam Lake as 20-40 g in early November 2020 were harvested 270-500 g until early June 2021 with a mortality rate varying between 1-10% (Table 1). The cage farms used 5 different aquafeed groups (CD/A, CD/B, CD/C, CD/D, CD/E). A compound diet with 6 diameters of 1.9 mm (D1), 3 mm (D2), 4 mm (D3), 4.5 mm (D4), 5 mm (D5), and 6 mm (D6) was used in these 5 different aquafeed groups. The order of the lowest and highest chemical compositions of 5 different aquafeed groups were like that 44-51 CP (crude protein), 17-22 CF (crude fat), 6.1-10.6 CA (crude ash), 0.9-2.4 CF (crude fibre) for D1 and D2, 38.7-45 CP, 20-25.2 CF, 6.8-11 CA, 1.7-2.7 CF for D3 and D4, 44-45 CP, 20-21 CF, 8.6-11 CA, 1.7-2.4 CF for D5, and 37-45 CP, 20-25.3 CF, 9.5-11 CA, 0.9-2.8 CF for D6. The feed ingredients used in diets generally vary as fish meal, poultry meal, blood meal, krill meal, hydrolysed feather protein, fish oil, soybean oil, soybean meal, soybean concentrated, wheat, wheat flour/middlings, wheat gluten, corn gluten/protein, sunflower meal, sunflower cake, guar protein, yeast extract, vitamins, and minerals. Each diet has different feed ingredients content.

Cultural Energy (CE, Mcal kg^{-1}) and Carbon Footprint (CF, kg CO_2e kg^{-1}) Expended Analyses

The CE and CF values of the compound diets were determined by the method given by Diken & Koknaroglu (2022) and Diken et al. (2022) (Feedipedia, 2002; IAFFD, 2020).

Based on the chemical analysis of the compound diets and the feed ingredients content, the CE values of the compound diets (Mcal kg^{-1}) were determined by multiplying the unit values of feed ingredients (Mcal kg^{-1}) with the usage percent rate of the feed ingredients (Tables 2, 3). It was calculated by multiplying the total consumed compound diet amount (Table 1) by the unit values of the feeds (Tables 2, 3), and the cultural

Figure 1. Karacaören-I Dam Lake (Türkiye) (Google Earth, 2022)

Figure 2. Karacaören-I Dam Lake rainbow trout cage farms

energy expended on consumed compound diet (CECD) values are given in Table 4, and the carbon footprint expended on a consumed compound diet (CFCD) values are given in Table 5. CE and CF expended calculations for the transport of compound diet, juvenile and harvested fish (cultural energy expended on transportation-CET, carbon footprint expended on transportation-CFT) were calculated by multiplying the distance and amount given in Table 1 by the unit values in Tables 2 and 3, and the results are given in Tables 6 and 7. The sustainability management of KRTC according to CECD, CFCD, CET, and CFT is given in Table 8.

Results and Discussion

Karacaören Dam Lake-I Rainbow Trout Cage Farming

In the production period of 2020-2021, 22 Karacaören Dam Lake-I rainbow trout cage farms (KRTC) produced 83.31% of their total annual project capacity (Table 1; Figures 1, 2). While the annual project capacity of inland water species was 215,022 tonnes and the production of inland aquaculture species was 136,042, 135,732 tonnes of this amount was met from trout production (GDFA, 2022). KRTC is the basin where production is above the average of Türkiye.

Table 2. Cultural energy values for input and output of compound diet and transportation of Karacaören-I Dam Lake rainbow trout cage farming

Items	Unit	Mcal unit^{-1}	References
CE expended on consumed compound diet			
Feed ingredients			
fish oil	kg	2.38	Chatvijitkul et al. (2017) & Davulis et al. (1977)
soybean oil	kg	2.24	Chatvijitkul et al. (2017) & Smith et al. (2007)
fish meal, anchovy	kg	4.45	Chatvijitkul et al. (2017) & Davulis et al. (1977)
krill meal	kg	17.95	Ecoinvent v3
blood meal	kg	5.45	Ecoinvent v3
poultry meal	kg	2.32	Chatvijitkul et al. (2017) & Davulis et al. (1977)
hydrolysed feather protein	kg	0.05	Ecoinvent v3
corn gluten/protein	kg	2.98	Chatvijitkul et al. (2017)
soybean meal	kg	0.93	Chatvijitkul et al. (2017) & Smith et al. (2007)
soybean concentrated	kg	5.43	Ecoinvent v3
sunflower meal	kg	0.68	Ecoinvent v3
sunflower cake*	kg	0.68	Ecoinvent v3
wheat	kg	0.95	Chatvijitkul et al. (2017) & Davulis et al. (1977)
wheat flour/middlings	kg	1.84	Chatvijitkul et al. (2017)
wheat gluten	kg	2.98	Chatvijitkul et al. (2017)
guar protein**	kg	0.93	Chatvijitkul et al. (2017)
yeast, extract	kg	28.32	Ecoinvent v3
vitamins	kg	0.09	Chatvijitkul et al. (2017)
minerals	kg	0.09	Chatvijitkul et al. (2017)
pellets production	kg	0.51	Hognes et al. (2011)

***CE expended* (Mcal kg^{-1})**							
Aquafeed	***1.9mm***	***3mm***	***4mm***	***4.5mm***	***5mm***	***6mm***	***Mean ± SD***
A		3.76	3.61		3.61	3.61	3.65 ± 0.08
B		3.60	3.57		3.57	3.57	3.58 ± 0.01
C		3.38	3.29		3.29	3.67	3.41 ± 0.18
D		3.34	3.22		3.34	3.11	3.25 ± 0.11
E	5.40	2.78		3.23		2.77	3.55 ± 1.25

CE expended on transportation			
Items	**Unit**	**Mcal unit^{-1}**	**References**
Truck	km.kg	0.00083	Pimentel (1980)

Note: CE = cultural energy. * Since sunflower meal and cake are derived from the same process, sunflower meal data is a very good approximation to sunflower cake data. ** Since guar is a typical Indian crop, the values of soybean meal have been used.

Table 3. Carbon footprint (kg CO_2e) values for input and output of compound diet and transportation of Karacaören-I Dam Lake rainbow trout cage farming

Items	Unit	kg CO_2e unit^{-1}	References
CF expended on consumed compound diet			
Feed ingredients			
fish oil	kg	0.99	Hognes et al. (2011)
soybean oil	kg	2.024	Schmidt (2015)
fish meal, anchovy	kg	0.99	Hognes et al. (2011)
krill meal	kg	5.4	Parker & Tyedmers (2012)
blood meal	kg	2.45	Ecoinvent v3
poultry meal	kg	3.14	Hognes et al. (2011)
hydrolysed feather protein	kg	0.0244	Ecoinvent v3
corn gluten/protein	kg	1.061	O'Brien et al. (2014)
soybean meal	kg	0.541	Moe et al. (2014)
soybean concentrated	kg	3.20	Hognes e al. (2011)
sunflower meal	kg	0.468	Ecoinvent v3
sunflower cake*	kg	0.468	Ecoinvent v3
wheat, Chile	kg	0.425	Vellinga et al. (2013)
wheat flour/middlings	kg	0.913	Ecoinvent v3
wheat gluten	kg	2.08	Hognes et al. (2011)
guar protein**	kg	0.164	Ecoinvent v3
yeast, extract	kg	5.91	Ecoinvent v3
vitamins	kg	1.62	Rotz et al. (2019)
minerals	kg	1.62	Rotz et al. (2019)
pellets production	kg	0.13	Hognes et al. (2011)

***CF expended* (kg CO_2e kg^{-1})**

Aquafeed	*1.9mm*	*3mm*	*4mm*	*4.5mm*	*5mm*	*6mm*	*Mean ± SD*
A		1.07	1.05		1.05	1.05	1.05 ± 0.01
B		1.03	1.03		1.03	1.03	1.03 ± 0.00
C		1.14	1.12		1.12	1.19	1.14 ± 0.03
D		1.41	1.39		1.40	1.38	1.40 ± 0.01
E	1.47	0.89		1.08		0.96	1.10 ± 0.26

CF expended on transportation

Items	Unit	kg CO_2e unit^{-1}	References
	km.tonnes	0.236, 0.468, 0.722	Robertson et al. (2015)

Note: CF = carbon foot print. *Since sunflower meal and cake are derived from the same process, sunflower meal data is a very good approximation to sunflower cake data. ** Since guar is a typical Indian crop, the values of Indian soybean meal have been used.

A total of 3,236,720 tonnes of compound diets were used from 5 different aquafeed factories in KRTC. The shortest distance between cage farms to aquafeed factories is 315 km, the longest distance is 427 km, and the average distance is 387 km (Table 1). KRTC FCR values were the lowest at 0.91, the highest at 1.18, and the average at 1.08. The companies numbered 1, 9, and 14 from the cage farms have used 3 different aquafeed factories (Table 1). Fifteen KRTC juvenile fish needs were met from hatcheries established on the Göksu Stream flowing into the Karacaören Dam Lake. While the average distance of the hatcheries to the KRTC basin is 93.1 km, the distance of the hatchery with the longest distance to the cage farm is 650 km (Table 1). The distance between the processing factories to the cage farms is 150.3 km on average, with the shortest at 67 km and the longest at 450 km. Cage farm 2 sent the harvested fish in half and half to 2 different processing factories (Table 1).

The management strategies were similar due to the kinship of the cage farms because some different cage farms were owned by the same person or company (Table 1).

Table 4. Cultural energy expended on consumed compound diet (CECD) of Karacaören-I Dam Lake rainbow trout cage farming

Code		**Total cultural energy expended on consumed compound diet (Gcal)**							
Farm	***Aquafeed***	***1.9mm***	***3mm***	***4mm***	***4.5mm***	***5mm***	***6mm***	***Σ***	
1	A		105.29	187.54		389.51	581.34	1,263.69	3.4163
	C		11.83	21.41		44.46	73.85	151.55	0.3689
	E	18.89	18.10		43.62		55.85	136.45	0.4097
								Σ1,551.69	4.1949
2	A		26.32	198.36		245.25	270.50	740.43	3.9293
3	A		7.52	21.64		36.07	50.49	115.72	3.7706
4	D		16.70	31.20		49.40	73.30	171.59	3.9538
5	C		10.14	29.64		32.93	40.32	113.03	3.4673
6	B		15.30	30.39		57.20	69.71	172.59	3.9336
7	B		15.30	30.39		58.98	71.50	176.16	3.9366
8	B		14.40	28.60		53.62	64.35	160.96	3.9259
9	A		12.03	25.97		54.82	73.64	166.46	3.7338
	C		1.35	2.96		6.26	9.44	20.01	0.4024
	E	2.16	2.51		6.14		7.14	17.94	0.4489
								Σ204.41	4.5851
10	C		6.76	21.41		23.05	29.32	80.54	3.4494
11	B		7.20	21.45		35.75	51.83	116.23	4.1510
12	E	215.85	167.09		64.62			447.56	4.0687
13	D		66.78	386.35		668.96	497.77	1,619.85	3.4465
14	A		105.29	187.54		389.51	581.34	1,263.69	3.4163
	C		11.83	21.41		44.46	73.85	151.55	0.3689
	E	18.89	18.10		43.62		55.85	136.45	0.4097
								Σ1,551.69	4.1949
15	D		66.78	257.56		501.72	622.21	1,448.27	3.4982
16	D		183.65	321.96		501.72	68.44	1,075.76	3.4590
17	D		16.70	64.39		334.48	140.00	555.56	3.5162
18	A		8.01	122.34		80.61		210.97	3.2068
19	D		46.75	80.49		133.79	18.67	279.69	3.4530
20	D		13.36	25.76		20.07	21.78	80.96	3.5199
21	D		13.36	25.76		20.07	21.78	80.96	3.5199
22	B		3.60	17.87		17.87	35.75	75.09	3.2650
Σ/Average		255.78	992.03	2,163.37	157.99	3,800.56	3,659.99	11,029.72	3.7475

Note:  = CECD value per kg of rainbow trout aquaculture (Mcal).

Cultural Energy and Carbon Footprint Expended on Consumed Compound Diet

The average lowest and highest CE and CF expended values of the compound diets were calculated as 3.65 and 3.25 Mcal kg^{-1} and 1.03 and 1.40 kg CO_2e kg^{-1} (Tables 2, 3). This situation is related to the rate of use of feed ingredients depending on the chemical composition of the compound diets. It is similar to the difference in the embodied energy values of their feeds depending on the feed ingredients content reported by Chatvijitkul et al. (2017). The results were similar to the 3.40 Mcal kg^{-1} CE expended value of rainbow trout diets reported by Diken & Koknoroglu (2022), but higher than the 0.97 kg CO_2e kg^{-1} CF expended value reported by Diken et al. (2022). In addition, considering the report of Boissy et al. (2011), which states that depending on the diet content, the climate change effect (kg CO_2e) of a plant-based diet in trout feed is 6% lower than that of a fish meal-based standard diet, it can be concluded that the CF of diets can be improved in trout aquaculture. At the same time, it has been reported that the choice of different feed production systems and feed ingredients considering the distance effect should be evaluated in terms of environmental impact strategies to create the less global warming effect of aquaculture feeds (da Silva Pires et al., 2022). Although these approaches reveal the importance of plant-derived feed ingredients for the sustainability of trout diets, attention should be paid to the kg CO_2e unit^{-1} values of feed ingredients given in Table 3 in compound diet rations.

Table 5. Carbon footprint expended on consumed compound diet (CFCD) of Karacaören-I Dam Lake rainbow trout cage farming

Code		**Total Carbon Footprint Expended on Consumed Compound Diet (tonne CO_2e)**							
Farm	***Aquafeed***	***1.9mm***	***3mm***	***4mm***	***4.5mm***	***5mm***	***6mm***	***Σ***	
1	A		30.00	54.53		113.26	169.04	366.83	0.9917
	C		3.98	7.27		15.10	23.90	50.24	0.1358
	E	5.15	5.81		14.55		19.26	44.76	0.1210
								Σ 461.83	1.2485
2	A		7.50	57.68		71.31	78.65	215.14	1.1417
3	A		2.14	6.29		10.49	14.68	33.60	1.0949
4	D		7.04	13.86		21.07	27.63	69.60	1.6038
5	C		3.41	10.07		11.18	13.05	37.70	1.1566
6	B		4.37	8.75		16.47	20.08	49.67	1.1321
7	B		4.37	8.75		16.99	20.59	50.70	1.1330
8	B		4.12	8.24		15.44	18.53	46.33	1.1299
9	A		3.43	7.55		15.94	21.60	48.52	1.0884
	C		0.45	1.01		2.13	3.05	6.64	0.1489
	E	0.59	0.36		0.97		2.46	4.38	0.0982
								Σ 59.54	1.3355
10	C		2.27	7.27		7.83	9.49	26.86	1.1503
11	B		2.06	6.18		10.30	14.93	33.46	1.1950
12	E	58.81	53.65		21.56			134.02	1.2183
13	D		28.17	166.33		280.92	221.05	696.47	1.4819
14	A		30.00	54.53		113.26	169.04	366.83	0.9917
	C		3.98	7.27		15.10	23.90	50.24	0.1358
	E	5.15	5.81		14.55		19.26	44.76	0.1210
								Σ 461.83	1.2485
15	D		28.17	110.89		210.69	276.31	626.06	1.5122
16	D		77.47	138.61		210.69	30.39	457.17	1.4700
17	D		7.04	27.72		140.46	62.17	237.40	1.5025
18	A		2.57	39.85		25.27		67.70	1.0290
19	D		19.72	34.65		56.18	8.29	118.85	1.4672
20	D		5.63	11.09		8.43	9.67	34.82	1.5140
21	D		5.63	11.09		8.43	9.67	34.82	1.5140
22	B		1.03	5.15		5.15	10.30	21.62	0.9400
Σ /Average		69.69	350.19	804.63	51.63	1,402.09	1,296.98	3,975.21	1.2827

Note:  = CFCD value per kg of rainbow trout aquaculture (kg CO_2e)

Table 6. Cultural energy expended on transportation (CET) of Karacaören-I Dam Lake rainbow trout cage farming

Code		**Aquafeed**		**Hatchery**		**Processing Factory**		**Σ**
Farm	***Aquafeed***	***Σ Gcal***		***Σ Gcal*** CO_2e		***Σ Gcal*** CO_2e		
1[a,b,c]	A	110.13	0.2977	0.58	0.0016	19.52	0.0528	
	C	15.11	0.0408	0.31	0.0008	29.20	0.0789	
	E	14.96	0.0404					
		Σ140.20	0.3790	Σ0.89	0.0024	Σ48.71	0.1317	0.5131
2[b,c]	A	64.66	0.3431	0.46	0.0025	36.59	0.1942	0.5781
				0.01	0.0001	7.22	0.0383	
				Σ0.48	0.0025	Σ43.81	0.2325	
3[b]	A	10.09	0.3289	0.07	0.0024			
			0.01	0.0005				
			Σ0.09	0.0029		13.72	0.4470	0.7788
4[b]	D	17.72	0.4083	2.03	0.0469			
				1.46	0.0337			
				Σ3.50	0.0806	27.51	0.6340	1.1229
5[b]	C	11.42	0.3504	0.07	0.0023			
			0.30	0.0092				
			Σ0.37	0.0115		2.71	0.0831	0.4449
6	B	12.61	0.2875	0.12	0.0026	5.78	0.1317	0.4218
7	B	12.88	0.2877	0.12	0.0026	5.78	0.1291	0.4194
8	B	11.77	0.2629	0.12	0.0028	5.78	0.1409	0.4066
9[a,c]	A	14.57	0.3268			1.50	0.0337	
	C	2.00	0.0054			4.17	0.0936	
	E	1.98	0.0444					
		Σ18.55	0.3767	0.15	0.0033	Σ5.67	0.1272	0.5071
10[b]	C	8.13	0.3483	0.07	0.0032			
				0.30	0.0128			
				Σ0.37	0.0160	1.81	0.0773	0.4416
11	B	8.50	0.3035	0.01	0.0004	4.33	0.1547	0.4586
12	E	40.64	0.3694	19.09	0.1735	8.67	0.0788	0.6217
13	D	166.42	0.3541	8.72	0.0185	38.93	0.0828	0.4554
14[a,b,c]	A	110.13	0.2977	0.58	0.0016	19.52	0.0528	
	C	15.11	0.0408	0.31	0.0008	29.20	0.0789	
	E	14.96	0.0404					
		Σ121.36	0.3790	Σ0.89	0.0024	Σ48.71	0.1317	0.5131
15	D	149.77	0.3618	10.46	0.0253	35.03	0.0846	0.4717
16	D	109.11	0.3508	194.22	0.6245	28.06	0.0902	1.0656
17	D	56.58	0.3581	3.49	0.0221	13.62	0.0862	0.4664
18	A	20.93	0.3182	3.85	0.0585	15.43	0.2346	0.6113
19	D	28.36	0.3501	75.53	0.9325	8.02	0.0990	1.3816
20	D	8.32	0.3618	0.58	0.0253	1.98	0.0859	0.4729
21	D	8.34	0.3627	0.60	0.0259	1.98	0.0859	0.4745
22	B	5.49	0.2387	0.06	0.0025	2.21	0.0960	0.3372
Σ/Average		1,050.70	0.3400	323.69	0.0927	368.24	0.1566	0.5893

Note: = CET value per kg of rainbow trout aquaculture (Mcal). It includes calculations resulting from a = aquafeed factory distance difference and b,c = difference of vehicles used in transportation

Table 7. Carbon footprint expended on transportation (CFT) of Karacaören-I Dam Lake rainbow trout cage farming

Code		**Aquafeed**		**Hatchery**		**Processing Factory**		**Σ**
Farm	***Aquafeed***	***Σ tonne CO_2e***		***Σ tonne CO_2e***		***Σ tonne CO_2e***		
1[a,b,c]	A	31.32	0.0847	0.17	0.0004	5.55	0.0150	
	C	4.30	0.0116	0.09	0.0002	8.30	0.0224	
	E	4.25	0.0115					
		Σ39.87	0.1078	Σ0.25	0.0007	Σ13.85	0.0374	0.1459
2[b,c]	A	18.38	0.0976	0.13	0.0007	20.63	0.1095	
				0.01	0.0001	4.07	0.0216	
				Σ0.14	0.0008	Σ24.70	0.1311	0.2294
3[b]	A	3.53	0.1151	0.04	0.0014			
				0.01	0.0004			
				Σ0.06	0.0018	7.74	0.2521	0.3689
4[b]	D	5.04	0.1161	0.58	0.0133			
				0.42	0.0096			
				Σ0.99	0.0229	7.82	0.1803	0.3193
5[b]	C	3.97	0.1219	0.06	0.0020			
				0.17	0.0052			
				Σ0.23	0.0072	1.53	0.0468	0.1759
6	B	3.59	0.0818	0.03	0.0008	3.26	0.0742	0.1567
7	B	3.66	0.0818	0.03	0.0007	3.26	0.0728	0.1553
8	B	3.35	0.0816	0.03	0.0008	3.26	0.0794	0.1618
9[a,c]	A	4.14	0.0929	0.04	0.0009	0.43	0.0096	
	C	1.74	0.0127			1.19	0.0266	
	E	1.72	0.0126					
		Σ7.60	0.1183			Σ1.61	0.0362	0.1554
10[b]	C	2.31	0.0990	0.06	0.0028			
				0.17	0.0072			
				Σ0.23	0.0100	1.02	0.0436	0.1526
11	B	2.96	0.1059	0.01	0.0004	2.44	0.0872	0.1935
12	E	11.55	0.1050	5.43	0.0493	21.23	0.1930	0.3474
13	D	47.32	0.1007	2.48	0.0053	7.75	0.0165	0.1224
14[a,b,c]	A	31.32	0.0847	0.17	0.0004	5.55	0.0150	
	C	4.30	0.0116	0.09	0.0002	8.30	0.0224	
	E	4.25	0.0115					
		Σ39.87	0.1078	Σ0.25	0.0007	Σ13.85	0.0374	0.1459
15	D	42.59	0.1029	2.97	0.0072	9.96	0.0241	0.1341
16	D	31.02	0.0998	55.22	0.1776	7.98	0.0257	0.3030
17	D	16.09	0.1018	0.09	0.0063	3.87	0.0245	0.1326
18	A	5.95	0.0905	1.10	0.0166	4.20	0.0667	0.1738
19	D	8.06	0.0996	21.48	0.2651	2.28	0.0281	0.3928
20	D	2.37	0.1029	0.17	0.0072	0.56	0.0244	0.1345
21	D	2.37	0.1031	0.17	0.0074	0.56	0.0244	0.1349
22	B	1.56	0.0679	0.03	0.0014	1.92	0.0835	0.1528
Σ/Average		304.94	0.1004	92.35	0.0269	149.37	0.0723	0.1995

Note: = CFT expended per kg of rainbow trout aquaculture (kg CO_2e). It includes calculations resulting from a = aquafeed factory distance difference and b,c = difference of vehicles used in transportation

Table 8. Total cultural energy and carbon footprint expended values in Karacaören-I Dam Lake rainbow trout cage farming sustainability management

Cage Farm	Compound Diet		Transportation								TOTAL		Compound Diet (%)		CE:CF
			Aquafeed		*Hatchery*		*Processing factory*		*Σ*						
	CE	*CF*	*CE*	*CF*	*CE*	*CF*	*CE*	*CF*	*CE*	*CF*	*CE*	*CF*	*CE*	*CF*	
1	4.1949	1.2485	0.3790	0.1078	0.0024	0.0007	0.1317	0.0374	0.5131	0.1459	4.7080	1.3944	89.10	89.54	3.3763
2	3.9293	1.1417	0.3431	0.0976	0.0025	0.0008	0.2325	0.1311	0.5781	0.2294	4.5074	1.3711	87.17	83.27	3.2874
3	3.7706	1.0949	0.3289	0.1151	0.0029	0.0018	0.4470	0.2521	0.7788	0.3689	4.5494	14639	82.88	74.80	3.1078
4	3.9538	1.6038	0.4083	0.1161	0.0806	0.0229	0.6340	0.1803	1.1229	0.3193	5.0766	1.9231	77.88	83.40	2.6399
5	3.4673	1.1566	0.3504	0.1219	0.0115	0.0072	0.0831	0.0468	0.4449	0.1759	3.9121	1.3325	88.63	86.80	2.9360
6	3.9336	1.1321	0.2875	0.0818	0.0026	0.0008	0.1317	0.0742	0.4218	0.1567	4.3554	1.2889	90.31	87.84	3.3792
7	3.9366	1.1330	0.2877	0.0818	0.0026	0.0007	0.1291	0.0728	0.4194	0.1553	4.3560	1.2884	90.37	87.94	3.3810
8	3.9259	1.1299	0.2629	0.0816	0.0028	0.0008	0.1409	0.0794	0.4066	0.1618	4.3326	1.2918	90.61	87.47	3.3540
9	4.5851	1.3355	0.3767	0.1183	0.0033	0.0009	0.1272	0.0362	0.5071	0.1554	5.0923	1.4909	90.04	89.58	3.4155
10	3.4494	1.1503	0.3483	0.0990	0.0160	0.0100	0.0773	0.0436	0.4416	0.1526	3.8910	1.3029	88.65	88.29	2.9864
11	4.1510	1.1950	0.3035	0.1059	0.0004	0.0004	0.1547	0.0872	0.4586	0.1935	4.6097	1.3884	90.05	86.07	3.3200
12	4.0687	1.2183	0.3694	0.1050	0.1735	0.0493	0.0788	0.1930	0.6217	0.3474	4.6905	1.5657	86.74	77.81	2.9957
13	3.4465	1.4819	0.3541	0.1007	0.0185	0.0053	0.0828	0.0165	0.4554	0.1224	3.9019	1.6043	88.33	92.37	2.4322
14	4.1949	1.2485	0.3790	0.1078	0.0024	0.0007	0.1317	0.0374	0.5131	0.1459	4.7080	1.3944	89.10	89.54	3.3763
15	3.4982	1.5122	0.3618	0.1029	0.0253	0.0072	0.0846	0.0241	0.4717	0.1341	3.9699	1.6463	88.12	91.85	2.4114
16	3.4590	1.4700	0.3508	0.0998	0.6245	0.1776	0.0902	0.0257	1.0656	0.3030	4.5246	1.7730	76.45	82.91	2.5520
17	3.5162	1.5025	0.3581	0.1018	0.0221	0.0063	0.0862	0.0245	0.4664	0.1326	3.9826	1.6351	88.29	91.89	2.4357
18	3.2068	1.0290	0.3182	0.0905	0.0585	0.0166	0.2346	0.667	0.6113	0.1738	3.8180	1.2028	83.99	85.55	3.1743
19	3.4530	1.4672	0.3501	0.0996	0.9325	0.2651	0.0990	0.0281	1.3816	0.3928	4.8346	1.8601	71.42	78.88	2.5991
20	3.5199	1.5140	0.3618	0.1029	0.0253	0.0072	0.0859	0.0244	0.4729	0.1345	3.9929	1.6485	88.16	91.84	2.4222
21	3.5199	1.5140	0.3627	0.1031	0.0259	0.0074	0.0859	0.0244	0.4745	0.1349	3.9944	1.6489	88.12	91.82	2.4225
22	3.2650	0.9400	0.2387	0.0679	0.0025	0.0014	0.0960	0.0835	0.3372	0.1528	3.6022	1.0928	90.64	86.02	3.2964
Ave.	3.7475	1.2827	0.3400	0.1004	0.0927	0.0269	0.1566	0.0723	0.5893	0.1995	4.3368	1.4822	86.59	86.61	2.9682

Note: = Mcal or kg CO_2e expended corresponding per kg of rainbow trout aquaculture. Ave. = Average

The CD/A-4mm, 5mm, and 6mm diets with the same chemical compositions had lower CE and CF expended values compared to the CD/A-3mm diet (Tables 2, 3). This situation was due to the rate of use of fish meals, the high crude protein value of the A-3mm diet, and the low crude fibre. While the crude fibre ratios of the B diets were the same, the CE expended value was high due to the high crude protein value of the B-3mm diet (Table 2). Crude protein and crude fat total values were similar at a rate of 64% in CD/B-3mm and 65% in other B diets, respectively. While the CE value of the fish meal was higher than fish oil, the CF values were similar (Tables, 3, 4). Because of this situation, while the CE expended value of the CD/B-3mm diet was high, the CF values of all B diets were similar. The difference in the crude fibre values of the C diets affected the CE and CF expended values of the diets. The CE and CF expended values of the 6mm diet with the lowest crude fibre values were higher than the other C diets (Tables 2, 3). The crude fibre and crude protein ratios of D diets affected the CE and CF expended value. The 6mm diet with a high crude fibre value was the diet with low CE and CF expended value (Tables 2, 3). Compared to the CD/D-6mm diet, the 4 mm diet, which had a high crude protein value and a low crude fibre value had the lowest CE and CF expended values after this diet. The difference in feed ingredients used in the E diets affected the CE and CF expended values of the diets (Tables 2, 3). At the same time, the increase in the crude fibre ratio and the decrease in the crude protein ratio affected these values. The limiting effect of the crude fibre value of the diet formulations on the feed ingredient utilization rate primarily affected the CE and CF expended values of the diets. In addition, the feed ingredient differences of the same diet groups also affected the CE and CF expended values of the diets (Tables 2, 3).

Rainbow trout farming with different compound diets in KRTC 1, 9, and 14 fish farms increased the cultural energy expended on consumed compound diet (CECD) and carbon

footprint expended on consumed compound diet (CFCD) values per kg of rainbow trout aquaculture (Tables 4, 5). Although the FCR values of these farms were similar, the CECD and CFCD values increased to raise per kg of rainbow trout aquaculture due to the low amount of harvested fish from farm 9. While the CECD value of farm 22 using a B diet with low FCR values was low per kg of rainbow trout aquaculture, the high FCR value of farm 11 using the same diet increased this value (Tables 1, 4). The CECD value increased depending on the FCR value of the D compound diet with a low CE expended value (Table 1, 4). The increase in FCR values of farms 15, 16, and 17 using the D compound diet increased the CECD value per kg of rainbow trout aquaculture. Farms 13 and 19 using the D compound diet had a low CECD per kg of rainbow trout aquaculture.

The reason why the CECD value of farm 10 using compound diet C was similar to farms 13 and 19 per kg of rainbow trout aquaculture was due to the low FCR (Tables 1, 4). Farm 22 in the basin had a low CFCD value per kg of rainbow trout aquaculture (Tables 1, 5). The most important factor in this value was that although the CF expended value of compound diet B was low, the amount of diet consumed due to FCR was low (Table 1).

Farms 13 and 19 with low FCR had a low CECD per kg of rainbow trout aquaculture, but a high CFCD (Tables 1, 4, 5). This is due to the CF value of compound diet. As the FCRs of the farms using the same compound diets increased, the CECD and CFCD values of the compound diets consumed per kg of rainbow trout aquaculture increased (Tables 1, 4, 5). The CECD value per kg of rainbow trout aquaculture in the basin was high in farm 4, depending on the high value in the FCR (Tables 1, 5). FCR was the most influential factor over CECD and CFCD value per kg of rainbow trout aquaculture. In general, 5 and 6 mm compound diets from the grow-out diets of farms increased the CECD and CFCD values, and depending on these values, it increased the Mcal and kg CO_2e values per kg of rainbow trout aquaculture (Tables 1, 4, 5).

Depending on the nutritional habits of the cultivated species, the diversity of feed ingredients used in compound diets and the formulation differences affected the CE and CF expended values (Tables 1, 2, 3). In aquaculture, the feed had a high energy input of 53-86% (Pelletier et al., 2011; Diken & Koknaroglu, 2022). This rate was similar to broiler and layer hen production. The reason for this was the use of high-quality feed ingredients in the feed of chickens and laying hens (Koknaroglu & Atilgan, 2007; Akunal & Koknaroglu, 2021). The reason for the high CE expended values of carnivorous species such as rainbow trout was due to the need for feed ingredients (fish meal, fish oil, corn and wheat gluten, soybean concentrated, etc.) of animal origin and/or higher protein value in their diets. The CE expended values of diets belonging to carnivorous species such as rainbow trout were high due to feed ingredients with low CE expended value from other ruminant livestock (sheep) (Demircan & Koknaroglu, 2007; Demircan, 2008; Koknaroglu, 2008, 2010; Cinar & Koknaroğlu, 2019; Koknaroglu & Hoffman, 2019). According to Chatvijitkul et al. (2017), and Diken & Konaroglu (2022), the CE expended value of rainbow trout compound diets was between 2.93-3.40 Mcal kg^{-1}. In this study, the CE expended an average value of compound diets between 3.25-3.65 Mcal kg^{-1} (Table 3). The CE expended values for hybrid catfish, tilapia, pangasius, Atlantic salmon, whiteleg shrimp, back tiger shrimp from other aquatic species were reported as 1.17, 1.39, 1.27, 2.98, 2.17, 2.54 Mcal kg^{-1}, respectively (Chatvijitkul et al., 2017). The percentage of crude protein in European seabass compound diets was between 3.61-4.21 Mcal kg^{-1}, due to its relatively high value related to the rainbow trout diet (Diken et al., *unpublished*). The CE value of one kg of concentrated feed for beef cattle and dairy cattle was 1.13 Mcal kg^{-1} (Demircan, 2008; Koknaroglu, 2008) and 1.30 Mcal kg^{-1}, respectively.

Considering the 77.78% and 77.88% cultural energy and 72.60% carbon footprint values of the consumed compound diet, excluding the transportation values of rainbow trout cage farming (Diken et al., 2021, 2022; Diken & Koknaroglu, 2022), also in this study, the high rates of CE and CFP expended values of compound diet and consumed compound diet due to FCR support the result of feed-induced CE and CF budget increase. Flos & Reig (2017) reported that the feed had an energy share of 79% in intensive salmon cage cultivation. The CECD expended rate of earthen pond European seabass farming was calculated as 28.06% (Diken et al., *unpublished*). Together with these reports, the results of the current study revealed that CE and CF expended values based on feed should be considered as sustainability criteria in trout farming (Tables 5, 6).

In addition to a fish meal with high protein values, high emissions due to land use, such as soybean production, had affected salmonid feed emissions (MacLeod et al., 2020). Ziegler et al. (2021), reported that 85% of the total CF in salmon production was made up of feed. Similarly, the cage had a high share in rainbow trout farming compared to feed, and fish and feed transportation (Tables 4, 5, 6, 7, 8). The result of the study showed that the use of FCR and high-emission feed ingredients increased the CF expended value, supporting the finding that FCR increase in salmon fish farming and feed inputs with intense emissions caused an increase in emissions in production (Ziegler et al., 2021) (Tables 1, 3, 5). This and similar approaches enable us to understand the statements that CO_2e was reported to be used on feed labels as an indicator of the sustainability of the private aquaculture sector (Hatchery Feed & Management, 2021).

Cultural Energy and Carbon Footprint of Transportation

The compound diet transportation of cage farms 6, 7, 8, and 22 closest to the aquafeed factory in KRTC were the farms with low CET and CFT values per kg of rainbow trout aquaculture (Tables 1, 6, 7). Among these farms, farm 22, which had low transport distances, has the lowest CET and CFT values per kg of rainbow trout aquaculture (Tables 1, 6, 7). Farm 4 was the farthest from the aquafeed factory and processing factory (Table 1). CET was high per kg of rainbow trout aquaculture due to distance (Table 6). However, the CEF value for transportation per kg of rainbow trout aquaculture was not high (Table 7).

Farm 11 had the lowest CET and CFT values per kg of rainbow trout aquaculture in the basin, due to the low-capacity rainbow trout farming, the low need for juvenile fish, and the supply of juvenile fish from the close-range hatchery on the Göksu Stream located in the same basin (Tables 1, 6, 7). Since cage farms 1 and 14, which had the highest rainbow trout aquaculture in the basin, meet the need for juvenile fish from the hatcheries in the same basin, and the distance to the processing factory was below the average values of the basin, the CE and CF expended values of transportation were low (Table 1, 6, 7). Since cage farms 16 and 19 meet their juvenile fish needs from the same hatchery at the farthest distance, and the need for juvenile fish was high, the CET and CFT expended values were high (Tables 1, 6, 7). Cage farms 1, 2, 3, 6, 7, 8, 9, 11, 14, and 22, which provide juvenile fish needs from hatcheries on Göksu Stream, were farms with low CET and CFT values per kg of rainbow trout aquaculture (Tables 1, 6, 7).

CET and CFT of harvested fish per kg of rainbow trout aquaculture due to the proximity of farm 13 with the highest production to the processing factory were low (Tables 1, 6, 7). The CFT value of the harvested fish was high per kg of rainbow trout aquaculture since farm 22 transports the harvested fish with a low-capacity vehicle (Table 7).

If the distance of a farm to the aquafeed factory, hatchery, and processing factory in KRTC was below the average of the basin, the CET and CFT values per kg of rainbow trout aquaculture were low (Tables 1, 6, 7). The average values of 0.5893 Mcal kg^{-1} CET and 0.1995 kg CO_2e CFT per kg of rainbow trout aquaculture of farms 1, 14, and 15, which had the highest production in the basin, were below the basin average values (Tables 1, 6, 7). These results support the statement that the transport distance reported by Diken et al. (2021, 2022), affected the value of CE and CF expended transportation in trout farming. It has also been reported that CE expended transportation will increase by 2.22-3.08% in the simulation of the fact that the need for feed and juvenile fish in earthen pond European sea bass farming was provided from farther region enterprises (Diken et al., *unpublished*). Similarly, in the rainbow trout cage farming simulation study by Diken (2021), it was reported that the CE expended transportation value of the farms that met the compound diet requirement from longer distances, where the FCR ratios did not change, increased significantly. A private firm reported that they were planning to establish a feed facility on a salmon farm to reduce the carbon footprint of feed-related transportation (Hatchery International, 2021). As a result, it was reported that the transportation distance compared to the feed had a lower share of CE and CF expended but had an effect that should be taken into account in rainbow trout farming (Diken et al., 2021, Diken & Koknaroglu, 2022, Diken, *unpublished*).

Climate change is effective in the growth and food security of the aquaculture sector (Cubillo et al., 2021). It was reported that Norwegian salmon aquaculture in open cages had a lower CF value than RAS cultivation in the United States, but the CF value of imported Norwegian salmon offered for consumption in the USA increased due to transportation involving the transportation of Norwegian salmon to the USA (Liu et al., 2016). These reports draw attention to the CF value associated with transportation in aquaculture and support this report in terms of sustainability in the current study. These evaluations revealed that the CF global-scale approach to the sustainability of aquaculture, production should be handled on a national and local basis. There was a relationship between CF analysis and energy calculations (Flos & Reig, 2017) and Ziegler et al. (2021), who reported that cage salmon farming had a total CF feed share of 85% due to differences in aquaculture production systems. Similarly, it was reported that 90% of the CF of different aquaculture systems in India was from feed (Adhikari et al., 2013). These results are considered an important criterion to be considered in the sustainability of feed and feed-based studies in aquaculture. In the results of the evaluation of KRTC management in Table 8, it had been determined that the compound diet budget had an important place in the sustainability of rainbow trout farming, according to the average values of 86.59% CECD and 86.61% CFCD per kg of rainbow trout aquaculture. The 1.2-2.7 kg CO_2e value per kg live-weight gain of Atlantic salmon farming presented in the Pelletier & Tyedmers (2007), report is similar to the feed and transportation values of the study (Table, 8). It was important for CET sustainability that the hatcheries, where cage farms provide juvenile fish, were very close to Karacaören-I Dam Lake. In addition, when the report of Korkut et al. (2007), was examined, it could be stated that the distances of Türkiye aquafeed factories to the basin were below the Türkiye average, which is important in CET sustainability. Considering that the estimated distance of the Aegean aquafeed factory evaluated in the study in another dam lake where intensive production was made in Türkiye was around 3.5 times, the average distance of the Karacaören-I Dam Lake Basin, the cage farm compound diet transportation value of the other basin with the same production capacity would be calculated 3.5 times more.

Troel et al. (2004) reported that the increase in capacity had a positive effect on the energy used for unit production. Similarly, Demircan & Koknaroglu (2007) reported that the increase in farm size had a positive effect on energy use efficiency. According to these evaluations and the results of the study, production should be made according to the project capacity in terms of sustainability in KKTC. The 2.9682 CE:CF KRTC sustainability value of the catchment compound diet and transportation given in Table 8 should be taken into account in future studies.

Conclusion

One of the most important factors affecting the CE and CF expended values of rainbow trout farming was the CE and CF expended value and FCR of the compound diet, depending on the feed ingredients and usage rates. Depending on the FCR, with the value of CE and CF expended from the compound diet, transportation can be considered as a sustainability criterion in terms of production and food safety of rainbow trout farming. KRTC was in a sustainable position in terms of its distance from the aquafeed factory, hatchery, and processing facility. KRTC was in a sustainable position in terms of its distance from the aquafeed factory, hatcheries, and processing facilities. Aquaculture facilities need to produce according to their annual project production capacity in terms of reducing the CE and CF expended sustainability values per kg of rainbow trout aquaculture. In terms of aquaculture systems and aquaculture types, it is recommended to develop the sustainability index of the aquaculture species (species-specific) and aquaculture system (system-specific) CE and CF expended values.

Acknowledgments

The author would like to thank Dr. Hayati Koknaroglu, Dr. Hüseyin Sevgili, Dr. Joël Aubin, and Dr. Jacopo Bacenetti for their help during the study, and Fisheries Technician İsmail Can for fieldwork support.

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.

Funding

There was no funding for the research.

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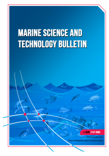
Mar. Sci. Tech. Bull. (2022) 11(4): 493-500
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1184231



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RESEARCH ARTICLE

First data on some biological aspects of the Caucasian goby, *Ponticola constructor* (Nordman, 1840) (Teleostei: Gobiidae) from the lower Çoruh River Basin (NE Türkiye)

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ARTICLE INFO

Article History:
Received: 04.10.2022
Received in revised form: 27.10.2022
Accepted: 28.10.2022
Available online: 22.12.2022

Keywords:
Borçka Dam Lake
Caucasian goby
Fulton's condition factor
Growth type
Length-weight relationship
Ponticola constructor

ABSTRACT

The present study describes for the first time some biological aspects (size composition, sex ratio, length-weight relationship and condition factor) of *Ponticola constructor* (Caucasian goby) inhabiting Borçka Dam Lake and its tributaries from the lower Çoruh River Basin located in the northeastern Türkiye Fish samples were caught from June 2017 to May 2018 by using trammel nets with various mesh sizes and by pulsed DC electrofishing device. A total of 145 Caucasian goby (78 females and 67 males) ranging from 6.8 cm to 13.4 cm TL were sampled during the study. The sex ratio of female to male was calculated as 1.16:1.00 which did not deviate from the expected ratio of 1:1 (χ^2 = 0.834, p>0.05). The total length-weight relationships were determined as W= 0.016TL$^{2.896}$ (r^2=0.995, P<0.05) for females, as W= 0.018TL$^{2.841}$ (r^2=0.993, P<0.05) for males and as W= 0.017TL$^{2.856}$ (r^2=0.994, P<0.05) for combined sex with the b-values were significantly different from isometric growth (b=3) indicating negative allometric growth of Caucasian goby for all sexes (Pauly's t- test, P<0.05). The regression analysis revealed that the coefficient of determination was r^2>0.99 showing a highly significant correlation between total length and weight. The differences in slopes (b values) of the LWR were statistically significant for both sexes (ANCOVA, P<0.05). Fulton's condition factor (K) values ranged from 1.124 to 1.312 in females and from 1.076 to 1.426 in males indicating a good growth condition in this habitat. No significant difference was observed in mean K values between females and males (t-test, P>0.05). The present study will provide a baseline on some biological parameters of *Ponticola constructor* to provide a guideline for the fisheries management authority and fisheries scientists for further investigations as well as presents the first known reference on the LWRs of this species for the FishBase database. Furthermore, the reported results may contribute to the conservation and sustainability of this species in the area.

Please cite this paper as follows:

Yeşilçiçek, T. (2022). First data on some biological aspects of the Caucasian goby, *Ponticola constructor* (Nordman, 1840) (Teleostei: Gobiidae) from the lower Çoruh River Basin (NE Türkiye). *Marine Science and Technology Bulletin*, *11*(4), 493-500. https://doi.org/10.33714/masteb.1184231

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Introduction

The length and weight data of fishes have commonly been studied to obtain biological information and has become one of the standard methods used in fisheries biology (Le Cren, 1951; Yesilcicek et al., 2015). Length-weight relationships (LWRs) and condition factor are of great importance in fishery assessment studies due to providing information about the growth of the fish, its general wellbeing, and fitness in the existing habitat (Yesilcicek et al., 2015; Jisr et al., 2018). The biometric studies which give information on fish species for an estimated assessment of their biomass are keystone tools for research and management (Zargar et al., 2012). In biometric studies, it is essential to determine the growth characteristics related to the length and weight of the fish (Morato et al., 2001), in addition to the condition of wellbeing of the species influenced by different biological and environmental factors. The length-weight relationships (LWRs) in fish also provide significant information about the general health, growth pattern, life history, habitat conditions, fish fatness and condition, as well as morphological characteristics of the fish (Schneider et al., 2000; Froese, 2006).

In fish populations, sex ratio and size structure provide basic information to assess reproductive potential and estimating stock size (Vicentini & Araújo, 2013). Size structure also plays an important role in maintaining reproductive potential and stability of a fish population. Hence, investigating the change of size structure may provide insight of how resilient a fish population can be (Tu et al., 2018).

Ponticola Iljin, 1927 is a genus of the family Gobiidae which was originally described as a subgenus of *Neogobius* and transferred to the genus level as a result of molecular analysis (Neilson & Stepien, 2009). The genus *Ponticola* is endemic to Black and Caspian Seas and does not occur in Marmara and Mediterranean Seas (Freyhof, 2011). While this genus comprises 18 valid species recorded from the Black Sea and Caspian Sea basins (Fricke et al., 2021), in Turkish freshwaters this genus is represented by only 6 species (Cicek et al., 2020). *Ponticola constructor* (Nordmann, 1840) also, commonly known as the Caucasian goby or Blackbelly river goby is a benthopelagic freshwater species which inhabits a wide variety of flowing waters from cold hill to foot hill streams and never found in brackish water. The Caucasian goby has a restricted distribution area in European and Asian Caucasus. It distributes in western tributaries of River Kuban in northern Caucasus and in coastal Black Sea drainages of southern Caucasus from Coruh to Psyrtskha (IUCN, 2008; Froese & Pauly, 2022). The Caucasian goby, *P. constructor* is globally listed as Least Concern (LC) category by the IUCN Red List of Threatened Species (IUCN, 2008), however, this species is considered as Endangered (EN) in inland waters of Türkiye due to dams' construction on rivers, habitat loss, water abstraction, eutrophication and pollution (Fricke et al., 2007).

There is no study in the available literature on the biological aspects, such as size structure, sex ratio, length–weight relationships (LWRs) and condition factor of *P. constructor* from different populations in its distribution area. To the best of the author's knowledge, this is the first study on some biological aspects of *P. constructor* both in Çoruh river basin and in its other distribution areas as well. The present study firstly describes the size structure, sex ratio, LWRs and Fulton's condition factor (K) of *P. constructor* inhabiting Borçka Dam Lake and its tributaries located in the lower Coruh river basin in the North Eastern Black Sea region of Türkiye. Hereby, the present study aims to provide a baseline on these issues of *P. constructor* to provide a guideline for the fisheries management authority and fisheries scientists for further investigations. This study also presents the first known reference on the LWR for the Caucasian goby for the FishBase database. Furthermore, the reported results may contribute to the conservation and sustainability of this species in the area.

Material and Methods

Study Area

This study was carried out in Borçka Reservoir and its tributaries, which is located on the lower Çoruh River Basin in North eastern Black Sea region of Türkiye (Figure 1). The reservoir has 419 hm^3 volume at normal water level and a surface area of 10,84 km^2. Coruh River has a total length of 431 km, within 410 km of borders of Türkiye and the last 21 km in Batumi (Georgia) where the river falls into the Black Sea (Yesilcicek & Kalayci, 2020).

Fish Sampling and Measurement

P. constructor samples were caught from June 2017 to May 2018 by using trammel nets with various mesh sizes and by pulsed DC electrofishing device from reservoir and its tributaries. Each fish specimen was measured in total length (TL) to the nearest 1 mm and weighed to the nearest 0.01 g body weight (W). Sex was determined via macroscopic observation of the gonads.

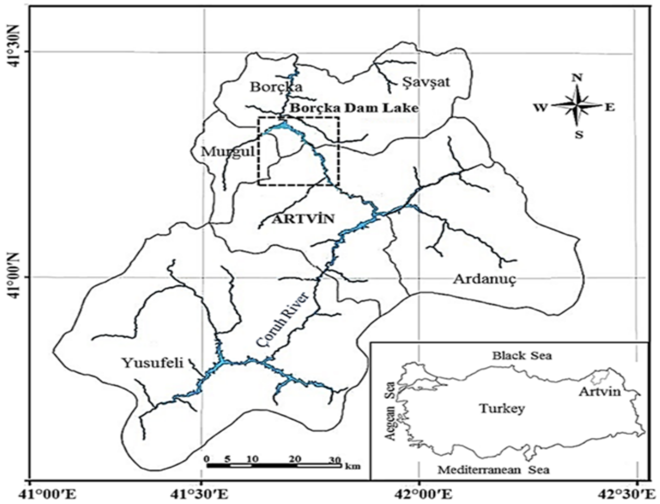

Figure 1. The map of the study area

Length-Weight (L-W) Relationship

The relationships between fish length and weight are expressed by equation (1):

$$W = aTL^b \quad (1)$$

which was converted to logarithmic form as equation (2):

$$\log W = \log a + b \log TL \quad (2)$$

where *W* is total body weight (g), *TL* is the total length (cm), *a*: intercept and *b*: slope regression coefficients. Then the length–weight relationship (LWR) parameters were estimated by linear regression using the log-transformed values of this equation (Ricker, 1973; King, 2007). The model fit to the data was measured by the coefficient of the Pearson r-squared (r^2) test (Froese, 2006).

Fulton's Condition Factor (K)

Fulton's condition factor (K) was calculated using the following formula;

$$K = \frac{W}{TL^3} \times 100 \quad (3)$$

where, *W* is total body weight (g) and *TL* is total length (cm) (Le Cren, 1951).

Statistical Analyses

The differences in mean Fulton's condition factor between sexes were tested by t-test. ANCOVA was used to determine if there was significant difference in slopes (b values) between sexes (Zar, 1999). To compare the slopes to confirm whether the b value was different from the isometric growth (b=3) for both sexes and combined sex, Pauly's t-test (Pauly, 1984) was performed using the formula as:

$$t = \frac{Sd_{logTL}}{Sd_{logW}} \frac{|b-3|}{\sqrt{1-r^2}} \sqrt{n-2} \quad (4)$$

where, Sd_{logTL} is the standard deviation of the *log TL* values, Sd_{logW} is the standard deviation of the *log W* values, r^2 is correlation coefficient, n is the number of specimens of *P. constructor* used in the calculation. If calculated t value is greater than the table *t* values for n-2 degrees of freedom the value of b is different from isometric growth (b=3) (Pauly, 1984; Bilgin et al., 2020). All tests applied in the study were performed at the 0.05 level of significance. Statistical analyses were performed using the SPSS and Microsoft Office Excel software.

Results

Length and Weight Distribution

A total of 145 *P. constructor* (Caucasian goby*)* individuals were sampled during the study. The total lengths (TL) of all samples examined were between 6.8 cm and 13.4 cm (mean 10.01±0.153 cm) and their body weight ranged from 4.14 to 28.91g (mean 13.21±0.580). Total length (TL) and weight (W) characteristics of the Caucasian goby, *P. constructor* by sexes were presented in Table 1.

Length - Frequency Distribution

The total length - frequency distribution of 145 Caucasian goby samples ranging from 6.8 cm to 13.4 cm TL was plotted based on 1 cm class intervals for female and male individuals (Figure 2). The length -frequency distribution showed that most of the individuals of males (67.2%, N=45), females (75.3%, N=58) and all samples (71%, N=103) distributed in the length classes between 7 and 10 cm.

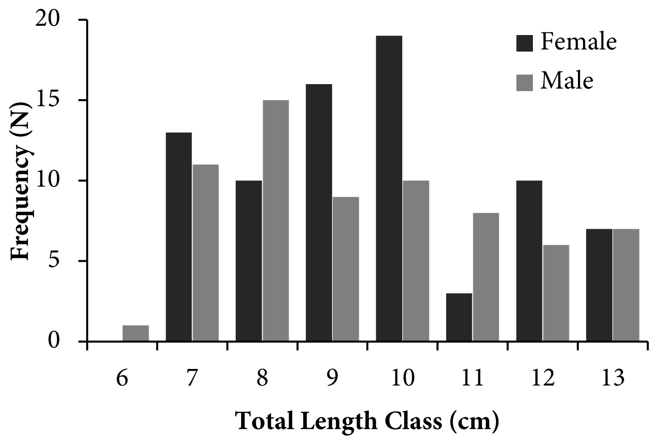

Figure 2. The length - frequency distribution of the Caucasian goby by sexes.

Length-weight relationship (LWR)

The total length-weight relationships were determined as $W= 0.016TL^{2.896}$ (r^2=0.995, N=78, P<0.05) for females, as $W=0.018TL^{2.841}$ (r^2=0,993, N=67, p<0.05) for males and as $W=0.017TL^{2.856}$ (r^2=0,994, N=145, p<0.05) for combined sex (Figure 3) with the b-values were significantly different from isometric growth (b=3) indicating negative allometric growth of Caucasian goby for all sexes (Pauly's t- test, p<0.05). The slopes (b value) of the length-weight relationship were statistically significant for both sexes (ANCOVA, p<0.05). The length-weight relationships (LWR) for *P. constructor* by sexes are presented in Figure 3. The correlation coefficient (r^2) for regression analysis indicated that the Caucasian goby has a high correlation (r^2>0.99) between the total length and weight variables for both sexes and combined individuals.

Descriptive statistics and estimated parameters of the length-weight relationship (LWR) of Caucasian goby by sexes from the lower Coruh River Basin, NE Türkiye were presented in Table 2.

Fulton's Condition Factor (K)

Fulton's condition factor (K) values of the Caucasian goby ranged from 1.124 to 1.312 in females and from 1.076 to 1.426 in males. No significant difference was observed in mean K values between females and males (t-test, *p>0.05*) (Table 3).

Table 1. Total length (TL) and weight (W) characteristics of the Caucasian goby, *P. constructor* by sexes

Sex	**N**	**Total Length (cm)**		**Body Weight (g)**	
		Min-Max	***Mean±S.E***	***Min-Max***	***Mean±S.E***
Female	78	7.2-13.4	9.88±0.210	4.72-28.91	12.51±0.788
Male	67	6.8-13.2	9.61±0.255	4.14-28.01	12.02±0.948
Combined	145	6.8-13.4	9.75±0.162	4.14-28.91	12.29±0.606

Note: N: Sample Size, S.E: Standard Error, Min.: Minimum, Max.: Maximum

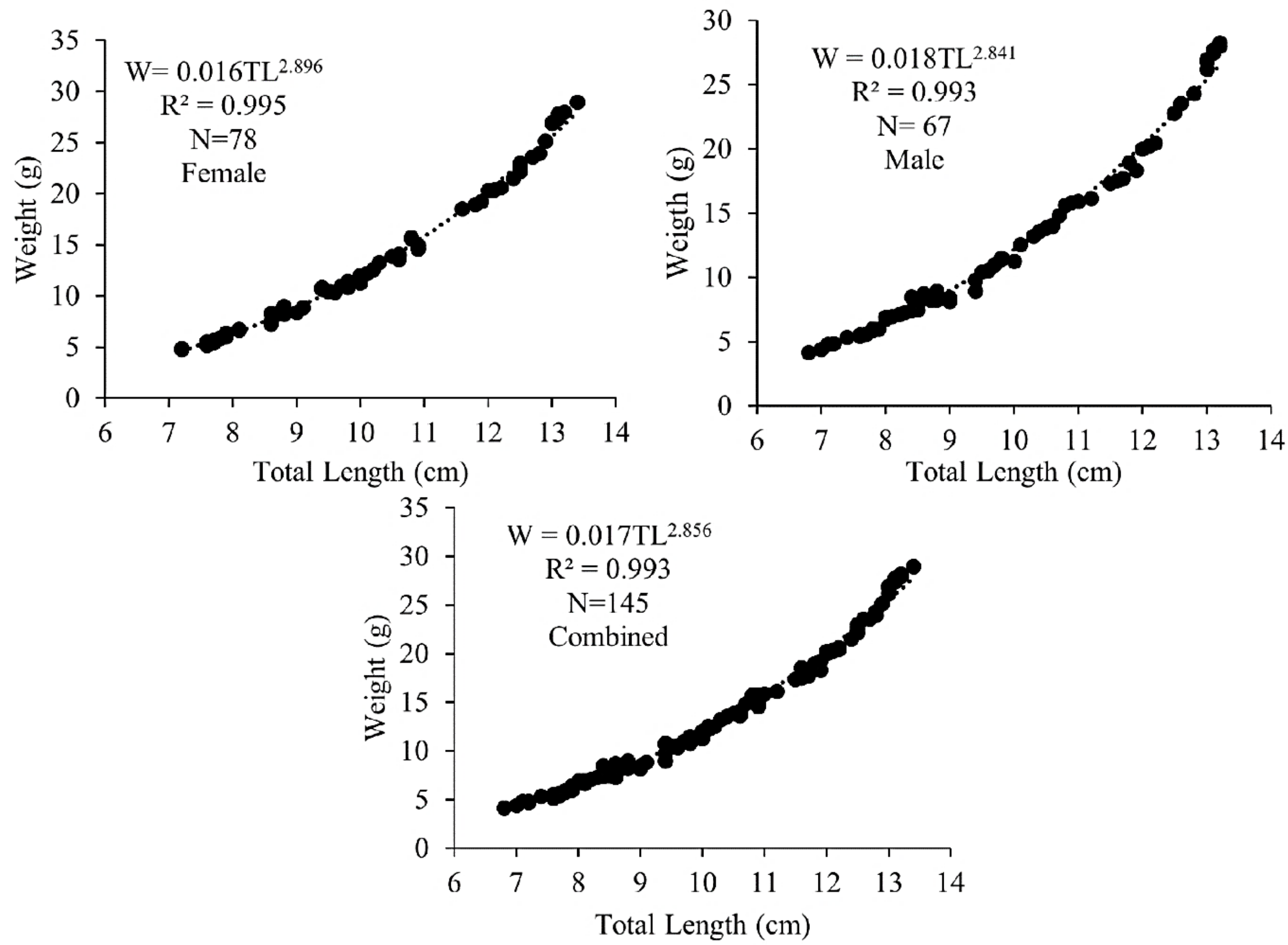

Figure 3. The length-weight relationships (LWR) for the Caucasian goby, *P. constructor* by sexes

Table 2. Descriptive statistics and estimated parameters of the length-weight relationship (LWR) of Caucasian goby, *P. constructor* by sexes from the lower Coruh River Basin, NE Türkiye

Sex	N	Parameters of LWR						
		a	*b*	*S.E. (b)*	*95% CI of b*	R^2	*Growth Type*	*Pauly's t-test*
Female	78	0.016	2.882	0.025	2.831-2.933	0.995	- Allometric	$p<0.05$
Male	67	0.018	2.841	0.033	2.762-2.896	0.993	- Allometric	$p<0.05$
Combined	145	0.017	2.851	0.021	2.809-2.893	0.994	- Allometric	$p<0.05$

Note: N: Sample Size, a: Intercept of the relationship, b: Slope; S.E.(b): Standard Error of b, R^2: Coefficient of determination, C.I.: Confidence Intervals)

Table 3. Fulton's condition factor (K) values of the Caucasian goby by sexes.

Sex	N	Kmin-Kmax	Kmean±S.E.	t-test
Female	78	1.124-1.312	1.202±0.006	$p>0.05$
Male	67	1.076-1.426	1.221±0.009	$p>0.05$

Sex Composition

During the study, of the sampled 145 *P. constructor* individuals, 53.8% (N=78) were females and 46.2% (N=67) were males, accordingly the ratio of female to male was calculated as 1.16:1.00 which did not deviate from the expected ratio of 1:1 ($\chi^2 = 0.834$, $p>0.05$).

Discussion

In this study, the size composition, sex ratio, length -weight relationship and Fulton's condition factor for the Caucasian goby, *P. constructor* was reported for the first time. There are no previous references for comparison the results of the present study. Therefore, all comparisons were made with the other species among the genus *Ponticola* reported in the available literature.

The values of the coefficient b of the LWRs for Caucasian goby, *P. constructor* in this study were 2.896 for females and 2.841 for males within the expected range of 2.5-3.5 (Froese, 2006), however, the b value of LWR may vary between 2 and 4 (Bagenal & Tesch, 1978). The growth pattern was negative allometric growth (-A growth) for Caucasian goby in both sexes. The value of b>3 indicates that the fish become plump as they increase in length and b <3 shows that the fish gets slimmer with increasing length (Jobling, 2002).

The b value of LWRs for Kura goby, *Ponticola cyrius* from Kura River, eastern Anatolia Region in Türkiye was reported as 2.9795 indicating isometric growth pattern by Çiçek et al. (2019). This value was reported for the same species as 2.938 (-A growth) from Shahrbijar River, Southern Caspian Sea basin in Iran (Asadi et al., 2017) and as 3.214 (+A growth) by Faradonbeh et al. (2015) from Totkabon River (southern Caspian Sea basin), Guilan in Iran. The b value for *Ponticola bathybius* from the Southern Caspian Sea basin in Iran was reported as 3.32 (+A growth) (Nikmehr et al., 2021). The b value of *Ponticola iranicus* from Sefidroud River was reported as 3.050 for females, as 2.893 for males and as 3.002 for all individuals (Mousavi-Sabet et al., 2016). The b value was determined for *Ponticola gorlap* as 3.64 (+A growth) from Tajan River, Iran (Jamali et al., 2015). The b value of the LWR for Aksu goby, *Ponticola turani* was reported as 2.86 for females and 2.85 for males from Terme Stream with negative allometric growth in both sexes (Yilmaz & Sakalli, 2020).

The parameters of the LWRs in fish may change depending on some factors such as species, habitat, sex, season, the number and length distribution of specimens, degree of stomach fullness, and gonad maturity (Bagenal & Tesch, 1978), also sampling techniques, food availability and feeding intensity (Le Cren, 1951), degree of muscular development, the amount of reserved fat and life history (Gupta & Banerjee, 2015). The LWRS may also be influenced by geographical location and environmental conditions in given year (Balon, 1984).

In the current study, the sex ratio for the Caucasian goby, *P. constructor* of female to male was calculated as 1.16:1.00 which did not deviate from the expected ratio of 1:1. The sex ratio of females to males was reported as 1.0: 0.52 for Aksu goby, *Ponticola turani* in Terme Stream from the northern Türkiye (Yilmaz & Sakalli, 2020). This ratio in Iranian Goby, *Ponticola iranicus* was reported as 1:1.32 in favor of males from the Southern Caspian Sea Basin (Mohammadi-Darestani et al., 2016). Knowledge on the sex distribution in fish populations is most important for the reproduction of the population. In many species, the sex composition (female: male ratio) is usually as 1:1. However, there may be deviations from this ratio in some species or some age groups (Erkoyuncu, 1995). The sex ratio in fishes may generally vary from species to species, between different populations of the same species and from year to year in the same population as well (Nikolsky, 1963). Furthermore, Mohammadi-Darestani et al. (2016) noted that the sex ratio of gobies can vary in different species and even in the same species under different environmental conditions.

The Fulton's condition factor (K) values calculated for the Caucasian goby, *P. constructor* in the current study varied from 1.124 to 1.312 in females (mean 1.202±0.006) and from 1.076 to 1.426 in males (mean 1.221±0.009) indicating that the species is in a good growth condition in the Borçka Dam Lake and its tributaries.

The mean Fulton's condition factor for Kura goby, *Ponticola cyrius* was reported as 1.43 from Kura River, eastern Anatolia Region in Türkiye (Çiçek et al., 2019), as 0.96±0.14 from Shahrbijar River, a tributary of Sefidrud River in the Caspian Sea basin (Guilan Province, North of Iran) (Asadi et al., 2017) and as 0.94±0.207 from Totkabon River (southern Caspian Sea basin), Guilan in Iran (Faradonbeh et al., 2015). This value was calculated as 1.00±0.18 for *Ponticola bathybius* from the Southern Caspian Sea basin in Iran (Nikmehr et al., 2021). Fulton's condition factor (K) values ranged from 0.72 to 1.48 in females (mean 1.14 ±0.09) and from 0.84 to 1.31 in males (mean 1.10±0.09) for Aksu goby, *Ponticola turani* from Terme Stream from the northern Türkiye (Yilmaz & Sakalli, 2020). The condition factor is an index reflecting the interaction between biotic and abiotic components in the physiological conditions of fishes. Therefore, this factor may vary among fish species in different localities (Blackwell et al., 2000) and the fluctuating in this index is also based on the seasonal variations of the gonads and feeding intensity (Biswas, 1993). Higher condition factor values (K≥1) indicate suitability of a specific water body for growth as well as a good level of feeding and appropriate environmental conditions in favor of fish (Ujjania et al., 2012; Abbasi et al., 2019). The differences in Fulton's condition factor (K) values among the present study and other studies previously reported above may be explained by the factors such as species, sampling period and techniques, feeding regime, food presence and utilization, habitat conditions and state of gonadal development.

Information on basic biological data of a fish population is of great importance to understand how fishes react under anthropogenic pressure or other environmental conditions (Trindade-Santos & Freire, 2015). The biological aspects of Gobies have been rarely studied in Northeastern parts of the Anatolia; however, *P. constructor* (Nordman, 1840) has not been previously studied both from the region and its other distribution areas. There is no information on biological aspects of this species; therefore, the main aim of the present study was to provide preliminary biological data on this species from the lower Çoruh River basin in terms of size composition, sex ratio, as well as length-weight relationship and condition.

Even if *P. constructor* is globally listed as Least Concern (LC) category by the IUCN Red List of Threatened Species (IUCN, 2008), however, this species is considered as Endangered (EN) in inland waters of Türkiye due to dams construction on rivers, habitat loss, water abstraction, eutrophication and pollution (Fricke et al., 2007), thus suggesting that this species should be subject to conservation measures through continuous monitoring of bioecological aspects of the species in the study area. Considering the ecological role of the Caucasian goby, which has no economic value in this ecosystem, investigation of the biological characteristics of this species will be important in terms of both the protection and sustainability of the species as well as the ecosystem in its distribution area.

Conclusion

In conclusion, the present study provides the first data on the size composition, sex ratio, length-weight relationships, and condition factor of Caucasian goby that would be useful for fishery managers and biologists studying in the field of fisheries biology. Potential conservation plans need much more data on the biology of the species. The results of the present study will provide a primary dataset to fisheries management authority and fisheries scientists for further investigations to fully understand the bio-ecological characteristics of this species in addition to take conservation measures and to ensure sustainability of its populations in the area.

Acknowledgements

The author would like to thank Dr. Cüneyt Kaya, and M.Sc. Yunus Dedeoğlu for their valuable helps in field samplings.

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

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Mar. Sci. Tech. Bull. (2022) 11(4): 501-514
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1195335

Marine Science and Technology Bulletin

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RESEARCH ARTICLE

Nutrient quality of cultured fish species in the Black Sea: Evaluation of fatty acids, amino acids and fillet colors

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ARTICLE INFO

Article History:
Received: 27.10.2022
Received in revised form: 19.12.2022
Accepted: 22.12.2022
Available online: 22.12.2022

Keywords:
Black Sea
Nutrient composition
Fatty acid
Amino acid
Fillet color

ABSTRACT

This study aims to examine the nutritional composition (amino acid and fatty acid) and fillet color of commercially produced cultured fish of the Black Sea. All fish species [(sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), turbot (*Scophthalmus maximus*), and large rainbow trout (*Oncorhynchus mykiss*)] were collected from the Black Sea's aquaculture sites in 2020. Total amino acid values of fillets were listed as sea bream>turbot>large rainbow trout>sea bass fillet. The highest omega-3/omega-6 ratio was in turbot fillets, and the highest omega-6/omega-3 ratio was in sea bass and sea bream fillets ($p<0.05$). Consequently, it was concluded that the fish cultured from the Black Sea have excellent nutritional values, including high amounts of unsaturated fatty acids and appropriate amounts of essential amino acids and the cultured fish in the Black Sea are of good quality, nutritious and beneficial for human consumption.

Please cite this paper as follows:

Kaya Öztürk, D. (2022). Nutrient quality of cultured fish species in the Black Sea: Evaluation of fatty acids, amino acids and fillet colors. *Marine Science and Technology Bulletin*, *11*(4), 501-514. https://doi.org/10.33714/masteb.1195335

Introduction

Aquaculture has developed into a major global industry since the introduction of intensive aquaculture in the 90s. With this development, worldwide aquaculture production reached 75.4 million tons in 2019 (FAO, 2021). This development in aquaculture has affected the Black Sea economically, socially and culturally for generations as well as the world. Today, the aquaculture sector is expanding and becoming an increasingly significant aspect of the Black Sea economy, with output quantities exceeding 700,000 tons in 2019 (FAO, 2022). The modern aquaculture sector in Türkiye emerged in the mid-1970s, with the beginning of commercial freshwater farming of rainbow trout (*Oncorhynchus mykiss*). A decade later, in the mid-1980s, Türkiye's first commercial sea bass (*Dicentrarchus labrax*), and sea bream (*Sparus aurata*), hatchery introduced the country to marine aquaculture (FAO, 2022). Türkiye's aquaculture industry is now a vibrant and competitive seafood production sector that is well integrated into international

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seafood markets. The sector also contributes significantly to national and international food security, as well as revenue, employment, and economic growth. Turkish aquaculture production is dominated by three species: rainbow trout, seabass and sea bream. For many years freshwater aquaculture (i.e., farming of rainbow trout) was the backbone of the sector; however, more recently, marine aquaculture (mainly seabass and seabream) has begun to take the lead in farmed fish output. Along with the growth of marine aquaculture along Türkiye's Black Sea coast, cage farming of large rainbow trout, branded as Turkish salmon, is becoming a major component of the economy (FAO, 2022). Aquaculture practices of turbot (*Scophthalmus maximus*), are vital to meet the high market demand. In addition to commercial turbot production in recent years, juvenile turbots were released into nature in order to increase the stock biomass (Maslova, 2002; Aydın, 2021).

The world's leading health authorities widely recommend consuming fish on a regular basis as part of a healthy diet due to their high nutrient content including protein, fat, vitamin, and minerals (WHO, 2003; EFSA, 2009). In the global fight against malnutrition, fish products are a much healthier food source than terrestrial meat products. On a global scale, fish products are the third most important source of dietary protein consumed by humans after cereals and milk, accounting for 17.1% of the total animal protein supply (VKM, 2014; Bogard et al., 2015; FAO, 2018). Fish has a higher protein content on an edible weight basis than most terrestrial meats, a lower caloric density, and is generally much leaner than red and processed meats, has the highest content of omega-3 polyunsaturated fatty acids of any animal food, and has a higher mineral and vitamin content than most terrestrial meats and processed meat products (Tacon & Metian, 2013; USDA, 2018). Furthermore, considerable scientific evidence exists regarding the direct health benefits of consuming fish, such as a lower risk of death from coronary heart disease and stroke, a long er gestation period and improved visual and cognitive development, improved neurodevelopment in infants and children when fish is consumed before and during pregnancy (Forouhi et al., 2018). Although it has been proven that consuming fish is so healthy, it is a known fact that the consumption of aquatic products is very low in developing countries. For example, the average per capita consumption of fish in Türkiye is very low compared with other European countries and in 2019 amounted to 6.3 kg/year. (Anonymous, 2021). This consumption amount is affected by factors such as socioeconomic status, lack of education, product availability, food safety, and especially the prejudice towards cultured fish. Many studies were carried out in which wild and cultured fish are compared and fillet quality values of cultured fish are determined in order to break down such prejudices (Fuentes et al., 2010; Baki et al., 2015; Colombo & Mazal, 2020; Tarricone et al., 2022). The current study aimed to analyze and compare the biochemical composition, in terms of amino acid, fatty acid, and fillet color, of available cultured fish fillets produced in the Black Sea (large rainbow trout, turbot, sea bass, and sea bream).

Materials and Method

Fish, Diets and Sampling Areas

In the study, sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), turbot (*Scophthalmus maximus)*, and large rainbow trout, (*Oncorhynchus mykiss)* were used cultured. All fish species were collected from aquaculture sites in the Black Sea (Sinop, Samsun, and Trabzon), in 2020. All collection sites are located in the Black Sea and species information, fish number, and site location are shown in Supplementary file Table S1. It was preferred that the cultured fish used in the study should be at least in portion weight in order to give clear results to the consumers. Large rainbow trout, sea bream and sea bass were produced in cage systems, while the turbot was produced in a recirculating aquaculture system. The fish were sampled by the operating personnel while they were harvested, therefore this manuscript does not need an ethical approval. Fish and diet samples were transported to Sinop University, laboratory of the Aquaculture and Sinop University Scientific and Technological Research Center (SUBITAM) by providing cold chain conditions with Igloo Island Breeze brand wheeled ice cooler with in-house ice accumulator. After biometric measurements were made in the laboratory, skinless and boneless fillets of fish were removed and stored in a deep freezer (WiseCryo/WUF-D500-80°C) until analysis. Biometric data (viscerosomatic, hepatosomatic and gonadosomatic index, carcass yield, and condition factor) used in the study were obtained from Owatari et al. (2022).

The diets were made by BioMar SAGUN (D1) (Aydın-Türkiye), Sürsan Inc. (D2) (Samsun- Türkiye) and Noordzee Inc. (D3) (Muğla- Türkiye) the commercial diet manufacturer, using a closed formula for large rainbow trout, turbot, sea bass and bream, respectively. (The biochemical, amino and fatty acid compositions of the diets are given Supplementary file Table S2).

Biochemical Analyses

The biochemical content (crude protein, crude fat, crude ash and dry matter) of the diet and fillet samples was evaluated using AOAC (1995) approved procedures. All biochemical analysis in fish fillets were made from the wet basis and three repetitions.

Amino Acid Analyses

Amino acid analyzes of diets and fillets were made by Sinop University Scientific Research and Application Center (SUBITAM). Amino acid analyses of diet and fish fillets were performed using the Jasem LC-MS/MS amino acid assay kit. The total amino acids and quality of amino acids were calculated from the following formulas given below (Li et al., 2009):

Fatty Acid Analyses

Fatty acid analyzes of diets and fillets were made by Sinop University Scientific Research and Application Center (SUBITAM). The fillet and diet samples were converted to methyl esters by derivatization of fat samples in a gas chromatography device (Thermo Scientific Trace 1310) for fatty acid analyses. Moreover, the quality of acquired fatty acids was established using peroxidisability index (PI) (Arakawa & Sagai, 1986), atherogenicity index (AI), thrombogenicity index (TI) (Ulbricht & Southgate, 1991) and hypo-cholesterolaemic/hypercholesterolaemic ratio (HH) (Santos-Silva et al., 2002).

Fillet Color

The color values of fillets of cultured fish of the Black Sea were assessed using Minolta Chroma Meter, standardized to a white plate as a reference before each measurement (standard values for white plate L*=91.97; a*=−1.4; b*=2.0, Standard C2-22326). L*, a* and b* values represent lightness, redness and yellowness, respectively. The Hue is a descriptor of what is generally understood to be the true color, and the chroma (C*) is the intensity or degree of saturation of the color. The angle of Hue and C* was calculated using a* and b* values (Hernández et al., 2009): $C^*=\sqrt{a^{*2}+b^{*2}}$ c and $Hue = \arctan(b^*/a^*)$.

Color measurement of fillet and skin of cultured fish of the Black Sea was done from three locations: 1st location: between behind of the operculum; 2nd location: under the dorsal fin; and 3rd location: front of the caudal fin.

Essential Amino Acids (EAA)= Histidine + Lysine+ Phenylalanine+ Methitonine+ Threonine+ Leucine+ Isoleucine+Valine+ Arginine

Semi-Essential Amino Acids (SEAA)= Histidine + Arginine

Non-Essential Amino Acids (NEAA)= Alanine+ Aspartic acit+ Glutamic acit+ Tyrosine+ Glicine+ Serine+ Proline

Branched-chain amino acid (BcAA)= Leucine+ Isoleucine+ Valine

Sulfur-containing amino acids (SAA)= Cystine+ Methinonine

Aromatic amino acids (ArAA)= Phenylalanine+ Tyrosine

Basic (alcaline) amino acids (BAA)= Lysine+ Arginine+ Histidine

Acidic amino acids (AAA)= Aspartikc acid+ Glutamic acid

Total saturated fatty acids (ΣSFA) = C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0

Total mono unsaturated fatty acids (ΣMUFA) = C14:1 + C15:1 + C16:1 + C17:1 + C18:1n-9c + C18:1n-9t + C20:1n-9c + C22:1n-9 + C24:1

Total poly unsaturated fatty acids (ΣPUFA) = C18:2n-6t + C18:2n-6c + C18:3n-3 + C18:3n-6 + C20:2 + C22:2 + C20:3n-6 + C20:5n-3 + C20:4n-6 + C22:6n-3

ΣOmega-3 = C18:3n-3+C20:3n-3+C20:5n-3+C22:5n-3+C22:6n-3;

ΣOmega-6 = C18:2n-6t + C18:2n-6c+ C18:3n-6+ C20:4n-6+ C20:3n-6

ΣOmega-9 = C18:1n-9c+ C18:1n-9t+ C20:1n-9c+ C22:1n-9

Atherogenicity Index (AI)= [(C12:0+(4 x C14:0)+C16:0)] / (MUFA+Omega-3+Omega-6)

Thrombogenicity Index (TI)= (C14:0+C16:0+C18:0) / [(0.5 x MUFA) + (0.5xOmega-6) + (3xOmega-3) + (Omega-3/Omega-6)]

Hypocholesterolemic/Hypercholesterolemic ratio (HH)= (C18:1n-9+C18:2n-6+C18:3n-3+C20:4n-6+C20:5n-3+C22:6n-3) / (C14:0+C16:0)

Peroxidisability index (PI)= (MUFAx0.025) + (C18:2n-6+C20:2) x 1 + [(C18:3n-6+C18:3n-3)x2] + [(C18:4n-3+C20:4n-6+C22:4n-6) x 4] + [(C20:5n-3+C22:5n-3) x 6] + [(C22:6n-3) x 8]

Statistical Analysis

The data were reported as average values with standard error (SE). The IBM SPSS 21 statistics package application was used for statistical analysis. Shapiro–Wilk normality and Levene's tests were used to determining the data's normality and equality of variance. The significance of the differences in the data was determined using one-way ANOVA, followed by Tukey's procedure for multiple comparisons. Regression analyses were used to examine the relationships between the values.

Results

Biometric Data

The study aimed to determine the meat quality values of the sea fish cultured in the Black Sea, at the weight reached by the consumer. The weights (g) and biometric data [(hepatosomatic (HSI), viscerosomatic (VSI) and gonadosomatic (GSI) index and carcass yield (CY), %)] of cultured fish in the Black Sea are given Table 1. In this study, average harvest weights of large rainbow trout, turbot, sea bass, and sea bream sampled at the same time were determined as 1556.36±140.81, 761.30±42.56, 533.18±56.76, and 759.36±32.40 g, respectively Gonad formation was observed only in turbot and large rainbow trout, and gonadosomatic index (GSI) values were calculated only for these fish. While the highest hepatosomatic index (HSI) values of cultured fish in the Black Sea were determined in sea bass and the lowest in large rainbow trout ($p<0.05$), Viscerosomatic index (VSI) values were determined to be the highest in large rainbow trout and the lowest in sea bream ($p<0.05$). The carcass yield (CY) values of four different cultured fish with high economic value was large rainbow trout>sea bass>sea bream>turbot.

Biochemical Composition

The biochemical composition of cultured fish fillet of the Black Sea is given in Table 2. The dry matter values (DM) of the cultured fish fillet of the Black Sea were as sea bream>large rainbow trout>sea bass>turbot, and the statistical difference was significant ($p<0.05$).

The crude protein (CP) ratio of fish fillets was highest in sea bream and lowest in turbot, and the CP ratios of turbot fillets were statistically different from the CP ratios of other cultured fish fillets in the Black Sea ($p<0.05$). The crude fat ratio (CF) was determined as highest in large rainbow trout fillets and lowest in turbot fillets ($p<0.05$).

Amino Acid Composition

The amino acid composition of cultured fish fillets of the Black Sea is shown in Table 3. The highest essential amino acid in cultured fish fillets was determined lysine, and the non-essential amino acid was glutamic acid. Except for ornithine (highest in large rainbow trout fillets) and cystine (highest in turbot fillets) in fish fillets, all amino acid values were highest in sea bream fillets. The proline value was determined to be similar in sea bream and turbot fillets.

Table 1. The weights (g) and biometric data [(hepatosomatic (HSI), viscerosomatic (VSI) and gonadosomatic (GSI) index and carcass yield (CY), %)] of cultured fish in the Black Sea

Fish Species	Weight (g)	HSI (%)	VSI (%)	GSI (%)	CY (%)
Large rainbow trout	1556.36±140.81	$1.68±0.16^{a}$	$16.48±1.33^{c}$	$7.16±3.29^{b}$	$44.23±1.66^{c}$
Turbot	761.30±42.56	$2.46±0.22^{b}$	$8.41±0.69^{a}$	$0.77±0.16^{a}$	$27.37±1.43^{a}$
Sea bass	533.18±56.76	$3.27±0.74^{c}$	$12.95±0.95^{b}$	-	$44.08±0.22^{c}$
Sea bream	759.36±32.40	$1.74±0.17^{a}$	$8.07±0.87^{a}$	-	$38.87±2.00^{b}$

Note: Each value means mean±standard error. Values expressed with different exponential letters on the same column are statistically different from each other ($p<0.05$).

VSI (%) = (Viscera weight, g/Total body weight, g) × 100

HSI (%) = (Liver weight, g/Total body weight, g) × 100

GSI (%) = (Gonad weight, g/Total body weight, g) × 100

CY (%) = (Edible fillet weight, g/Total body weight, g) × 100

Table 2. Biochemical composition of cultured fish fillets in the Black Sea

Biochemical Composition (%)	***Large Rainbow Trout***	***Turbot***	***Sea Bass***	***Sea Bream***
Crude protein	20.54±0.50^{b}	16.60±0.95^{a}	21.96±0.35^{b}	22.00±0.35^{b}
Crude fat	11.21±0.12^{c}	2.49±0.16^{a}	8.20±0.06^{b}	10.12±0.69^{bc}
Crude ash	4.15±0.02^{b}	3.82±0.11^{a}	3.85±0.13^{a}	4.26±0.10^{b}
Dry matter	35.94±0.42^{b}	22.98±0.35^{a}	35.08±0.49^{b}	36.41±0.35^{bc}

Note: Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$).

Table 3. Amino acid composition of cultured fish fillets in the Black Sea (g/100g)

Amino Acids (g/100g)	**Large Rainbow Trout**	**Turbot**	**Sea Bass**	**Sea Bream**
Alanine	1.22±0.01^{b}	1.12±0.01^{a}	1.20±0.01^{b}	1.42±0.01^{c}
Aspartic Acid	1.89±0.01^{a}	2.08±0.01^{b}	1.91±0.01^{a}	2.24±0.01^{b}
Methinonine	0.54±0.01^{a}	0.50±0.01^{a}	0.56±0.01^{a}	0.64±0.01^{b}
Glutamic Acid	2.29±0.01^{a}	2.49±0.01^{b}	2.51±0.01^{b}	2.78±0.01^{c}
Phenylalanine	0.71±0.01^{ab}	0.66±0.01^{a}	0.64±0.01^{a}	0.83±0.01^{c}
Lysine	1.68±0.01^{b}	1.63±0.01^{a}	1.72±0.01^{b}	2.24±0.01^{c}
Histidine	0.50±0.01^{bc}	0.29±0.01^{a}	0.40±0.01^{b}	0.63±0.01^{d}
Tyrosine	0.57±0.01^{ab}	0.54±0.01^{a}	0.50±0.01^{a}	0.65±0.01^{b}
Glysine	0.15±0.01^{a}	0.88±0.01^{c}	0.62±0.01^{b}	1.02±0.01^{d}
Valine	0.60±0.01^{ab}	0.64±0.01^{b}	0.53±0.01^{a}	0.78±0.01^{c}
Leucine	1.29±0.01^{b}	1.13±0.01^{a}	1.09±0.01^{a}	1.53±0.01^{c}
Isoleucine	0.37±0.01^{ab}	0.38±0.01^{ab}	0.30±0.01^{a}	0.51±0.01^{c}
Threonine	0.83±0.01^{bc}	0.76±0.01^{b}	0.66±0.01^{a}	0.92±0.01^{d}
Serine	0.86±0.01^{ab}	0.88±0.01^{b}	0.82±0.01^{a}	0.94±0.01^{c}
Proline	0.66±0.01^{a}	0.77±0.01^{b}	0.61±0.01^{a}	0.77±0.01^{b}
Ornithine	0.16±0.01^{a}	0.17±0.01^{a}	0.13±0.01^{a}	0.15±0.01^{a}
Cystine	0.15±0.01^{b}	0.20±0.01^{c}	0.08±0.01^{a}	0.13±0.01^{b}
Arginine	1.06±0.01^{b}	1.05±0.01^{b}	0.90±0.01^{a}	1.17±0.01^{c}
Total Amino Acids (g/100g)				
TAA	15.44±0.06^{a}	16.15±0.01^{b}	15.16±0.01^{a}	19.32±0.02^{c}
ΣEAA	7.57±0.01^{b}	7.04±0.01^{b}	6.80±0.01^{a}	9.24±0.01^{c}
ΣSEAA	1.56±0.01^{b}	1.34±0.01^{a}	1.30±0.01^{a}	1.80±0.01^{c}
ΣNEAA	7.87±0.05^{a}	9.12±0.01^{c}	8.37±0.01^{b}	10.08±0.01^{d}
EAA/NEAA	0.96±0.01^{c}	0.77±0.01^{a}	0.81±0.01^{b}	0.92±0.01^{c}
ΣBcAA	2.26±0.01^{b}	2.15±0.01^{b}	1.92±0.01^{a}	2.82±0.01^{c}
ΣSAA	0.69±0.01^{a}	0.70±0.01^{ab}	0.64±0.01^{a}	0.77±0.01^{bc}
ΣArAA	1.28±0.01^{b}	1.20±0.01^{a}	1.14±0.01^{a}	1.48±0.01^{c}
ΣBAA	3.24±0.01^{b}	2.97±0.01^{a}	3.02±0.01^{a}	4.04±0.01^{c}
ΣAAA	4.18±0.01^{a}	4.57±0.01^{b}	4.42±0.01^{b}	5.01±0.01^{c}
EAAI	0.88±0.01^{a}	0.85±0.01^{a}	0.83±0.01^{a}	0.97±0.01^{b}

Note: Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$).

Total amino acid values (ΣTAA) were listed as sea bream>turbot>large rainbow trout>sea bass and ΣTAA values of sea bream and turbot fillets were statistically significant ($p<0.05$). The high amino acid values of sea bream fillets were also reflected in essential (ΣEAA), semi-essential (ΣSEAA), non-essential (ΣNEAA), branched-chain (ΣBcAA), sulfur-containing (ΣSAA), aromatic (ΣArAA), basic (ΣBAA), and acidic (ΣAAA) amino acid values and essential amino acid index (ΣEAAI) values. The EAA/NEAA values were higher in large rainbow trout fillets ($p<0.05$).

Fatty Acid Composition

The fatty acid composition of cultured fish fillet of the Black Sea is presented in Table 4. The most determined saturated fatty acid in all cultured fish fillets in the Black Sea was C16:0, and the highest C16:0 was in turbot fillets, and the lowest was in large rainbow trout fillets. ($p<0.05$). The total saturated fatty acid (ΣSFA) value was highest in turbot fillets and lowest in large rainbow trout fillets, and the statistical difference between ΣSFA values of fillets was significant ($p<0.05$). C18:1n-9c value, which is the most monounsaturated fatty acid (MUFA) in fillets, was ranked as Rainbow trout>sea bream>sea bass>turbot, and the C18:1n-9c value in turbot fillets was statistically significant ($p<0.05$). The C18:1n-9c value determined at high values in fillets also affected the amount of ΣMUFA and the order of ΣMUFA in fillets was as in C18:1n-9c. The most determined polyunsaturated fatty acids in fillets were C18:2n-6c and C22:6n-3 (DHA). The C22:6n-3 (DHA) ranking was realized as turbot>large rainbow trout>sea bass>sea bream and the statistical difference between C22:6n-3 values in fillets were found to be significant ($p<0.05$). The C20:5n-3 (EPA) value was highest in the turbot fillets and lowest in the large rainbow trout ($p<0.05$). Total polyunsaturated fatty acids (ΣPUFA) in fillets were the highest turbot and the lowest sea bream fillets, and the statistical difference was significant for PU/FA values of turbot and sea bass fillets ($p<0.05$). The highest ΣOmega-3, ΣOmega-6, and ΣOmega-9 values were in turbot bass and large rainbow trout fillets, respectively. The highest omega-3/omega-6 ratio was in turbot fillets, and the highest omega-6/omega-3 ratio was in sea bass and sea bream fillets ($p<0.05$). Atherogenicity (AI) and peroxidisability (PI) index values of turbot fillets were higher than fillets of other cultured fish in the Black Sea ($p<0.05$). The thrombogenicity index (TI) values of sea bream fillets were higher than fillets of other cultured fish in the Black Sea, but the statistical difference was not significant ($p>0.05$). The highest Hypocholesterolemic/Hypercholesterolemic ratio (HH) was determined in large rainbow trout fillets and the statistical difference was found to be significant ($p<0.05$). The highest EPA+DHA value was in turbot fillets ($p<0.05$), while the highest EPA/DHA ratio was determined in sea bass fillets ($p<0.05$).

Fillet Color

Table 5 shows the L*, a*, b*, C* and Hue values in three different measurement regions (1st location: between behind of the operculum; 2nd location: under the dorsal fin; and 3rd location: front of caudal fin) of large rainbow trout, turbot, sea bass, and sea bream fillets cultured in the Black Sea. The highest L* (lightness) values in all measurement regions were determined in turbot fillets ($p<0.05$). The highest a* (redness), b* (yellowness), C* (chroma), and Hue (true color) values were determined in large rainbow trout fillets at all measurement regions. The average L* values of the fillets were in the order of turbot>large rainbow trout>sea bream>sea bass, and the L* values of the turbot and large rainbow trout fillets were to be statistically significant ($p<0.05$).

Discussion

A deeper insight into the biochemical, amino acid, and fatty acid composition and color parameters of four culture fish species (large rainbow trout, turbot, sea bass, and sea bream) from the Black Sea region, was given through this study. Although the market weight of cultured fish is determined under the name of "portion weight" (250-350 g), consumer preferences play an important role in the weights of fish offered to the market. For example, rainbow trout is sold in 1kg apart from its portion weight or in the 3-5 kg range called "Turkish salmon", and sea bass and sea bream are sold over 500g in order to be similar to the wild type.

Carcass yields (CY) differ between species (Table 1). Our results indicate that large rainbow trout has the largest CY of the species studied at almost 44.23±1.66% of the body weight, compared to turbot fillets which were the lowest at 27.37±1.4% of body weight ($p<0.05$). Malcorps et al. (2021) reported that CY could increase or decrease only depending on the fish species. So much so that while the CY of sea bass and rainbow trout with a fusiform body shape approaches 50% (Baki et al., 2015; Kaya Öztürk et al., 2019; Tarricone et al., 2022), this rate varies between 30% and 50% in large-headed sea bream (Kaya Öztürk et al., 2020), and decreases to 20% in a flat-bodied turbot (Malcorps et al., 2021).

Table 4. Fatty acid composition of cultured fish fillets in the Black Sea (%)

Fatty Acid	Large Rainbow Trout	Turbot	Sea Bass	Sea Bream
C12:0	0.08±0.01^{a}	0.09±0.01^{a}	0.06±0.01^{a}	0.09±0.01^{a}
C13:0	0.02±0.01^{a}	0.07±0.01^{b}	0.02±0.01^{a}	0.02±0.01^{a}
C14:0	2.91±0.02^{a}	5.31±0.11^{c}	3.23±0.06^{b}	3.46±0.02^{b}
C15:0	0.41±0.01^{a}	1.10±0.01^{c}	0.50±0.01^{ab}	0.48±0.01^{a}
C16:0	12.31±0.09^{a}	13.80±0.21^{c}	12.77±0.10^{b}	12.85±0.04^{b}
C17:0	0.47±0.01^{a}	0.79±0.01^{c}	0.63±0.02^{b}	0.61±0.01^{b}
C18:0	6.54±0.08^{b}	4.60±0.01^{a}	6.78±0.08^{b}	7.54±0.08^{c}
C20:0	0.74±0.03^{a}	0.78±0.01^{a}	0.87±0.01^{b}	0.96±0.01^{c}
C21:0	0.02±0.01^{a}	0.06±0.01^{b}	0.02±0.01^{a}	0.02±0.01^{a}
C22:0	0.49±0.01^{a}	0.64±0.01^{c}	0.41±0.01^{a}	0.58±0.04^{b}
C23:0	0.04±0.01^{a}	0.04±0.01^{a}	0.06±0.01^{a}	0.15±0.05^{b}
C24:0	0.08±0.01^{b}	0.07±0.01^{b}	0.02±0.01^{a}	0.04±0.01^{a}
ΣSFA	24.11±0.24^{a}	27.35±0.29^{c}	25.37±0.26^{ab}	26.77±0.15^{b}
C14:1	0.17±0.01^{a}	0.42±0.01^{c}	0.21±0.01^{a}	0.30±0.01^{b}
C15:1	0.06±0.01^{a}	0.17±0.01^{b}	0.06±0.01^{a}	0.07±0.01^{a}
C16:1	0.37±0.01^{a}	0.98±0.02^{c}	0.57±0.02^{b}	0.60±0.01^{b}
C17:1	0.48±0.01^{a}	0.84±0.02^{c}	0.60±0.01^{b}	0.68±0.01^{b}
C18:1n-9c	25.26±0.42^{b}	17.45±0.22^{a}	24.99±0.17^{b}	25.02±0.06^{b}
C18:1n-9t	1.16±0.08^{a}	3.06±0.09^{c}	2.50±0.03^{b}	2.24±0.03^{b}
C20:1n-9c	4.57±0.19^{c}	2.25±0.05^{b}	1.25±0.03^{a}	1.20±0.01^{a}
C22:1n-9	3.87±0.05^{c}	2.45±0.04^{a}	2.82±0.02^{b}	3.09±0.01^{bc}
C24:1	1.16±0.01^{a}	1.08±0.03^{a}	1.10±0.01^{a}	1.76±0.01^{b}
ΣMUFA	37.11±0.37^{b}	28.71±0.03^{a}	34.11±0.20^{b}	34.94±0.06^{b}
C18:2n-6t	0.44±0.01^{b}	0.11±0.01^{a}	0.64±0.02^{c}	0.64±0.01^{c}
C18:2n-6c	12.60±0.19^{a}	13.08±0.11^{b}	13.77±0.06^{b}	12.88±0.03^{a}
C18:3n-3	6.01±0.09^{c}	3.02±0.07^{a}	6.04±0.04^{c}	5.81±0.01^{b}
C18:3n-6	0.35±0.01^{a}	0.31±0.01^{a}	0.62±0.02^{b}	0.60±0.01^{b}
C20:2	3.03±0.04^{c}	1.99±0.04^{a}	2.79±0.01^{bc}	2.43±0.01^{b}
C20:3n-3	1.57±0.02^{c}	0.52±0.01^{a}	1.02±0.02^{b}	1.45±0.01^{c}
C20:3n-6	0.35±0.01^{b}	0.19±0.01^{a}	0.32±0.01^{b}	0.44±0.03^{c}
C20:4n-6	1.69±0.02^{a}	1.64±0.03^{a}	1.69±0.02^{a}	1.67±0.02^{a}
C20:5n-3	3.81±0.05^{a}	7.31±0.01^{d}	5.28±0.05^{c}	4.31±0.03^{b}
C22:2	0.09±0.03^{a}	0.08±0.01^{a}	0.08±0.01^{a}	0.14±0.01^{a}
C22:6n-3	9.83±0.14^{c}	15.61±0.02^{d}	8.26±0.14^{b}	7.87±0.02^{a}
ΣPUFA	39.77±0.52^{a}	43.86±0.29^{b}	40.50±0.06^{ab}	38.23±0.07^{a}
ΣOmega-3	21.22±0.30^{a}	26.46±0.10^{b}	20.60±0.15^{a}	19.44±0.04^{a}
ΣOmega-6	15.43±0.21^{a}	15.33±0.16^{a}	17.03±0.08^{c}	16.22±0.05^{b}
ΣOmega-9	34.87±0.37^{c}	25.21±0.05^{a}	31.56±0.23^{b}	31.54±0.08^{b}
n3/n6	1.38±0.01^{b}	1.73±0.01^{c}	1.21±0.01^{a}	1.20±0.01^{a}
n6/n3	0.73±0.01^{b}	0.58±0.01^{a}	0.83±0.01^{c}	0.83±0.01^{c}
EPA/DHA	0.39±0.01^{a}	0.47±0.01^{b}	0.64±0.01^{d}	0.55±0.01^{c}
EPA+DHA	13.64±0.19^{b}	22.92±0.03^{c}	13.54±0.18^{b}	12.18±0.03^{a}
AI	0.32±0.01^{a}	0.50±0.01^{b}	0.36±0.01^{a}	0.38±0.01^{a}
TI	0.24±0.01^{a}	0.23±0.01^{a}	0.26±0.01^{a}	0.28±0.01^{a}
PUFA/SFA	1.65±0.02	1.60±0.03^{b}	1.60±0.02^{b}	1.43±0.01^{a}
HH	3.52±0.05^{b}	2.89±0.05^{a}	3.42±0.05^{b}	3.21±0.02^{a}
PI	138.01±1.94^{b}	197.87±0.59^{c}	135.90±1.21^{b}	125.15±0.30^{a}

Note: Each value means mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$).

Table 5. The L*, a*, b*, C* and Hue values of cultured fish fillets in the Black Sea

Values	Large Rainbow Trout	Turbot	Sea Bass	Sea Bream
1st location (The behind of the operculum)				
L*	50.88 ± 2.18^{by}	65.85 ± 1.93^{cx}	43.37 ± 0.90^{ax}	46.90 ± 1.33^{ax}
a*	10.00 ± 2.22^{bx}	0.98 ± 0.94^{ax}	0.79 ± 0.20^{ax}	0.72 ± 0.80^{ax}
b*	16.07 ± 1.41^{dx}	4.71 ± 1.24^{cx}	-0.35 ± 0.50^{ax}	0.33 ± 0.67^{bx}
C*	19.47 ± 1.99^{dx}	5.22 ± 1.26^{cy}	1.45 ± 0.25^{ax}	2.67 ± 0.74^{bx}
Hue	1.05 ± 0.10^{cx}	0.01 ± 0.51^{ax}	0.31 ± 0.45^{bx}	0.48 ± 0.24^{bx}
2nd location (Under the dorsal fin)				
L*	48.65 ± 0.70^{ax}	65.70 ± 0.81^{bx}	45.66 ± 1.14^{ax}	46.65 ± 1.15^{ax}
a*	12.27 ± 1.10^{cx}	0.93 ± 0.66^{ax}	3.68 ± 0.62^{by}	3.69 ± 0.98^{by}
b*	18.18 ± 0.96^{dy}	4.07 ± 0.83^{cx}	2.75 ± 0.72^{by}	1.34 ± 0.46^{ay}
C*	22.39 ± 1.27^{cy}	4.62 ± 0.94^{bx}	4.62 ± 0.50^{by}	3.88 ± 0.61^{ay}
Hue	1.02 ± 0.03^{dx}	-0.13 ± 0.22^{ax}	0.51 ± 0.49^{cy}	0.27 ± 0.08^{bx}
3rd location (Front the caudal fin)				
L*	53.62 ± 3.77^{ay}	62.48 ± 0.95^{bx}	51.40 ± 0.82^{ay}	49.61 ± 1.23^{ax}
a*	11.34 ± 2.44^{dx}	0.71 ± 0.58^{ax}	4.25 ± 0.65^{by}	6.18 ± 1.59^{cz}
b*	18.14 ± 0.92^{cy}	5.01 ± 1.09^{by}	3.41 ± 0.57^{ay}	2.77 ± 1.18^{az}
C*	21.99 ± 1.75^{bxy}	5.17 ± 1.14^{ay}	5.52 ± 0.69^{ay}	6.80 ± 1.94^{az}
Hue	1.05 ± 0.09^{cx}	0.49 ± 0.57^{aby}	0.68 ± 0.10^{by}	0.38 ± 0.05^{ax}
Average				
L*	51.05 ± 1.60^{b}	64.67 ± 1.10^{c}	46.81 ± 1.10^{a}	47.72 ± 0.76^{a}
a*	11.20 ± 1.27^{c}	0.88 ± 0.44^{a}	1.98 ± 0.52^{b}	2.58 ± 0.99^{b}
b*	17.46 ± 0.69^{c}	4.60 ± 0.54^{b}	1.08 ± 0.55^{a}	1.14 ± 0.57^{a}
C*	21.28 ± 1.08^{c}	5.00 ± 0.56^{c}	2.74 ± 0.61^{a}	3.68 ± 0.91^{b}
Hue	1.04 ± 0.05^{b}	0.13 ± 0.30^{a}	0.33 ± 0.19^{a}	0.39 ± 0.08^{a}

Note: Each value represents the mean±standard error. Values expressed with different exponential letters on the same line are statistically different from each other ($p<0.05$).

a, b: The differences between the means with different letters on the same line within the group are statistically significant ($p<0.05$).

x, y: The differences between the means with different letters in the same column in different regions are statistically significant ($p<0.05$).

The average a* and Hue values were the highest in large rainbow trout fillets and the lowest in turbot fillets ($p<0.05$). The average b* and C* values were the highest in the large rainbow trout fillets and the lowest in the sea bass fillets, and the difference was statistically significant ($p<0.05$).

Analysis of the biochemical composition showed that the turbot fillets had a statistically significant ($p<0.05$) lower dry matter (DM), crude fat (CF), crude ash (CA), and crude protein (CP) contents than analyzed other fish fillets. In addition, the biochemical composition values of turbot fillets were lower than previous studies (Manthey-Karl et al., 2016; Hernandez-Urcera et al., 2017). In the remaining three fish species fillets, the CP and CF values were higher than those presented in earlier studies values (Regost et al., 2001; Poontawee et al., 2007; Davidson et al., 2014; Baki et al., 2015; Kaya Öztürk et al., 2019, 2020; Tarricone et al., 2022). The biochemical composition of fish fillets did not correlate with the composition of the feed which the fish were fed ($r=0.15$, $p=0.714$); (Supplementary file Table S2), because it is not the only parameter that affects the biochemical composition of a fish species. In general, the biochemical compositions of fish vary with the season of cultivation (Petrović et al., 2015). Because fish are ectothermic poikilotherms, the CF in a certain period of the year can be attributed to the ongoing physiological process of conserving the fat stock, which occurs in autumn after intense feeding, or the process of spending the fat stock, which occurs during winter. In early summer, when environmental conditions change, primarily as an increase in temperature, the fish metabolism accelerates and the energy taken from food is used for fish growth. In addition, Grigorakis (2007) reported that a variety of variables, including age, sex, and environmental variables like temperature, salinity, etc., may have an impact on biochemical composition. Therefore, in this study, although the sea bass and sea bream were fed the same type of feed and in the same environmental condition, their biochemical composition contents differed significantly.

Adequate dietary amino acid supply is critical for animal and human health, growth, development, and survival (Wu, 2009). Amino acids have traditionally been classed as nutritionally essential (EAA) or non-essential (NEAA) for mammals, birds, and fish based on growth or nitrogen balance (Le Plénier et al., 2012). When the results were expressed per 100 g of product, the ΣEAA ranged between 6.80±0.01 g in sea bass fillet to 9.24±0.01 g in sea bream fillet. Although they were fed the same diets, the differences in the amino acid contents between sea bass and bream and were statistically significant. The EAA composition measured for the cultured fish fillets in the present study and generally matched previously reported fillets' EAA values, with lysine and leucine, consistently being dominant in all species (Kaushik, 1998; Peres & Oliva Teles, 2008; Sanchez-Lozano et al., 2011; Baki et al., 2015; Yıldız & Ofori-Mensah 2017; Colombo & Mazal, 2020). The EAA requirement for an adult human weighing 70 kg is about 5.6g per day (Gawedzki, 1997). The EAA results in the present study indicated that 100g of cultured fish from the Black Sea, met the daily requirement for EAA. In addition, Wang & Han (2017) reported that the EAA content and ratio is an essential factor for learning protein quality. According to FAO/WHO (1973), the ratio of EAA to ΣTAA should be at least 40% in a quality protein. In the current study, the EAA/TAA ratios of large rainbow trout, turbot, sea bass, and sea bream fillets were determined as 49.02, 43.59, 44.85, and 47.82%, respectively, and the protein quality of all fillets was suitable for human consumption according to FAO/WHO (1973).

The fatty acid composition data demonstrate that C18:1n-9 was the most significantly represented fatty acid in four species studied, followed by C18:2n-6 and C16:0. The results of this study are similar to previous studies that demonstrated an increase in C:18 fatty acids, such as C18:1n-9, C18:2n-6, and C18:3n-3, in cultured fish, in response to the use of vegetable oils in their feed (Benedito-Palos et al., 2009; Strobel et al., 2012). Among the unsaturated fatty acids, 18:1n-9 and C18:2n-6 contribute the most to the enrichment of aromatic components (Elmore et al., 1999) and are regarded as having high nutritional value since they protect against cardiovascular disease (Hornstra, 1999). There were statistically significant differences in the fatty acid content of the fillets of the fish species studied ($p<0.05$). The value of SFA (C13:0, C14:0, C15:0, C16:0, C17:0; C21:0 and C22:0) was the highest in the turbot fillets and this result was similar to Manthey-Karl et al. (2016) and Pleadin et al. (2017)'s study. The large rainbow trout, sea bass, and sea bream fillets had a significantly higher value of MUFA than the turbot fillets, which is attributable to their C18:1n-9 contents. The reason for the high oleic acid values in fish fillets is generally explained by over-adding vegetable oil sources in fish diets (Francis et al., 2007a, 2007b; Pettersson et al., 2010). In this study, the presence of high levels of C18:1n-9 in the diets of large rainbow trout, sea bass and sea bream (Supplementary file Table S2) supports the aforementioned studies. A significantly lower proportion of C18 PUFAs (C18:2n-6t, C18:3n-3, C18:3n-6) was found in the turbot fillets (except C18:2n-6c). In previous studies, the mentioned fatty acids were at low values in turbot fillets (Manthey-Karl et al., 2016; Pleadin et al., 2017). The fillet of turbot analyzed in this study contained less SFA and MUFA, and at the same time, more omega-3 and more PUFA than previously reported levels, resulting in a more favorable omega-3/omega-6 ratio than obtained by other researchers (Sérot et al., 1998; Pleadin et al., 2017). Many studies have demonstrated that fish consumption protects against coronary heart disease (He, 2009; Mozaffarian & Wu, 2011; Tacon et al., 2020). Simopoulos (2002) claimed that the omega-3/omega-6 ratio is a reliable index for comparing the relative nutritional values of species. High omega-3/omega-6 ratios are crucial for human health because they can reduce or prevent fetal programming and cardiovascular disease in adults (Marangoni et al., 2020; Shrestha et al., 2020). According to Sargent (1997) although the optimum omega-3/omega-6 ratio should be 1:5, Testi et al. (2006) reported that this ratio should be a minimum 1:1. In this study, the omega-3/omega-6 ratio of fish cultured in the Black Sea (the lowest sea bream, the highest turbot fillet, see Table 4) was higher than 1:1. It is thought that this result is an indicator of the environmental condition and feed quality in aquaculture. The consumption of fish and the intake of n-3 long-chain PUFAs are advised in distinct ways (especially EPA and DHA). While The American Heart Association (2022) recommends eating two servings of fish (especially oily fish such as salmon, and rainbow trout) twice a week to meet the dietary needs of EPA and DHA for human nutrition, the German Nutrition Association recommends a smaller once-a-week fat content portions of fatty fish (70 g) and larger portions of lean fish (80-150 g) (Berglaiter, 2012). Generally, most of official organizations recommend two meals of fish each week (around 140-240 g per meal) (Comi et al., 2022). This recommendation, would deliver about 500 mg of combined EPA and DHA per day, which is the amount that the majority of nations and organizations advise for optimum general health and decreased cardiovascular risk. Nevertheless, the European Food Safety

Authority (EFSA) has set a minimum daily value of 250 mg of EPA and DHA for adults (EFSA, 2009). Therefore, approximately 55, 92, 54, and 49g of large rainbow trout, turbot, sea bass, and sea bream, respectively must be ingested daily in order to meet the lowest-level EFSA recommendation.

Dietary fats are generally fatty acids that may play positive or negative roles in preventing and treating diseases. In nature, fatty acids occur in the form of mixtures of SFA, MUFA and PUFA, so their nutritional and/or medicinal values must be determined (Chen & Liu, 2020). The, IA, IT and HH (Ulbritcht & Southgate, 1991) are the most commonly used to assess the composition of fatty acids as they outline significant implications and provide clear evidence. Pleadin et al. (2017) reported that lipids with AI<1 and TI<1 are beneficial to human health. According to Tonial et al. (2014), MUFA and PUFA have more significant health advantages since they lower the risk of cardiovascular disease. The AI and TI values determined in the present study were lower than 1 and the statistical difference was not significant except for the AI value of the turbot fillet. In many studies, the AI and TI values of the fish fillets are lower than 1 and are in the similar value range with the data in this study (Cieślik et al., 2017; Khalili Tilami et al., 2018; Tarricone et al., 2022). Based on the above-mentioned discussion, it was decided to use HH, another nutritional quality measure, to learn more about how fatty acids affect cholesterol levels. It is ideal if the HH index has a greater value, such as >3 (Santos-Silva et al., 2002; Testi et al., 2006). In this investigation, the HH values varied from 2.89±0.05 in the turbot fillets to 3.52±0.05 in the large rainbow trout fillets. Because these three species had smaller amounts of SFA than turbot fillets, the HH ratios for large rainbow trout, sea bass, and sea bream were much greater than those for turbot.

Color is a significant attribute of fish freshness quality and is also used as an indirect estimate of chemical components and sensory characteristics of food (Cheng et al., 2015). Cultured sea bass and sea bream fillets were darker than turbot and large rainbow trout fillets, regardless of measuring regions, as can be observed by the significantly lower L* values ($p<0.05$) (Table 5). In some studies, it has been stated that the L* value determined in fish fillets is related to the moisture content of the fish fillets (Hernández et al., 2009; Fuentes et al., 2010), but this relationship was not found in this study. The a* value in large rainbow trout fillets was significantly higher than the other three cultured fish fillets and was statistically significant ($p<0.05$). Because sea bass, sea bream, and turbot are white-fleshed, it is normal for the a* value to be high for large rainbow fillets. The average a* value of large rainbow trout fillets was 11.20±1.27, which was lower than the studies on rainbow trout color parameters (Regost et al., 2001; Poontawee et al., 2007; Kaya Öztürk et al., 2019). The redness of the fillet in Salmonid species is an important quality parameter that gives a different image to this species. In previous studies, this difference in a* value reported that the redness values of fillet depend on the amount of carotene in fish feeds, pigment types, lipid source and level (Marty-Mahé et al., 2004; Lerfall et al., 2017).

Conclusion

Globally, aquaculture continues to be one of the fastest-growing food-producing sectors. However, preconceptions about the nutritional values of farmed fish influence consumption. Therefore, fish consumption is very important for balanced nutrition and minimizing health problems. This study established that cultivated fish from the Black Sea have excellent nutritional values, including high amounts of unsaturated fatty acids and the appropriate quantity of essential amino acids, and that they are of good quality, nutritious, and beneficial for human consumption.

Acknowledgments

I thank Sagun Aquaculture Company (in Sinop), Kızılırmak Aquaculture Company (in Samsun), and Central Fisheries Research Institute-SUMAE (in Trabzon) for providing the experimental fish and feed samples.

Compliance With Ethical Standards

Conflict of Interest

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

Ethical Approval

Not applicable. The fish were sampled by the operating personnel while they were harvested, therefore this manuscript does not need ethical approval.

Data Availability

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

Supplementary Materials

Supplementary data to this article can be found online at https://doi.org/10.33714/masteb.1195335

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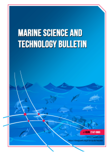
Mar. Sci. Tech. Bull. (2022) 11(4): 515-532
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1185059



Marine Science and Technology Bulletin

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REVIEW ARTICLE

Role of seaweeds in drug induced nephrotoxicity

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ARTICLE INFO

Article History:
Received: 08.10.2022
Received in revised form: 17.11.2022
Accepted: 16.11.2022
Available online: 23.12.2022

Keywords:
Nephrotoxicity
Medicinal herbs
Synthetic drugs

ABSTRACT

Kidney is an important organ which is necessary for the body to perform various important functions which include blood purification, expelling metabolic wastes and managing water and electrolytes balance in the body. In this era of modern science, many synthetic drugs are used on patients to examine their therapeutic properties. Unfortunately, some drugs cause negative effects resulting in renal damage. Drug induced nephrotoxicity results in serious clinical syndromes, such as chronic kidney disease (CKD) and acute kidney injury (AKI). Synthetic drugs not only cure diseases but also cause some side effects in the human body. Instead of looking for synthetic drugs to cure diseases, it is necessary to consider natural drugs that maximize side effects of synthetic drugs and reduce therapeutic consequences with the most effective and dynamic healing effects. Research and utilization of marine algae have increased markedly from the last several decades. Seaweeds have also been used as drugs or drug sources over a large number of years going back into folk medicine. Since consumption of seaweeds as human food or animal feeds is increasing rapidly. In the current review, we have summarized the information regarding the drugs which cause nephrotoxicity and marine algae as seaweeds used for the treatment of nephrotoxicity.

Please cite this paper as follows:

Sohail, N., & Farhat, H. (2022). Role of seaweeds in drug induced nephrotoxicity. *Marine Science and Technology Bulletin*, *11*(4), 515-532. https://doi.org/10.33714/masteb.1185059

Introduction

The presence of any kidney injury caused by medication directly or indirectly is called drug induced nephrotoxicity. Symptoms vary from acute or chronic in which decreased Glomerular Filtration Rate (GFR) to nephrotic syndrome and Hydro Electrolytic Disorders (HED) associated with tubular damage and glomerular filtration (Kane-Gill & Goldstein, 2015). Other serious clinical syndromes include AKI and CKD which ultimately lead to End Stage Renal Disease (ESRD) (Awdishu & Mehta, 2017). According to epidemiological studies showed that nephrotoxicity to be the third most common cause of acute kidney disease, which is going to be worse in recent decades due to the more frequently use of drugs

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that can cause kidney damage (Sales & Foresto, 2020; Sohail et al., 2021). It is estimated that 20% of nephrotoxicity is raised by drugs and 66% increased by the extent of drug usage (Kim & Moon, 2012; Yousaf et al., 2022). Numerous renal toxicities include acute and chronic interstitial nephritis, proximal renal tubular acidosis (Patel & Sapra, 2020).

Metabolic Functions of Kidney

Kidney is an important organ in our body which performs several important functions like blood purification, expelling metabolic wastes and managing water and electrolytes balance in the body (Ekinci Akdemir et al., 2017). Kidney dysfunction causes decline in the kidney by many aspects like an infectious disease, abnormal functioning of the immune system, high sugar level, malignant growth etc. (Chielle et al., 2015; Nisha et al., 2017). Besides their excretory role, their role in drug and chemical biotransformation is also crucial because drug elimination and detoxification both are main functions of the kidney which makes it an important organ (Garza et al., 2022). The transport mechanisms in the kidney are well known by electrolyte and acid-base disorders are also interrupted by drugs. If a person is already suffering from kidney diseases, then their medication should be carefully examined in order to prevent them from further nephrotoxic agents. Hence, precaution for kidney harm should be at forefront for methodologies to fight against drug-induced nephrotoxicity (Patel & Sapra, 2020). Despite nephrotoxicity, hepatotoxicity is also caused by a number of drugs i.e.; acetaminophen (AAP), methotrexate (Yan et al., 2018; Sajith et al., 2020) that ultimately cause liver damage (Rane et al., 2016). Liver also significantly contributes in biotransformation of drugs and toxins for the fulfillment of many functions such as carbohydrate, fat and protein metabolisms. Liver is also the main site of drug-induced damage. From ages, an upward trend has been witnessed in kidney and liver diseases worldwide (Ekinci Akdemir et al., 2017). Nephrotoxicity and hepatotoxicity are the two major concerns to the pharmaceutical companies and also to the Food and Drug Administration (FDA) (Beger et al., 2010; Zhang et al., 2012).

Role of Drugs in Nephrotoxicity

Researchers reported in literature that a lot of synthetic drugs with wide range of antibiotics or chemicals which causes nephrotoxicity and hepatic failure (Figure 1) (Perazella, 2018) including, cisplatin, acetaminophen (AAP), and, carbon tetrachloride (CCl_4) (Zamzami et al., 2019; Sohail et al., 2019, 2021). The most significant class of antibiotics executing toxic effects on kidney function is aminoglycosides which include gentamicin. Nephrotoxicity occurs in 10% to 25% of the cases who take gentamicin for 3 to 5 days (Saleh et al., 2016). Moreover, it decreases antioxidant enzyme activity and raises the reactive oxygen species (ROS) production (Erjaee et al., 2015). Vancomycin is an antimicrobial drug which causes nephrotoxicity in 10% to 20% of patients. Similarly, Cisplatin, (cis-diammine-dichloroplatinum (II), CDDP) is also the most significant nephrotoxic drug which is filtered at the glomerulus freely and absorbed into tubular cells of the kidney via transport-mediated process (Ciarimboli, 2014). It is normally used for the treatment of solid tumors that causes acute kidney damage after assemblage in the renal tubules (Sherif, 2015). For more than 25 years, it has been recognized that cisplatin causes nephrotoxicity and may cause various diseases including AKI, inadequate reabsorption in the proximal renal tubules, chronic kidney damage, deficiency of magnesium and calcium (Miller et al., 2010; Sohail et al., 2021). Although, from both clinical observations and animal experiments there is evidence which indicates that AKI is an independent risk for CKD as well (Shi et al., 2018). Commonly, the beginning of renal inadequacy starts after a few days of cisplatin dosage, evident by increased kidney profile (urea, creatinine & BUN). Another common occurrence is hypomagnesaemia, especially after dosage of cisplatin; nephrotoxicity has been identified even in the complete absence and reduction in glomerular filtration rate which is a high risk factor for cisplatin (Miller et al., 2010). As the dose of cisplatin increases the rate of nephrotoxicity also increases (Glezerman & Jaimes, 2016). Likewise, doxorubicin is another anti-cancer nephrotoxic drug which imbalances the functions between free radicals and antioxidants and therefore causes injuries in renal tissue (Ayla et al., 2011). Even though, usage of non-steroidal anti-inflammatory drugs (NSAIDs) i.e., AAP is linked with adverse renal effects which are approximately 1 to 5% among all patients (Solomon et al., 2017). The pharmacological effects of NSAIDs are dose and duration dependent, which predisposes the involvement of specific organs, and after liver the second one most affected is the kidney (Lucas et al., 2019). AAP is usually available as an over the counter drug and is widely available over the counter (OTC) in the UK and Australia (Lau et al., 2016). It is useful in mild to moderate pain of headache, myalgia and postpartum pain (Fokunang et al., 2018). It causes acute renal failure in 1–2% of patients (Canayakin et al., 2016; Hiragi et al., 2018). It is one of the most commonly consumed medications and its toxicity causes acute liver failure (ALF) which is reported in the United States (Rubin et al., 2018). NAPQI (N-Acetyl-p-benzoquinone) is present in the microsomal P450 enzyme system, which oxidize the major part of AAP taking away by

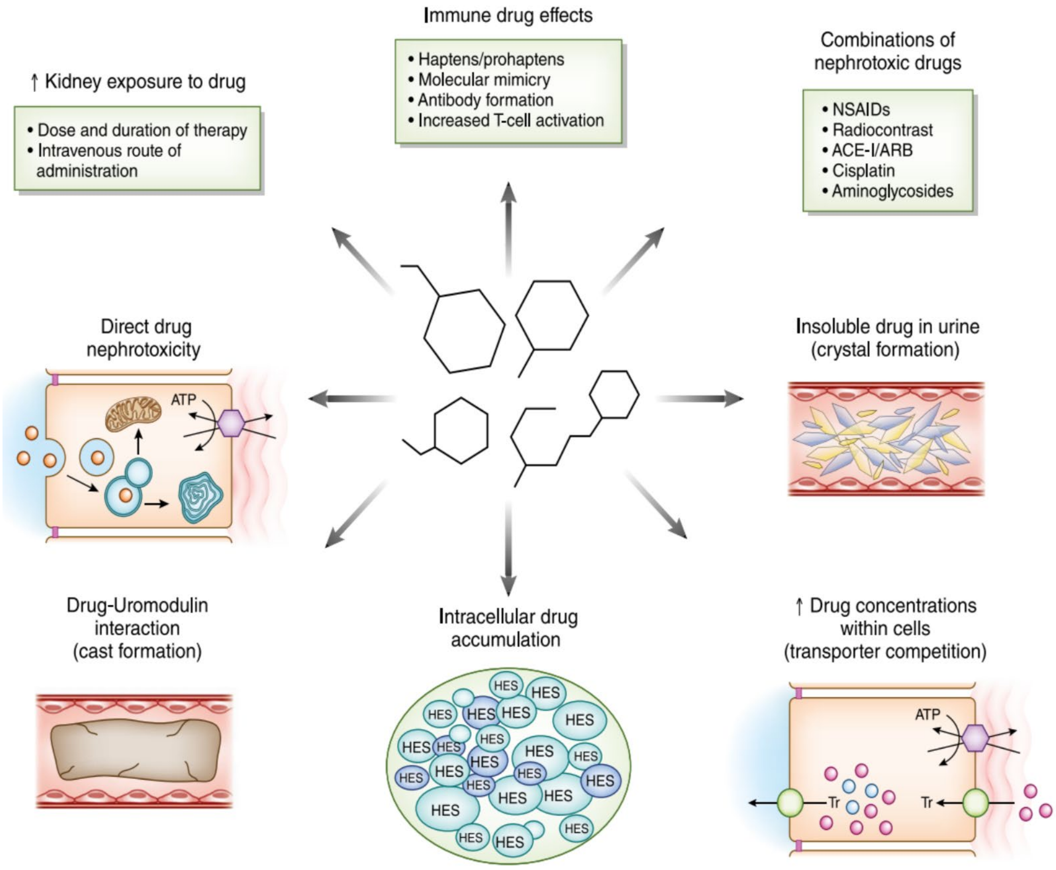

Figure 1. A prescribed mechanism of drug factors associated with increased risk for nephrotoxicity (Perazella, 2018).

GSH (intracellular glutathione) in therapeutic doses (Athersuch et al., 2018). The main symptom of acute kidney injury is acute necrosis in renal tubules caused by AAP toxicity by examining the level of kidney parameters (urea & creatinine) (Mazhar & Akram, 2016). When the drug toxicity is elevated in any organism, the free radicals are produced and oxidative stress performs a significant act in AAP which causes progressive kidney failure and also severe liver damage (Kheradpezhouh et al., 2010; Wang, 2017). Although these effects, warnings, and associations have been documented, acetaminophen remains a safe and effective medication when used correctly. The current manufacturer dose recommendation is limited to between 3 and 3.25 grams in 24 hours, depending on the formulation. However, toxicity is rare at less than 150 mg/kg for an adult or 200 mg/kg for a child (Gerriets et al., 2018).

Biomarkers of Drug Induced Nephrotoxicity

Biomarkers of kidney toxicity

Creatinine, urea and blood urea nitrogen (BUN) are the basic kidney parameters for the detection of nephrotoxicity in clinical laboratories. Healthy kidneys filter creatinine and other waste items which leave from our body in the form of urine. Therefore, determination on level of serum creatinine is necessary whether acute or chronic kidney diseases evaluation (Ostermann & Joannidis, 2016). BUN occurs in the liver as an end product and 15% excreted in the gastrointestinal (GI) tract and remaining through the kidney. The raising in BUN can also show kidney injury (Zachariah et al., 2019).

Beside these parameters, electrolytes also perform main functions in the kidney. The balance of electrolytes and fluidity is controlled by the kidney but if there is any dysfunction it causes acute or chronic kidney failure. Electrolytes include magnesium (Mg), phosphorus (P), potassium (K), sodium (Na) and calcium (Ca) which can be affected and lead to a range of side effects (Dhondup & Qian, 2017). Patients with CKD and ESRD experience both hyperkalemia and hypokalemia (Clase et al., 2020). The Na concentration is important for maintaining balance between acid-base and fluid and for neuromuscular function. The imbalance of Na level particularly those who have CKD, because kidneys have the ability to manage dilution and concentration level that gets damaged and finally causes kidney disease (Lim et al., 2016). Hyponatremia could also be a consequence of diuretic usage in these patients (Wolfe et al., 2010). Mg concentrations are also maintained by the kidney. The magnesium levels deteriorate when renal function does not perform properly (Cunningham et al., 2012). However, those patients who have CKD and ESRD have normally low magnesium levels (hypomagnesaemia) (Alhosaini et al., 2014).

Furthermore, Ca and P have reciprocal connection between each other and get imbalance during kidney injury. The symptoms associated with hypocalcemia include; Muscle cramp, disturbance in the brain, removal of mineral salts from bones, congestive heart failure etc. (Garrard & Jones, 2018).

On the other hand, it is not only possible that nephrotoxic drugs have a toxic effect on the kidney only but it also ultimately causes hepatotoxicity including hepatocellular damage, cholestasis and even tumor production (Patel et al., 2016). List of blood markers for estimating hepatotoxicity is given below:

Biomarkers of liver toxicity

Liver markers include enzymes in which Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST), Alanine aminotransferase (ALAT), Alkaline phosphatase (ALP) and bilirubin (Total bilirubin & direct bilirubin). ALAT is found in various organs like kidneys, liver, heart, and muscles. It occurs in cytoplasmic form and breaks the transamination reaction. Any sort of hepatic damage can increase the level of ALAT (Aulbach & Amuzie, 2017). It may be because of hepatitis, ischemic hepatic damage that causes liver damage. AST function is to replace the amino groups from glutamate to oxaloacetate to form aspartate which is used in the urea cycle as a by-product of nitrogen. Aspartate is utilized in the urea cycle and it's rising in patients with cirrhosis and even in hepatic injury that usually raise ALAT (Fikry & Ahmed, 2019). ALP is an enzyme which is found in renal tubules, liver etc. The main function of ALP is transport of the lipid in small intestines and bones calcification. Bones around 50% activate the ALP. The raise level of ALP usually occurs by acute viral hepatitis and others include; hepatic injury etc. Conversely, if ALP level is low then it causes hypothyroidism, zinc (Zn) deficiency, hypophosphatasia etc. (Fikry & Ahmed, 2019). LDH is an enzyme which performs a function to break the conversion of lactate to pyruvate. This enzyme is present in a vast variety of cells in the body. LDH content is present in various organs like; heart, kidney, liver, and muscle. Its production has been shown to be increased under hypoxic conditions in different cell lines (Yada et al., 2016).

Bilirubin is present in every person's blood and feces in the form of yellow pigment which proceeds by the liver. The liver can't process the bilirubin in case of an excess amount of bilirubin or inflammation in the liver. It is also reported that disturbance in bilirubin might increase the chances of certain heart diseases (Kwak et al., 2012; Fujiwara et al., 2018).

Beside these markers, lipid profile (Triglyceride & total cholesterol) and glucose are also key biochemical markers of nephrotoxicity and hepatotoxicity (Gill, 2016). The metabolism of lipids is actually the catabolic and anabolic of lipids in cells either to break down or store the fats. These fats are relatively consumed from food or they are synthesized in animal's liver for energy purposes. In the human body, excess amounts of lipid are present in the form of triglycerides and total cholesterol (Baynes, 2014). Furthermore, the system of kidney filtration can be damaged by raised levels of glucose because it goes into the bloodstream that may cause the kidneys to filter an excessive amount of blood (Triplitt, 2012).

Antioxidants markers

Antioxidants can mediate at first in the pathogenesis of renal damage by completely removing ROS. The research study in cells and tubules of nephrons and AKI in animal models have recognized nephron-defensive agents with antioxidant activities that decrease oxidative harm in the kidney (Panizo et al., 2015; Ratliff et al., 2016). In animal models the research clearly showed the increased level of oxidative stress and reduced tissue antioxidant status after nephrotoxicity (Dennis & Witting, 2017). Moreover, oxidative stress and ROS are considered to be driving factors in other chronic diseases that introduce the AKI include heart disease and diabetes (Ratliff et al., 2016).

Inflammatory cytokine in drug induced nephrotoxicity

Inflammation is the body's immune response which performs a significant function in progression of AKI (Black et al., 2019). Cytokines are nonstructural proteins which include interferon, chemokines, interleukins and tumor necrosis factors (Ferreira et al., 2018) which are transported in the form of paracrine and autocrine pathways. They are involved in different forms of diseases and disturbance in the immune system by anti and pro-inflammatory systems (Spangler et al., 2015). The pro-inflammatory effects in cytokines include TNF-α (tumor necrosis factor), IL-17 (interleukin-17), IFN-γ (interferon) and anti- inflammatory cytokines effect includes IL-4, IL-1ra and IL-10 and (Su et al., 2012). The inhibition in cytokines is linked with recovery of cisplatin which causes nephrotoxicity (Perse & Veceric-Haler, 2018). In this manner, new molecules are searched out that regulate kidney injury which is an important research zone that may lead to therapies to inhibit cisplatin-induced kidney injury (Humanes et al., 2017). Pro and anti-inflammatory cytokine relations work alone and sometimes merge with different types of cytokines responsible for up or down regulation of the rest of cytokines and some cytokines may have both inflammatory effects. It is typical for various cell types to emit a similar cytokine or for a single cytokine to follow up on a few diverse cell types (Pleiotropy) (Monastero & Pentyala, 2017).

Role of Medicinal Plant in Nephrotoxicity

God created everything on this earth which has a positive effect in contrast to humans who made things/medicines. To consider the side effect of drugs causes nephrotoxicity so researchers are looking for alternatives particularly coming from natural sources. There are some medicinal plants which are utilized to reduce the multiple illnesses for a very long time while they are being acceptable, reasonable, safe and accessible (Kasole et al., 2019). Natural or herbal therapeutics field has become one of the most famous trends because herbal products not only contain multicomponent therapeutics rather it also achieved a significant role in the health system for both humans and animals throughout the world (Pathak & Das, 2013). Numbers of evidence have reported that supplements from herbal medicine have potential to become a valuable complementary therapy in the treatment of various renal diseases (Roozbeh et al., 2013; Vahekeni et al., 2019).

Chinese herbal medicine (CHM) is a Traditional Chinese medicine (TCM), which has begun to attract academic attention in the world of western medicine. In past years, CHM aimed to develop new drugs for different varieties of diseases which affect human beings (Gu & Pei, 2017). China and other countries usually use CHM and it is ready to use in both the traditional and modern world (Pan et al., 2011). TCM theory is generally utilized in Asia and progressively worldwide to avoid CKD. The theoretical system of CHM is clearly different from conventional medicine and based on the differentiation of syndromes and patterns. The prescriptions are structured independently according to the patient's constitution and disease progression (Huang et al., 2018).

Medicines have many side effects which are used for the treatment of kidney damage, so herbal medicine performed a pivotal role in the prevention of many kidney diseases which are given in Table 1.

Role of Seaweeds in Drug Induced Nephrotoxicity

Over the last decade, researchers have developed an interest to focus on the significant role of algae which is a main source of a wide range of metabolites. Marine algae utilization and research have increased significantly in recent decades (Milledge et al., 2019). Marine algae have begun to gain attention as rich sources of various bioactive compounds with biomedical potential and great pharmaceuticals. It produces various compounds with great pharmacological activities such as anti-inflammatory, anticancer, antifungal, antimicrobial, antiviral etc. (Wali et al., 2019). Marine organisms survive in complex communities and live in close association with other organisms in competitive and hostile environments (Martins et al., 2014). They produce complex secondary metabolites as a response to ecological pressures such as predation and tide variations, competition for space. They serve as a source of natural products with therapeutic and nutritional properties. Moreover, the biomedical efficacy of several bioactive compounds derived from marine organisms has been experimentally demonstrated. Nowadays, researchers have demonstrated the various therapeutic properties of marine algae, which are verified *in-vivo* as well as *in-vitro* (Martins et al., 2014; Kim et al., 2018).

In Asian countries, seaweeds (marine algae) have been used as food for a long time. The estimated range of seaweeds is probably around 45,000 species. Researchers have been studying seaweed which is used as an alternative source for decades and found that it is one of the important resources for new therapeutics (Nedumaran & Arulbalachandran, 2015; Babu & Rengasamy, 2017). Seaweeds are also receiving attention for their bioactive metabolites like alkaloids, glycosides, flavonoids, saponins, tannins and steroids (Khalid et al., 2018). They may possess antiviral (Gheda et al., 2016), antibacterial (Shannon & Abu-Ghannam, 2016), antifungal (Mickymaray & Alturaiki, 2018), anticancer (Gutiérrez-Rodríguez et al., 2018; Sohail et al., 2021), antioxidant (Tariq et al., 2015), hypoglycemic (Akhtar et al., 2019), hypocholesterolemic (Ruqqia et al., 2015), hepato and renoprotective (Sohail et al., 2019, 2021) activities.

Phaeophyceae (Brown seaweed)

Brown seaweeds have the potential to provide an alternate source for solving many biomedical problems, including oxidative damage (Gomez-Zavaglia et al., 2019). *Sargassum*, a genus of brown seaweed, commonly known as sea holly or gulf-weed belongs to the family *Sargassaceae* and contains approximately 400 species (Kanimozhi et al., 2015). Fucoxanthin is the main pigment in brown seaweeds, which is one of the most abundant carotenoids in nature (10% of estimated total carotenoid production) (Pangestuti & Kim, 2011). However, different strains of brown seaweed have different profiles and compositions of fucoxanthin. Previous studies reported that fucoxanthin has anti-cancer, anti-diabetic, anti-oxidation, anti-carcinogenic, anti-inflammatory, anti-obesity and hepato-protective activities as well as cerebrovascular and cardiovascular protective effects (D'Orazio et al., 2012; Zhang et al., 2015).

Table 1. Effective role of medicinal plants used for the treatment of nephrotoxicity

S.No.	Plant	Part of Plant	Animal	Outcome	References
1	*Enicostemma littorale*	Whole plant	Rat	• Improvement in blood biochemical parameters • Increase antioxidant parameters • Amelioration in kidney histology	Bhatt et al. (2011)
2	*Ficus hispida*	Whole plant	Rat	• Amelioration in urine and blood biochemical parameters • Improvement in kidney histology and oxidative stress in both prophylactic and curative groups	Swathi et al. (2011)
3	*Nigella sativa*	Seeds and oil	Rat	• Normalization of the renal function • Improvement in oxidative stress • Amelioration of apoptotic markers • Amelioration in kidney histology	Salama et al. (2011)
4	*Cassia occidentalis*	Leaves	Rat	• Improvement in urine and blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Gowrisri et al. (2012)
5	*Phaseolus radiates*	Seeds	Rat	• Amelioration in blood biochemical parameters	Chaware (2012)
6	*Punica granatum*	Fruits	Rat	• Amelioration in kidney histology and blood biochemical parameters which were better when the extract co-administered with gentamicin which-induced nephrotoxicity	Ali & Saeed (2012)
7	*Khaya senegalensis*	Stem bark	Rat	• Amelioration in blood biochemical parameters • Betterment in hematological parameters • Amelioration in kidney histology	El Badwi et al. (2012)
8	*Ginkgo biloba*	Leaves	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Okuyan et al. (2012)
9	*Heliotropium Eichwaldi*	Root	Mouse	• Amelioration in blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Sharma & Goyal (2012)
10	*Moringa oleifera*	Leaves	Rat	• Amelioration in blood biochemical parameters • Decreased in kidney lipid peroxidation • Amelioration in kidney histology	Ouédraogo et al. (2013)
11	*Tephrosia purpurea*	Leaves	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress and histology of kidney in both curative and preventive groups	Jain et al. (2013)
12	*Nigella sativa*	Thymoquinone	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Kensara (2013)
13	*Vaccinium myrtillus*	Whole plant	Rat	• Improvement in oxidative stress • Amelioration in kidney histology	Pandir & Kara (2013)
14	*Curcuma longa*	Curcumin	Mouse	• Amelioration of kidney function • Decrease in intercellular adhesion molecule-, renal tumor necrosis factor-α and monocyte chemoattractant protein-1 • Improvement of kidney histology	Ueki et al. (2013)
15	*Curcuma longa*	Curcumin	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Palipoch et al. (2013)
16	*Cissampelos pareira*	Whole plant	Rat	• Amelioration in urine and blood biochemical parameters • Improvement in oxidative stress	Sekhara Reddy et al. (2014)
17	*Costus afer*	Leaves	Rat	• Amelioration in blood biochemical parameters • Amelioration in kidney histology	Ezejiofor et al. (2014)
18	*Elaeocarpus ganitrus*	Seeds	Rat	• Amelioration in urine and blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Kakalij et al. (2014)
19	*Myristica fragran*	Fruits	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress	Nivetha & Prasanna (2014)

Table 1 (continued)

S.No.	Plant	Part of Plant	Animal	Outcome	References
20	*Pistacia khinjuk*	Fruits	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress	Ghaedi et al. (2014)
21	*Tamarindus indica*	Fruits	Rat	• Amelioration in urine and blood biochemical parameters • Amelioration in kidney histology	Ullah et al. (2014)
22	*Punica granatum*	Flowers	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Motamedi et al. (2014)
23	*Turmeric plant*	Curcumin	Rat	• Increase anti-inflammatory cytokine levels • Decrease antioxidant expression	Soliman & Ismail (2014)
24	*Citrus medica*	Fruits	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Al-Yahya et al. (2015)
25	*Silybum marianum*	Silymarin	Human	• Reduction in kidney marker: creatinine, and urea 2 weeks after cisplatin administration	Momeni et al. (2015)
26	*Silybum marianum*	Silymarin	Human	• Oral administration of silymarin could not prevent cisplatin associated tubular dysfunction and nephrotoxicity in clinical study.	Shahbazi et al. (2015)
27	*Viscum articulatum*	Oleanolic acid	Rat	• Amelioration in urine and kidney biochemical parameters • Amelioration in kidney histology	Bachhav et al. (2012)
28	*Nigella sativa*	Seeds	Rat	• Amelioration in urine and blood biochemical parameters	Hosseinian et al. (2016)
29	*Plantago major*	Whole plant	Rat	• Reduction in kidney marker: creatinine, urea and in electrolytes: potassium in all extract treated animals • Raising in sodium in the extract 600 mg/kg group • Increase of superoxide dismutase and reduction in malondialdehyde concentration activity in extract 1200 mg/ kg group • Increase of catalase activity in all extract-treated animals	Parhizgar et al. (2016)
30	*Nigella sativa and Curcuma longa*	Nigella sativa: Seeds Curcuma longa: rhizome	Rat	• Improvement in oxidative stress	Mohebbati et al. (2017)
31	*Matricaria chamomilla*	Whole plant	Rat	• Normalization of the renal parameters • Improvement in oxidative stress • Amelioration of apoptotic markers • Amelioration in kidney histology	Farouk et al. (2017)
32	*Lemongrass*	Citral	Rat	• Amelioration antioxidant activity	Uchida et al. (2017)
33	*Caesalpinia bonduc*	Whole plant	Rat	• Improvement in blood biochemical parameters • Amelioration in kidney histology	Talukdar et al. (2018)
34	*Ficus carica*	Leaves	Rat	• Improvement in blood biochemical parameters • Improvement in oxidative stress markers	El-Sayed et al. (2019)
35	*Crocus sativus*	Saffron	Rat	• Amelioration in blood biochemical parameters • Improvement in oxidative stress • Amelioration in kidney histology	Zarei & Elyasi (2022)
36	*Mangifera indica*	Whole plant	Rat	• Amelioration in blood biochemical parameters • Improvement in kidney tissue damage	Akter et al. (2022)
37	*Nigella sativa*	Oil	Rat	• Improvement in kidney parameters • Increase in catalase and superoxide dismutase activities • Amelioration in kidney histology	Jaswal et al. (2022)

Table 2. Protective role of seaweeds for the treatment of nephrotoxicity in rats

S.No	Seaweeds	Class	Outcomes	References
1	*Colpomenia sinuosa*	Brown algae	Nephroprotective effects against carbon tetrachloride (CCl_4)	Ramarajan et al. (2012)
2	*Sargassum polycystum*	Brown algae	Nephroprotective and hepatoprotective activity against diabetic effect	Motshakeri et al. (2014)
3	*Ulva lactuca*	Green algae	Nephroprotective and antioxidant activity against D-Galactose	Yang et al. (2021)
4	*Turbinaria ornate, Padina pavonia*	Brown algae	Renoprotective activity against azoxymethane	Mahmoud et al. (2014)
5	*Ulva fasciata*	Green algae	Nephroprotective activity against anti-cancer drug, cisplatin and anti-bacterial drug, gentamicin	Rizk et al. (2016) Abd El Raouf et al. (2017) Sohail et al. (2021).
7	*Porphyra yezoensis*	Brown alga	Nephroprotective activity against cisplatin	Kim et al. (2018)
8	*Stokeyia indica*	Brown alga	Renoprotective and hepatoprotective activity against acetaminophen (AAP)	Taj et al. (2019)
9	*Spirulina platensis*	Green algae	Nephroprotective activity against cisplatin	Zakaria et al. (2019)
10	*Sargassum swartzii*	Brown alga	Nephroprotective and Hepatoprotective activity against acetaminophen (AAP) and CCl_4	Hira et al. (2019)
12	*Halymenia porphyroides, Sargassum ilicifolium*	Red algae Brown algae	Renoprotective and hepatoprotective activity against acetaminophen (AAP)	Sohail et al. (2019)
13	*Sargassum angustifolium*	Brown algae	Renoprotective effect against gentamicin induced nephrotoxicity	Pourkhalili et al. (2021)
11	Chondrus canaliculatus	Red algae	Antioxidant, nephro and hemato-protective against maneb	Jaballi et al. (2019)
14	*Sargassum cinereum*	Brown algae	Renoprotective and hepatoprotective activity against Cyclophosphamide	Khalil et al. (2020)
15	*Sargassum fusiforme*	Brown algae	Reno and hepatoprotection against cisplatin	Natarajan et al. (2022)
16	*Laminaria japonica*	Brown algae	Renoprotective effect against glycerol-induced acute kidney injury	Li et al. (2017)

Rhodophyta (Red seaweed)

The Rhodophyta (red algae) are a distinct eukaryotic lineage. The red color of these algae is due to the pigments; phycoerythrin and phycocyanin. It characterizes other pigments, chlorophyll *a* (without chlorophyll *b*), beta-carotene, and other unique xanthophylls (Barkia et al., 2019). Abundantly cultivated edible red seaweed, *Eucheuma cottonii*, contains large amounts of polyunsaturated fatty acids, minerals, polyphenols, dietary fiber, vitamins, proteins, antioxidants and phytochemicals and has medicinal properties (Kania et al., 2013). *E. cottonii* showed the highest *in-vivo* antioxidant and anti-hyperlipidemic activities, elevated erythrocyte GSH-Px, and reduced plasma lipid peroxidation of high-fat diet rats toward the values of normal rats. *Gracilaria birdiae* (GB) is grown in northeast Brazil and used for food. However, little research has been done on the economic potential of this alga. Over 300 species of *Gracilaria* have been identified, of which 160 are taxonomically recognized (Sakthivel & Devi, 2015). *G. birdiae* protects both the kidney and liver of rats from CCl_4 which cause nephrotoxicity and hepatotoxicity possibly due to their antioxidant capacity (Barros-Gomes et al., 2018).

Chlorophyta (Green seaweed)

Ulva is a green alga, commonly known as sea lettuce, is widely distributed beyond coastal regions of the world and is widely used in the biotechnology, food and pharmaceutical industries (Torres et al., 2019). Green seaweeds contain high amounts of polyphenols such as epigallocatechin gallate, epicatechin, catechin, and gallic acid (Vinayak et al., 2011). Some beneficial green edible seaweed is; *Ulva reticulata, Ulva lactuca,* etc. Ethyl acetate extract of *U. lactuca* showed the highest antifungal activity than that of other extracts against filamentous fungi and yeast. *Ulva reticulata* is widely distributed in the Indo-west Pacific region, Southwest Asia, Northern Pacific Ocean, Southeast Asia and Western and Eastern Indian Ocean. In southern India, *U. reticulata* is highly concentrated along the coastline of Tamil Nadu, especially Mannar Bay, Rameshwaram to Kanyakumari (Lalitha & Dhandapani, 2018).

Pharmacological studies indicate that fucoidan is a sulfated polysaccharide extracted from brown seaweeds that inhibits renal fibrosis and glomerular sclerosis by reducing the accumulation of extracellular matrices. It also maintains the glomerular basement membrane and glomerular structural integrity, improves glomerular filtration function, and protects renal glycosaminoglycans from abnormal degradation (Wang et al., 2019). Josephine et al. (2006, 2007) reported that isolated sulphated polysaccharide reduced blood urea nitrogen and serum creatinine level and attenuated renal tubular damage in renal glomerular injury in rats. Polysaccharides from green seaweeds, *Caulerpa racemose* (sea grapes) have been reported to be the kidney protective effects in diabetic models (Cao et al., 2021). The chemical constituents like fatty acids, alkaloids, phenolic compounds, terpernoids, were identified by GC-MS from green seaweeds like *Ulva fasciata* has nephroprotective activity (Abd El Raouf et al., 2017). The protective roles of seaweeds in nephrotoxicity are given in Table 2.

Conclusion

Worldwide kidney diseases have become a major cause for disability and even leading to death in the worst circumstances. Drugs which are usually used to treat diseases but its overdosing causes side effects and complications in the kidney and liver so there is a vast interest to search for alternative medicine which especially comes from natural sources without any side effects. The World Health Organization (WHO) reported that 80% of people rely on natural sources for some part of their primary health management needs. The main sources of these successful compounds are plants and microbes from the marine and terrestrial environments. Thereby, researchers are working on marine based natural products particularly edible seaweeds such as sea lettuce showed a promising role in attenuation of drug induced nephrotoxicity. In-addition they might be considered as potential candidates for natural based drug therapy as they minimize adverse effects of drugs particularly cisplatin and AAP. Further works are currently underway on seaweeds and other plants to discover effective medicines with fewer consequences.

Acknowledgements

I would like to acknowledge my supervisor Prof. Dr. Syed Ehteshamul-Haque (now late), Biological Research Centre (BRC), University of Karachi, for his support and advise.

Compliance With Ethical Standards

Authors' Contributions

NS: Designed the study, Wrote the first draft of the manuscript.
HF: Support and help to complete my work with efficient manner.
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.

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Mar. Sci. Tech. Bull. (2022) 11(4): 533-539
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1182270

Marine Science and Technology Bulletin

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SHORT COMMUNICATION

First record of *Rhynchorhamphus naga* Collette, 1976 (Beloniformes: Hemiramphidae) from Kerala, India, South Eastern Arabian Sea

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ARTICLE INFO

Article History:
Received: 30.09.2022
Received in revised form: 21.12.2022
Accepted: 22.12.2022
Available online: 28.12.2022

Keywords:
Hemiramphidae
South-west coast
Rhynchorhamphus naga

ABSTRACT

Species, under the genus *Rhynchorhamphus* (Family: Hemiramphidae), are widely distributed marine groups of fishes. Among four species described from India, only two species, i.e., *Rhynchorhamphus georgii* and *R. maabarica,* are reported along the Indian coast. However, during the present study, a specimen collected from the Western Indian Ocean, Chetty harbour, Kerala, the south-west coast of India, has been identified as *Rhynchorhamphus naga* (Collette, 1976), based on morphology and molecular characters. The species is characterized by D-14; A-14; Pec-9; Pev-6; GR-50 and a prolonged beak (171.82% HL). The species has been reported from Western Central Pacific and several other countries like Brunei Darussalam, Cambodia, Malaysia, Philippines, Singapore, Thailand and Vietnam considered to be endemic to that region. This finding reveals that the species has a wider distribution, as we recorded the species from Kerala, south-west coast of India along the Indian Ocean.

Please cite this paper as follows:

Behera, A., Gowda, J., Kumar, A. P., & Jaiswar, A. K. (2022). First record of *Rhynchorhamphus naga* Collette, 1976 (Beloniformes: Hemiramphidae) from Kerala, India, South Eastern Arabian Sea. *Marine Science and Technology Bulletin*, *11*(4), 533-539. https://doi.org/10.33714/masteb.1184165

Introduction

Rhynchorhamphus naga Collette, 1976, a minor epipelagic fish belonging to the family Hemiramphidae, is distributed widely in fresh, brackish and marine habitats. *R. naga* has been reported from the Gulf of Thailand and East Indies (Collette, 1976), Indonesia, Brunei Darussalam, Cambodia, Malaysia, Philippines (Cantor, 1849), Singapore, Thailand and Vietnam (Froese & Pauly, 2019) (Figure 1). The congeneric species *Rhynchorhamphus georgii* has a wide range of distribution in India (Varghese, 2005). Collette (1976) examined species of the genus *Rhynchorhamphus* in various museums and confirmed one from the Gulf of Thailand and the Java Sea as *Rhynchorhamphus naga* among two unrecognized species. The earlier literature on the distribution of species of the family

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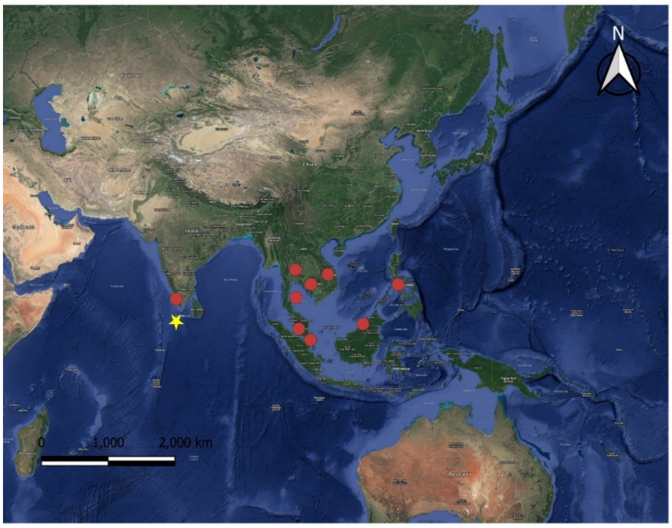

Figure 1. Distribution of *Rhynchorhamphus naga* Collette, 1976 in the World Ocean. The red circles indicate the records in different countries i.e., Brunei Darussalam, Cambodia, Malaysia, Philippines, Singapore, Thailand and Vietnam recently, from India Kerala - marked by yellow star).

Hemiramphidae has reported the distributional record of *R. georgii* (Figure 2) and *R. malabarica* from the Arabian Sea, especially in the south eastern region excluding *R. naga* (Varghese, 2005). However, a species collected from the the south eastern Arabian Sea region, Kerala, India did not match with the species distributed in the region. The identity of the species was confirmed by using standard identification keys (Collette, 1976), for morphomeristic traits and molecular tools (Ward et al., 2005) as *Rhynchorhamphus naga* (Figure 3) and thus, the present communication deals with the first record of *R. naga* from India.

Material and Methods

The fishes were caught by traditional ring seines (mesh size 8-10 mm), locally known as 'choodavala' from a depth of (7-8 m) in December 2018 in Chetty Fishing Harbour (9°37'25.4814" N, 76°17'44.7246" E), Kerala, India. Specimens were identified using a standard key (Collette, 1976), photographed and morphological traits were studied in fresh conditions. The COI gene (655bp) from the mitochondrial region was amplified by using targeted Primers such as FISH F2, FISH R2 (Ward et al., 2005). The thermal conditions for amplifying the COI region were set as an initial denaturation for 3 min at 94°C, followed by 35 cycles of 30 s at 94°C for denaturation, 30 s at 54°C for annealing, 60 s at 72°C for extension, with a final extension at 72°C for 10 min. The amplicons were purified and sequenced in both directions using PCR primers. The consensus sequences were blasted (BLASTN) against NCBI, and deposited in NCBI, Gene Bank (Accession Number *R. naga*-MN855100) (Figure 4).

Results

The collected specimen of hemiramphid was identified as *R. naga* (Collette, 1976) based on morphological and molecular characters (Table 1). Body slender (267.65 mm SL) with a prominent beak (171.82% of HL) but comparatively shorter than *R. georgii*. Nasal pectoral length 59.34% of HL. The upper jaw length (25.18% of HL) with prominent scales. Pre dorsal length is 51.62% of the standard length. Rays on dorsal fin 14; anal fin 14; pectoral fin 9; pelvic fin 6; gill rakers on first gill

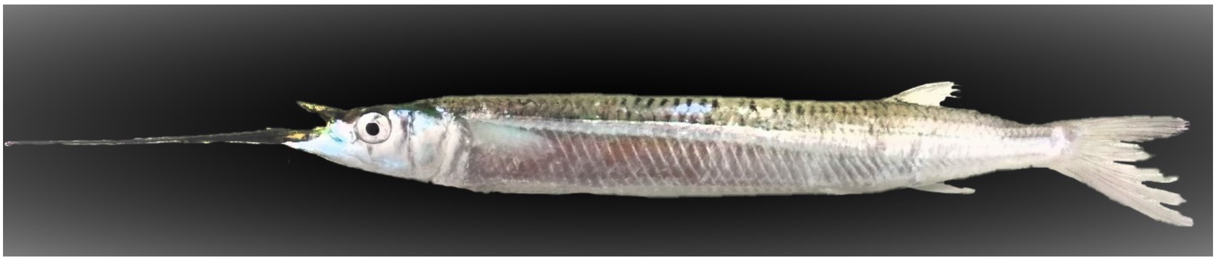

Figure 2. *Rhnchorhamphus georgii*

Figure 3. *Rhynchorhamphus naga*

Table 1. Morphometric and meristic parameters of species of genus *Rhynchorhamphus*, expressed as a percentage of standard length and head length

Parameters	**SPECIES**				
	R. naga		***R. georgii***		***R. malabarica***
Author	Present (n=1)	Collette, 1976	Present (n=15)	Collette, 1976	Collette, 1976
Characters (% of HL)					
ED	20.15%		17.49%-23.95%		
SNL	43.75%		42.61-51.64%		
BL	171.82% (1.71)	1.52-3.29 (mean x= 2.08)	134.05%-181.78%	1.23-2.64 (x's 1.48-1.75)	1.23-2.64 (x's 1.48-1.75)
UPJL	25.19%		24.97%-32.06%		
IOD	23.65%		24.11%-32.09%		
IND	12.38%		12.63%-18.85%		
NPL	59.35%		59.60%-71.83%		
Characters (% of SL)					
PDL/SL	55.06%%		53.18%-69.99%		
PAL/SL	59.23%		56.52%-71.71%		
PPvL/SL	10.38%		27.86-63.99%		
PPeL/SL	18.36%		16.85%-23.10%		
DFBL/SL	10.95%		10.48%-14.40%		
AFBL/SL	8.42%		7.30%-10.74%		
PEBL/SL	1.79%		1.78%-2.97%		
DFL/SL	6.39%		6.09%-8.57%		
AFL/SL	5.41%		4.52%-6.75%		
PeFL/SL	8.06%		8.50%-11.0%		
BD/SL	7.05%		7.86%-9.88%		
BW/SL	5.58%		4.90%-6.73%		
DAD/SL	7.02%		7.57%-9.06%		
CPL/SL	5.54%		3.06%-4.67%		
CPW/SL	3.28%		3.35%-6.77%		
Meristic characters					
Dorsal fin rays	14	14 or 15	14-16		
Anal fin rays	14	14 or 15	13-15		
Pectoral fin rays	9	10-12	9-11		
Pelvic fin rays	6		5-6		
Gill raker	50	47-59 (x 52.4)	45-58		
Pre dorsal scale	42	37-43 (Usually 40)	41-45		

Note: (Standard length or Sl; Snout length SNL; Eye Diameter ED; Upper jaw length UJL; Beak length BL; Inter orbital Depth IOD; Inter Nasal Depth IND; Nasal Pectoral length NPL; Head length HL; Pre dorsal length PDL; Pre Anal length PAL; Pre pelvic length PPvL; Pre pectoral length PPeL; Dorsal fin length DFL; Dorsal fin Base length DFBL; Anal fin Length AFL; Anal fin Base length AFBL Pectoral fin Length PeFL; Body Depth BD; Body Width BW; Dorsal Anal Distance DAD; Caudal peduncle Length CPL; Caudal peduncle Width CPW)

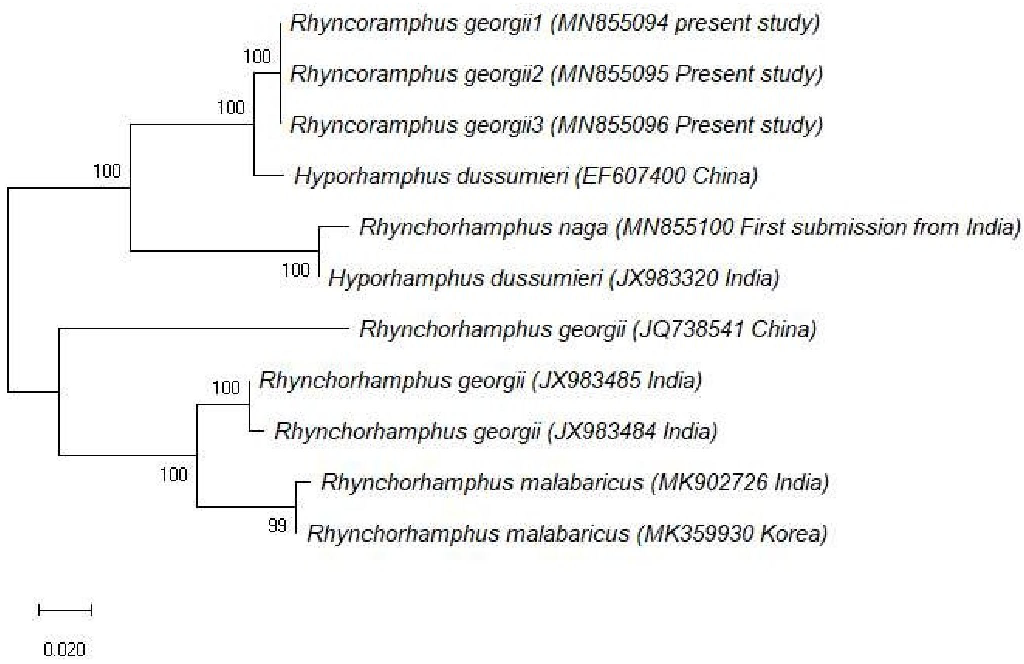

Figure 4. Maximum likelihood phylogenetic tree of *R. naga* based on DNA sequences of the mitochondrial COI gene

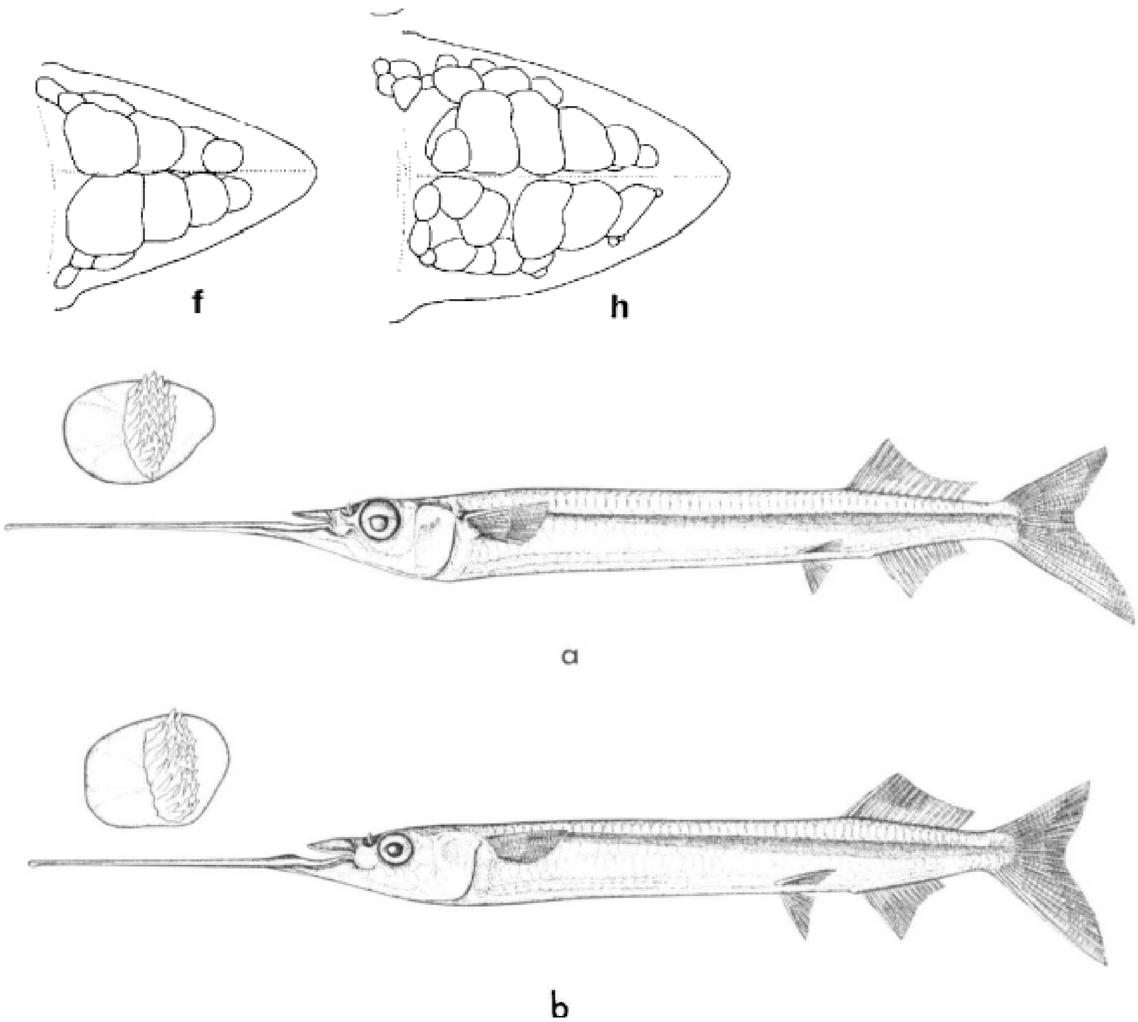

Fig. 5. The most peculiar distinguishing features of *Rhynchorhamphus naga* (Collette, 1972) (f, a) & *R. georgii* (Collette, 1972) (h, b)

arch 50, predorsal scales 42. Body with prominent silvery stripe, passing from behind the opercular region to the origin of the caudal fin. Caudal fin slightly forked. Both the jaws with adense, minute and conical teeth; the tongue and vomer are toothless. Body scales large, thin, cycloid and deciduous. In fresh condition, abdomen appears bluish, beak yellowish blue, a prominent lateral stripe passing through the lateral side of the body and slightly broadened towards the caudal region; lower lobe of caudal fin bluish while upper lobe yellowish blue, and blue marking on anal fin and below the abdomen.

Though there are very minute differences among the species of the genus, the species can be differentiated based on the lowest number of gill rakers (41), a narrow as well as shorter upper jaw and a broad prominent silvery stripe along the body, and slightly triangular shaped head.

Table 2 Comparison of morph-meristic characters of *R. naga* reported by authors from different geographical areas

Morphometric Traits	**Authors**	
	Present (n=1)	**Collette (1976)**
Characters in proportion to HL		
LJL/HL	1.71	1.52-3.29
IOD/OD	1.17	0.84-1.21
Meristic Characters		
DFR	14	14
AFR	14	15
PEFR	10	10-12
PVFR	6	-
PDS	42	37-43
GR (1st Gill arch)	50	47-59
GR (2nd Gill arch)	41	40-53

Note: HL-Head Length, LJL-Lower jaw Length, IOD-Inter Orbital Distance, IOD-Inter Orbital Distance, OD-Orbit Diameter)

Discussion

The Indo-Pacific region is considered to be a distributional hub, particularly for the genus *Rhynchorhamphus* (Collette, 1976). Collette (1976) has described *R. naga* from the Gulf of Thailand and the East Indies. There is no reported evidence of a distributional record of *Rhynchorhamphus naga* from the Indian Ocean region. Further, the overlapping of morpho-metric characters among the species under the genus *Rhynchorhamphus* creates ambiguity. There are very minute differences among the species of the genus (closely resembles *R. georgii*), but the species can be differentiated based on the lowest number of gill rakers and narrower as well as shorter upper jaw (compared to *R.georgii*) and a broad prominent silvery stripe along the body, slightly triangular shaped head. Thus, even if the species was distributed in the region, it was not correctly identified and reported earlier. The incorrect species identification based on morphological characters that has been deposited in public sequence databases such as GenBank leads to further taxonomic complications, thus there is a necessity for taxonomic verification of deposited sequence data based on taxonomic characters of voucher specimens (Silpa et al., 2021). However, the identity of *R. naga* was confirmed with the barcoding along with the original description. The species has been confirmed through NCBI-BLAST. Further, though the species has shown genetical similarity with *Hyporhamphus dussumieri*, there is a clear distinction between the genera *Rhynchorhamphus* and *Hyporhamphus,* especially the presence of fimbriae with nasal papilla in *Rhynchorhamphus* (Figure 5). Comparison of the morpho-meristic observations (Table 2), recorded during the present and earlier study (Collette, 1976) shows close similarity in the character of the species. Among the species under the genus *Rhynchorhamphus*, *Rhynchorhamphus naga* consists of fewest number of gills rakers. The proportion of IOD/OD 1.17 and LJL/HL 1.71 were found to be in the range of earlier studies. The standard length of the specimen was recorded to be 285.48 mm, comparatively higher than the earlier observation (62-177 mm). This variation may be due to a change in temperature which is considered to be an extrinsic factor for influential growth (Denechaud et al., 2020). The present report confirms the extension of the distribution range of *R.naga* from the Gulf of Thailand and East Indies to Kerala, the south-west coast of India, South eastern Arabian Sea. The east and west coast of Gulf of Thailand has been influenced by Pacific Ocean, the South China Sea, the Indian Ocean, the Andaman Sea, respectively. There is a possibility of migration of fish from the Gulf of Thailand to India through the Straits of Malacca due to surface water current (Kimura et al., 2009; Satapoomin, 2011; Klangunurak et al., 2012). Climatic variability associated with the change has been reported as a responsible factor for the distributional pattern of fishes (Rijnsdorp et al., 2009; Vivekanandan, 2011; Mohanty et al., 2017; Campana et al., 2020).

Acknowledgements

This work was carried out under the Masters study of the main author, at ICAR-Central Institute of Fisheries Education, Andheri, Mumbai, India. The authors would like to extend their gratification to the Director, ICAR-CIFE for providing support and facilities for carrying out this work.

Compliance With Ethical Standards

Authors' Contributions

AB & AKJ: Designed the study.
AB & JTM: Collection of the specimens.
AB: Wrote the first draft of the manuscript.
AB, JTM & APK: Performed and managed statistical analyses.
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

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

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