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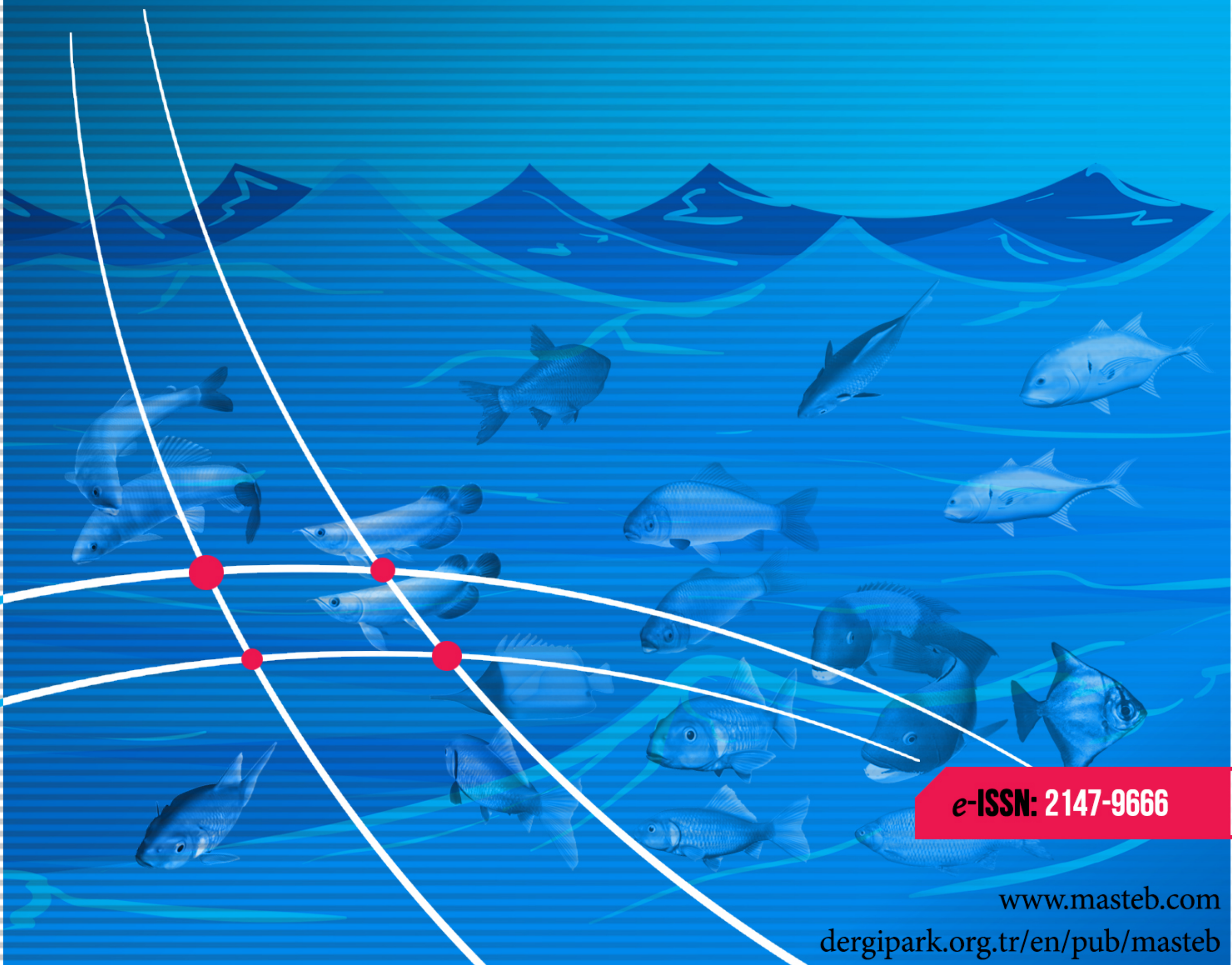

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RESEARCH ARTICLE

Spatio-temporal distribution of aquatic biodiversity in Gorai Creek, Sub-Urban Mumbai, India

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ABSTRACT

This study was conducted to assess the faunal diversity in Gorai Creek of Mumbai, India from August 2019 to July 2020. The data collected month wise were pooled together and transformed into three seasons [Monsoon (June to September), Winter (October to February), and Summer (March to May)] and this seasonal data were used for the study of biodiversity pattern. Using the average seasonal species occurrence data as input the following biodiversity indices, S, N, d, J', H'(loge), 1-Lambda, Delta, Delta*, Delta+, sDelta+, Lambda+, Phi+ & sPhi+ were calculated by using PRIMER (v7.0). It revealed that 90 species of fauna under 70 genera, 50 families, 29 orders, 6 classes and 3 phyla were found to occur in the creek. The order-wise representation of identified fauna was found to be 7 ichthyofaunas, 4 pelecypods, 6 gastropods, 2 cephalopods, 1 shrimp and 1 stomatopod along with 8 avifauna. The present study results indicated that the Gorai Creek ecosystem is endowed with moderate biodiversity that needs to be conserved.

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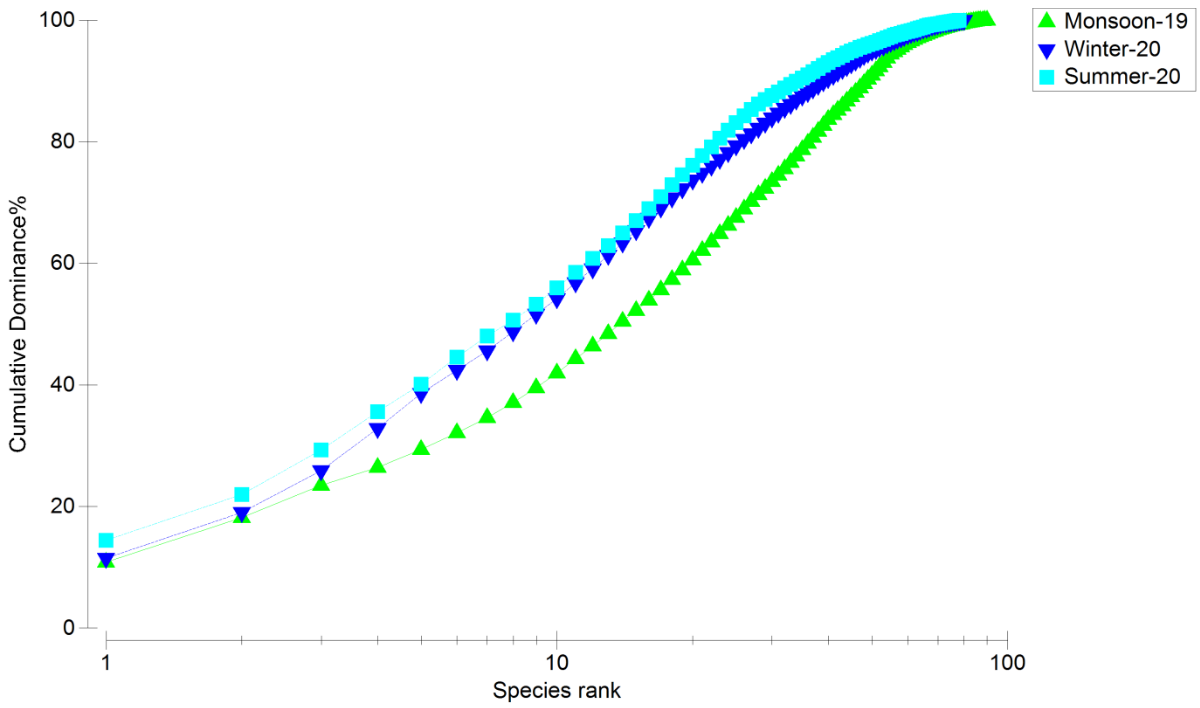

Figure 6. K-dominance plot among different seasons at Gorai Creek, Mumbai

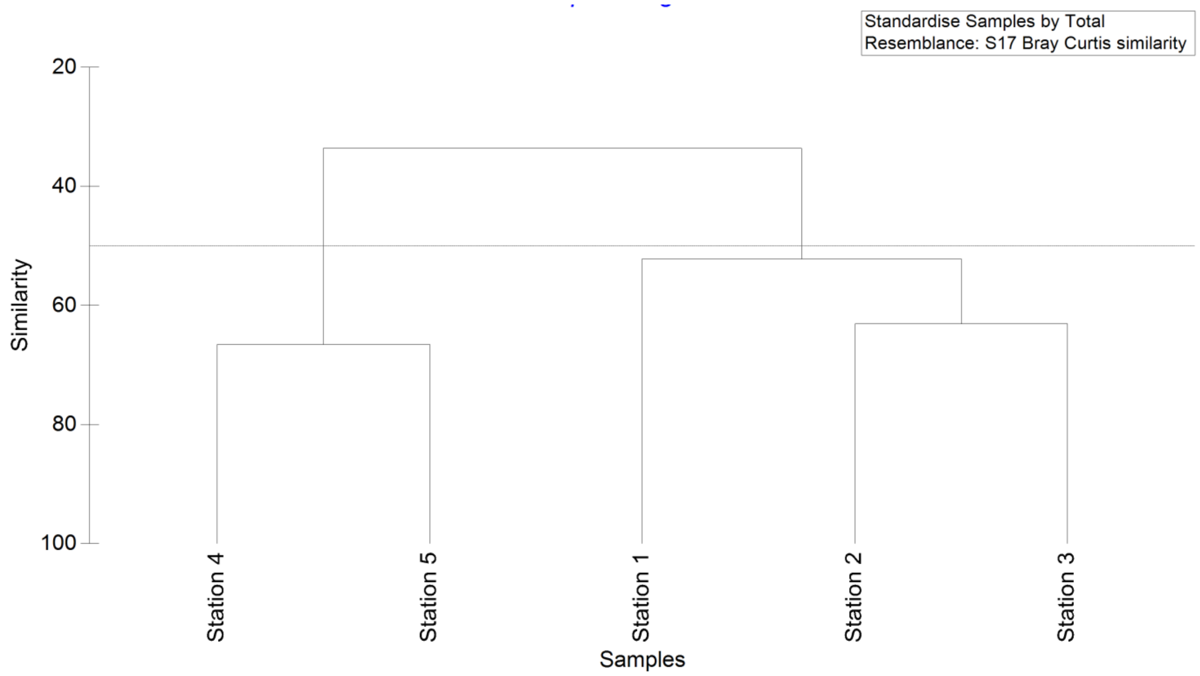

Figure 7. Dendrogram showing similarities between spatio-temporal variations based on the composition of species identified in Gorai Creek, Mumbai

Discussion

A total of 15 species of avian fauna belong to 8 orders, 11 families were recorded from the Gorai Creek, all are those species often hovered in coastal wetland areas. Still, the diversity shows more diminutive than the avian fauna recorded by Lad & Patil (2014) recorded 131 avian species belonging to 45 families including resident and migratory birds from Bhayander and Naigaon, Maharashtra. Out of 45 families observed in the study area, family Scolopacidae and Accipitridae represented 12 species each followed by Ardeidae and Sylviidae with 8 and 7 species, respectively while family Laridae, Motacillidae and Rostratulidae with 6 species each. It was seen that, 68% of the species were resident while 32% were migratory. However, the present study was comparatively less to the avian species recorded along with the wetland areas of Gorai Creek. The reason attributed for low diversity of avifauna is attributed due to the loss of habitat by reclamation of land for

Introduction

Coastal ecosystems occupy more than 70% of the global water surface area with rich aquatic diversity, abundance and dynamic resource distribution that determines the health and stability of these ecosystems (UNEP, 2006). Estuaries are unique coastal ecosystems utilized by various group of aquatic biota and also man-kind (Qasim, 1973; Ansari et al., 1995; Whitfield, 1999). In recent times, the biodiversity study has been focused to address the issues of declining fauna along with the ecological concerns (Thiel et al., 1995). Claridge et al. (1986) stated that the functionalities of an estuarine creek are the congregation of biota, since it is the place for shelter with rich food; despite fluctuating environmental conditions. The species diversity in estuaries varies with the environmental variables on spatio-temporal scale (Blaber & Blaber, 1980). The monsoon mediated environmental fluctuations in the creek system are the drivers of the spawning and migratory pattern of the aquatic biota (Potts et al., 2015); Nevertheless, the ichthyofaunal exploration (Weinstein & Heck, 1979) with respect to spatial and seasonal variations in species diversity and abundance (James et al., 2008) helps to understand the effect of external perturbations on these coastal ecosystems (Hook, 1991).

With a total area of 1121 ha, Gorai Creek of Mumbai, is one of the important creeks located along the Northwest coast of India, which connects the people socio-culturally through livelihood, transportation, recreation and religious value. A small-scale traditional dol net fishery within the coastal region, is considered to be an economic activity that is predominantly correlated to the rich ichthyofaunal diversity (Sreekanth et al., 2019). The dol net fishery is found to be a significant subsistence activity for the artisanal and motorized fishers in creeks along the Mumbai coast along with the gill net fishery, and hook and line fishery. There are limited studies on phytoplankton, zooplankton, economically important fin fish and shell fish species from this creek. Furthermore, there are no detailed attempts to understand the biodiversity, water and soil quality parameters, and their ecological interactions in this ecosystem.

On the other hand, the creek waters and estuarine complexes are highly prone to diverse anthropogenic factors. These anthropogenic stressors as predicted by the demographic pressure is executed by more than 50% of the population inhabiting coastal areas besides the complications arising from domestic discharges, industrial effluents, indiscriminate fishing, pollution and urban land use pattern (González-Sansón et al., 2022). In order, to characterize fishery, and fish biodiversity in a coastal ecosystem, it is essential to analyze spatio-temporal variation in species diversity, the economic profile of the fishery, ecosystem characteristics, trophic flows and ecological interaction between water-soil parameters and functional fish groups. Thus, the present study was carried out to study the spatio-temporal patterns in biodiversity of the fauna in Gorai Creek along with the ecological interactions using soil and water quality parameters.

Material and Methods

Study Area

In the present study, fish were collected from the five sampling sites [Station 1: Interior of the creek towards freshwater inlet - (19.2311, 72.8186); Station 2: Mid-way near to mangrove areas - (19.2214, 72.8071); Station 3: Near to Essel World- Global Vipassana Pagoda (19.2124, 72.8048); Station 4: Near to off mangrove areas (19.2063, 72.7992); Station 5: Near to Gorai beach (9.1997, 72.7952) towards the mouth of creek] using a dol net from Gorai Creek in Mumbai (Figure 1) from September 2019 to August 2020.

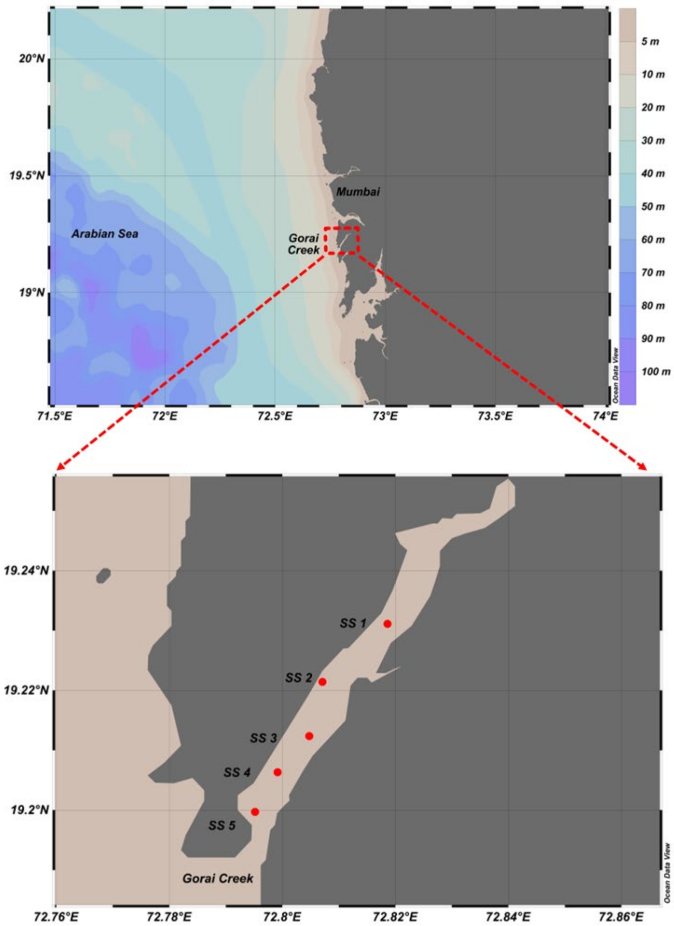

Figure 1. Map showing the study area, Gorai Creek with five selected sampling stations, Mumbai, Maharashtra

Fish Sampling Design and Gear Employed

Sampling was done at each station once in a month by using Dol net. The number of species caught was noted and the collected data were pooled together station-wise as well as season-wise for the calculation of biodiversity indices. Total species occurrence and abundance data were calculated by pooling together the total number of individual species collected from all the five stations during the study period. The period signifies one complete season of dol net fishery status except period od closed season (Monsoon fishing ban) from 10th June to 15th August or Narayali Poornima.

Species Identification

The collected fish samples were sorted and identified up to species level. The collected fishes were washed in clean portable water and preserved in formaldehyde solution for further studies. The preserved fishes were sorted into taxonomic groups and identified at fish biology laboratory of Fish Taxonomy Lab, Fisheries Resource Management, Fisheries Resources, Harvest and Post-Harvest Management Division, ICAR-Central Institute of Fisheries Education, Mumbai. The identification of fishes was carried out with the help of standard literature (Fischer & Bianchi, 1981) and updated with recent literature (Nelson et al., 2016). For Avifauna, bird watching and recording has been carried out with the aid of a binocular and camera with zoom lenses. Recorded birds were identified by using standard literature Ali (1996) and Monga (2003).

Water Quality Analysis

The physicochemical parameters of water such as pH, dissolved oxygen (DO), alkalinity, hardness, total suspended solids (TSS), total dissolved solids (TDS), nitrite, nitrate, phosphate, ammonia, biological oxygen demand (BOD) and chemical oxygen demand (COD) were analyzed as per the standard procedure (APHA, 2012). Surface water temperature was measured using a Celsius mercury thermometer calibrated up to 0.1°C. Water pH was measured on-site by OAKTON eco-tester pH 1 (0.0 to 14.0). The salinity of water for different stations was measured with the help of a handheld refractometer ATAGO S/Mill-E (0-100%).

Biodiversity Assessment

Seasonal and spatio-temporal variability in biodiversity indices such as Shannon-Wiener species diversity (H'), Margalef's species richness (d), Pielou's evenness (J'), Taxonomic diversity (Δ), Taxonomic distinctness (Δ*), Average taxonomic distinctness (Δ+), Variation in taxonomic distinctness (ʌ+) and total phylogenetic diversity (sPhi+) were calculated using computer software package PRIMER v6.1.9 (Clarke & Warwick, 1998; Clarke & Warwick, 1999; Clarke & Gorley, 2006)

Results

During the study, 90 species of fauna were identified in Gorai Creek, Mumbai under 70 genera, 50 families, 29 orders, six classes and three phyla. The phyla-wise; class-wise; order-wise; and family-wise distributions of recorded species (Figures 2 and 3) indicated the seasonal and spatio-temporal variations (station-wise, season-wise and month-wise) in various biodiversity indices (Tables 1-3). The seasonal and spatio-temporal variations of Shannon-Wiener diversity (H'), were found to be highest at station 5 (3.847) followed by station 4 (3.703) and lowest at station 2 (3.305) and station 1 (3.264). The seasonal values of Shannon-Wiener diversity were found to be in the range of (3.905, 3.636 and 3.530) for the 2019 monsoon, 2020 winter and 2020 summer seasons. The seasonal and spatio-temporal variations of species richness (d) were recorded to be highest at station 5 (16.069), followed by station 4 (15.200) and lowest at station 2 (9.337) and station 1 (8.034). The seasonal variations among the different sampling stations were presented in Table 1. The Pielou's evenness (J') index values were found to be highest for monsoon (0.868) and lowest for summer (0.810). During the present study, the spatial variation among the different stations were recorded as, Station 1 > Station 5 > Station 2 > Station 4 > Station 3.

Table 1. Seasonal variations of biodiversity indices

Seasons	S	N	d	J'	H' (loge)	Delta	Delta*	Delta+	sPhi+
Monsoon-19	90	3168	19.326	0.868	3.905	91.847	93.932	97.466	5975
Winter-20	80	1736	17.155	0.830	3.636	94.370	97.567	97.563	5475
Summer-20	78	1332	16.720	0.810	3.530	93.882	97.747	97.178	5050

Note: S, Total No. of species; N, Total No. of individuals; d, Margalef's species richness; J', Pielous's species evenness; H', Shannon-Wiener diversity index; Delta, Taxonomic diversity; Delta*, Taxonomic distinctness; Delta+, Average taxonomic distinctness index; sPhi+, Total phylogenetic diversity

Table 2. Spatial variations of biodiversity indices

Stations	S	N	d	J'	H'(loge)	Delta	Delta*	Delta+	sPhi+
Station 1	38	615	8.034	0.897	3.264	93.730	97.463	97.048	2750
Station 2	44	623	9.337	0.873	3.305	92.309	96.324	97.146	3100
Station 3	55	962	11.726	0.861	3.452	92.111	95.521	97.054	3850
Station 4	71	1729	15.200	0.869	3.703	93.584	96.527	97.123	4900
Station 5	75	2308	16.069	0.891	3.847	92.967	94.867	97.108	5000

Note: S, Total No. of species; N, Total No. of individuals; d, Margalef's species richness; J', Pielous's species evenness; H', Shannon-Wiener diversity index; Delta, Taxonomic diversity; Delta*, Taxonomic distinctness; Delta+, Average taxonomic distinctness index; sPhi+, Total phylogenetic diversity

Table 3. Monthly variations of biodiversity indices

Months	S	N	d	J'	H'(loge)	Delta	Delta*	Delta+	sPhi+
August. 19	81	920	17.372	0.898	3.946	93.861	95.372	97.037	5400
September. 19	78	812	16.720	0.907	3.951	94.065	95.522	97.211	5375
October.19	44	499	9.337	0.844	3.194	91.655	96.836	96.459	3125
November. 19	41	436	8.686	0.783	2.908	89.202	96.830	96.341	3000
December. 19	40	346	8.469	0.841	3.104	91.549	97.455	96.378	2950
January. 20	43	264	9.120	0.866	3.257	93.614	97.708	96.872	3225
February. 20	33	191	6.949	0.847	2.961	91.386	98.323	97.301	2650
March. 20	46	478	9.772	0.822	3.149	92.618	98.301	96.787	3200
April. 20	47	381	9.989	0.880	3.388	94.339	97.728	97.109	3275
May. 20	55	473	11.726	0.813	3.256	91.337	96.976	97.559	3950
June. 20	54	717	11.509	0.802	3.198	84.663	91.254	95.056	3475
July. 20	54	719	11.509	0.862	3.437	87.884	91.715	95.318	3550

Note: S, Total No. of species; N, Total No. of individuals; d, Margalef's species richness; J', Pielous's species evenness; H', Shannon-Wiener diversity index; Delta, Taxonomic diversity; Delta*, Taxonomic distinctness; Delta+, Average taxonomic distinctness index; sPhi+, Total phylogenetic diversity

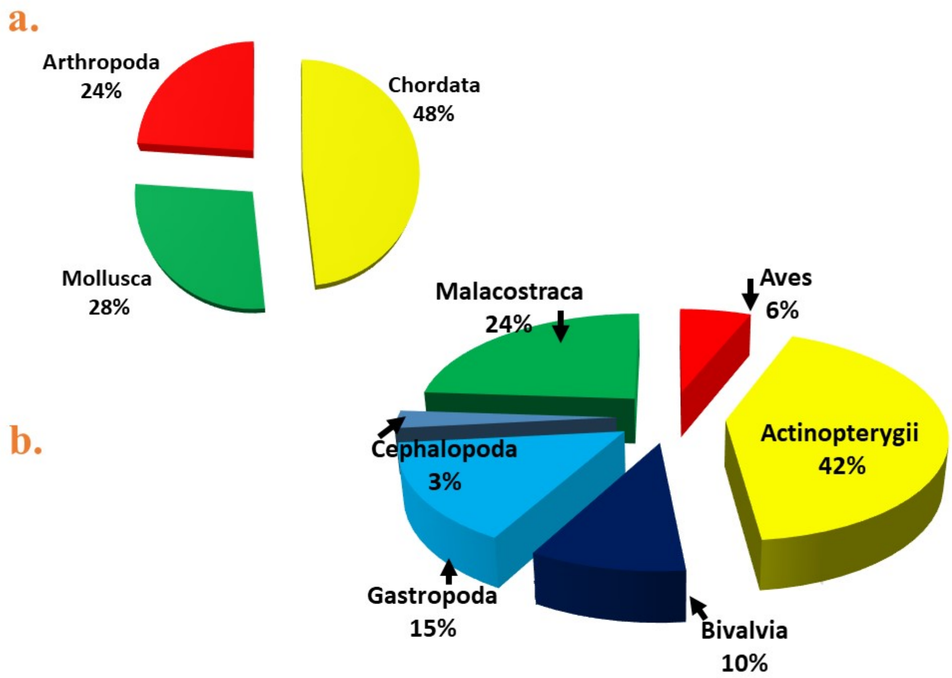

Figure 2. a. Phyla-wise, b. Class-wise representation of species from Gorai Creek, Mumbai

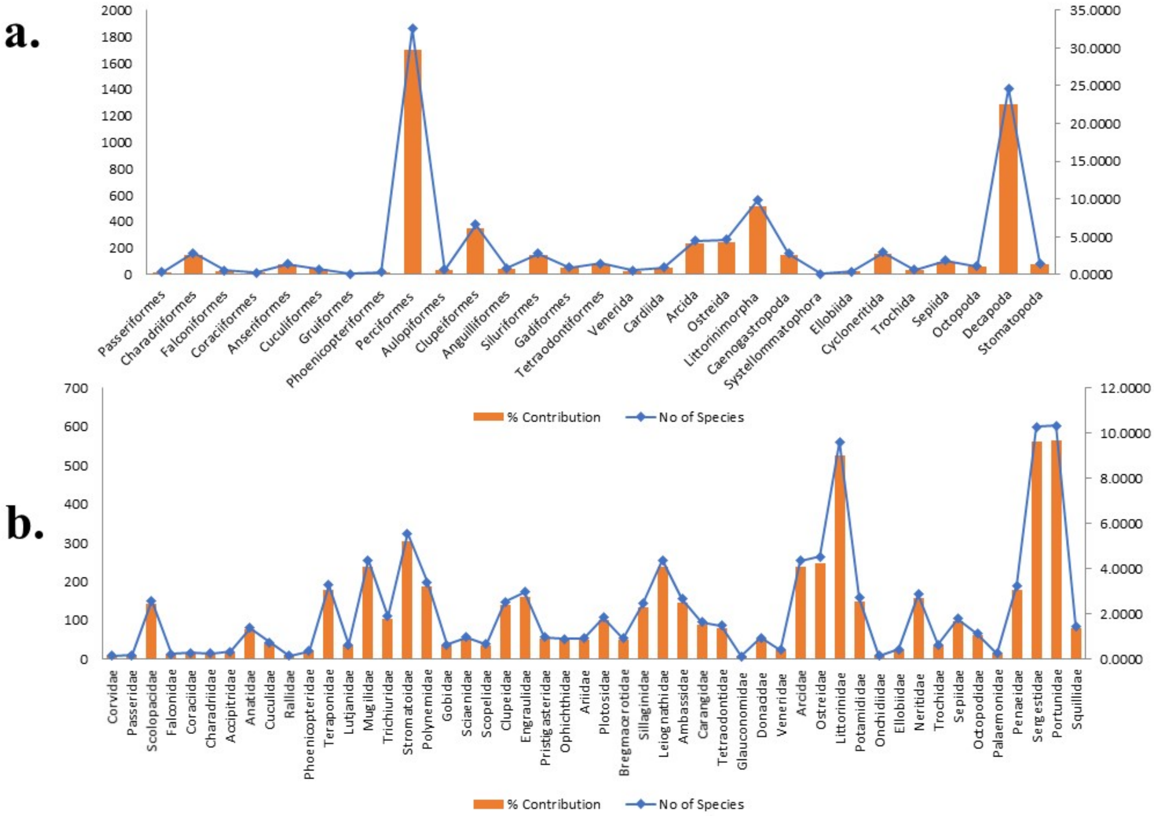

Figure 3. a. Order-wise, b. family-wise representation of species from Gorai Creek, Mumbai

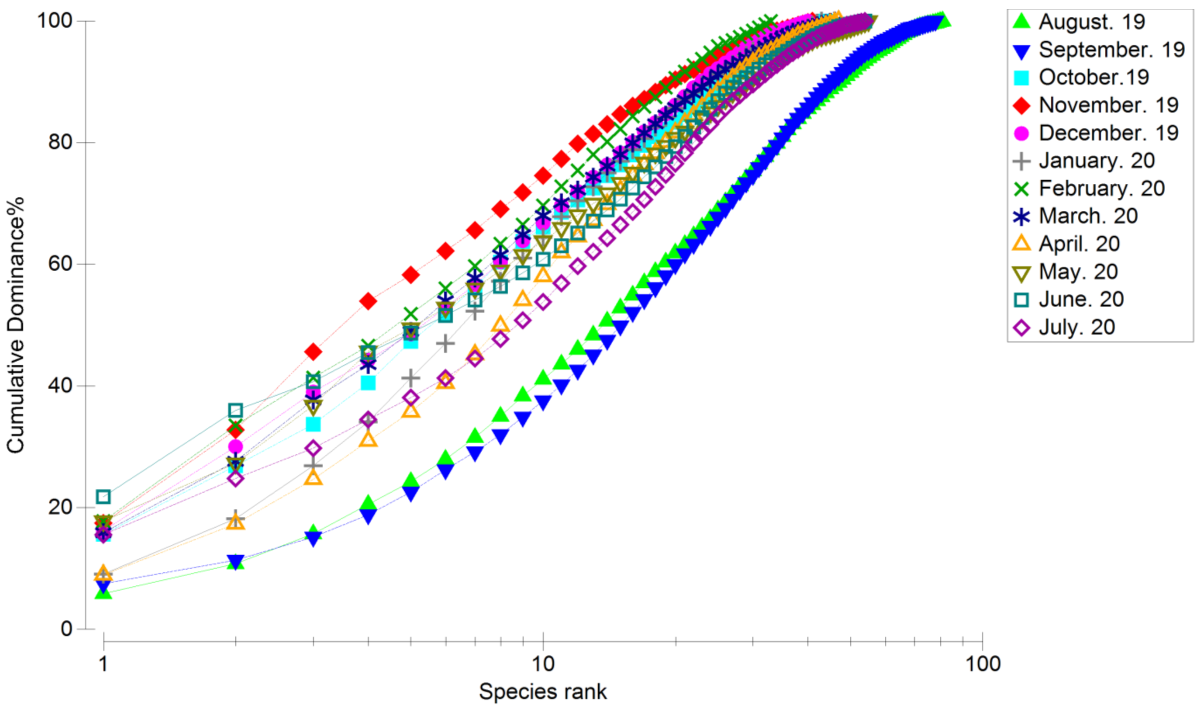

Figure 4. K-dominance plot among different months at Gorai Creek, Mumbai

The estimated taxonomic diversity (Δ) values for the sampling stations were recorded highest in winter (94.370) followed by summer (93.882) and lowest in monsoon (91.847). The seasonal variation of estimated taxonomic diversity values was found to be in the descending order as, winter (94.370) > summer (93.882) > monsoon (91.847). The seasonal variation in taxonomic distinctness was observed to be in the range of 93.932 to 97.747. Among the five sampling stations studied, the taxonomic distinctness (Δ*) was found to be the lowest (94.867) in station 5 and the highest (97.463) in station 1. The overall trend indicated lower average taxonomic distinctness during the monsoon period with increment in winter and highest in the summer period. The seasonal variation in taxonomic distinctness (Λ+) among the studied sampling stations was recorded in the order of winter (97.563) > monsoon (97.466) > summer (97.178). The spatial variation in taxonomic distinctness among the studied sampling stations (Table 3) was in the following descending order, station 2 (97.146) > station 4 (97.123) > station 5 (97.108) > station 3 (97.054) > station 1 (97.048).

The total phylogenetic diversity (sPhi+) value for station 1 is 2750, station 2 (3100) and station 3 (3850), station 4 (4900) and station 5 (5000). The seasonal variation in total phylogenetic diversity ranged from 5475 to 5050. The results of the K-dominance curve were obtained by plotting percentage of cumulative abundance against species rank K on a logarithmic scale. Among the sampling stations, station 5 and station 4 indicated relatively larger cumulative abundance than the other station waters. As the percentage contribution of each species was added, that curve extended horizontally before reaching the cumulative 100%. The result from the K-dominance curve was plotted season-wise. Cumulative relative abundances were higher in monsoon followed by winter and summer. The month-wise D dominance plot showed that the cumulative abundance was rich during August, 2019 and September, 2019 and poor during February, 2020 (Figure 4). The spatio-temporal variation in the K dominance plot among the sampling stations indicated that the flow of cumulative abundances is the flow of station 1>2>3>4>5 (Figure 5). The seasonal variation recorded for Gorai Creek resulted in high during monsoon, 2019 and less during summer, 2020 (Figure 6).

Bray-Curtis similarity is useful in quantifying the compositional similarity between the stations (Figure 7) and seasons (Figure 8). The seasonal variations revealed that the winter and summer seasons have formed clusters together and the monsoon season forms separate clusters for the study area. The seasonal variability in the BC similarity index for individual sampling stations was found to be higher (66.55%) between station 4 and station 5, and 63.13% between station 2 and station 3. Contrary to this, the lowest similarity (23.24%) was observed between station 1 and station 5, followed by 28.50% of station 2 and station 5.

The data collected were pooled for the normal probability test (PAST v3.1.1) and found to be a normal distribution of Aves (0.9237), Actinopterygii (0.9739), Bivalvia (0.9708), Gastropoda (0.9857), Malacostraca (0.8953) and Cephalopoda (0.8995), presented in Figure 9. The correlation matrix reveals (SPSS v22.0) that there are significant differences observed among the environmental variables and the species recorded. The Pearson's correlation showed that there is a positive relation between DO and pH (0.963^{**}), nitrite and water temperature (0.916^{*}) & alkalinity (0.960^{**}), alkalinity (0.986^{**}) & nitrite (0.969^{**}); Soil pH and Bivalvia (0.955^{*}), Gastropoda (0.972^{**}) in which is represented in PCA Plot (Figure 10) with factor loadings plot for the corresponding eigen values and in Table 5 (*Correlation is significant at the 0.05 level, 2-tailed; **Correlation is significant at the 0.01 level, 2-tailed).

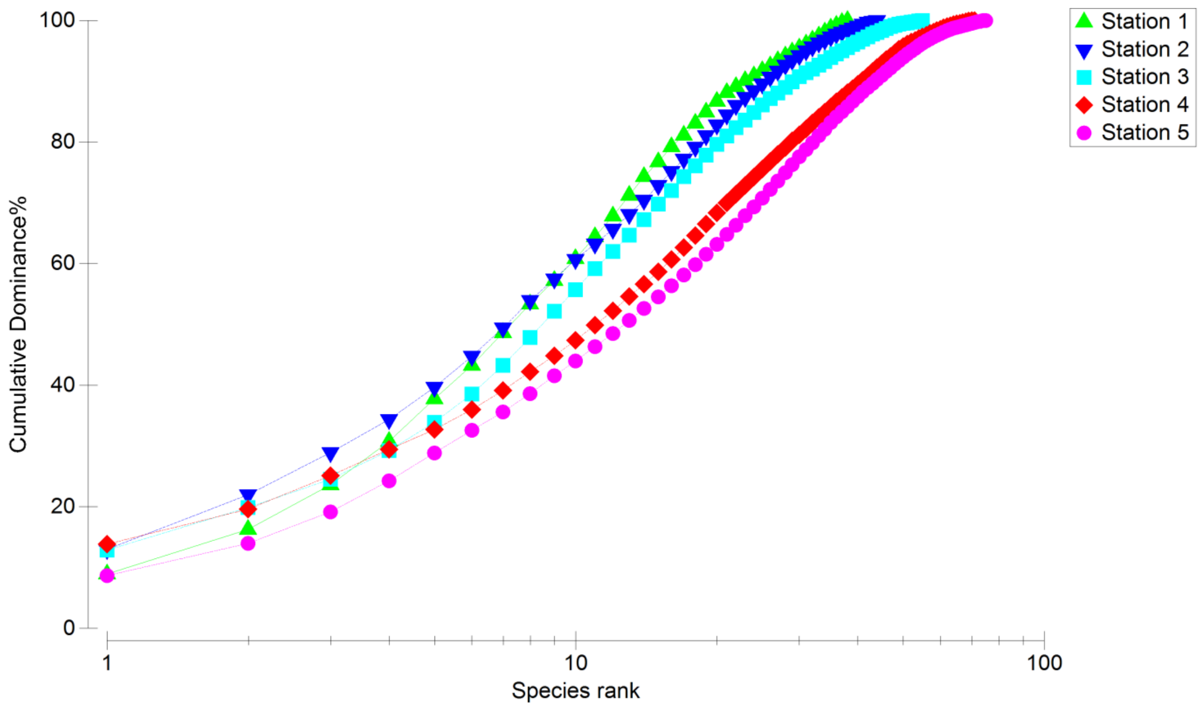

Figure 5. K-dominance plot among different spatio-temporal variations at Gorai Creek, Mumbai

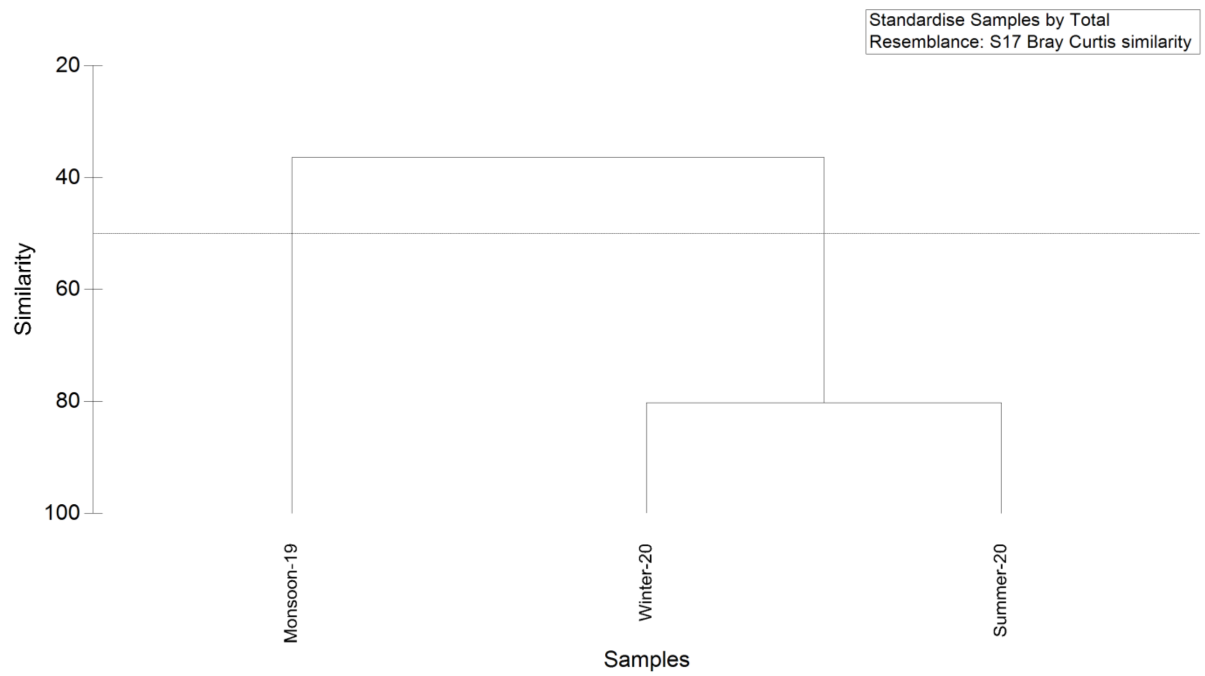

Figure 8. Dendrogram showing similarities between seasonal variations based on the composition of species identified in Gorai Creek, Mumbai

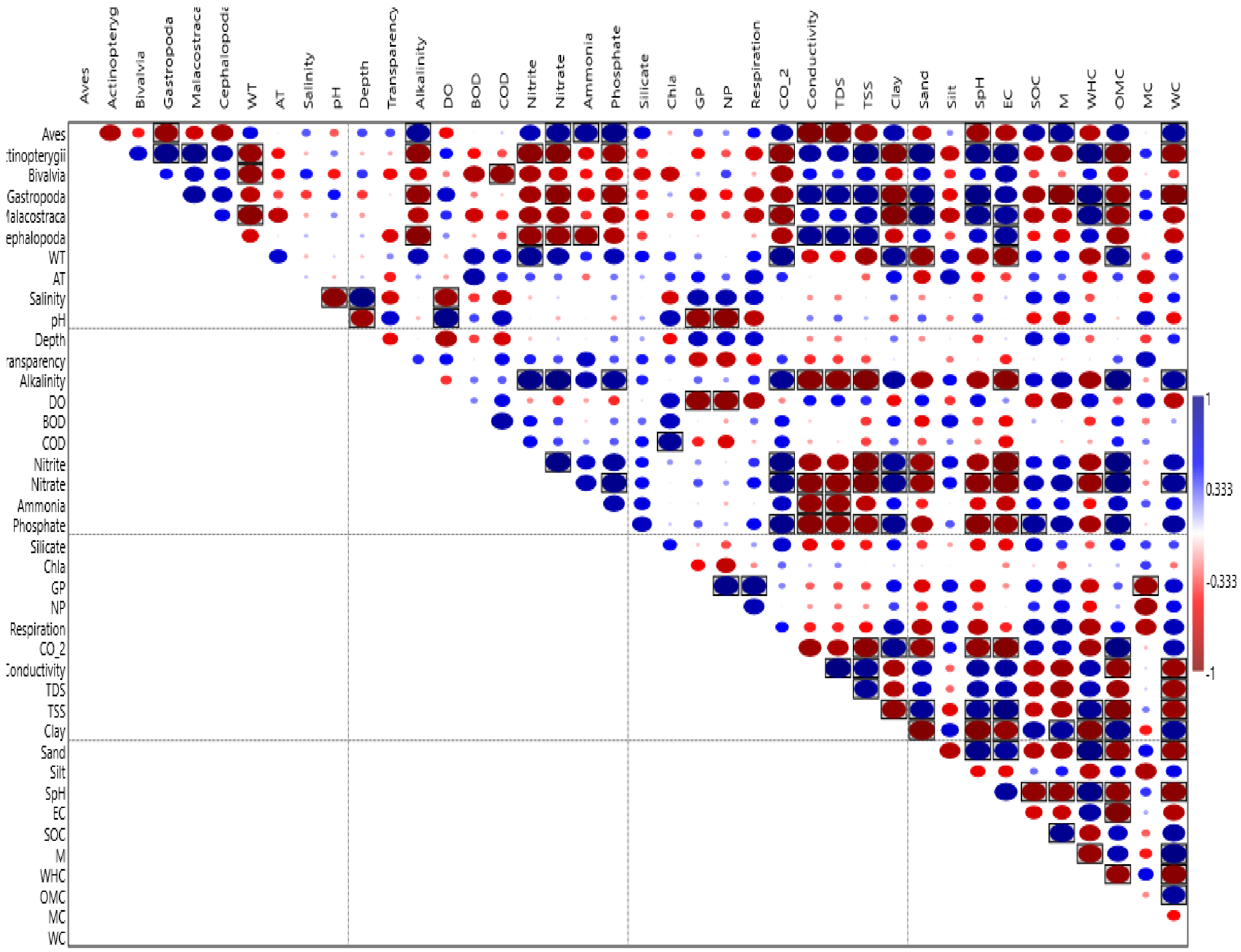

Figure 9. Correlation matrix for the species recorded and environmental variables

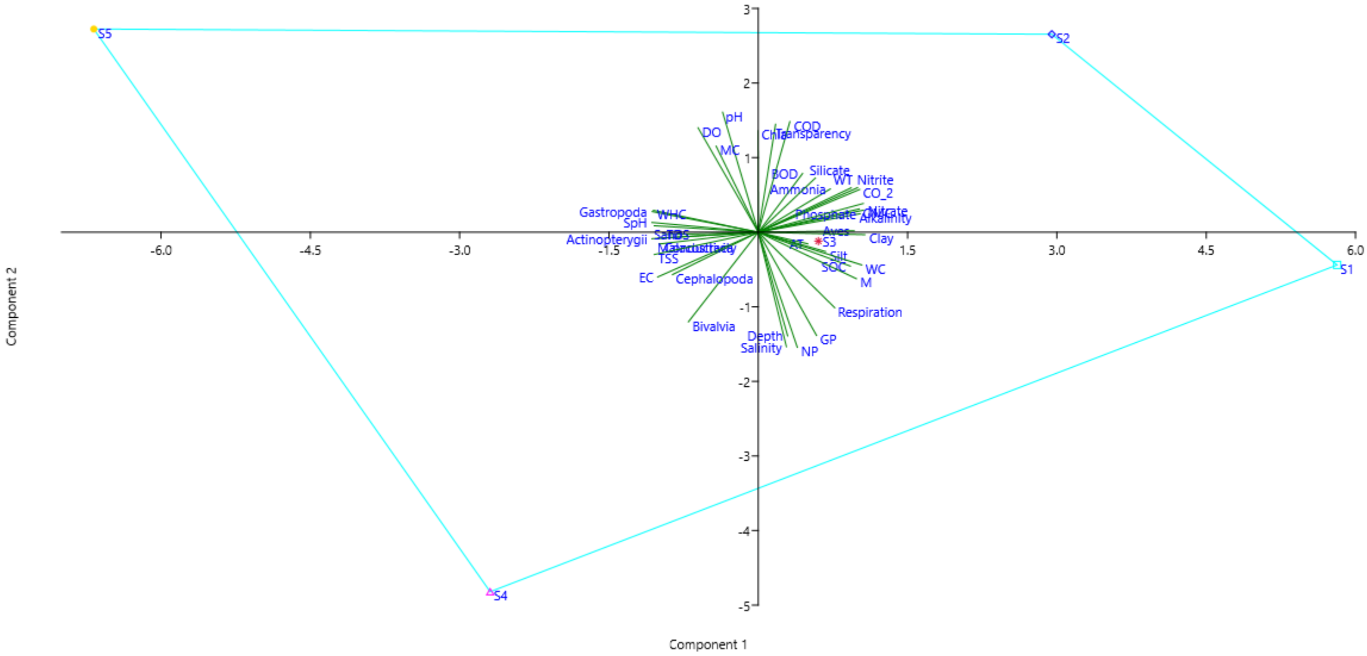

Figure 10. PCA plot for various environmental variables recorded during study period

the construction of residential complexes and the reduction of nesting sites (Lad & Patil, 2014). Chauhan et al. (2008) documented the avian fauna in the Gorai proximity and recorded 66 species. Based on the category made by Chauhan et al. (2008) the current study revealed that three species are migrant and uncommon, one species is resident and uncommon, and one species is migrant and uncommon. Nevertheless, the diversity is more compared to the findings of Sinnarkar et al. (2013) conducted in Mahim Bay, Mumbai.

On a global and Indian scale, a lot of studies have been undertaken to file the fish diversity, fishery and their spatio-temporal patterns in various coastal ecosystems (Tremain & Adams, 1995; Ansari et al., 1995). The ichthyofauna has been studied by several marine biologists and fisheries professionals along the Indian coast who documented 603 species from the Laccadive archipelago by Jones & Kumaran (1980), and 1367 species by Venkataraman & Wafar (2005) along the Indian coast.

The present study reveals the availability of 35 fish species, 5 bivalves, 15 gastropods, 2 cephalopods, and 18 malacostraca. The recorded diversity is comparatively less than the other creek ecosystem of Maharashtra reported by Lad & Patil (2013) and Garima et al. (2020). In the present study, the ichthyofaunal diversity indices were optimum, but the number of recorded species was less. The highest ichthyo diversity and evenness occurred in station 5 during the monsoon season, which may be due to less pollution pressure compared with the other sites and the availability of nutrients during the rainy season. Perciformes and decapods contribute the maximum catch. Station 1 is located near the mouth of Gorai Creek adjacent to the sea, which could expect more diversity. The economic activities like jetty construction, fishing harbour and pollution discharged by the tourist from the adjoining beach causes a substantial negative impact on the site. Garima et al. (2020) studied the ichthyofauna diversity of Karanja and Dharmatar creek which uncovered the effect of anthropogenic activities on the studied site.

Lad & Patil (2013, 2016a, 2016b) recorded 53 species belonging to 23 families and 6 orders. The study also reveals that the fish diversity along the estuarine area of Bhayander and Naigaon was satisfactory in comparison to the fish diversity of other estuaries. The fish diversity offers good support to the livelihood of fishermen residing in the adjoining villages. But, the mangrove forest ecosystem of the various estuaries is under threat due to various anthropogenic activities and it is necessary to take some constructive steps to maintain the mangrove ecosystem that indirectly helps in the maintenance of fish diversity. Lad & Patil (2016a) recorded 23 species of meiobenthos belonging to 8 phyla from Bhayander and Naigaon. The influence of environmental variables on fish diversity, assemblages, feeding and breeding grounds was reported by numerous researchers (Madhupratap et al., 2001;

Janureguizar, 2004; Shirodkar et al., 2012). The monsoon, winter and summer seasons are characterized by unique changes in the wind pattern, current shifts, water turbulence, river discharges and temperature along the coastal regions (Madhupratap et al., 2001; Shirodkar et al., 2012). Similar to the observations made by Qasim & Sen Gupta (1981) this ecosystem also experiences seasonal fluctuations in water quality parameters. In the purview of Ansari et al. (1995) and Shamsan (2008), the fish species in the mangrove-associated estuary, have their own strategy for breeding, larval development, and feeding migrations to match the environmental situation. Hence, many finfish and shellfish species are highly dependent on estuarine ecosystems for completing their life history and survival. The current study highlighted the spatio-temporal variation of fish assemblages assesssed by biodiversity indices and correlated with environmental variables of the tropical coastal creek ecosystem. The reports by Ansari et al. (1995) and Shamsan (2008) revealed that, the estuaries are endowed with rich aquatic biodiversity and species assemblages. Yet, Gorai Creek is found to be moderate in the line of research, hindered by anthropogenic activities and plastic pollution. Therefore, Gorai Creek plays an important role in the conservation and replenishment of aquatic life and a suitable practical fisheries management strategy is required for the sustainable fisheries management of the creek. Community and co-management practices with proper monitoring of fish catch, reporting systems, fishing holidays, seasonal and regional closures could be considered the practical solution for the sustainable utilization of the resources (Sreekanth et al., 2018).

Conclusion

The present study indicates that Gorai Creek is endowed with moderate biodiversity of flora and fauna. The water and soil quality parameters revealed that the health condition is impacted by the non-biodegradable debris and domestic sewage pollution. The creek ecosystem and its associated biodiversity are subjected to various anthropogenic stressors on a wide range in Mumbai, Maharashtra. Though the study interprets the biological aspect, the data generated could be used for framing the mixed fisheries management as ecological indicators which encompass biological, physio-chemical and socio-economic dimensions for resolving the fisheries complexities in the tropical multispecies paradigm. The study also recommends the valuation of the essential economic benefits of the creek could reveal the exact efficiency and prompt measures for formulating policies and implementing rules and regulations.

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Compliance With Ethical Standards

Authors' Contributions

Author AKJ designed the study, SC wrote the first draft of the manuscript, SBS has done literature analyses, SGB performed and managed statistical analyses, GD and BBN corrected the draft. 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. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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RESEARCH ARTICLE

Length-weight relationships and relative condition factor of *Spicara flexuosum* (Rafinesque, 1810) inhabiting the Black Sea and the Turkish Straits System

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ABSTRACT

Length-weight relationships and condition factor of *Spicara flexuosum* collected from İstanbul, Rize, Çanakkale and Sinop-Kastamonu by using commercial gill nets and hand-line fishing methods were evaluated between 2013 and 2014 in this study. The overall length-weight equation ($W=0.0236\ L^{2.7334}$) indicated negative allometric growth from all sampling periods. Samples collected from Istanbul and Çanakkale exhibited positive allometric growth while *S. flexuosum* showed negative allometric growth in Rize and Sinop-Kastamonu in December 2013 and January 2014 respectively. The relative condition factor among regions varied from 0.99 to 1.08, indicating a state of wellbeing among all locations. This study provides the baseline data of length-weight relationships and condition factor analyses for *S. flexuosum* species from Turkish coastal waters for future management purposes for this species.

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Introduction

Spicara spp. (Picarels) are members of the Centracanthidae or Sparidae family groups and are small-to medium-sized fish (Froese & Pauly, 2022). Morphologic misidentification which was previously observed for the *Spicara* genus, especially *Spicara flexuosa* (Rafinesque, 1810) and *Spicara maena* (Linnaeus, 1758) (Salekhova, 1979; Vidalis & Tsimenides, 1996) has been eliminated based on previous studies in the literature (Chiba et al., 2009; Imsiridou et al., 2011; Minos et al., 2013;

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Şalcıoğlu et al., 2021). *Spicara flexuosa* is now genetically identified and gained valid species status (Şalcıoğlu et al., 2021; Şalcıoğlu, 2022), recalled as *S. flexuosum* (Rafinesque, 1810) in the FishBase (Froese & Pauly, 2022).

Different meristic and morphometric studies were previously available (Vasilieva & Salekhova, 1983; Rizkalla, 1996; İlkyaz et al., 2007) in the literature about *Spicara* species. Some studies were based on biology, growth, nutrition (Soykan et al., 2010; Dalgıc et al., 2021), length-weight relationship (İşmen et al., 2007; Soykan et al., 2010; Özvarol, 2014) and condition factor (Mytilineou & Papaconstantinou, 1991; Dulčić et al., 2000; Mater et al., 2001) of this fish species.

Length–weight relationship (LWR) studies of fish species determine important resources for the management of fish stocks. (Martin-Smith, 1996; Froese et al., 2011). Fisheries biologists need length–weight relationship (LWR) data to understand the rational management of fishing resources and the most widely used tools for fisheries data (Yedier et al., 2019). Previous studies have shown that LWRs are generally different not only between species but also among different stocks of the same species in relation to region, season and age group (Gerritsen & McGrath, 2007; Froese et al., 2011; Demirel & Murat-Dalkara, 2012).

The relationship between the length and weight of fish provides information on the weight variation of individuals in relation to their length (Condition factor, K). The condition factor of the fishes is generally used for comparison of two or more populations living in similar or different ecological conditions based on the food or climate (Weatherley & Gill, 1987). Furthermore, these data are valuable resources for fisheries management and conservation of fish stocks (Jisr et al., 2018).

Length-weight relationships of *Spicara flexuosum* populations were previously evaluated in FishBase for Greece coasts (Petrakis & Stergiou, 1995; Moutopoulos et al., 2013) for Italian coast (Giacalone et al., 2010) for Portugal coast (Borges et al., 2003). However, none of the studies have been found in FishBase for *S. flexuosum* species in Turkish coastal waters with different locations and periods, although some studies have been found in the literature (Mater et al., 2001; Özvarol, 2014; Dalgıç et al., 2021; Samsun & Erdoğan Sağlam, 2021). The aim of this study is to provide seasonal determination of length-weight relationship and condition factor data of *S. flexuosum* sampling from Turkish Straits System and Black Sea coastal waters for future fisheries management purposes.

Material and Methods

Fish Sampling

S. flexuosum specimens were obtained by a random sampling method between 2013 and 2014 from commercial fishing by using gill nets (mesh sizes from 16 mm to 20 mm) from the Southeast Black Sea coast (Rize-Fener) (December 2013), (41.0387900 N, 40.4949340 E), Sinop-Kastamonu Central Black Sea coast (January 2014) (41.969994 N, 34.532061 E); and using handline fishing methods for Istanbul (February 2014) (40.5382480 N, 29.4102180 E) and Çanakkale (Dardanos) (June 2014) (40.101300 N, 26.375620 E). Fish samples were identified according to main morphological characteristics (Whitehead et al., 1986). A total of one hundred ten individuals were sampled, total length (TL) measurements were conducted by a Vernier caliper at 1 mm intervals, and the body weight (BW) of the specimens were weighed on a balance with a sensitivity of 0.001 g.

Length-Weight Relationship and Condition Factor

Body length (TL) and body weight (BW) were calculated by the

$$W = a \times L^b \quad (1)$$

where, W is the total weight (g). The parameters a and b were estimated by linear regression;

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

L is the total length (cm), a is the intercept (Coefficient related to the body) and b is the slope of log-transformed linear regression (Le Cren, 1951).

The model fit to the data was measured by the coefficient of the Pearson r-squared (r^2) test (Froese, 2006).

Fish growth is expressed by the values of the exponent b; when b=3, weight is isometric. When the value of b is greater than 3, the weight increase is allometric (Positive allometric if b>3, when the b value is smaller than 3, negative allometric if b<3). (Morey et al., 2003). The t test was used, if b was statistically significantly different from 3 (Pauly, 1984). 95% confidence interval (C.I.) of b values were also evaluated.

The relative condition factor (K_n) was calculated according to the following equation by Le Cren (1951):

$$K_n = \frac{W}{a \times L^b}, \quad (3)$$

where, *W* is the observed weight, and $a \times L^b$ is the calculated weight from the length-weight relationship.

This equation is used to reduce and eliminate the allometry of fish (Bagenal & Tesch, 1978) and is preferred to the Fulton's condition factor, which assumes isometric growth (Fulton, 1911). Student's t-test was used to evaluate test differences between the mean condition factor values of all individuals (Zar, 1999). All statistical analyses were conducted using Microsoft Excel 2013 and IBM SPSS Statistics version 22.0 for Windows package software (IBM Corp., Armonk, NY, USA).

Results

Descriptive statistics of the length-weight relationships and condition factor results are given in Table 1. Parameters were calculated according to locations.

All values of *a* ranged from 0.00834 to 0.0694. The b values ranged from 2.2979 to 3.2124. The estimated b values felt within the range reported in FishBase (2.627–3.696) for *S. flexuosum* (Froese & Pauly, 2012) except from Sinop-Kastamonu samples. Among all locations, the determination coefficients ranged from 0.85 to 0.96 (Table 1). *p* values were found to be significantly different ($p<0.05$) according to Pauly' test; therefore, growth is "positive allometric" for Istanbul and Çanakkale and "negative allometric" for Rize and Sinop-Kastamonu locations. Considering all individuals, growth is considered negative allometric (b=2.7334), ($p<0.05$), (Figure 1a-e).

Length values ranged from 11.04 to 18.84 cm, 14 to 18.6 cm, 11.4 to 16.2 cm and 12.31 to 19.50 cm; weight values ranged from 15 to 102, 27.15 to 73.78, 16.35 to 48.06 g and 24.42 to 68.48 g for the Istanbul, Rize, Çanakkale and Sinop-Kastamonu samples, respectively. Of all the regions examined, the minimum total length was found in Istanbul (11.04 cm), and the maximum total length (19.50 cm) was found in Sinop-Kastamonu and the minimum (15 g) and maximum weight (102 g) were collected from İstanbul (Bosporus Strait) in February.

The relative condition factor values ranged from 0.9990 (±0.06) (Canakkale) to 1.0800 (±0.01) (Sinop-Kastamonu).

Discussion

LWR parameters could be influenced by different factors, such as age, feeding condition, gonad maturity, sex, and size (Froese, 2006; Freitas et al., 2022). Moreover, spatial variation in fish growth is influenced by water quality, food resources, and physiological conditions of the sea, such as salinity and temperature (Moutopoulos & Stergiou, 2002; Gerritsen & McGrath, 2007).

Table 1. The mean total length and weight values, parameters of the length-weight equations and relative condition factors of *Spicara flexuosum* (SD. Standard deviation)

Region	Time	N	L, mean S.D.	W, mean S.D.	WL equation	*a*	*b*	95% C.I. of *b* (S.D)	R^2	Growth type	K_n. S.D.
Rize	December, 2013	27	16.16 (±1.12)	44.59 (±9.88)	$W=0.0125\ L^{2.9336}$	0.0125	2.9336	2.735-3.433 (±0.19)	0.88	A (-)	1.0030 (±0.07)
Sinop-Kastamonu	January, 2014	34	16.76 (±5.08)	46.03 (±31.15)	$W=0.0694\ L^{2.2979}$	0.0694	2.2979	1.729-3.435 (±0.07)	0.96	A (-)	1.0800 (±0.01)
İstanbul	February, 2014	23	16.54 (±1.86)	71.39 (±60.81)	$W=0.00834\ L^{3.2124}$	0.00834	3.2124	2.958-3.880 (±0.32)	0.85	A (+)	1.0139 (±0.17)
Çanakkale	June, 2014	26	13.92 (±1.31)	32.95 (±9.30)	$W=0.009\ L^{3.1061}$	0.009	3.1061	3.022-3.523 (±0.12)	0.95	A (+)	0.9990 (±0.06)
Total		110	15.27 (±5.98)	41.74 (±37.81)	$W=0.0236\ L^{2.7334}$	0.0236	2.7334	2.469-3.297 (±0.25)	0.76	A (-)	0.8799 (±0.02)

Note: * A (+): Positive allometric growth, A (-): Negative allometric growth.

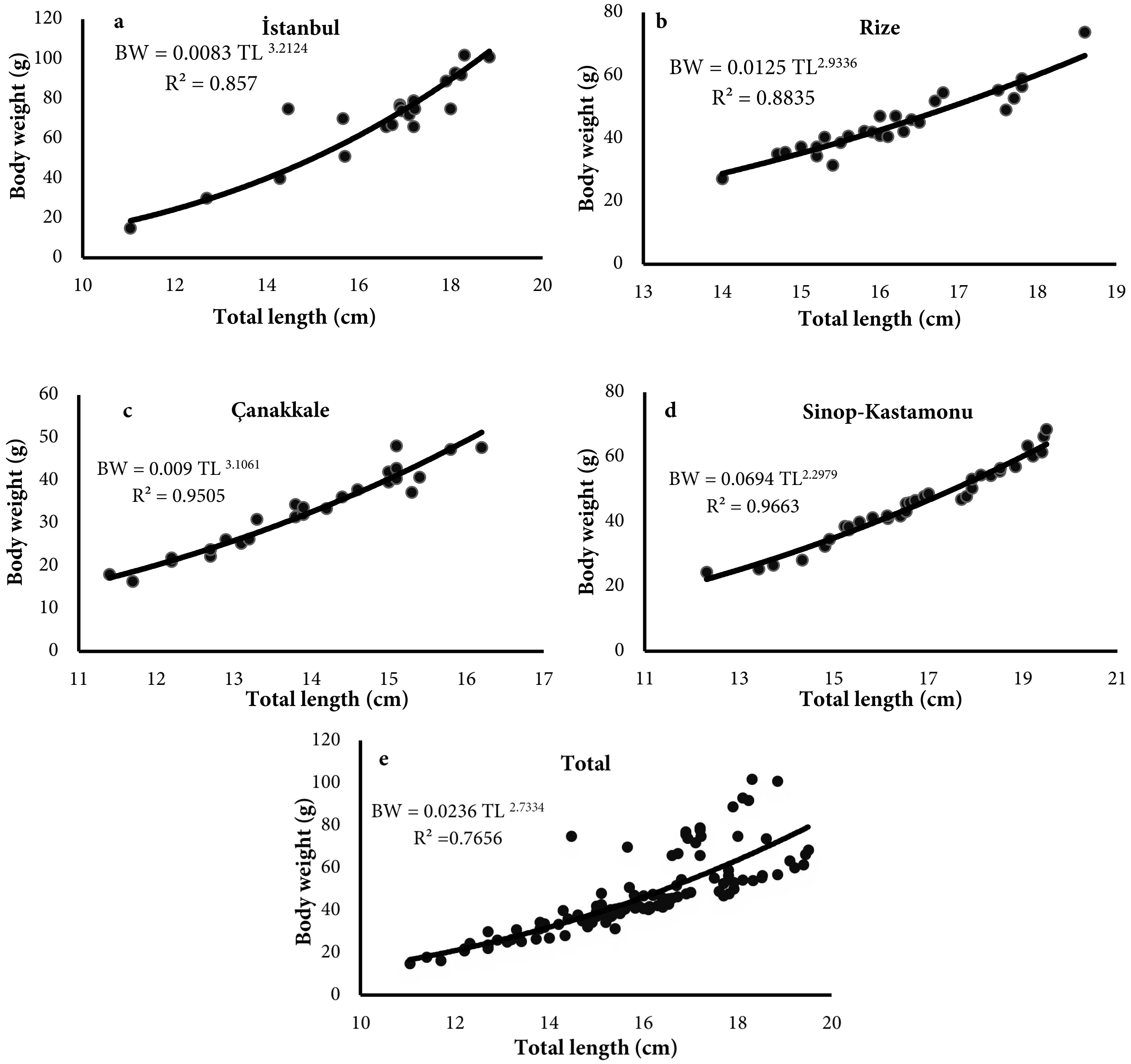

Figure 1. Length-weight relationship for *Spicara flexuosum* populations a) İstanbul, b) Rize, c) Çanakkale, d) Sinop-Kastamonu, e) Total samples

The LWR relationships of three *Spicara* species were previously examined by several researchers along the Mediterranean regions. İşmen (1995) reported the total length of all *S. smaris* specimens varied between 10.0 and 18.5 cm in the Black Sea. For *S. maena* species, minimum and maximum total lengths have been reported ranging from 7.5 (Soykan et al., 2010; Central Aegean Sea) to 22 cm (Karakulak et al., 2006; North Aegean Sea) along the Turkish coastal waters. Considering minimum and maximum length values for *S. flexuosum* along the Mediterranean coasts, total lengths ranging from 6.5 (Mytilineou & Papaconstantinou, 1991; Greece) to 20 cm (Giacalone et al., 2010; Italian coasts) (Table 2). On the other hand, minimum and maximum length and weight of *S. flexuosum* were found ranging from 8.7 (Dalgıç et al., 2021; Rize, Eastern Black Sea) to 22.5 cm (Samsun & Erdoğan-Sağlam, 2021; Ordu, Southeastern Black Sea) (Table 2) and 7.1 to 129.94 g (Dalgıç et al., 2021), respectively, along the Turkish coasts. The minimum-maximum total length (11.04 cm-19.50 cm) and weight (15-102 g) values of *S. flexuosum* in this study were nearly identical to those of other studies from the Turkish coast.

Parameters of the b values were within the range from 2.2979-3.2124 in this study. b values varied between 2.594 (Mater et al., 2001; Aegean Sea) to 3.389 (Petrakis & Stergiou, 1995; Greece coast) for *S. flexuosum* (Table 2). The b values have been found nearly identical for our results along the

Turkish coasts (Özvarol, 2014; Dalgıç et al., 2021; Samsun & Erdoğan-Sağlam, 2021) Greece (Mytilineou & Papaconstantinou, 1991), Portugal (Borges et al., 2003) and Italian coast (Giacalone et al., 2010) in recent years (Table 2). Moreover, the (LWR) results of this study Rize (b=2.9336) and Sinop-Kastamonu (b=2.2979) are in agreement with that of Dalgıç et al. (2021), who found negative allometric growth (b=2.9727) of *S. flexuosum* in the eastern Black Sea.

Determination coefficient values for each region were found to be greater than 0.85, which are in agreement with previous studies of *S. flexuosum* along the Turkish coasts, (Mater et al., 2001; Özvarol, 2014; Dalgıç et al. 2021; Samsun & Erdoğan-Sağlam 2021), Greece (Mytileniou & Papaconstantinou, 1991; Petrakis & Stergiou, 1995) Portugal (Borges et al., 2003) and Italy (Giacalone et al., 2010) (Table 2).

The b values, which vary according to species, age, and sex, reflect the shape of the fish. Furthermore, the reproductive period of the fish, such as gonad development and the availability of food in their surrounding area, can influence the b value (Jobling, 2002). The spawning period of *S. flexuosum* is generally observed in spring and summer seasons (March to June) along the Mediterranean and Turkish coastal waters (Çiçek et al., 2007, Soykan et al., 2010). The length at first maturity of *S. flexuosum* was reported by Mytilineou (1987) to be 9.1 mm. Considering b values, samples from Rize and Sinop-Kastamonu samples exhibited negative allometric growth. There are some factors such as season, density dependent factors (Giacalone et al., 2010) (food resources) and reproductive seasons of fish that can be directly affect growth performance. Rize and Sinop-Kastamonu samples were collected in December and January (winter) out of the spawning period, and available food might not be good enough for consumption in winter. Furthermore, overfishing pressure of the fish (Turkish coastal waters) might be negatively influenced growth characteristics (Oğuz et al., 2012). The b values can also be affected by environmental conditions, sampling season and locations and size composition of the samples (Froese, 2006). Sampling from fish with different geographical locations could also be directly affected b values as previously observed *Raja clavata* (Thornback ray) from Mediterranean (b=3.262) (Başusta et al., 2012) and Aegean Sea (b=2.82) (İlkyaz et al., 2008) as stated in the review by Gündogdu et al. (2016).

Table 2. The LWR parameters of *Spicara flexuosum* in the literature and this study.

Authors	Region	n	Length range (cm)	a	b	R^2	Growth Type*	Condition factor
Mytilineou & Papaconstantinou (1991)	Greece	692	6.5-15.6	0.00037 F* 0.00006 M*	2.79 F* 3.17 M*	0.95 0.96	A (-) F* A (+) M*	1.39±0.02 F* 1.46±0.02 M*
Petrakis & Stergiou (1995)	Greece	441	11.9-17.7	0.00490	3.389	0.97	A (+)	-
Mater et al. (2001)	Türkiye (Aegean Sea)	412	9.20-15.50	0.0411	2.594	0.92	A (-)	1.255
Borges et al. (2003)	Portugal	45	10.2-19	0.00972	3.076	0.98	I	-
Giacalone et al. (2010)	Italy	6043	6.5-20	0.0141	2.89	0.94	A (-)	-
Özvarol (2014)	Türkiye (NE Mediterranean)	440	9-17.3	0.0260	2.655	0.81	A (-)	-
Dalgıç et al. (2021)	Türkiye (Black Sea)	599	8.7-21.8	0.0118	2.972	0.94	A (-)	-
Samsun & Erdoğan Sağlam (2021)	Türkiye (Black Sea)	318	11-22.5	0.0079	3.0915	0.94	A (+)	1.01
This study	Rize (Black Sea)	27	14-18.6	0.0125	2.9336	0.88	A (-)	1.0030 (±0.07)
This study	Sinop-Kastamonu (Black Sea)	34	12.31-19.50	0.0694	2.2979	0.96	A (-)	1.0800 (±0.01)
This study	İstanbul (TSS)	23	11.04-18.84	0.00834	3.2124	0.85	A (+)	1.0139 (±0.17)
This study	Çanakkale (TSS)	26	11.4- 16.2	0.009	3.1061	0.95	A (+)	0.9990 (±0.06)

Note: *I: Isometric growth, A (+): Positive allometric growth, A (-): Negative allometric growth, F: Female, M: Male. TSS: Turkish Straits System.

Considering the *a* values, it can be seen that all a values (Table 1) fall within the range of 0.00834 to 0.0694. Froese (2006) pointed out that a value is directly related to the growth rate of the fish. According to this statement, *a* increases as the *b* value decreases, and *b* increases as the *a* value decreases thus having an inverse relationship (Froese, 2006). This statement has also confirmed in our study, for Istanbul (a=0.00834; b=3.2124, Istanbul); and (a=0.0694; b=2.2979) Sinop-Kastamonu locations.

The condition factor of the fish varies with sex, size, season and degree of gonad development (Heincke, 1908) and also influenced by different ecological conditions, age and feeding behaviors of the fish and are generally decreased after spawning seasons (Froese, 2006). As opposed to negative allometries, positive allometric growth and high condition factors of Istanbul (1.0139±0.17) and Çanakkale (0.9990± 0.06), as observed in this study, could be influenced by gonadal maturity and available food resources. However, considering all stations, (İstanbul, Çanakkale, Rize, Sinop-Kastamonu) mean calculated condition factor value is smaller than one (≤0.879). Condition factor values (1.275±0.122) have been previously evaluated for *S. maena* species in the Central Adriatic (Dulčić et al., 2000) and *S. flexuosum* in the Greece coasts (1.39±0.02) (Mytilineou & Papaconstantinou, 1991) and Türkiye (Central Aegean Sea) (Mater et al., 2001) (1.255), which were found to be greater than those in this study. On the other hand, condition factor value has been found as 1.01±0.005 which was found to be nearly identical value from our study in recent years in Black Sea (Samsun & Erdoğan-Sağlam, 2021). Little seasonal variation in the condition factor was observed among all regions in this study, as stated by Mytilineou & Papaconstantinou (1991) in Greece. Variation in condition factor might also be affected by increasing age (Mytilineou & Papaconstantinou, 1991), which was not considered in this study.

Conclusion

This study provided LWRs and the condition factor data for *Spicara flexuosum* species collected from Turkish coastal waters. *S. flexuosum* showed negative allometric growth in Rize and Sinop-Kastamonu locations in winter months. On the other hand, species exhibited positive allometric growth in İstanbul and Çanakkale in early spring and summer months. Condition factor values for each location were found to be equal to or greater than one, showing the wellbeing of fish. Overall condition factor value was found to be smaller than one in this study. On the other hand, samples from Istanbul were better in February (early spring) than in other periods and regions based on both LWRs and condition factor results.

Currently, no regulation is implemented for *S. flexuosum* fisheries in Turkish coastal waters (e.g., minimum landing size); therefore, this study can help authorities and researchers establish future regulations of *S. flexuosum* in the Turkish coastal waters as well as other regions around the world.

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Compliance With Ethical Standards

Authors' Contributions

AŞŞ: Designed, prepared and wrote the draft, performed and managed all statistical analyses.
AYS: Provided Sinop-Kastamonu data.
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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SHORT COMMUNICATION

Role of heat shock protein influencing bioactive compounds from mangrove tropical estuarine microalgae for enhancement of copepod egg production in culture system

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ABSTRACT

In silico investigations of the natural bioactive compounds in the microalgae from mangrove tropical estuaries showed an influence on heat shock protein -70 production. Incorporation of algae with such compounds in the diet of copepod high density culture might lead to enhanced egg production. For this study, the structure of the ligands (bioactive compounds from microalgae in the region of the mangrove estuary) and X-ray crystal structure of hsp-70 complex was taken from PDB (3P9Y) with a resolution of 2.10 Å. The molecular docking study was performed using GOLD software. In the present study, a total of ten bioactive compounds showed good molecular interaction with hsp-70 protein. Among these bioactive compounds, Quercetin from the microalga, *Chlamydomonas eugametos* exhibited the highest molecular interaction and this compound is potential for enhancement of hsp-70 protein compared to other bioactive compounds and is considered a good nutrient enrichment for copepod culture as well as enhancement of hsp-70 protein against ROS and adverse environmental conditions. Successful high density copepod culture might lead to scaling up of hatchery rearing of marine finfish larvae.

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Introduction

Estuarine mangroves ecosystem plays an important role in biodiversity, energy flow and maintaining functioning food chains with phytoplankton (microalgae) as primary producers (Saifullah et al., 2015). A microalga initiates the marine food chain by serving as food to primary consumers such as zooplankton, shellfish, and finfish (Altaff, 2020). The distribution and abundance of commercially important fish and shellfish and their larvae are dependent on some species of microalgae as their main food source. In aquaculture, microalgae are used as a direct food source for various filter feeding larval stages of marine organisms. They are also used as direct food source in the mass culture of copepods (Altaff & Janakiraman, 2015). Cultivation of microalgae is mandatory in the hatchery as it is a basic and nutritious diet for live feed organisms, specifically for copepods. Copepods are of a great ecological importance, being ideal food for larvae of many species of fish. The marine copepods are considered most suitable for the economically valuable cultivable finfish species, as they are a valuable source of protein, lipid (especially HUFA, 20:5 n-3 and 22:6 n-3), enzymes (amylase, protease, exonuclease and esterase), which are essential for larval survival, growth, digestion and metamorphosis (Aman & Altaff, 2004).

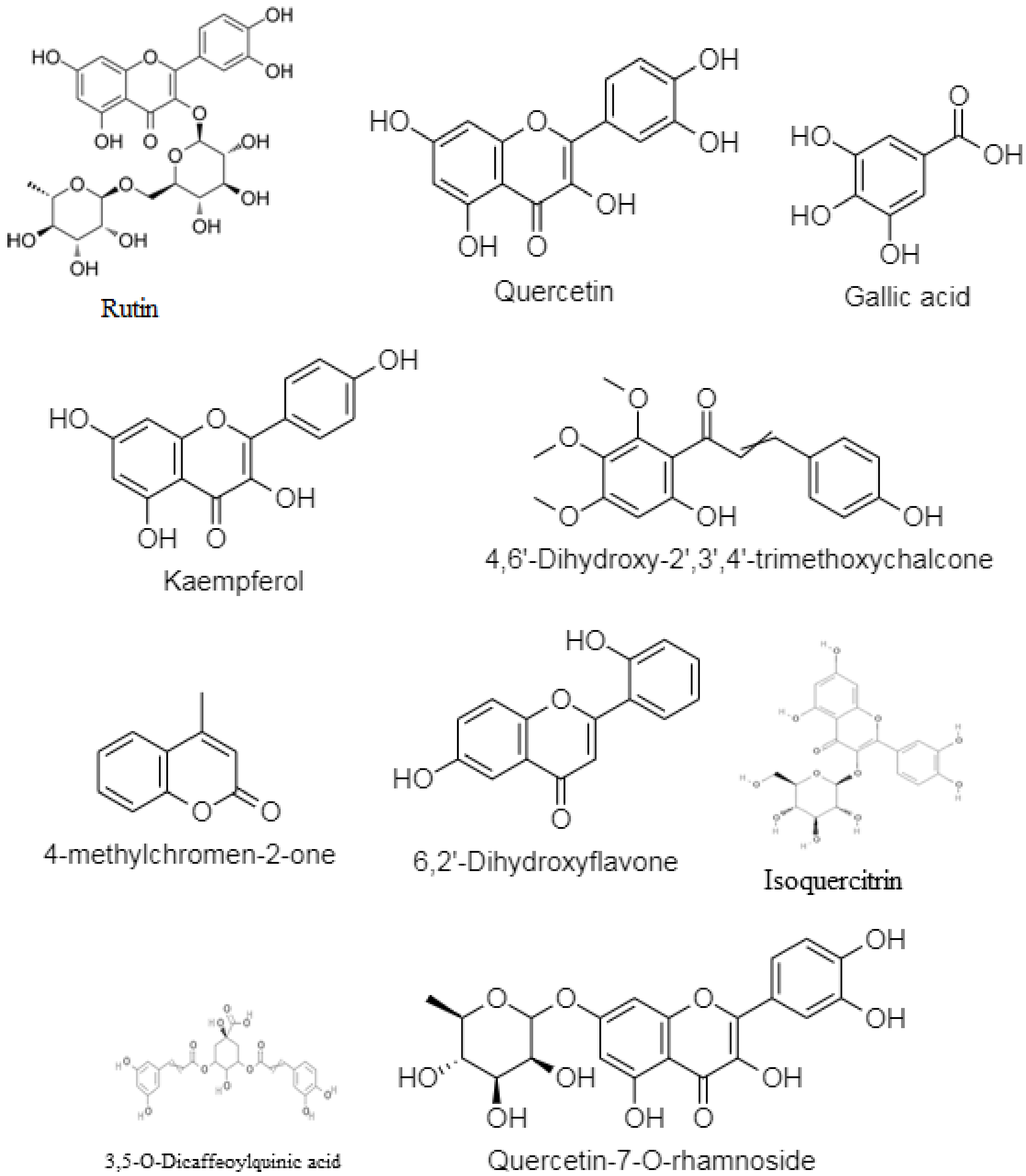

Figure 1. Structure of the ligands of bioactive compounds from microalgae

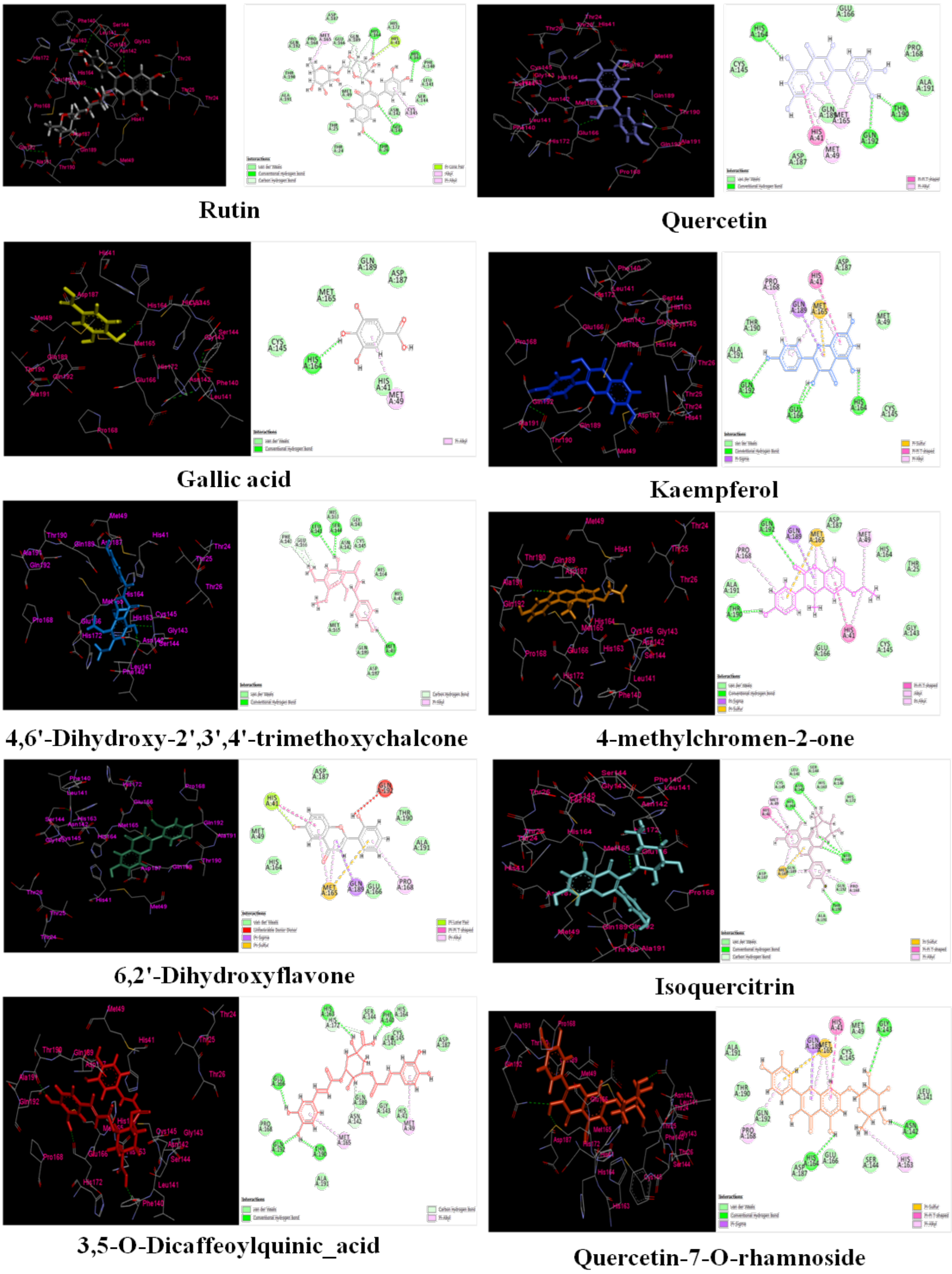

Figure 2. Molecular Interaction of Bioactive compounds with specific protein hsp-70

Under unfavorable conditions some copepod species can produce thick-shelled dormant eggs or resting eggs and with a wider tolerance to temperature and salinity changes. The induction of heat shock proteins (hsps) is considered as an important protective, eco physiologically adaptive, and genetically conserved response to environmental stress in all organisms. Among the hsps, the heat shock protein 70 (hsp-70) production is the response of copepods in shallow waters to protects them against the adverse environmental conditions such as temperature and pH which otherwise leads to damage the cellular macromolecules through reactive oxygen species (ROS) (Nilsson et al., 2014). Based on the above rational, in the present study an attempt is made to search for hsp -70 enhancement bioactive compounds from microalgae in the region of mangrove tropical estuaries using *in silico* modeling. These molecular interaction studies are not reported to our knowledge in any copepod. Present study aims to enhance and regulate fundamental cellular processes during high density culture of marine copepod.

Table 1. Ligands of bioactive compounds from microalgae and their molecular formula

Name of the Bioactive Compounds	Molecular Formula	Bioactive Compound Sources	Reference
Rutin	$C_{27}H_{30}O_{16}$	*Chlorella vulgaris*	Barkia et al. (2019)
Quercetin	$C_{15}H_{10}O_7$	*Chlamydomonas eugametos*	Birch et al. (1953)
Gallic acid	$C_7H_6O_5$	*Euglena cantabrica*	Jerez-Martel et al. (2017)
Kaempferol	$C_{15}H_{10}O_6$	*Microcystis aeruginosa*	Cao & Li (2018)
4,6'-Dihydroxy-2',3',4'-trimethoxychalcone	$C_{18}H_{18}O_6$	*Nannochloropsis oculata*	Sanjeewa et al. (2016)
4-methylchromen-2-one	$C_{10}H_8O_2$	*Porphyridium purpureum*	Su et al. (2016)
6,2'-Dihydroxyflavone	$C_{15}H_{10}O_4$	*Dunalliela* sp.	Lv et al. (2014)
Isoquercitrin	$C_{21}H_{20}O_{12}$	*Schizochytrium aggregatum*	Lv et al. (2014)
3,5-O-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	*Dunaliella salina*	Rismani & Shariati (2017)
Quercetin-7-O-rhamnoside	$C_{21}H_{20}O_{11}$	N*anno chloropsis*	Saifullah et al. (2015)

Material and Methods

The bioactive compounds (ligands) of Rutin from *Chlorella vulgaris*, Quercetin from *Chlamydomonas eugametos,* Gallic acid from Cyanobacteria *Euglena cantabrica*, Kaempferol from Cyanobacterium *Microcystis aeruginosa*, 4,6'-Dihydroxy-2',3',4'-trimethoxychalcone from *Nannochloropsis oculata*, 4-methylchromen-2-one from *Porphyridium purpureum*, 6,2'-Dihydroxyflavone from *Dunalliela* sp., Isoquercitrin from *Schizochytrium aggregatum*, 3,5-O-Dicaffeoylquinic acid from *Dunaliella salina*, and Quercetin-7-O-rhamnoside from N*annochloropsis* sp. were selected for this study (Figure 1). The details of the ligands of bioactive compounds are presented in Table 1. These bioactive compounds occur commonly in the microalgae of mangrove tropical estuaries (Saifullah et al., 2015). For this study, the structure of ligand (bioactive compounds) and X-ray crystal structure of hsp-70 complex was taken from PDB (3P9Y) were received from the databases of PubChem and Protein Data Bank (PDB), respectively. Molecular Docking was performed by GOLD software and detailed methodologies are given in our earlier publication (Altaff & Vijayaraj, 2021).

Results and Discussion

In aquaculture, microalgae constitute direct food source for the larval stages and adults of various filter feeding organisms (Altaff, 2020). They are also used as a direct food source in the production of rotifers, Artemia and copepods which in turn are used as food for the carnivorous larvae of many marine fish species. The microalgae are rich in several chemical compounds such as amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes and cyclic polysulphides and are used in several biological applications (Skjanes et al., 2013; Altaff, 2020). Likewise, the microalgae are one of the largest producers of biomass in the estuarine environments. As primary producers the microalgae form the base of the food chain in the estuary and due to the different environmental conditions in the estuarine region, they produce many bioactive compounds which might be considered suitable diet for enhancing copepod reproductive potential in culture system.

Table 2. Bioactive compounds binding interaction with specific protein hsp-70

Ligand (Bioactive compound)	Binding score (Kcal/mol)
Rutin	38.57
Quercetin	46.59
Gallic acid	29.70
Kaempferol	41.67
4,6'-Dihydroxy-2',3',4'-trimethoxychalcone	41.32
4-methylchromen-2-one	44.02
6,2'-Dihydroxyflavone	40.20
Isoquercitrin	46.29
3,5-O-Dicaffeoylquinic_acid	40.40
Quercetin-7-O-rhamnoside	41.72

In recent years, the molecular docking techniques greatly improved the efficiency of research while reduced the cost of research work considerably. It has become a key tool in computer assisted program to predict the binding affinity and analyze the interactive mode of secondary metabolites of bioactive molecules (Vijayaraj et al., 2019, 2021). In the present study, all the bioactive compounds showed good binding interaction with specific protein of hsp-70. The interaction binding energy recorded in the bioactive compounds is presented in Table 2. The molecular interaction of Rutin (38.57 kcal/mol), Quercetin (46.59 kcal/mol), Gallic acid (27.70 kcal/mol), Kaempferol (1.67 kcal/mol), 4,6'-Dihydroxy-2',3',4'-trimethoxychalcone (41.32 kcal/mol), 4-methylchromen-2-one (44.02 kcal/mol), 6,2'-Dihydroxyflavone (40.20 kcal/mol), Isoquercitrin (46.29 kcal/mol), 3,5-O-Dicaffeoylquinic acid (40.40 kcal/mol) and Quercetin-7-O- rhamnoside (41.72 kcal/mol) is depicted in figure-2. Among these bioactive compounds, the bioactive compound, Quercetin from microalga, *C. eugametos* exhibited highest molecular interaction and this compound has higher potential for enhancement of hsp-70 protein compared to other bioactive compounds. *Chlamydomonas* sp. constitute one of the microalgae cultured for use as live feed in aquaculture (Duerr et al., 1998; Sivakumar et al., 2011).

Eggs of many copepods enter in to a resting state, called quiescence, to overcome unfavorable environmental conditions (Sørensen et al., 2007). Under favorable conditions, subitaneous eggs are produced that are characterized by hatching within a few days after spawning (Marcus, 1996). In response to adverse environmental conditions, subitaneous eggs enter a quiescent state, where embryonic development is delayed until exposure to more favorable environmental conditions (Danks, 1987). Quiescence can be defined as a direct inhibition of development due to adverse conditions. Quiescence is a disruption of the embryogenesis that is hypothesized to be associated with transcriptional quiescence and reduced protein synthesis (Hofmann & Hand, 1994; Stuart & Brown, 2006). Thus, the metabolic rate is suppressed during quiescence. Quiescence can be induced in copepod eggs by several adverse conditions (Pedler et al., 1996; Clegg, 1997; Nielsen et al., 2006). Abrupt changes in salinity (Holmstrup et al., 2006), low temperatures (Drillet et al., 2006) and anoxia (Nielsen et al., 2006) have been shown to induce quiescence in order to store copepod eggs over time. When a copepod embryo undergoes quiescence, it requires a number of stabilizing factors (Rhee et al., 2009). Heat shock protein 70 is a family of proteins where some of which are constitutively expressed, while others are induced by several types of stress conditions, such as high and low temperatures, anoxia, reactive oxygen species (ROS), high culture density and osmotic stress. hsp-70 functions as a chaperone molecule that prevents stress-induced misfolding of proteins by facilitating correct folding pathways (Feder & Hofmann, 1999). Under adverse environmental conditions, increased synthesis of hsp-70 is important for the survival of copepods. Aruda et al. (2011) identified several, including four forms of hsp-70 in the calanoid copepod, *Calanus finmarchicus*. hsp-70 has been shown to induce stress alleviation in active *C. finmarchicus* in surface waters, but not in the diapausing animals in deeper waters (Aruda et al., 2011). Voznesensky et al. (2004) and Rhee et al. (2009) found that hsp-70 gene expression is elevated when copepods are exposed to elevated temperatures. The heat shock response of *C. finmarchicus* in shallow waters protects proteins against the higher temperatures experienced under these environmental conditions (Aruda et al., 2011). Changes in available cellular oxygen can result in higher levels of ROS, which in turn causes oxidative stress. ROS includes super oxide ($O2^{-}$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (•OH) that is responsible for most damage to cellular macromolecules (Kumaran, 2017, 2018). Because iron is a catalyst in the production of potentially damaging ROS, the amount of free iron in the cell must be minimized to reduce the amount of cellular damage. Quercetin is the most abundant dietary flavonoid found in the natural resources. Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. Quercetin from *Dunaliella tertiolecta* has been reported and these flavonoids acted as a protector of microalgae cells from metal toxicity and unfavorable environment conditions. The extracts containing this compound also showed antioxidant activity (Ferdous et al., 2021). In 1953, Birch et al. described the flavanol quercetin from microalgae *Chlamydomonas eugametos*, where the latter is used as sex hormone. Use of this microalga in the diet of high density culture of calanoid copepods might reduce stress of the copepods as well as enhance their reproductive potential leading to optimize egg production.

To our knowledge there is no published report available on the influence of hsp-70 in the enhancement of copepods egg production. However, only few copepod species have so far been subject in the *in vivo* model among the marine copepods. *Acartia tonsa* response to the heat shock protein was more pronounced at low salinity model (Nilsson et al., 2014; Petkeviciute et al., 2015) and similar impact of sublethal stress of *A. tonsa* using solar UV radiation was also reported (Tartarotti & Torres, 2009). Likewise, Rhee et al. (2009) reported that the *hsp-70* gene expression is elevated when copepods are exposed to elevated temperatures. Our Docking study indicate use of diet containing Quercetin for copepods in culture system might optimize their egg production by combating adverse culture condition through production of hsp-70.

Conclusion

In the present study the bioactive compound, Quercetin from microalgae, *C. eugametos* is showing highest binding energy and good molecular interaction towards hsp-70 production. Hence the microalgae (*C. eugametos*) *in* the diet of copepod culture might alleviate stress and could provide enhanced egg production leading to high density culture which in turn promote marine finfish larval rearing. Further *in vivo* investigations should be carried out on copepods for confirming the egg production with this microalgal diet.

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Compliance With Ethical Standards

Authors' Contributions

KA: Principal Investigator, Research Supervisor, Experimental Design, Prepared and approved the manuscript.
VR: Performed the experiments, data collection and interpretation, prepared draft manuscript.
MJ: Co-Principal investigator, data analysis and preparation of draft 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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REVIEW ARTICLE

Review on invasive alien species (IAS): Challenge and consequence to the aquatic ecosystem services

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ABSTRACT

The invasive alien species (IAS) are widely recognized as a significant threat to marine biodiversity and severely affect ecosystem services. There has been no measurable global assessment of their impacts and routes of introduction. This review highlights some aspects of invasive species and their impacts on the ecosystem in general. For many roots like global transportation, biological pest control, climate changes, and sometimes commercial, invasive species are introduced into the new environments. Afterward, the invasive species are rapidly dominant over the indigenous species because of their first growth, rapid reproduction, ecological competence, and phenotypic plasticity, consequently, altering the structure of Ecosystems and deterring the biological and physical organization of the system. Many policies have been introduced to stop the destruction produced by invasive animals and plants and to prevent upcoming invasions. Some critical components of getting rid of invasion are concern about transporting wildlife to new areas, Ballast water in tankers, aquarium species, and shipping.

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Introduction

Invasive Alien Species?

An invasive alien species (IAS) is an immigrant animal in an ecosystem that causes severe ecological or economic impairment to the environment and sources a major threat to local biodiversity by altering or shifting with the native habitats or species (Molnar et al., 2008; Riley et al., 2008; Pejchar & Mooney, 2009). IAS species have been transferred in a new bio-geographical area by human activity at a historical scale

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(Audemard et al., 2002). Those species can be familiarized to a new habitat via the ballast water of ocean-going ships, deliberate and unintentional releases of aquaculture species, aquarium samples or bait, and other means (Molnar et al., 2008) and initiating extinction of local floras and faunas, dipping biodiversity, instructing with indigenous organisms for partial resources, and fluctuating habitats. For instances, the American blue crab (*Callinectes sapidus*, Figure 1A,) is a predatory invasive species that capable to survive very extreme environments and has high fecundity rates. Now this species has widely spread throughout the Mediterranean Sea especially in the coast of Spain, Italy and France (Stasolla & Innocenti, 2014). This American crab has been recorded first in Ebro Delta in 2012, then it has rapidly expanded to the Mediterranean Sea and its adjacent rivers and wetlands. The massive invasion capacity of this crab established this species as a potential threat to the marine biota. This crab also makes threats on traditional fishing boat which is one of the most affected sectors of southern Spain fisheries sectors as the crab accidentally destroys the fishing nets. Another dominant candidate, the zebra mussels (*Dreissena polymorpha*, Figure 1B) are small freshwater shellfish mainly indigenous to the lakes and rivers that move into the Caspian, and Black seas in eastern Europe, are now widespread throughout the entire European water bodies and The Mediterranean Sea. They are frequently moving into new waterways attached with boats and also with ballast water discharged from commercial ships. This mussel was first introduced at 1980s, since causing millions of dollars in economic loss and altering ecosystems intensely.

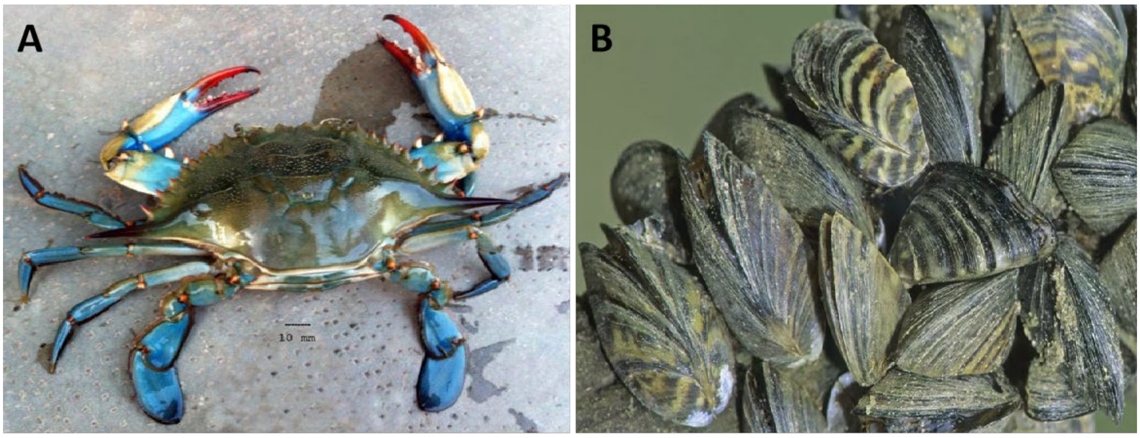

Figure 1. Two prominent invasive species in European water bodies. **A**) The American blue crab, An invasive species in the Mediterranean Sea. **B**) Zebra mussels originated from US and are now invasive to European water bodies (Figure adopted from the newsletter of Meghan Holmes, writer, New Orleans, LA).

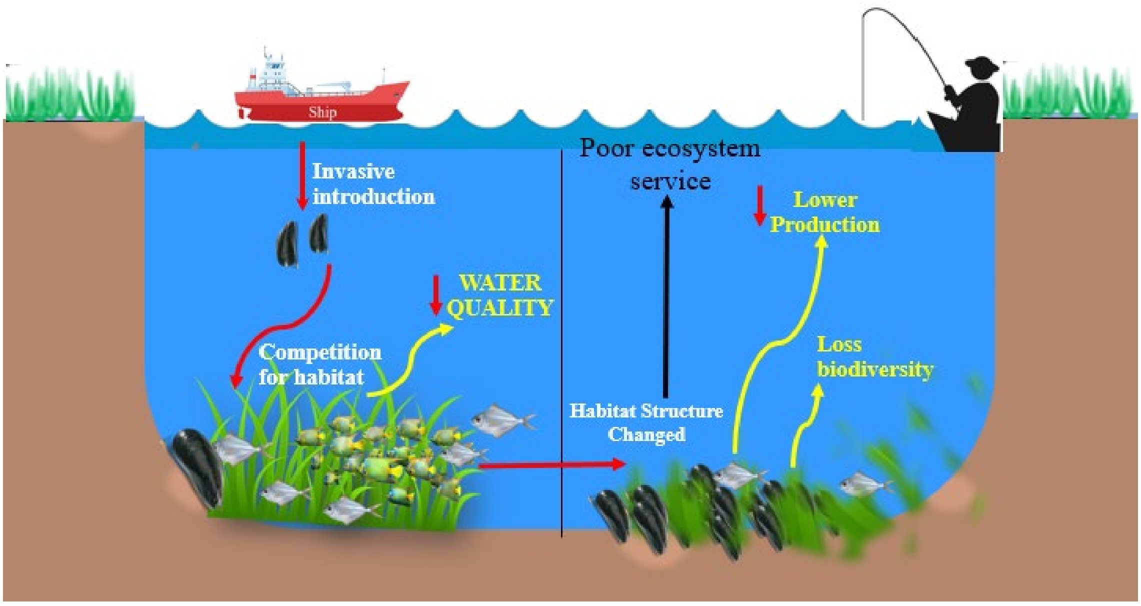

Figure 2. *Caulerpa taxifolia* an invasive assassin species for other algal species, seagrasses, and sessile invertebrate communities in the Mediterranean Sea (Photo modified from the Mark Hoddle, Extension Specialist and Director of Center for Invasive Species Research, University of California Riverside, USA).

Table 1. Origin and availability of some major invasive species with their ecological and economic impact. Source: Global Invasive Species Database (GISD, 2021)

Name of Invasive Species	Origin	Place of Invasion	Ecological Loss	Economic Loss
Gambusia holbrooki	Southeastern United States	Australia and Europe	Predating on amphibian eggs has negative influence on some frog species' choice of breeding habitat	Losing the biodiversity in Australia by predating the tadpoles of gold frog.
Hypophthalmichthys molitrix	Asia	Worldwide	Reducing native diversity by competing for and depleting zooplankton populations, altering the food web.	Carrying and transmitting the disease Salmonella typhimurium.
Hypophthalmichthys nobilis	Asia	Worldwide	Depleting zooplankton populations	Spreading disease through escape and introduction
Poecilia reticulata	Brazil, Guyana, Venezuela, Caribbean Islands	Worldwide except Antarctica	Threat to native cyprinids and killifishes	Carrier of exotic parasites
Cyprinus carpio	Eastern Russia and China	North America and Europe	Strong effects on benthic communities, stirring up bottom sediments during feeding, resulting in increased siltation and bio turbidity	Impacting growth rates and stocks of other fish by competition with carp, Providing protein in some third world countries
Eichhornia crassipes	South America	Africa, Asia, North America, Australia and New Zealand	Clogging up rivers makes it nearly impossible for animal life to move through them. Blocking sunlight and oxygen from getting to other plants below the water causing the ecosystem to change dramatically.	Reducing water flow thereby caused flooding. Impacting fishermen, sports-fisherman, water-skiers, and swimmers in recreational waters.
Elodea canadensis	Elodea canadensis	Europe	Negative impact on the functioning of the aquatic ecosystem will outcompete native aquatic plants.	It can impede water flow and adversely affect recreation activities
Sargassum muticum	Japan	Washington State, Europe	Forming a screen that prevents light, capturing nutrients, and disfavoring phytoplankton	Creating problems in shellfish farming, and getting entangled with the boat's propellers.
Dreissena polymorpha	Black, Caspian, Aral and Azov seas	Russia, Europe and North America	Severely impact native plankton, which reduces food for fish	fouling of intake pipes, ship hulls, navigational constructions and aquaculture cages
Asterias amurensis	China, Japan, Korea	Australia	Blamed for the decline of the critically endangered spotted handfish	In Japan, sea star outbreaks cost the mariculture industry millions of dollars

Invasive Species: Concerns to Habitat Degradation?

With the quick change in climate and different emerging contaminant, world biodiversity is at immense risk (Martin et al., 2010; Rizzo et al., 2017; Pierucci et al., 2019). The invasive species are achieving extra importance for the declination of oceanic diversity because of their unquantified hazard to ecosystem services (Thomsen et al., 2014; Walsh et al., 2016).
The interactions between invasive species and native dwellers cause drastic changes in marine biotic and abiotic factors of the ecosystem. These changes have continuously transformed marine habitats around the world. Most importantly, these invaders displace native species, food webs, food chain and, alter the change community assembly and also alter essential processes, such as the sedimentation process and biogeochemical cycle (Molnar et al., 2008; Piazzi & Balata, 2009; Rizzo et al., 2017; Pierucci et al., 2019). Therefore, in a normal community, species grow together into an ecosystem balances the limit the population growth of any one species (Crooks, 2005; Simberloff, 2005; Sih et al., 2010; Narwani et al., 2013; Rizzo et al., 2017). These balances include predators, parasites, herbivores, diseases, and other organisms interacting for similar resources and limiting environmental factors (Cook et al., 2007; Crowl et al., 2008). For a better illustration of the consequence of invasion, *Caulerpa taxifolia* invasion in the Mediterranean Sea can be a good instance. *Caulerpa taxifolia* is an invasive alga native in tropical waters with populations naturally occurring in the Caribbean, Gulf of Guinea, Red Sea that is causing serious environmental problems in the Mediterranean Sea (Arnaud-Haond et al., 2017; De Montaudouin et al., 2018; Mannino et al., 2019). However, after the introduction, *Caulerpa* has colonized thousands of hectares of sea bottom in the Mediterranean. It is found from France to Croatia. Its range in the Mediterranean will likely continue to expand (Arnaud-Haond et al., 2017). In literature, there are several conceptually different ambiguous terms used in the context of invasion ecology. There are many probable routes and pathways of invasive introduction. A simple conceptual diagram is helpful to represent the possible route of introduction and their interaction with the native dwellers, and the consequence of the ecosystem summarized through a conceptual diagram (Figure 2).

Invasive Species: Why Concerned for Ecological Alteration?

For sure, IAS is widely accepted as one of the leading direct causes of biodiversity loss. Introduction of a new species in an ecosystem may have diverse cumulative effect of this system. It is not always meaningful that the introduced species has negative consequences for the environment. Something its blessing for the biota of the native environment through the association of the both individual. For instance, several fish species have been introduced into the Great Lakes for recreational fishing which was subsequently spread in water bodies and causing problem to the natives' inhabitants (Gozlan et al., 2010; Rothlisberger et al., 2012). Although they have not enough recorded evidence of adverse impacts on leisure opportunities and a food source (Griffiths et al., 1991). However, many species have altered the existing environment in order to makes it more favorable for them while adverse for natives' dwellers, which is known as ecological facilitation (Botts et al., 1996; Gallien et al., 2012; Douda et al., 2013; Pierucci et al., 2019). For instance, *Spartina alterniflora* invasion in the Bay of Arcachon, France that quickly changed the bottom structure with the speedy proliferation of the plant (Figure 3).

Figure 3. a). Change the environment by rapid proliferation and high growth of invasive *Spartina alterniflora*, that causes the decline of Spartina maritime in basin de Arcachon, France, Modified from Proença et al. (2019), U.M.R., 5805 EPOC, University of Bordeaux, Talence Cedex, France, b). Conceptual mechanism associated with different Invasive alien species (IAS), corresponding to their settlement and spread in varying environment (Rai & Singh, 2020).

How Do Invasive Species Introduce?

The introduction of invasive species, which often differ functionally from the components of the recipient community, generates ecological impacts that propagate along with the food web (Simberloff, 2005; Gallardo et al., 2016). There are several techniques for the introduction of invasive species in the marine environment, such as deliberate commercial introductions, transport on ships' hulls either of sessile, boring or vagile species including escapes from aquariums and the discard or release back into the sea of bait and edible species, related intended introductions, movement through seawater canals linking biogeographically distinct water bodies and most important the ballast water (Clarke Murray et al., 2011, 2014; Gollasch et al., 2019). Many of invasive species were intruded as unsuccessful attempts to control other harmful species from the water bodies. For example, In the 1800s, rats that introduce to the Virgin Islands on ships infested the sugar cane fields, initiating massive crop damage. The reasons for invasive species introduction are Ballast water, fouling ships, deliberate commercial introduction, Aquarium business (Padilla & Williams, 2004), and some accidental introduction (Figure 4). For example, at present, global shipping transport over 90% of the world's commodities in intercontinental traffics that introduces 40% of invasive species from different mainland (IMO, 2007; Phillips, 2009).

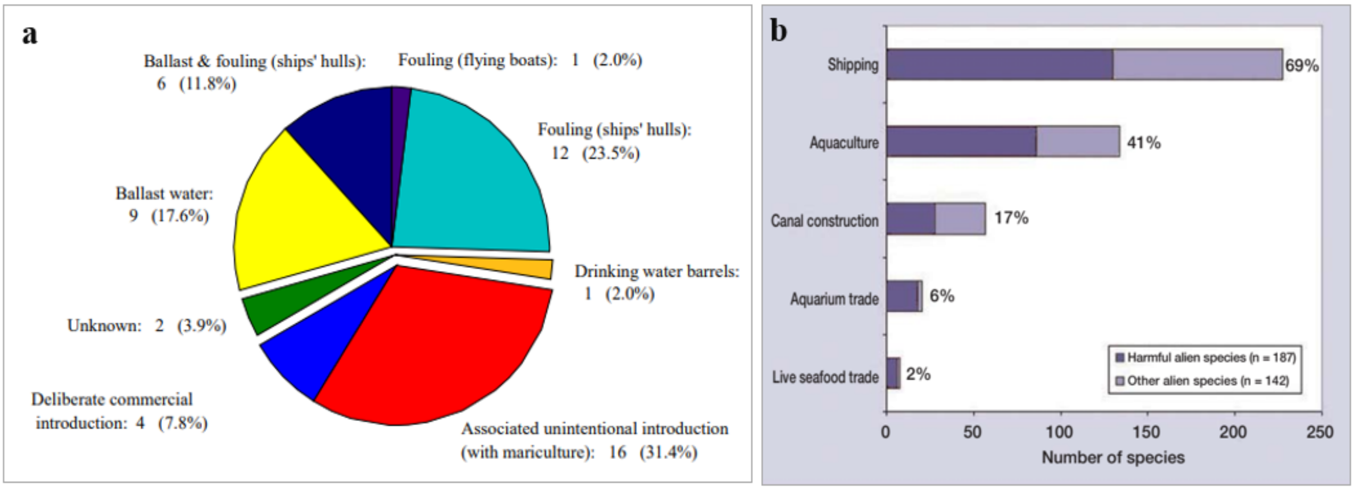

Figure 4. This figure indicates the probable method of introduction of invasive species in British water. a). Numerals indicate number of species involved and the percentage of total introductions (Eno, 1997), b). Number of marine alien species known or likely to be introduced by the most common human-assisted pathways (Adopted from Molnar et al., 2008).

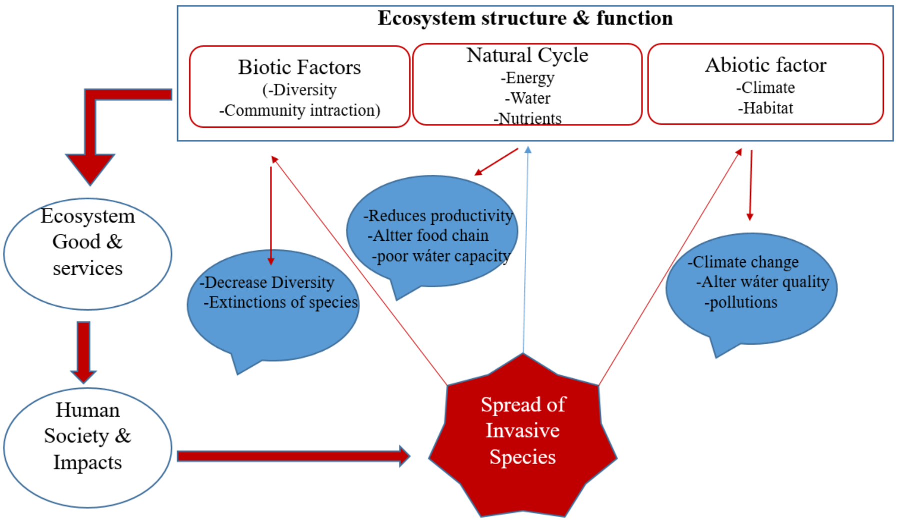

Figure 5. Mechanisms of ecosystem service alteration by invasive species. Those species mainly alter biotic and abiotic environment first and rapidly change the ecosystems (Figure modified from Charles & Dukes, 2008).

Methods of Ecosystem Modification

Ecosystems are characterized by biological, chemical and physical structure and functions, leading to ecosystem services' production and maintenance. The significant attention of ecosystem ecology is on functional processes and ecological mechanisms that maintaining the function and services produced by ecosystems including the alteration of primary production of biomass, decomposition and trophic relations. Ecosystem function corresponds to the process of energy transfer among the food webs and the central process that allows biochemical cycling and biochemical cycling. The invasive species mainly alter the ecosystem services (Figure 5) (Charles & Dukes, 2008; Traill et al., 2010). Most often these unwanted species may change community structure through exploitation, competition for resource uses. Their effects on other species including predation, herbivory, parasitism, and mutualisms and can modify the abundance of species with certain key traits which influence ecosystem processes (Chapin et al., 2000; Callaway & Ridenour, 2004; Ruitton et al., 2005; Charles & Dukes, 2008).

Impact of Invasive Species

Impacts on Habitat and Ecosystem

Invasive species are acting as major driver of global change having a number of harmful impacts on the areas that they were intruded. Several studies have been reported that they can rapidly modify the biodiversity and ecosystem functions in a virgin ecosystem (Pejchar & Mooney, 2009; Linders et al., 2019). The most significant of these is the widespread loss of habitat. Invasive species can change the food web in an ecosystem by destroying or replacing native food sources (Molnar et al., 2008). The invasive species may provide little to no food value for wildlife. But this invasive species can ridiculously alter the abundance or diversity of species that are important habitats for native wildlife. Many species establish in a new habitat with few disturbances, whereas others alter entire ecosystems or put native species at risk of extermination (Molnar et al., 2008; De Montaudouin et al., 2018; Linders et al., 2019). For example, the Burmese pythons the top predators in the Everglades are responsible for the decimation of local mammal and bird populations (Dorcas et al., 2012). They convert the habitat through their engineering effect and have strong invasive activity to the habitat (De Montaudouin et al., 2018; Linders et al., 2019). The resulting shell debris provides a hard substratum for attachment of juvenile *Crepidula fornicata*, perpetuating the population, and other epifauna, such as ascidians, ultimately destroying the entire natural ecosystem (Readman & Rayment, 2016; De Montaudouin et al., 2018).

Impacts on Biodiversity and the Environment

Invasive species are threats to the biodiversity including floras and faunas and overall ecosystem health by disrupting existing biological communities and ecological processes (Molnar et al., 2008; Linders et al., 2019; Proença et al., 2019). For instance, Asian carp recruited into the United States outcompete native fish for both habitat and foods, leading to significant alterations in natural fish populations that makes the ecosystem less diverse. And always these less diverse ecosystems are more vulnerable to further disturbances such as diseases and natural disasters (Dale et al., 2001; Molnar et al., 2008; Proença et al., 2019). In spites of having some economic and ecological benefits, alien invasions may result in extensive modification in the ecosystem structure, composition, functions and the distribution of the biota, leading homogenization of the world's fauna and flora's diversity (Charles & Dukes, 2008; Molnar et al., 2008; Traill et al., 2010; Proença et al., 2019).

Impact on Hydrodynamics, Nutrient and Water Cycling

Most often, the introduction of alien species alters the hydrodynamics of the existing ecosystem consequently changes the energy flows and trophic relationship of living biota. A study on golden apple snail (*Pomacea canaliculata*), a herbivore snail observed dramatically decreased aquatic plant populations in wetlands with implications for water parameters and purification (Carlsson et al., 2004; Moulin et al., 2007; Martin et al., 2010). Along with this, the nutrient cycling in aquatic system also be altered by invasive plants that fix nitrogen, and release compounds that alter nutrient availability or retention, including nitrogen and phosphorus, and change topsoil erosion or fire frequency (Dukes & Mooney, 2004; De Montaudouin et al., 2018).

Impact on Human Health

Invasive species are also harmful for human health. Many studies found that invasive zebra mussels produce toxins likes PCBS and PAHs in their tissues. When other predatory organisms prey on these mussels, the toxins are transfer into the food chain and finally can goes through the higher trophic level. Discharged ballast water from ships sometimes brings harmful bacteria like cholera. Invasive animals can also be vectors for these deadly disease (Gollasch et al., 2019). Encroachment of humans into previously remote ecosystems has exposed exotic diseases such as HIV (Pimentel et al., 2005) to the broader population. Throughout recorded history, epidemics of human diseases, such as malaria, yellow fever, typhus, bubonic plague, spread via some invasive vectors (Elton, 2020). A recent example of an introduced disease is the spread of the West Nile virus, which killed humans, birds, mammals, and reptiles (Lanciotti et al., 1999; Molnar et al., 2008). Examples of various types of impact of aquatic invasive alien species on human health are shown in Table 2.

Table 2. Various types of impact of aquatic invasive species on human health (Data modified from Pyšek & Richardson, 2010).

Type of Impact	Saltwater Invasive Invertebrates	Freshwater Invasive Animals
I. Cause or vector of human diseases or ailment	*Alexandrium catenella* (poisoning from consumed shellfish can lead to death), *Styela clava* (respiratory problems from sprays damage tissues)	*Eriocheir sinensis* (in native range, a host for the lung fluke parasite, causing diseases of lungs and other body parts), *Procambarus clarkiia* (host for trematodes that are potential parasites of humans)
Causes injuries	*Balanus improvisus, Ensis americanusa* (sharp shells inflict cuts), *Siganus rivulatus, Rhopilema nomadicaa* (painful stings)	*Dreissena polymorpha* (sharp shells inflict cuts), *Plotosus lineatus* (injuries caused by the barbed and venomous dorsal spine)
Causes allergies		*Cercopagis pengoi* (may cause allergic reactions in fisherman when they clean their nets)
Accumulation of toxins and their transfer to human food		*Neogobius melanostomus, Procambarus clarkiia* (heavy metals and cyanotoxins)
Impedes recreational activities and tourism	*Alexandrium catenella* (causing red tides)	

Invasive Species Impacts on the Economy

Not only to the biodiversity, invasive species have severe effects on global economy. Millions of dollars need to spend every year on eradicating invasive species and restoring the habitats they have invaded. For example, figures for the total cost of IAS in the USA range from US$131 billion cumulative to US$128 billion annually, and also China spent US$14.45 billion for this purpose. Economic losses can also occur through the loss of recreational and tourism revenues (Simberloff, 2001; Pimentel et al., 2005; Xu et al., 2006; Pejchar & Mooney, 2009). They can damage a wide array of environmental services that are important to recreation, including, but not limited to, water quality and quantity, plant and animal diversity, and species abundance. For instance, silver carp and common carp can be harvested for human food and exported to markets already familiar with the product in many developing Asian countries. According to the European Commission, the estimated annual economic losses (Euro) of some developed countries are given in the Table 3.

Table 3. Estimated annual economic losses (Euro) of EU and some developed countries (Data modified from the report of European Commission, 2013)

Country	Predictable Losses (trillion/year)
Globally	€ 1
US	€ 91
EU	€ 12
China	€ 11
New Zealand	€ 2
UK	€ 12

Control Measures for Invasive Species

There are several strategies have been taken to reduce the damage caused by invasive species along with to prevent future invasions. However, managing invasive alien species is particularly challenging in the ocean because marine ecosystems are extremely interconnected across broad spatial scales. Extermination of aquatic invasive species was only achieved when species were spotted early, and management responded swiftly (Pyšek & Richardson, 2010; Giakoumi et al., 2019). Many international and local laws and regulations have also been introduced to combat the future expansion of invasives. (Hunter & Hart, 2013). Usually, invasive control strategies in the marine environment follow a species-by-species approach. Though, trait-based prevention and management could result in more effective conservation outcomes as a set of management actions could benefit multiple invasive species sharing common traits. Furthermore, a comprehensive approach to invasive species management should consider, such as the expected impacts of these species on native ecosystems, the available technical intervention options, their expected likelihood of success and their cost, the risks associated with management, and the extent of public support and stakeholder support for the proposed interventions (Pyšek & Richardson, 2010; Ojaveer et al., 2014; Giakoumi et al., 2019).

The various options of management include-

Physical or Mechanical Control - involving physical removal of the invasive species by harvesting or using barriers or traps. These techniques mainly suitable for the removal of invasive plants.

Chemical Control - Can be executed by using pesticides, herbicides, insecticides, fungicides and other recommended chemicals. As well as adverse effects of using these chemicals in the ecosystem need to be considered.

Biological Control - This method is the most purposeful and ecologically sustainable approach of controlling invasion. In this process many biological individuals are used to reduce the invasive species populations.

Conclusion

Invasive species is a global problem and consider a prime disaster to species diversity. Depending on diverse taxa, geographic locations, and ecosystem types invasive species can alter ecosystem services by affecting populations, community interactions, ecosystem processes, biotics and abiotic variables. Almost all ecosystem services can be destructively impacted by invasive species, whereas some minor positive impacts exist. Sometimes the developed country can overcome the consequence of invasive activity, but it's really difficult for developing one, therefore, the ecosystem and species richness are a significant risk to those nations. It is high time to take initiatives to control invasive species distribution for a sound ecosystem.

Compliance With Ethical Standards

Authors' Contributions

JB: Manuscript design, writing, editing, analysis.
RG: Writing, editing, reading,
SSI: Draft checking, editing, and writing.
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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RESEARCH ARTICLE

Implications from the meteorological data effects on water level fluctuations of the Lake Van (Eastern Anatolia/Türkiye)

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ABSTRACT

Lake Van is located in the eastern part of Türkiye and forms the largest soda lake in the world. In this study, we present the relationship between instrumental data which belongs to Lake Van level changes and meteorological parameters by performing a multilevel analysis. The data set consists of monthly average levels of Lake Van and monthly meteorological parameters (temperature, precipitation and wind speed) between 1944-2019 years. Two different multilevel linear models; random intercept, random intercept and slope model were used. Unstructured (UN) covariance structure and Maximum Likelihood (ML) were used for repeated measurements and estimation, respectively. Log-likelihood (ll), Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC) and AIC Corrected (AICC) were used for the selection of the best model. The random intercept and slope model (Model II) is explained as the best model for the lake level changes in this study. Statistical results in this study indicated that the temperature and wind speed are the key parameters controlling the Lake Van water level fluctuations, whereas the precipitation effect is minimal due to the type of precipitation (snowfall). For this reason, temperature, wind speed and also the type of precipitation (snowfall or rain) must be considered for disaster modelling in settlements of Lake Van and similarly closed basin lakes.

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Introduction

Lake levels fluctuate naturally in response to tectonic activity, erosion, changes in groundwater level, precipitation, drought and/or human-induced activities (land use, irrigation activities, dams, etc.). Climate change is the most important cause of lake level changes and is due to the precipitation-evaporation change over the drainage basin. In the absence of natural and human-induced effects, the water level in closed lakes represents the balance of input (precipitation, runoff, and groundwater) and output (evaporation and seepage). Close and deep lakes are very sensitive to climate changes especially in arid/semi-arid region. Lake Van has an altitude of 1648 m above sea level, extending for 130 km WSW-ENE on the Eastern Anatolian high plateau, Türkiye (Figure 1). It is the fourth-largest terminal lake (area 3570 km^2, volume 607 km^3, maximum water depth 450m) and the largest soda lake in the world (Degens & Kurtman, 1978). Lake Van is surrounded by semi-active volcanoes of Nemrut (2948 masl) and Süphan (4058 masl). Due to evaporation processes, hydrothermal activity, and chemical weathering of volcanic rocks, the lake water is extremely alkaline (alkalinity 155 $meql^{-1}$, pH 9.81, salinity 21‰; Kempe et al., 1991). The origin of the lake is attributed to the cut of the outlet of an ancient river at ca. 600 ka as a combination of the presence of thick ignimbrites underlying South of Tatvan and Muş Basin and the growth of the Nemrut Volcano between Van and Muş basins (Sumita & Schmincke, 2013; Cukur et al., 2014a; Schimincke & Sumita, 2014).

Lake Van Basin is located at the crossroads of the North Atlantic, Siberian High Pressure and the mid-altitude subtropical high-pressure systems (Türkeş & Erlat, 2003; Akçar & Schlüchter, 2005). The basin has a continental climate characterized by cold and wet winters and dry summers (Landman et al., 1996a, 1996b; Wick et al., 2003). The annual precipitation of the lake's catchment is about 400mm/year (Landman et al., 1996a, 1996b; Wick et al., 2003). During the winter season, the precipitation type is snowfall which is typical in Eastern Anatolia (Kadıoğlu et al., 1997). The most freshwater that enters the lake occurs in spring with snowmelt and rain. The annual average temperature is 9-10°C. The driest and warmest period is between July and September, while evaporation is at its maximum during July-August (Kadıoğlu et al., 1997). The water that enters the Lake Van which is hydrographically closed is 2.1 km^3 via rivers and 1.68 km^3 via precipitation per year and an average of 3.78 km^3 of water is lost by evaporation (Landman et al., 1996a; Reimer et al., 2008). While the water body of Lake Van shows stratification during the summer with the effect of temperature, it mixes during the winter (Peeters et al., 2000). The lake is classified as a meromictic lake with a monomictic upper (70-100 m) layer (Kipfer et al., 1994).

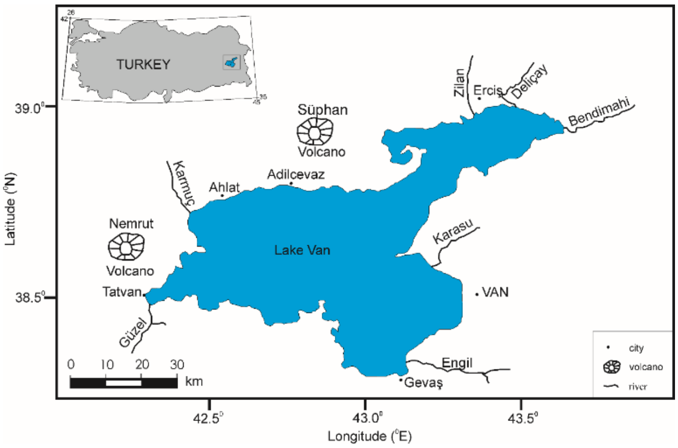

Figure 1. Map of Lake Van showing the major inflowing rivers and the volcanoes

An International Continental Drilling Project (ICDP)-PaleoVan Project was realized in Lake Van in 2010 to investigate its geological evolution and climate changes. Approximately 600,000 years of geological evolution and climatic variability of the region were revealed in this project (Litt et al., 2014). Earlier works show that the lake water levels have fluctuated drastically due to the climate changes over the last 20,000 years (Kempe & Degens, 1978; Lemcke, 1996; Landman et al., 1996a, 1996b; Kempe et al., 2002; Wick et al., 2003, Litt et al., 2009). Based on exposed terraces around the lake, it has been reported that the lake level rose to 107 meters from its current level in the past (Valeton, 1978; Kempe et al., 2002; Kuzucuoğlu et al., 2010). The seismic profiles obtained from Lake Van reveal that the lake level has fluctuated dramatically during the last 600.000 years (Çukur et al., 2014a, 2014b; Damcı et al., 2012).

Lake Van's water level fluctuates unsteadily today as it was in the past. Therefore, many studies have been conducted to explain the reasons for the level changes. The 2 m rise in the lake level recorded between 1944-1974 was attributed to sunspot cycles occurring at intervals of 10-11 years (Kempe et al., 1978). Afterwards these level changes associated with precipitation, evaporation, and temperature (Saraçoğlu, 1990; Gürbüz, 1994; Kadıoğlu, 1995; Gürer & Yıldız, 1996; Batur, 1996; Sezen, 1996). In addition to climatic factors, it has been reported that the groundwater controls the lake level (Kaynak, 1995; Batur, 1996; Gürer & Yıldız, 1996; Düzen, 2011). While investigating the causes of lake level changes, it was revealed that 50% regeneration in deep water occurred until 1994, according to studies on some trace elements (Kipfer et al., 1994). In the following years, trace element studies continued in the water column, and it was stated that in response to the lake level rise that started in 1994, the intensity of deep-water exchange decreased significantly, and anoxic conditions developed below 300 meters (Kaden et al., 2010). It was observed that the He concentration of the water samples from Lake Van in 2004 was higher than in 1990/91 (Litt et al., 2009). This revealed that the mixing (oxygenation) in deep water slows as the level rises. Fort this reason, it has been interpreted that the lake level changes in response to climate change affect the mixing dynamics over time (Litt et al., 2009).

The increases in the lake level had a significant impact on the settlements located around Lake Van, especially in the 90s, and forced the people living in that area to migrate. On the other hand, in Tatvan and Van Piers which are the important trade routes of the region, the railway tracks, warehouses belonging to the enterprise and the basements of the lodgings were completely submerged (Deniz & Yazıcı, 2003). These human and economic negativities in the vicinity of the lake shore have been tried to be eliminated by taking a series of measures.

The effects of climate change not only mean the increase in temperature in that region and therefore drought, but also the change in weather events (especially precipitation type and duration). It is insufficient to explain the lake level change with the annual average amounts of temperature and other meteorological parameters. To see the effect of meteorological events on the lake level, making an evaluation using monthly is one of the solutions to the problem. Considering the monthly changes means that seasonal changes can also be easily interpreted. With such evaluations, more sensitive approaches are obtained and thus the corrects interpretation of the parameters is ensured. The predictability of the impact of climate change on the Lake Van level will enable early measures to be taken against possible problems that may arise over time. The aim of this study is therefore to examine the water level changes of Lake Van, which may affect more than one settlement in our region with the effect of climate change. To show the which parameters control the lake water level changes, we present the relationship Lake Van water level changes data and meteorological parameters by performing a multilevel analysis.

Material and Methods

The dataset of the study includes the average lake level of Lake Van between 1944 and 2019 and monthly average temperature, precipitation, and wind speed variables in the region of the same years. Lake level data were obtained from the Lake Van - Tatvan Station affiliated with the Turkish State Hydraulic Works Survey, Planning and Allocations Department. Monthly average temperature, precipitation and wind measurements were obtained from the Turkish State Meteorological Service.

Two different multilevel linear regression (MLM) models were used to analyze the data (Goldstein, 2011). The first model is the random intercepts model, and second model is the random intercept and slope model.

Model I: The equation of the random intercept model is

$$Y_{ij} = \beta_0 + \beta_1 X_{1ij} + \beta_2 X_{2ij} + \beta_3 X_{3ij} + u_{0j} + e_{ij} \qquad (1)$$

Where; Y_{ij}, lake level of the ith year and jth month; $X_{1ij}, X_{2ij}, X_{3ij}$, temperature, precipitation and wind speed values measured in the ith year and jth month; β_0, random intercept point and β_1, β_2, and β_3, coefficients; u_{0j}, level-2 error term and e_{ij} shows level-1 error term. Error terms are normal distributed, and level-2 and level-1 errors are independent of each other. $u_j = u_{0j} \sim N(0, \sigma^2_{u_0})$ and $e_{ij} \sim N(0, \sigma^2_e)$ assumptions are made.

Model II: The equation of the random intercept and slopes model is

$$Y_{ij} = \beta_0 + \beta_1 X_{1ij} + \beta_2 X_{2ij} + \beta_3 X_{3ij} + u_{0j} + u_{1j} + e_{ij} \qquad (2)$$

The difference between Model II from the Model I is included in the second random error term model which assumes temperature values change from year to year randomly. Therefore, the random error term u_1j is added to the model. In this model, it could be a correlation among level-2 errors, however it is independent of level-1 errors. In other words, $u_j = [u_{0j}, u_{1j}]^T \sim N(0, \Omega_u)$, $\Omega_u = \begin{bmatrix} \sigma^2_{u_0} & \sigma^2_{u_{01}} \\ \sigma^2_{u_{01}} & \sigma^2_{u_1} \end{bmatrix}$ and $e_{ij} \sim N(0, \sigma^2_e)$ the assumption is still existing.

In Model I and Model II, ML (Maximum Likelihood: Maximum Likelihood) estimation method and UN (non-structural) covariance structure were used. For model selection, Log-likelihood ll, Akaike Information Criteria AIC, Bayesian Information Criteria BIC and Akaike Information Criteria AIC Corrected AICC (Akaike, 1974; Schwarz, 1978; Hurvich & Tsai, 1989) were used.

SAS (v9.4) and MLwiN (v2.02) statistical package programs were used while analyzing the data (Rashbash et al., 2010).

Results and Discussion

Lake Van Level Fluctuations Between 1944 and 2019

The minimum and maximum values of the lake level obtained from the State Hydraulic Works between 1944-2019 are given in Figure 2.

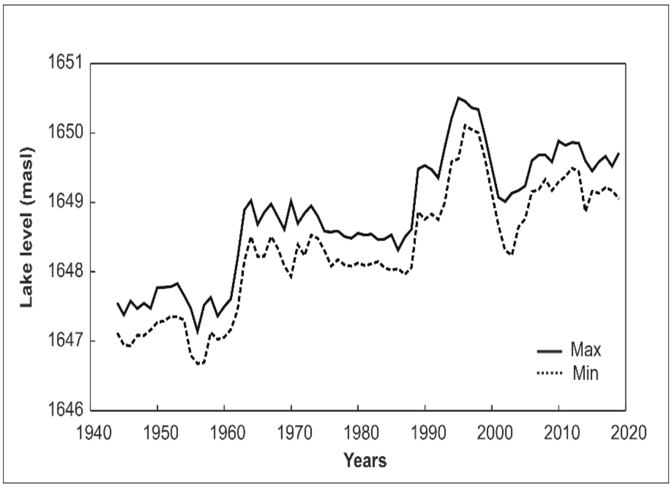

Figure 2. Lake Van minimum and maximum water level (Turkish State Hydraulic Works, 2019)

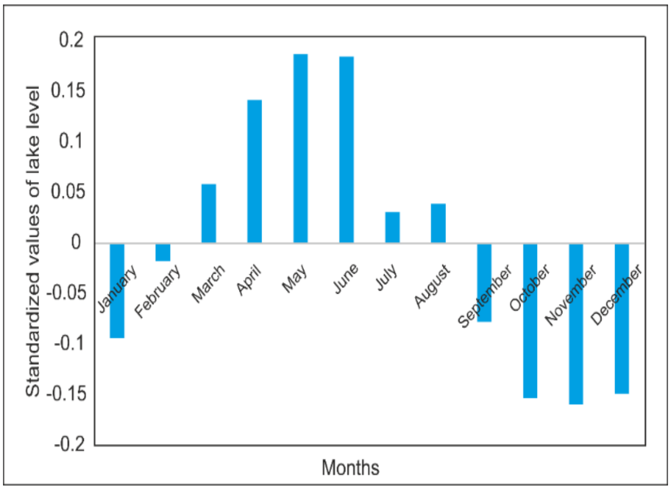

Figure 3. Monthly average changes of standardized lake level data (1944-2019)

The lake level fluctuates unsteadily according to instrumental data (Turkish State Hydraulic Works, 2019). The lake level increased between 1960-1965 and 1987-1997. Between 1965-1987 the level decreases relatively. In total 76 years of lake level data have been standardized and the monthly changes in the lake level are given in Figure 3.

The lake level was above the average between March-August, and the maximum lake level was recorded in May. The minimum value of the lake level, which fell below the average between September and February, was observed in November. In this study, which investigates how much this fluctuation can be explained by temperature, precipitation, and wind speed, the standardized data (monthly average temperature, precipitation, wind speed (Turkish State Meteorological Service, 2019) and lake level (Turkish State Hydraulic Works, 2019) are also presented together (Figure 4).

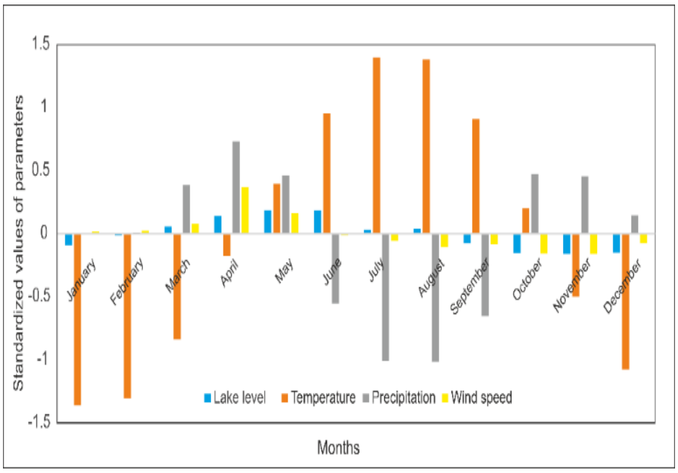

Figure 4. Monthly average changes of standardized lake level, precipitation, temperature, and wind speed data (1944-2019)

The standardized data show that the temperature in January is well below the average, the precipitation and wind effect are almost equal to the average and the lake level is below the average (Figure 4). This situation is similar in February and the lake level increases relatively but still below the average (Figure 4). In March, when the lake level rises above the average for the first time, the temperature is below the average, but increases compared to February (Figure 4). However, wind speed and precipitation are above the average. In April, when the precipitation is at its highest level above the average, the temperature increases compared to the previous month, precipitation and wind speed reach the highest level of the year, and the lake level continues to rise (Figure 4). All standardized data are recorded above the average and in May and the lake level is at its highest level (Figure 4). The effect of precipitation amount and wind speed decreases compared to the previous month. The temperature continues to increase in June when the wind speed decreases to its average value and the precipitation falls below the average (Figure 4). Precipitation rate is the lowest and the temperature is highest at maximum in June and August (Figure 4). Lake level is about its average level and wind speed drops below the average. In September, the temperature decreases compared to August but is above the average (Figure 4). Although the wind speed has increased compared to the previous month, it is below the average. The lake level drops below the average from this month and this decrease is observed at the maximum level in November (Figure 4). The precipitation rate which rises above abruptly above the average in October, decreases until November, but it is observed above the average till January (Figure 4).

Sezen (1996) reported that there is a positive relationship between the lake level fluctuation and precipitation and that precipitation falling into the basin causes an increase in the lake level in the following months. It has also been revealed that the lake level change is related to the variations in precipitation, snow melt, and temperature (Gürbüz, 1944; Saraçoğlu, 1990). Düzen (2011) suggested that groundwater controls the lake level changes as well as climatic patterns. Based on standardized instrumental data, we show that the lake level is at its minimum level in November albeit it is below the average, starts to rise from December, and rises above the average in March. Lake Van is located on a high plateau and is adjacent to several mountain glaciers (Akcar & Schlüchter, 2005). It is fed mostly by river input and precipitation. The predominant precipitation type is snowfall in the basin because of its location. In line with the increase in temperature, the transformation of precipitation type into the rain and the increase in wind speed, the snow-mass in the higher elevations begin to melt and the lake level rises above the average in March. The water input to the lake peaks in May with the increase of snowmelt and the water level reaches its maximum then begins the decrease in June. The lake level is still above the average between July and August due to the continued snowmelt at higher elevations. Despite the increase in the amount of precipitation compared to the previous month, the lake level drops below the average in September and this decrease continues until November due to the precipitation type is snowfall. The decrease in the lake level in autumn can be explained by the cold weather in the region and the fact that the snow doesn't turn into water, and thus the decrease continues until the temperature increase. The lake level fluctuation cycle can be summarized throughout the year like this according to the length.

Statistical Models

In multi-level models, the analysis is started with the random intercept model (Model-I). The data structure is two-level, in other words, the months are classified over the years. The intraclass correlation coefficient is calculated as 92% used by the ICC formula (Raudenbush & Bryk; 2002; Goldstein, 2003). These results reveals that the lake level fluctuates significantly over the years, and this should be considered.

Temperature, which is one of the variables used to explain the lake level fluctuations, varies randomly according to the years. Therefore, in Model II, it was assumed that the temperature changed from year to year randomly and the data were analyzed accordingly. The goodness of fit results (ll , AIC, BIC, AICC respectively) obtained to determine which model explains lake level changes are given in Table 1 for both models.

Table 1. Fit criteria for two-level linear regression models

Models	$-2ll$	AIC	BIC	AICC
Model I	375.6	387.6	401.5	387.7
Model II	281.7	289.7	289.8	299.0

Model II is the random intercept and slope model. The fit criteria results (ll , AIC, BIC and AICC, respectively) obtained using the ML estimation method for this model were significantly smaller than the fit criteria of Model-I. The results revealed that the best model for the study data is Model II. Because the model with the smallest fit criteria is reported as the model that best explains the change (Akaike, 1974; Schwarz, 1978; Akkol & Denizhan, 2016; Akkol et al., 2018). The estimation, standard error, and significance level obtained by using Model II are presented in Table 2.

Table 2. The analysis results of Model II

Fixed effect	$\bar{X} \pm SE$	t	Random effect	$\bar{X} \pm SE$	z
Intercept (β_0)	1648.32±0.1071	15396.80***	$\sigma^2_{u_0}$	0.66900±0.11060	6.05***
Temperature (β_1)	0.005346±0.0019	2.83**	$\sigma^2_{u_1}$	0.00021±0.00005	4.787***
Precipitation (β_2)	0.000403±0.0003	1.38	$\sigma^2_{u_{01}}$	0.00218±0.00157	1.39
Wind Speed (β_3)	0.07819±0.0782	3.21**	σ^2_e	0.04279±0.00230	18.61***

Note: $\bar{X}$: mean, *SE*: Standard error, *t*: t value, *z*: z value, **: P<0.01, ***: P<0.001

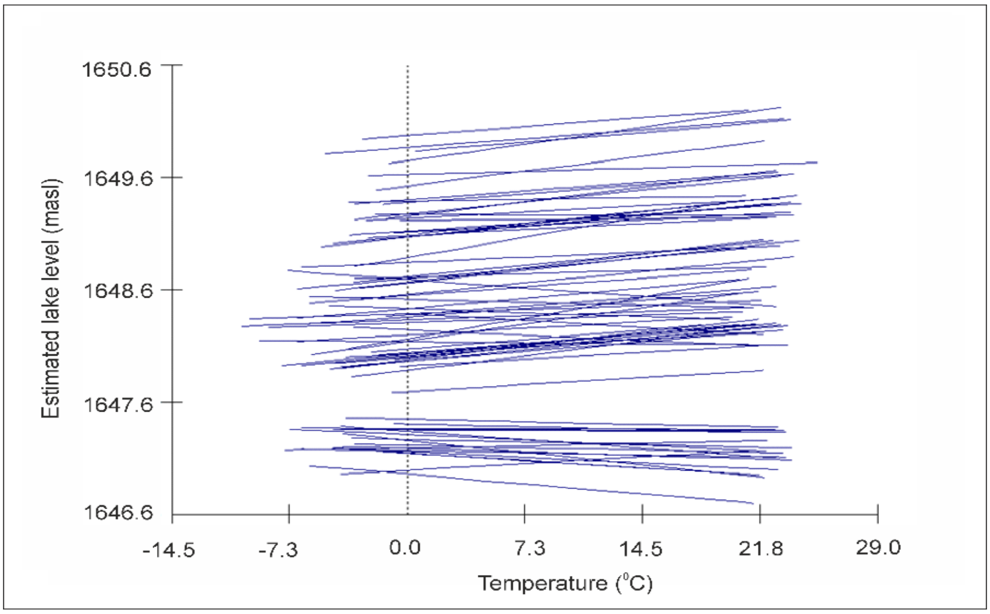

Figure 5. Estimated lake level and temperature relationship (1944-2019)

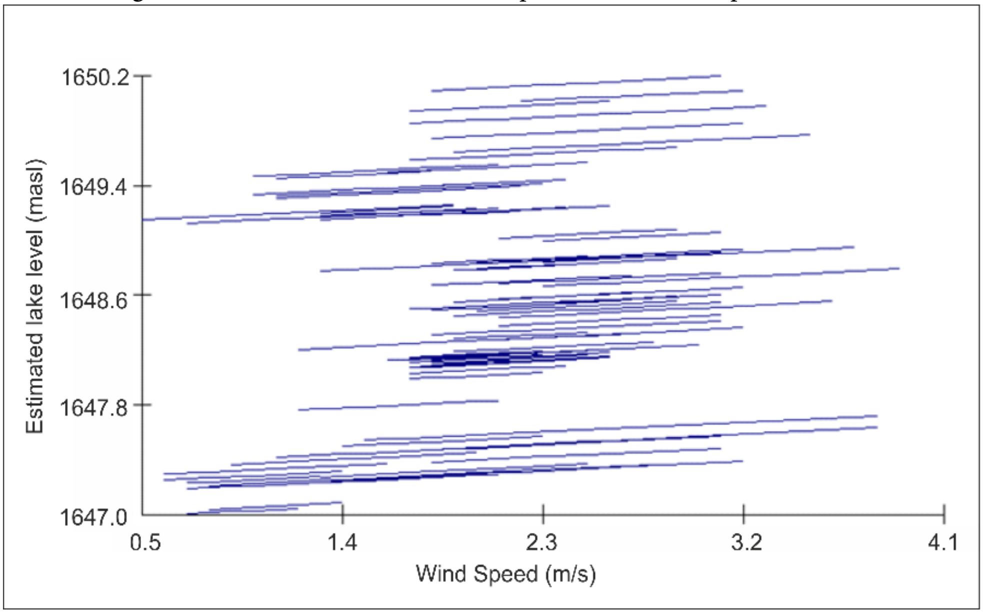

Figure 6. Estimated lake level and wind speed relationship (1944-2019)

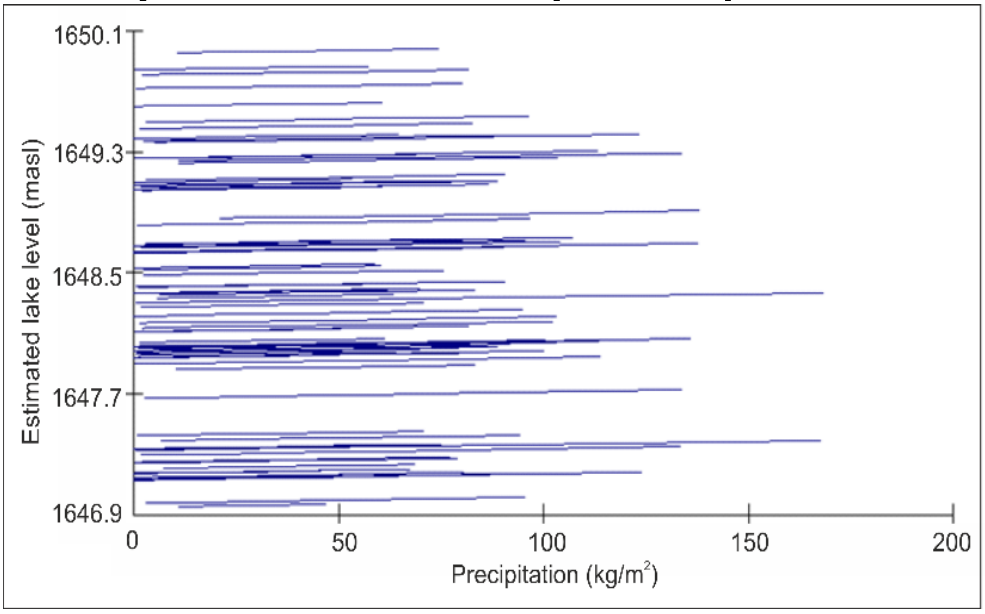

Figure 7. Estimated lake level and precipitation relationship (1944-2019)

The intercept (1648.32) from the fixed effect in Table 2 was found to be significant (P<0.001). In other words, the lake water level is 1648.32 units on average, which is an important amount. However, the effect of temperature and wind speed were found to be significant (P<0.01). An increase of 1°C in temperature causes an increase of 0.005346 m in lake level and an increase of 1 m/s of wind speed causes a 0.07819 m increase in lake level. The variations of the intercept and slope ($\sigma^2_{u_0}$ ve $\sigma^2_{u_1}$, respectively) and the covariance between the intercept and the slope ($\sigma^2_{u_{01}}$) were found to be significant (Table 2). It is seen that the random effects in Table 2 ($\sigma^2_{u_0,}\sigma^2_{u_1}$ and $\sigma^2_{u_{01}}$) are significant (P>0.001). When interpreting fixed and random effects together for intercept, the average lake level is 1648.32 units, and this value varies significantly from each other for each year. For temperature the fixed and random effect are interpreted the average temperature is 0.005346°C and the average temperature value has change significantly from year to year.

We examined the effects of temperature, precipitation, and wind speed to explain the fluctuation of lake level for the last 76 years. The estimated lake level values obtained from Model-II and the temperature relationship are presented in Figure 5. The lake level has a different average value for each year. The fact that the mean effect of temperature on the lake level and the change around the mean was found to be significant is explained by the fact that each regression line has a separate slope (Figure 5). This is explained by regression lines with different intercepts and slopes.

The estimated values for lake level obtained by model-II and wind speed relationships are presented in Figure 6.

The effect of the wind speed is found to be significant, and it is explained by the fact that the regression lines have slopes. The effect of wind speed on lake level changes can occur with the Seiche event and can only be effective in a part of the lake (Rabinovich, 2009). Especially strong winds cause the changes in lake level. Since Lake Van is a very large lake in terms of area and volume, the strong winds in the basin may cause lake level change. It is also possible that the wind speed indirectly accelerates the snow melt and increases the lake level.

The relationship between the estimated lake level and precipitation is given in Figure 7.

The absence of slope of the regression lines indicates that the precipitation effect was minimal (Figure 7). This can be explained by the fact that the lake level in January for each year was different, but the amount of precipitation has an insignificant effect on the lake level.

Conclusion

Previous studies have shown that there have been fluctuations in the lake level of Lake Van due to the tectonism, volcanism, and climate changes for the last 600,000 years. Especially in the studies on current lake level changes on Lake Van, it has been reported that there is a close relationship between meteorological parameters and lake level changes. In this study, we examined the relationship between meteorological parameters (temperature, precipitation, and wind speed) and lake level fluctuation by using statistical models.

The statistical models which are obtained from this study indicated that the lake level is affected by temperature and wind speed, but not by precipitation. Since Lake Van is located at 1648 meters above sea level, precipitation type is mainly snowfall and the drainage basin consists of high mountains, the most freshwater input to the lake occurs in spring with mixing of snowmelt and rain. This situation is explaining why precipitation effect seems to be insignificant. Therefore, while it is expected that the lake level would decrease due to the evaporation with the temperature, the increase in temperature has been associated with the increase of lake level by activating the water resources that feed the Lake Van. Another important result obtained from this study is that wind speed has a significant effect on the lake level (snowmelt).

Compliance With Ethical Standards

Authors' Contributions

AFM: Designed the study and collect the lake level data, wrote the first draft of the manuscript.
SA: Performed and managed statistical analysis.
OHD: Collected the meteorological data.
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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RESEARCH ARTICLE

Microplastic occurrence in the gill and gastrointestinal tract of *Chelon ramada* (Mugilidae) in a highly urbanized region, İskenderun Bay, Türkiye

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ABSTRACT

Microplastic pollution in marine ecosystems has become a significant, global concern which attracting the attention of academics and policy makers. This study provides information regarding the microplastic occurrence in the gill and gastrointestinal tract (GIT) of *Chelon ramada* (Risso, 1827). A total of 158 MPs were extracted from the gill and GIT with a mean of 1.9±1.8 particle/individual in gill and 3.4±2.1 particle/individual in GIT. Fiber was the most commonly extracted microplastic type (79%), followed by fragments (16%), film (4%) and pellet (2%). Mean size of extracted MPs from the organs of *Chelon ramada* was found as 1251±1602 µm. Black, transparent, red and blue MPs were extracted from the organs and dominance of black and transparent MPs were observed in the gill and GIT, respectively. This study is providing the first data regarding the microplastic ingestion of *Chelon ramada* and the results obtained in this will help to understand the relationship between anthropogenic influences and microplastic ingestion.

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Introduction

Following its invention, plastic materials have become an indispensable material that is used in all areas of daily lives. Unfortunately, used plastic materials end up in marine environments and become permanent pollutants due to low degradability (Ferreira et al., 2020; Reboa et al., 2022). As a result, 96% of marine litter consists of plastic materials (Iñiguez et al., 2016).

Plastic materials are divided into 5 different size categories, from largest to smallest, megaplastics (>1 m), macroplastics (1 m- 25 mm), mesoplastics (25-5 mm), microplastics (<5 mm),

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and nanoplastics (<1 μm) (GESAMP, 2019). Among all, microplastics (MPs) and nanoplastics are the most concerning ones due to the wide distribution in marine litter. In fact, Suaria et al. (2016) estimated that more than 92% of plastic materials found in the Mediterranean Sea surface waters belong to the microplastic size range. These small size particles may fragment from larger particles with the impact of photodegradation or weathering processes (Eriksen et al., 2014), or be produced in this size range such as granules, pellets. In addition to anthropogenic influences, natural processes like winds, runoff, rivers and marine currents play important roles in the transport and accumulation of MPs which lead to the presence of MPs even in remote regions (Peng et al., 2018; Napper et al., 2020).

As a consequence of widespread and abundant distribution of MPs, almost 800 marine species are encountered with plastic materials either with entanglement or ingestion (Worm et al., 2017). Among all, fish are the most concerning species; since, it creates a pathway for upper trophic levels including humans. Even though the link between fish and humans is not definite, recent studies reporting the presence of MPs in human body may confirm the claims (Braun et al., 2021; Mohamed Nor et al., 2021; Zhang et al., 2021a)

Once microplastic particles enter the fish body, they may cause physical damage or congestion in the digestive system (Walkinshaw et al., 2020; Miloloža et al., 2021). Even though some portion of ingested MPs could be evacuated from the body, smaller size MPs may translocate into other tissues (McIlwraith et al., 2021). Besides, plastic additives, potentially toxic chemicals absorbed into MPs may be released into different tissues which creates chemical damage to fish and upper trophic levels (Bucci et al., 2020).

Previous studies showed that microplastic ingestion in fish varies depending on many bio-ecological factors such as age, special niche, fish feeding behavior, habitat and contamination level of the surrounding environment (Atamanalp et al., 2022). In general, a higher microplastic ingestion rate was reported in the gyre regions (Markic et al., 2018), freshwater drainage areas (Kılıç & Yücel, 2022), heavily industrialized or urbanized regions (Naidoo et al., 2016), porting areas (Reboa et al., 2022) compared to cleaner environments.

İskenderun Bay is located in the northeastern part of the Mediterranean Sea and has intense pollution problems depending on urbanized and industrialized coastal area, marine traffic issues and intense porting activities and tourism. Since the coastal area of İskenderun Bay is surrounded by many industrialized centers and ports, the bay serves as a polluter collector and distributes them to the open sea (Preston-Whyte et al., 2021). In addition, frequent dredging activities applied in the ports lead to sediment resuspension (Preston-Whyte et al., 2021). Besides, marine traffic and the existence of a variety of boats and vessels act like a plastic and microplastic source (Nel et al., 2017). As a result of all these anthropogenic activities, severe microplastic contamination is present in the İskenderun Bay which was also confirmed by previous study (Güven et al., 2017).

As a consequence of global microplastic pollution concern, a significant number of studies have been devoted to monitor microplastic pollution in the marine ecosystem and its interaction with marine biota. In this context, species belonging to the Mugilidae family were proposed as bioindicator species which might be used to understand both MPs pollution level in the sampling area and potential harm to the marine biota (Naidoo et al., 2016; Zhang et al., 2021b; Wootton et al., 2021; Reboa et al., 2022; Kılıç & Yücel, 2022). This study was designed to evaluate microplastic occurrence in the gill and GIT of Mugilidae (*Chelon ramada)* in the heavily urbanized region, İskenderun Bay. In literature, data regarding the microplastic occurrence in the gastrointestinal tracts (GIT) of fish from İskenderun Bay exists (Güven et al., 2017; Kılıç & Yücel, 2022); however, there is no information regarding the microplastic ingestion of *Chelon ramada.* Results obtained in this study will fill the information gap.

Material and Methods

Fish Sampling

Fish were purchased in February 2022 from Dörtyol region (n=15) and August 2022 from İskenderun region (n=15). Information regarding fishing place and fishing vessel was obtained from fishermen. Fishing was carried out using purse seine in İskenderun Bay (Figure 1). It is confirmed that all selected specimens were recently caught, wild and local. Selected fish samples were wrapped with tin foil, placed in an ice bag and transported to the laboratory immediately.

Chelon ramada is pelagic-neritic species and it has a wide distribution range including Eastern Atlantic, Mediterranean Sea and Black Sea (Froese & Pauly, 2022). Adults are usually observed in lagoons, near shore waters and lower reaches of rivers, and they were resistant to polluted waters (Kottelat & Freyhof, 2007). While juveniles usually feed on zooplanktons, adults feed on epiphytic algae, pelagic eggs, larvae, detritus, small benthic or planktonic organisms (Kottelat & Freyhof, 2007; Froese & Pauly, 2022). In turn, they were consumed by carnivorous fish, birds and humans which create a potential pathway for microplastics to higher trophic levels (Naidoo et al., 2016).

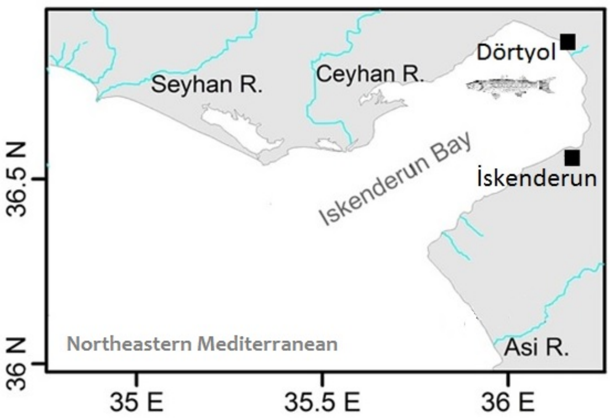

Figure 1. Location map indicating the sampling place of *Chelon ramada*

Microplastic Extraction

Once fish samples were transported to the laboratory, total length and weight were recorded (Table 1). Then, dissection preparation was carried out and all dissection equipment, glass beakers and laboratory surfaces were cleaned with pure water to eliminate contamination risk. Fish samples were rinsed with pure water to clean the body surface. Next, the abdominal was opened and the gastrointestinal tract and gill of each specimen were dissected, weighed and placed in a glass beaker, separately. To prevent contamination, all beakers and fish samples were covered with tin foil when they were not processing. After each dissection procedure, dissection equipment was rinsed with pure water. To degrade the organic material, 20 mL of 30% H_2O_2 per gram of organ were added into a glass beaker (Renzi et al., 2019) and heated on a hot plate at 60°C at least for 8 hours. Once the majority of the organic material was degrated, the remaining supernatant was filtered by 50 µm pore size filters. Filters were placed into sterile petri dishes for microscopic examination.

Microscopic Examination

Microscopic examination was conducted using Olympus SZX7 microscope with an attached Olympus DP 20 digital camera. During the examination, suspicious particles were checked with a hot needle to confirm their plastic nature. Then, information related to the physical features of extracted MPs such as color, type, size was noted. Filters which include MPs larger than 1 mm were placed to sterile petri dishes for Fourier transform infrared (FTIR) spectroscopy.

Fourier Transform Infrared (FTIR) Spectroscopy

MPs which are suitable in size were analyzed by Fourier transform infrared spectroscopy (FTIR) to detect the origin of extracted microplastics. FTIR analysis was carried out on a SHIMADZU QATR10 FTIR spectrophotometer equipped with a single reflection attenuated total reflectance (ATR) accessory. The spectrum range was 4000–400 cm^{-1} and a resolution of 4.0 cm^{-1} with 32 scans for each measurement. The polymer type identification was done by comparing absorbance spectra to references from the SHIMADZU library. Polymers that showed more than 70% spectral similarity were considered in the results section.

Contamination Prevention

To prevent airborne contamination, following precautions in addition to the described ones in the microplastic extraction section were taken. First of all, each step of the study (both digestion and microscopic examination) was carried out in private laboratories and doors and windows were kept closed to minimize the air flow (Torre et al., 2016; Bessa et al., 2019). Filters were checked for the existence of contamination prior to use. Only the authorized personnel were allowed to enter the laboratories, and they always wore nitrile gloves and cotton lab coats. Pure water and chemicals used in the microplastic extraction step were filtered through 50 µm pore size filters before use. In order to check the existence of airborne contamination, wet blank filters were placed in the laborites during analysis. No microplastic particle was detected at the blank filters.

Data Analysis

Since the normality of the data could not be validated with Kolmogorov-Smirnov and Shapiro-Wilk test, spearman's rank correlation coefficient was used to test the relationship between physical parameters and MPs abundance. To evaluate the differences in the MPs abundance among organs, Kruskal Wallis test was employed. All statistical analysis was performed by PAST software.

Results

In this study, 30 specimens of *Chelon ramada* (15 species in each station) were analyzed in terms of the presence of microplastic particles in the GIT and gill organs. Microplastic particles were detected in the gill of 22 specimens, comprising 73% of sampled fish; on the other hand, MPs were detected in the GIT of all examined specimens (Table 1). A total of 158 MPs were extracted from the gill and GIT of *Chelon ramada* with a mean of 1.9±1.8 particle/individual in gill and 3.4±2.1 particle/individual in GIT. A maximum of 5 and 8 MPs were detected in the gill and GIT of a single specimen, respectively (Figure 2). Fiber was the most commonly extracted microplastic type (79%) and followed by fragments (16%), film (4%) and pellet (2%) (Figure 3). Mean size of extracted MPs from gill and GIT of *Chelon ramada* was found as 1755±1881 µm and 1246±1235 µm with a minimum of 216 µm and 225 µm, respectively. Black, transparent, red and blue MPs were extracted from the organs and dominance of black and transparent MPs were observed in the gill and GIT, respectively (Figure 3). Statistical analysis showed that neither fish length, fish weight, nor wet weight of digestive tract or wet weight of gill exhibited a statistically significant relationship with number of microplastic particles present in the organs (Figure 4). Kruskal Wallis test showed that there were no significant differences in the MPs abundance between organs ($p>0.05$). FTIR analysis determined the majority of the identified polymers as polyethylene terephthalate, polyester, polyamide, and polypropylene.

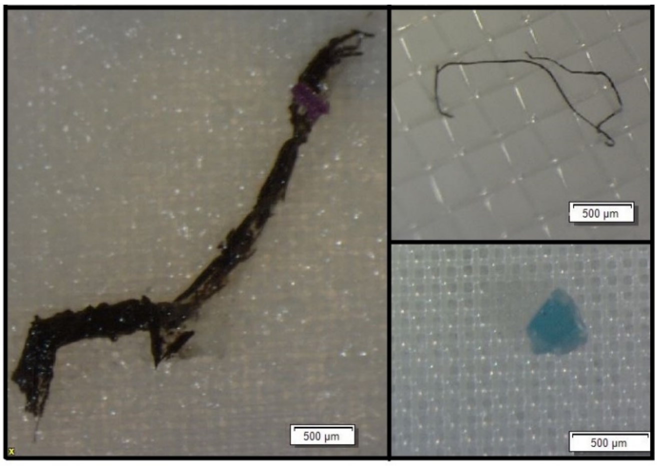

Figure 2. Examples of extracted microplastic particles from *Chelon ramada* samples in İskenderun Bay

Discussion

In this study, microplastic occurrence in the GIT and gill of *Celon ramada* was examined to evaluate the microplastic pollution in a heavily polluted region, İskenderun Bay. The selection of *Chelon ramada* was done due to its feeding strategy and habitat. Since it is benthopelagic species and usually fed from both the benthic environment and water column (Whitfield et al., 2012; Reboa et al., 2022), the results obtained in this study will represent the microplastic pollution status of different habitats.

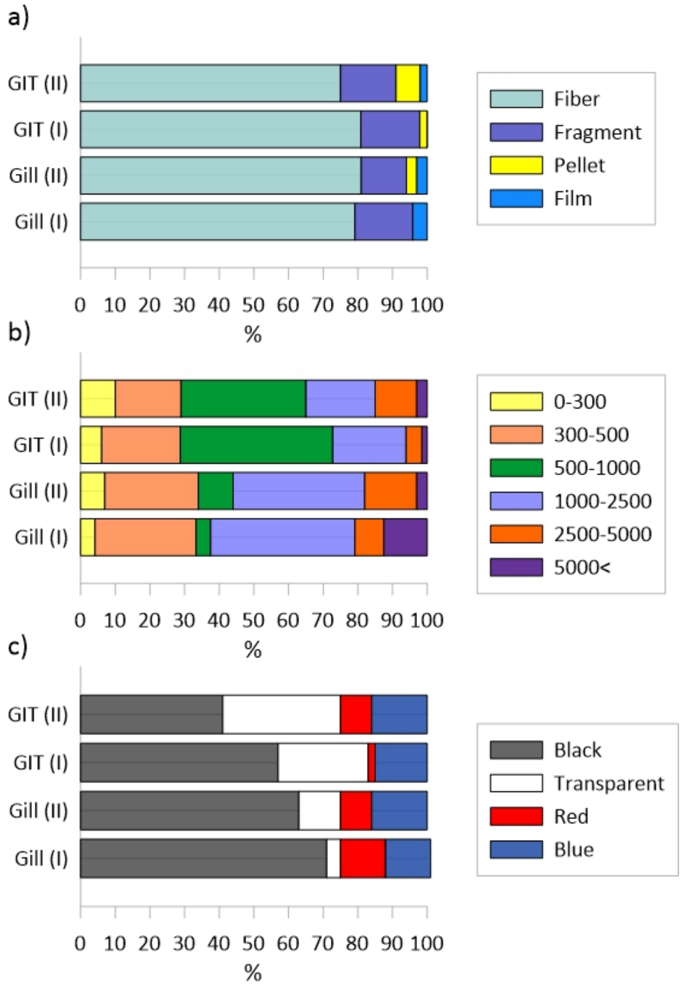

Figure 3. Characteristic features of extracted microplastic particles in percentage (%) from the organs of *Chelon ramada* in terms of (a) type, (b) size (in µm), (c) color where I denotes for Dörtyol station, II denotes for İskenderun station

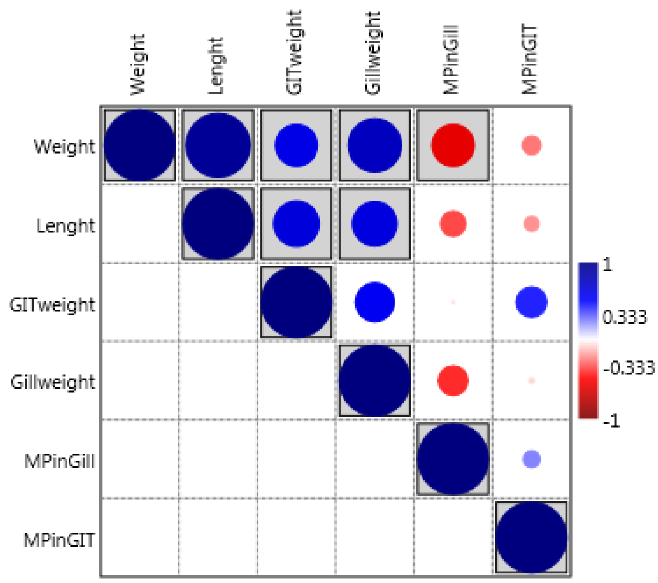

Figure 4. Diagram indicating the results of correlation analysis (dots inside the gray boxes indicate correlation is significant at 0.05 significance level)

Table 1. Morphological features (total length, weight, gill weight, GIT weight) of examined *Chelon ramada* samples together with estimated MPs abundance (particle/individual) and MPs occurrence rates (%) in the organs (I: Dörtyol station, II: İskenderun station)

					Gill			GIT		
	Mean length (cm)	*Mean weight (g)*	*Gill weight wet basis (g)*	*GIT weight wet basis (g)*	*%*	*MPs/fish*	*MP/fish for positive samples*	*%*	*MPs/fish for all fish*	*MP/fish for positive samples*
I	29.1±2.0	212.8±44.2	6.7±1.8	9.6±3.9	60	1.7±1.6	2.4±1.4	100	3.1±2.5	3.1±2.5
II	28.7±1.8	208±55.7	5.8±1.2	8.7±3.5	92	2.1+1.9	2.6+1.8	100	3.7+1.6	3.7+1.6

Table 2. Recent literature indicating the mean MPs abundance (particle/individual) and microplastic occurrence (%) in the GIT of fish from Mugilidae family

Species	**Collection site**	**MPs abundance in GIT**	**IR**	**Dominant color**	**Predominant type**	**Reference**
Chelon ramada	İskenderun Bay, Mediterranean Sea	3.1±2.5	100	Transparent	Fiber	This study
Mugil cephalus	Hong Kong	3.8	73	White	Fiber	Naidoo et al. (2016)
Liza aurata	Mediterranean Sea	3.26	44	Blue	Fiber	Güven et al. (2017)
Mugil cephalus	China	3.7±1.0	100	Transparent	Fiber	Jabeen et al. (2017)
Mugil cephalus	Hong Kong	4.3	60	Green	Fiber	Cheung et al. (2018)
Mugil cephalus	Sydney Harbor	4.6(±1.2)	64	-	Fiber	Halstead et al. (2018)
Ellochelon vaigiensis	South Pacific subtropical gyre	4.3+1.7	48.5	Black, blue, white, colorless	Fragment	Markic et al. (2018)
Mugil cephalus	South Pacific subtropical gyre	2.0+0.6	13.6	Black, blue, white, colorless	Fragment	Markic et al. (2018)
Mugil cephalus	Indonesia	10.07±6.4	100	Transparent and blue	Fiber	Hastuti et al. (2019)
Crenimugil seheli	Indonesia	9.17±11.9	100	Transparent and blue	Fiber	Hastuti et al. (2019)
Chelon richardsonii	South Africa	1.8	40	Transparent or brown	Fiber	McGregor & Strydom (2020)
Mugil cephalus	China	5.2		White	Fiber	Zhang et al. (2020)
Mugil cephalus	China	1.2	42	Black	Fragment	Borge-Ramirez et al. (2020)
Mugil cephalus	China	10±9	97	Black	Fiber	Guilhermino et al. (2021)
Mugil cephalus	Australia	0.94±0.18	50	-	Fiber	Wootton et al. (2021)
Chelon saliens	Caspian Sea	4.2+2.8		Black-Grey	Fiber	Nematollahi et al. (2021)
Mugil cephalus	India	7.8±4		-	Fiber	Saha et al. (2021)
Mugil cephalus	İskenderun Bay, Mediterranean Sea	5.9±3.2		Black	Fiber	Kılıç & Yücel (2022)
Mugil cephalus	Samandağ, Mediterranean Sea	46.4±11.9		Transparent	Fiber	Kılıç & Yücel (2022)
Chelon auratus	Port of Genoa, north-western Mediterranean Sea	28±11	57.1	Transparent, white, black	Filaments and Fragments	Reboa et al. (2022)
Chelon auratus	Fishpond of S'Ena Arrubia, north-western Mediterranean Sea	53±48	23.8	Transparent, white, black	Filaments and Fragments	Reboa et al. (2022)
Mugil incilis	Colombia	1.2	10.1	Colorless, black, green	Fragment	Garcés-Ordóñez et al. (2022)

In this study, all of the examined *Chelon ramada* contained MPs in the GIT. So, it can be concluded that *Chelon ramada* is prone to microplastic pollution in highly urbanized İskenderun Bay. Previous studies conducted in the urbanized regions of China (Jabeen et al., 2017; Guilhermino et al., 2021), Hong Kong (Naidoo et al., 2016), Indonesia (Hastuti et al., 2019), Türkiye (Kılıç & Yücel, 2022) reported almost 100% microplastic ingestion rate in the fish from Mugilidae family which is similar to this study (Table 2).

Microplastic abundance in the GIT of *Chelon ramada* was coherent to other species from the Mugildae family like *Liza aurata* from Mediterranean Sea (Güven et al., 2017), *Mugil cephalus* from İskenderun Bay (Kılıç & Yücel, 2022), China (Jabeen et al., 2017; Zhang et al., 2021b), Sydney Harbor (Halstead et al., 2018), Hong Kong (Naidoo et al., 2016), South Pacific (Markic et al., 2018), *Chelon saliens* from Caspian Sea (Nematollahi et al., 2021), *Ellochelon vaigiensis* from South Pacific (Markic et al., 2018). On the other hand, almost 10-fold higher MPs abundance was reported in the GIT of *Mugil cephalus* from Samandağ (Kılıç & Yücel, 2022), *Chelon auratus* from the north-western Mediterranean Sea (Reboa et al., 2022). Variability in the microplastic ingestion rate may result from the density of MPs in marine litter of sampled environment.

Similarly, previous studies conducted in a similar region (northeastern Mediterranean Sea) reported different microplastic ingestion rates. For example, Güven et al. (2017) investigated 1337 specimens belong to 28 species and 14 families from the northeastern Mediterranean Sea and a mean of 2.36 particle/individual were extracted from the either stomach or intestine of examined specimens. Kılıç & Yücel (2022) estimated the mean microplastic abundance in the GIT of *Mullus barbatus, Mullus surmuletus* and *Saurida undosquamis* as 2.9 particle/individual, 5.5 particle/individual, 3.4 particle/individual, respectively. Therefore, even though the sampling region is similar, different microplastic ingestion rates were reported in the literature. Drawing a clear picture to reflect to variations in the microplastic ingestion is a challenging task due to differences in employed species, employed methodology, sampling period (Hastuti et al., 2019).

Microplastic ingestion was reported to be related to habitat of fish (Zhang et al., 2020). Güven et al. (2017) reported higher MPs ingestion rates in pelagic fish species from the northeastern Mediterranean Sea. On the other hand, higher MPs ingestion rates was reported in demersal fish species from the South China Sea (Koongolla et al., 2020). Differently, higher microplastic ingestion rate was reported in the benthopelagic fish species from the Mediterranean Sea (Bessa et al., 2018; Kılıç & Yücel, 2022). High microplastic ingestion rate observed in this study may be related with the benthopelagic habitat of studied species and coherent to the previous study conducted in this region (Kılıç & Yücel, 2022).

Vulnerability of specie to microplastic pollution is highly correlated with the feeding strategy. Previous studies showed higher microplastic ingestion rates in plankton feeder species (Kılıç & Yücel, 2022). Considering the encounter of MPs with planktonic organisms (Setälä et al., 2014; Lima et al., 2015), microplastic ingestion could also be done by trophic transfer. In addition, while *Chelon ramada* swallow their prey, they also filter an amount of water which might result from unselective ingestion of MPs (Hastuti et al., 2019). For these reasons, species which has this type of feeding behavior was reported to be more vulnerable to MPs pollution (Rummel et al., 2016).

Gills are another pathway of MPs entrance into the fish body; yet, literature examining the MPs occurrence in the gill is highly limited. A recent study reported the 90% microplastic occurrence in the gill of *Mugil cephalus* from İskenderun Bay and Samandağ coast of the Mediterranean Sea with a mean abundance of 3.5±1.9 particle/individual and 4.2±2.4 particle/individual, respectively (Kılıç & Yücel, 2022). Considering the high MPs occurrence (60%) observed in the gill of *Chelon ramada* together with the previous report, it is logical to assume that İskenderun Bay is severely polluted by MPs.

Even though, there was no significant difference detected in the MPs abundance between organs ($p>0.05$), higher MPs occurrence and abundance observed in the GIT may indicate the ingestion of MPs by mistake. Alternatively, this condition may reflect the different spatial constraints of organs considering the size and distribution of MPs (Kılıç & Yücel, 2022).

The type of microplastic particle affects the likelihood of their ingestion (Boerger et al., 2010). For example, a recent study reported that fibers were more often ingested by grazers and omnivores, while fragments by benthic and pelagic predators (Markic et al., 2018). Previous studies reported the dominance of fragment type particles in the surface waters of İskenderun Bay (Güven et al., 2017); however, the majority of the ingested microplastics was found to be fibers in this study (Figure 3). Therefore, these conflicting results may indicate that type of ingested MPs is related with the feeding behavior of *Chelon ramada* rather than the ambient environment. Coherent to this study, previous studies employing fish from Mugilidae family also reported the dominance of fiber particles in the GIT (Naidoo et al., 2016; Güven et al., 2017; Jabeen et al., 2017; Cheung et al., 2018; Halstead et al., 2018; Hastuti et al., 2019; McGregor & Strydom, 2020; Zhang et al., 2021b; Guilhermino et al., 2021; Saha et al., 2021; Nematollahi et al., 2021; Wootton et al., 2021; Kılıç & Yücel, 2022)

The size of MPs is the main factor determining the ingestion potential of a particle. In fact, as the size of a MPs decreases, its bioavailability to the marine biota increases (Shim et al., 2018). In addition, tiny microplastics or nanoplastics may translocate into different tissues (McIlwraith et al., 2021) which increases the health risk concern depending on the consumption of these species. In this study, MPs extracted from the gill were commonly belong to 1-2.5 mm size class. On the other hand, the majority of ingested MPs were small microplastics which are less than 1 mm in size (Figure 3). Previous studies also showed the dominance of small size MPs in the GIT of wild fish (Hastuti et al., 2019; Jonathan et al., 2021; Nematollahi et al., 2021; Kılıç & Yücel, 2022; Reboa et al., 2022).

Previous studies showed that fish tended to ingest MPs which are closer to the color of their prey (Hastuti et al., 2019). In this study, the ratio of transparent MPs over other detected colors (black, red and blue) was significantly high which is a consequence of dominance of transparent and white colors in most of the plankton and algae species. However, black color particles were dominantly found in the gill tissue. This conflicting results in the dominant color among organs may indicate the ingestion of MPs accidentally for food (Wang et al., 2020). Recent studies employing mugilids showed the dominance of white or transparent colors in the GIT similar to this study (Naidoo et al., 2016; McGregor & Strydom, 2020; Zhang et al., 2021b; Kılıç & Yücel, 2022; Rebeo et al., 2022).

FTIR analysis showed that polyethylene terephthalate, polyester, polyamide and polypropylene are the major polymer types extracted from the organs of *Chelon ramada* which is coherent to the previous studies conducted in the different parts of the ocean (Güven et al., 2017; Halstead et al., 2018; Sayed et al., 2021; Atamanalp et al., 2022; Kılıç & Yücel, 2022). Major sources of identified polymers are reported as textile industry (Atamanalp et al., 2022), plastic bags and bottles (Suaria et al., 2016) which indicate the impact of anthropogenic influences in the study area.

Chelon ramada is a highly popular and preferred food source in the locals of İskenderun Bay and Turkish people. In this study, all examined specimens contained MPs in their GITs. Even though Turkish customers usually prefer to remove GIT before consumption, there are still some people who prefer to consume fish with its organs which leads to the trophic transfer of MPs. Also, as mentioned previously, small size MPs may translocate to the muscle tissue which increases the possible health risk concerns depending on the fish consumption (Ma et al., 2021). Besides, plastic materials are made from hazardous polymers like polycyclic aromatic hydrocarbon (PAH), polychlorinated biphenyl (PCBs), petroleum hydrocarbon, bisphenol, organochlorine pesticides which may lead to deterioration of human immune system and disruption of the endocrine system (Teuten et al., 2009; Smith et al., 2018). Furthermore, MPs may release toxic chemicals that are adsorbed on the surface upon the entrance of the body (Bucci et al., 2020) which create a potential gate for toxic chemicals into the human body. Considering the reported presence of MPs in the human body (Zhang et al., 2021a) together with high MPs presence observed in this study, concerns about the consumption of fish raise.

Conclusion

This study was undertaken to evaluate the danger of MP pollution in a highly urbanized İskenderun Bay. High microplastic occurrence observed in the GIT and gill of *Chelon ramada* emphasized the severity of pollution status and highlighted the anthropogenic influences in the region. These findings showed that necessary national legislation need to be undertaken not only for the benefit of the environment but also for its potential impacts on human health. More comprehensive research is required to understand the fate and transport of MPs in the urbanized regions and its transfer to marine biota.

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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RESEARCH ARTICLE

Investigation of cluster-based cyclone track pattern within the Bay of Bengal

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ABSTRACT

Bangladesh is a highly disaster prone flat land country in south Asia. 80% of the disaster comes from cyclonic disaster around this area. To investigate the damage risk due to the cyclonic event around the Bay of Bengal associated with the cyclone track (CT) is an important issue. The present study has extensive analysis on generating a most favorable track along the Bay of Bengal from the MRI-AGCM cyclone track data. We have investigated present (1978-2003) and future (2075-2099) track data from the MRI-AGCM data set to ensure the synthetic track for the present and future climate conditions of Bangladesh. A k-mean clustering technique has been applied to investigate the synthetic track for the present and future climate condition. This work may insight the changes in cyclone track patterns in both the present and future climate conditions with the global warming scenario. This study has found that the Sundarbans and its adjacent areas are the risky coastline area of the landfall zone and for the global warming scenario it will be shifted to the Odisha area in India.

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Introduction

Bangladesh is a riverine delta-shaped country in south Asia. Since the country is the world's number one densely populated country, the number of deaths by natural disasters is higher than other countries in the world. According to the Intergovernmental Panel on Climate Change (IPCC, 2014) the sea level rising trend is 1.5 mm/y for Bangladesh. In future, Bangladesh will be one of the worst ruined countries due to its disaster prone and low elevation geography. Moreover, the tropical storm is a poisonous snake for this country. Therefore, to investigate its path is highly desirable which can reduce the damages and the death toll for human beings. This region faces an increasing number of tropical cyclones associated with the surge and torrential rain causes coastal flooding that can increase the death toll. Most of the notable cyclones around the coast of Bangladesh are 1970, 1985, April 1991, 1997, SIDR 2007, Nargis 2008, AILA 2009 and MORA 2017 (Mizanur Rahman et al., 2011). In the Bay of Bengal, cyclones always move in the north-westerly direction to hit the coast of Bangladesh and India. For this region, the destruction due to the storm surge flooding is a serious concern along the coastal regions of India, Bangladesh and Myanmar. Numerical modeling of storm surges associated with tropical cyclones in the Indian seas has been confined to the Bay of Bengal (Ramsay et al., 2012; Saha, 2015; Gayathri et al., 2016; Al Mohit et al., 2018a, 2018b; Sahoo & Bhaskaran, 2018; Paul & Ali, 2019; Li et al., 2020; Mishra & Vanganuru, 2020; Murty et al., 2020; Rehman et al., 2021; Al Mohit & Towhiduzzaman, 2022; Uma & Sannasiraj, 2022). These studies were adequate for storm surge simulation, but inadequate for track forecasting in this region. Due to the funneling shape of the coastal region of Bangladesh, increasing the probability of storm path makes this region more devastating and that's why it's important to find out the probabilistic storm track. According to (Szczyrba, 2022), there are 26 average number of cyclones occurred globally and 17 of the Pacific Ocean (PO), 10 of the Atlantic Ocean (AO), 9 of the South Indian Ocean (SIO), 5 of the North Indian Ocean (NIO) and 3-5 of the Bay of Bengal (BoB) region per year. The probability of landfall depends upon the storm track and its need to better understand in order to identify the aspect of its life cycle. The worth mentioning work of cyclone track analysis (Chen et al., 2013; Ying et al., 2014; Zhu et al., 2016; Gao et al., 2018; Giffard-Roisin et al., 2020; Wang et al., 2022) was carried out at some different basin around the world. All of the studies were concentrated for the individual basin with their aspects. Their constructed cluster range was 3 to 6 which was not optimal for the accurate prediction. Several methods have been developed to forecast the cyclone track position around the NIO for disaster management. Some useful track prediction techniques were developed by (Gao et al., 2018) and it was found that the track prediction error has not reduced in this area. The dynamical track forecasting model has some difficulties to forecast accurately such as advective, adiabatic and frictional effects. Giffard-Roisin et al. (2020) forecasted the track for 24 hours and 72 hours using deep learning, but the studies could not escape large forecasting errors.

Currently, the clustering method is a useful tool to fit the geographical shapes of the projected tracks. The present study concerned the k-mean clustering method to investigate the more probabilistic track along the Bay of Bengal with present and future climate conditions. K-mean clustering developed on the regression mixture model that tends to find clusters of comparable spatial extent, while clusters arrange expectations and maximization through different shapes. The past study of cluster technique on track detection has been carried out from the regression mixture model (Kowaleski & Evans, 2016). Current research eliminates the limitations of previous research and the second approach of the regression mixture model has been implemented in the present study. We have found the probable future track shifted to the Odisha area in India.

In this study, we have applied the updated regression mixture model approach to the MRI-AGCM present data (1978-2003) and future (2075-2099) data set and find the synthetic track along this region. This article is organized through different sections. Section 2 represents the data pattern and policy. Section 3 provides the information about material and methods. Section 4 shows the discussion about the outcomes and conclusion.

Material and Methods

Data and Location

Japan Meteorological Agency (JMA) and the Meteorological Research Institute (MRI) jointly develop a new operational numerical weather prediction model known as MRI-AGCM (Murakami et al., 2012). A semi Lagrangian three-dimensional advection scheme was used for accelerating the time integration (Kitoh & Endo, 2021) with 20 km horizontal grid spacing and 60 level altitude 0.1 hPa vertical grid spacing. The time-slice method was used for the higher resolution (20 km) AGCM experiment. The time-slice method has two layers, the global warming projection system that consists of an

Atmospheric-Ocean General Circulation Model (AOGCM) and the higher part of the vertical level AOGCM generated by AGCM. The present day climate condition simulates 25 years' data from 1978 to 2003 associated with observed sea surface temperature (SST) (Varghese et al., 2020). The future climate data simulation was conducted from 2075 to 2099 years' data. All these works were based on the boundary SST data and the data were prepared with three different stages. At the first step, the Couple Model Intercomparison Project 3 (CMIP3) multi-modeled data set was used to project the Multi-Model Ensemble MME of SST. The second stage represents the linear trend in MME of SST that projected the MIP3 data set. At the third step, a difference between 20th century experiment of the Intergovernmental Panel on Climate Change (IPCC) fourth assessment report (IPCC, 2007a, 2007b) and future simulation under the special report on Emission Scenarios (SRES) A1B emission Scenario (Nakicenovic et al., 2014) in MME of SST. After receiving the simulated data from AGCM, we have separated the necessary data for our study domain. Our study domain is bounded by 50N-270N latitude to 700E-1000E longitude and this area was classified with four different areas according to the classification of cyclone lifestyle. The Figure 1 shows the study domain area for the current research which includes the Bay of Bengal and Indian region.

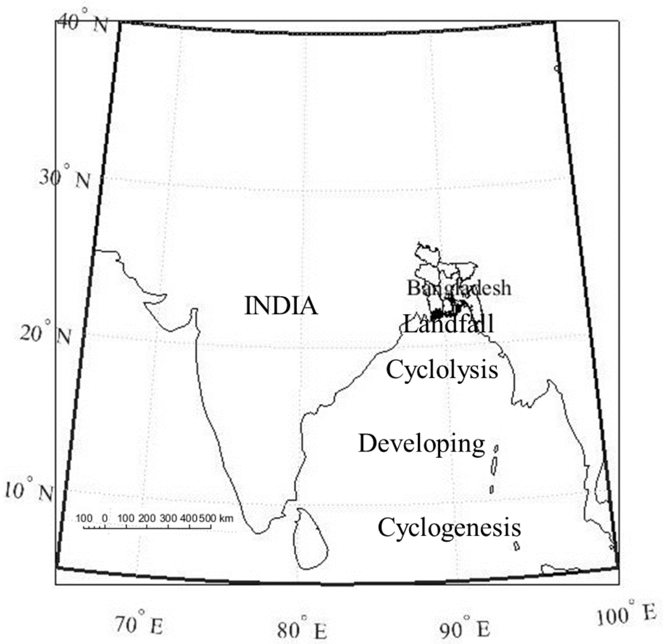

Figure 1. Study domain regions with different classified areas

We have isolated the individual cyclone track from the present (1978-2003) and future (2075-2099) data set which was used for the analysis. Current climate conditions mean storm data from 1978 to 2003. On the other hand, storm data from 2075 to 2099 are identified as future storms. Basically, climate change is assumed to be due to global warming by expecting a 2-degree.

Methodology

To find the homogenous group of objects for a data set is important. So, the clustering method is useful for finding the homogenous group of data. The present research concerns the useful clustering method known as k-mean cluster. The improved k-mean clustering method describes as

$$J = \sum_{i=1}^{C} \sum_{k=1}^{K} (\mu_{ik})^m \|X_k - C_i\|^2 \quad (1)$$

Where, $\mu_{ik} = \left[\sum_{j=1}^{C} \left(\frac{\|X_k - C_i\|^2}{\|X_k - C_j\|^2}\right)^{\frac{2}{m-1}}\right]^{-1}$ and $C_i = \frac{\sum_{k=1}^{K}(\mu_{ik})^m X_k}{\sum_{k=1}^{K}(\mu_{ik})^m}$

Here, μ_{ik} is the membership coefficient, C_i is the ith cluster center.

The k-mean algorithm represents the process of the fundamental working procedure. Choosing a data randomly from the data set to consider as a centroid and then compute the distance from each observation and centroid. When the allocation of an object to another cluster decreases within- the cluster variation and the data is assigned to that cluster. After that, their center is changed based on the next feasibility. The previous process is repeated until there is no change in cluster membership. The next section describes the process to utilize the method to find the cluster of the track data. This process may be helpful and useful because the time requirements facilities are linear, less affected by outliers and the homogeneity, heterogeneity makes optimizing the final solution. Furthermore, we may apply to very large data sets since this process has no computation demand (Xu et al., 2020).

Determining the optimal cluster is important because the morphology of the k-mean cluster depends on the choice of cluster number. According to Kaufman, Silhouettes are an important factor for measuring the gumminess and validate the cluster data speardness. Basically, the considerable silhouette coefficient values are from -1 to +1 range and the value determines the cluster number. In this study, silhouette value analysis shows that the five numbers of clusters are important for the cluster analysis.

We have applied this process for all the designated areas like genesis, cyclolysis and landfall. The same cluster number is used in all the areas like genesis, cyclolysis, landfall to reduce the complexity of cluster technique. The fewest number of data shows the negative silhouette values that represent the inhomogeneity with that cluster data. We have several times run the procedure of clustering to minimize the negative silhouette values. Finally, reduce track points which are outlier. In this study, the mean silhouette values are represented as cluster3<cluster<4<cluster5 and we consider the mean silhouette values is 0.5445 to get the best solutions.

Numerical procedure

The aim of this study is to find the k-mean cluster based synthetic track detection from the available track data. So, for this reason we have collected the data from the Atmospheric Global Circulation Model (AGCM) 1978 to 2003 (Present) and 2075 to 2099 (Future) global track data. The track data is six hourly interval data with the information on wind speed and barometric pressure. The track data was then separated from the original data set to be useful for the target region. After that, we find the individual tracks for different years. The twenty-five years track data 1979 to 2003 was then separated in each year to better analyze their activities. Using the same approaches, we have applied the future track data for investigating the future cyclone activity and the synthetic track with future climate conditions. Based on this same theory, the number of storm events in the current climate condition is upwards, but the rate of cyclone genesis in the future will be higher due to the future climate condition and the number of storm events is slightly lower. From the eye point of the storms in each cluster, it appears that the genesis is shifting to higher latitudes. However, various studies have shown that the Power Dissipation Index (PDI) and Accumulated Cyclone Energy (ACE) of the future storm will be a little higher (Knutson et al., 2010). And that's why we want to find a synthetic track that will explain the direction of a storm for a lot of importance for Bangladesh. For this reason, find a more probabilistic cluster zone from the k-mean cluster. After that, find the probability density from each data point of the probabilistic cluster zone by using the equation (2).

$$P(x) = f(x|\mu,\sigma) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \text{q} \quad (2)$$

where μ and σ represent the mean and variance of the data point.

Finally, find the maximum probabilistic point from the cluster zone and the similar process applies for the individual cyclone area like developing area cyclolysis area and landfall area. Based on those probabilistic points we make a new synthetic track which is a cluster base probabilistic synthetic track. Both present and future climate conditions we have found the synthetic track for the Bangladesh region.

Results

We have some output and some valuable information after analyzing the cyclone track data. From the data of MRI-AGCM3.2, we found the Event, genesis occurrence and their trend of a cyclone that struck the Bay of Bengal Coast. At first, we separated the global data to a regional data (study area). After that we have separated the cyclone track data from each climatic condition data. For the present scenario and the future scenario of three cases C0, C1, C2, we have separated the cyclone track data. After that, we have analyzed the data for various criteria. Before that we have checked the track information with observed track information. We have checked the seasonal behavior of observed data and the simulated data.

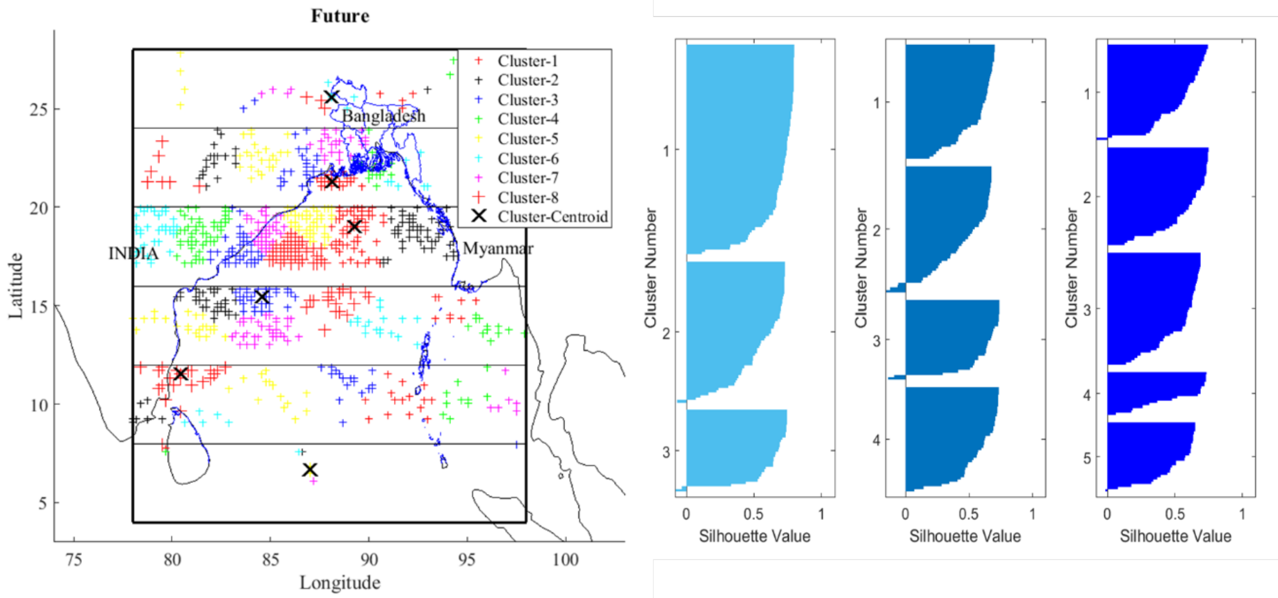

Figure 2. k-mean clustering and silhouette value

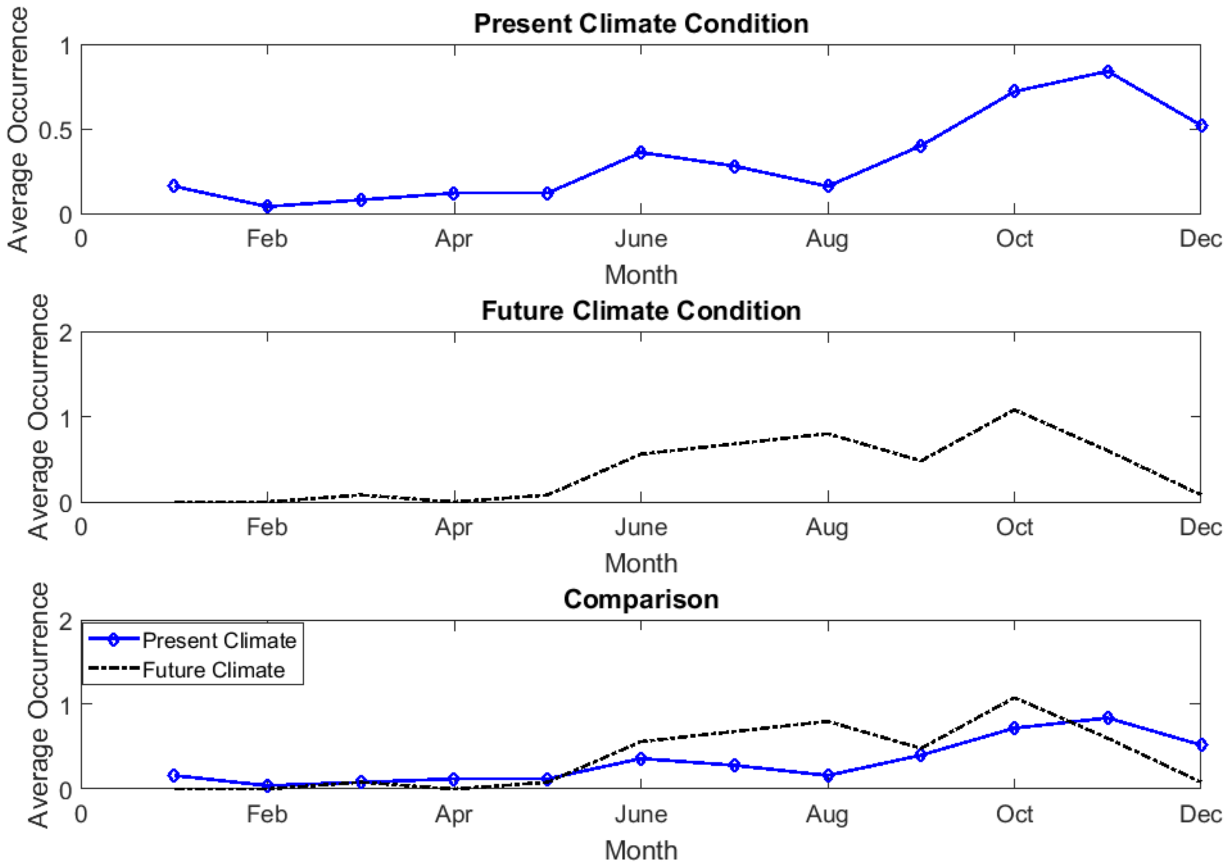

Figure 3. Cyclone occurrences along the Bay of Bengal are associated with different climate conditions and their comparison

Figure 3 demonstrates the yearly averaged monthly event of the storm where it is reflected that the month of November for the Bangladesh region is a very probable month for the cyclone. On the other hand, February is the lowest probable month for a cyclone event.

The Figure 4 shows the Event, genesis occurrence and their trend in present and future climate conditions. The left column and right column are cyclone activity for the present and future climate condition, and the consecutive downward figure shows the Events, occurrence trend and genesis trend respectively. The result shows the increasing trend of cyclone genesis in the future that is lower than the present climate condition. We also review the storm's monthly activity to make a clearer view, which shows the cyclone seasonality.

Basically, we will create a suitable synthetic track based on current and future climate conditions which will play an important role for the people of the coastal region of Bangladesh. This study also found that, the sea surface temperature SST which plays an important role in increasing the event along the coastal region of Bangladesh. There is some correlation between SST and the frequency and intensity of tropical cyclones in Bangladesh (Ying et al., 2014). The figure 5 represents the SST changes along the Bay of Bengal. This figure can help to understand the cyclone event under the present and future climate conditions.

The left of the figure is based on the current climate of 2003 and the right-side figures are on future climate conditions of the year 2099. To ensure the future SST, the simulations used to obtain data on future storms simulated changes in SST assuming global temperature increases of up to 2 degrees. From the figure we have seen that the SST of current climate conditions is the upward trend that represents the trend of growing uncomfortably than the future SST behavior. According to the concept, we have applied mathematical and statistical methods to find the path of a cyclone for present and future climate scenarios. Table 1 shows the estimated path of a cyclone. Here we have used only the Longitude and latitude information to find the cyclone path.

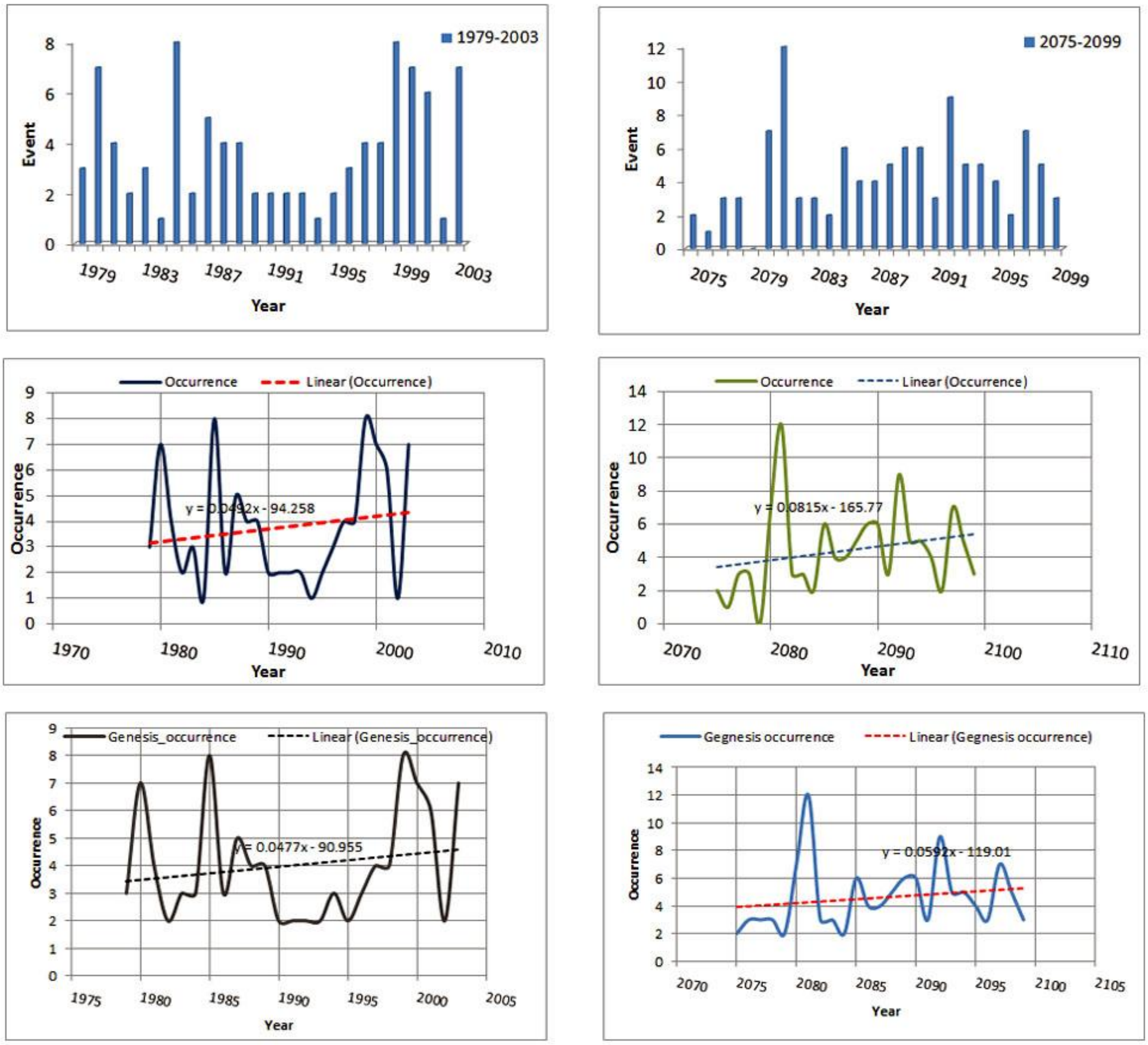

Figure 4. A simple statistical activity analysis of present and future climate conditions along the Bay of Bengal

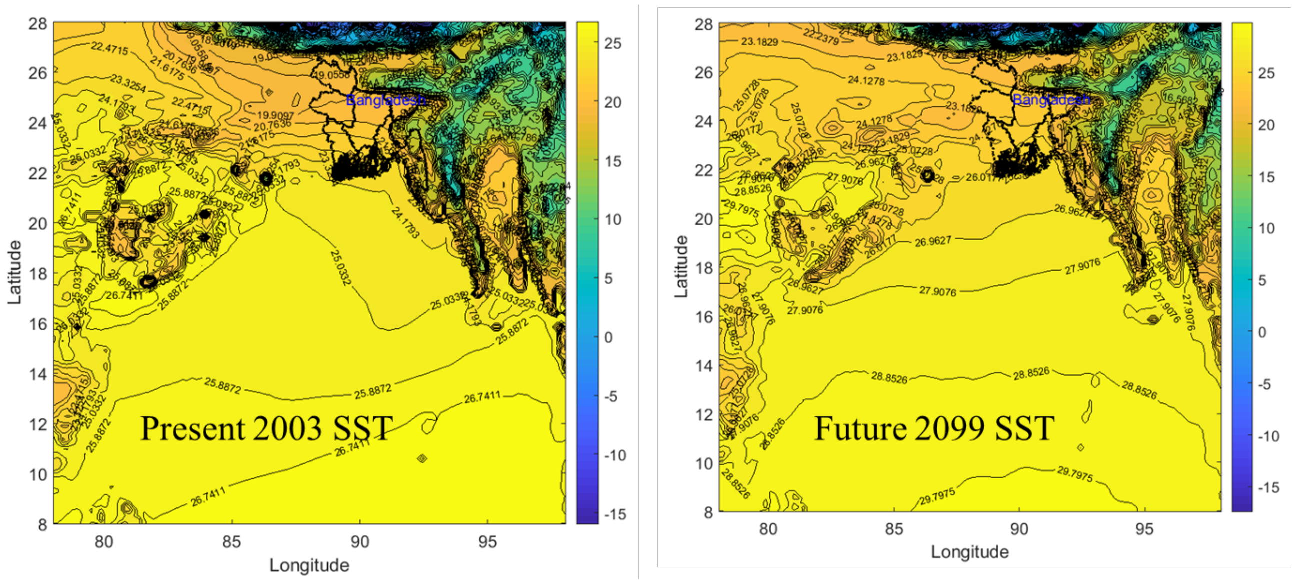

Figure 5. SST condition at present and the future climate scenario

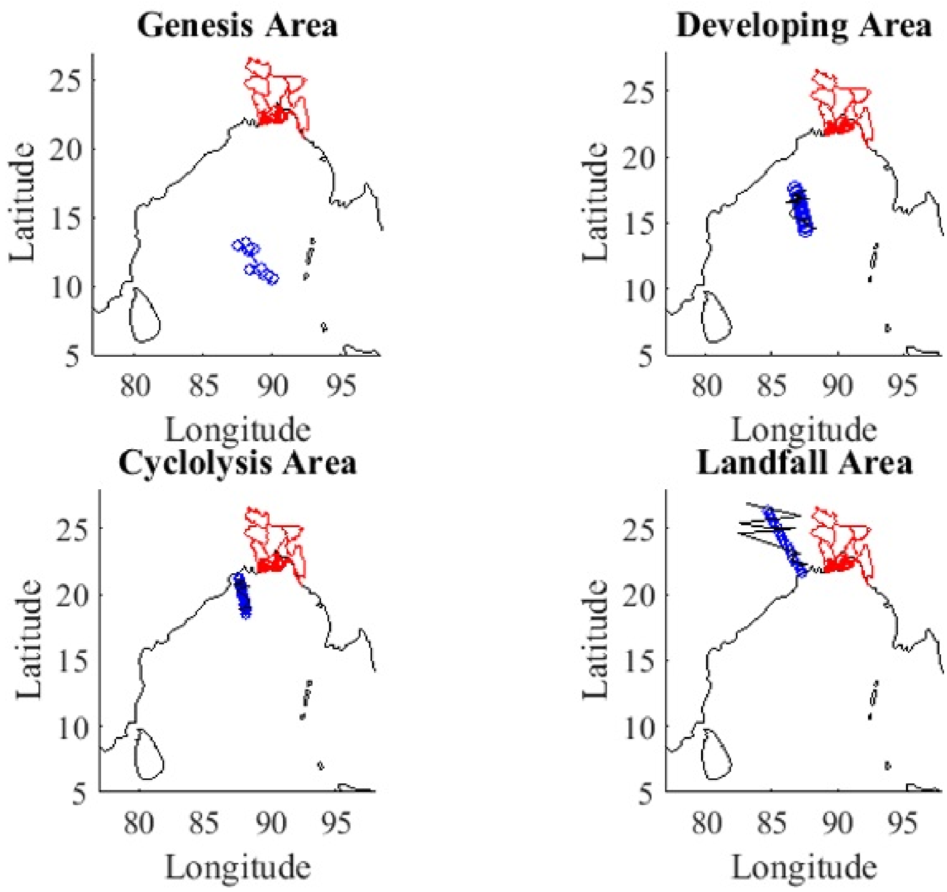

Figure 6. Clustering data of cyclone track data in different stages for present climate condition

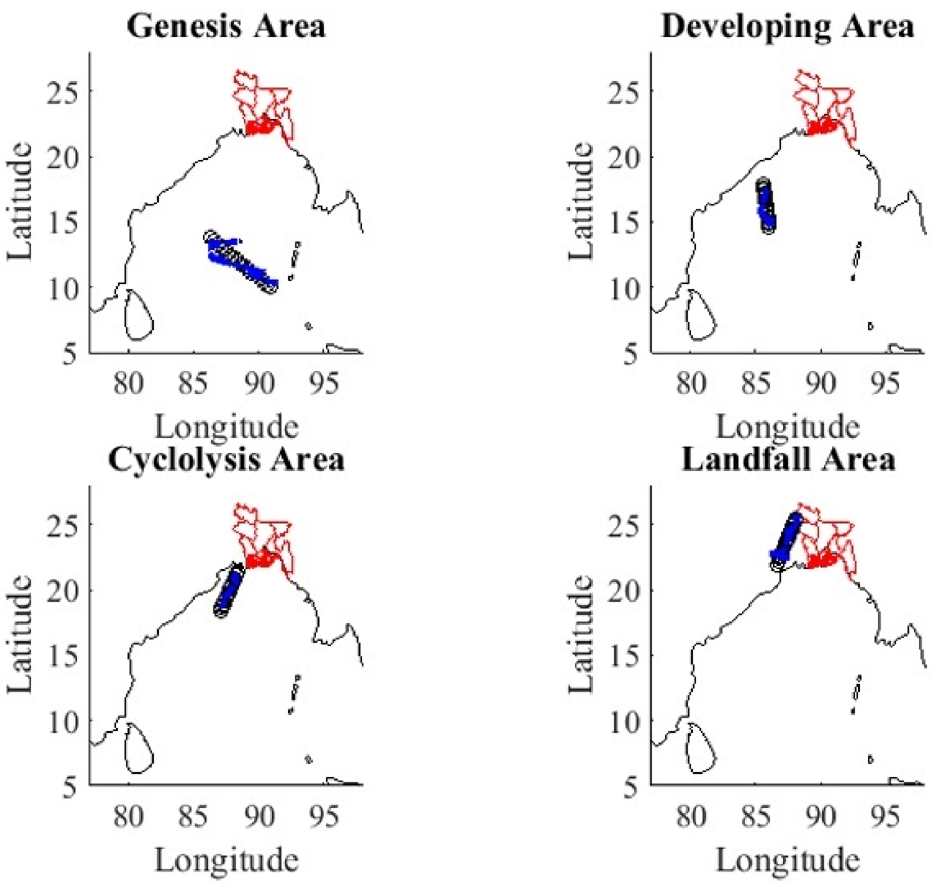

Figure 7. Clustering data of cyclone track data in different stages for future climate condition

Table 1. The estimated path of a cyclone

Life Cycle of Storm	Eye Point	Present Climate	Future Climate	Future Climate C1	Future Climate C2	Future Climate C3
Cyclogenesis	Latitude	7.41	6.65	7.31	7.31	7.41
	Longitude	96.00	87.00	84.56	90.28	89.62
Developing	Latitude	10.97	11.53	11.43	9.8	10.41
	Longitude	90.17	80.44	96.56	80.71	81.56
Mature	Latitude	13.97	15.47	15.37	15.09	15.66
	Longitude	81.56	84.56	85.69	84.75	83.81
Cyclolysis	Latitude	18.84	19.03	18.28	18.09	18.66
	Longitude	81.19	89.25	84.46	84.94	83.81
Landfall	Latitude	21.84	21.28	22.22	22.22	22.03
	Longitude	88.50	88.12	89.81	87.56	86.25

Discussion

In our research we were looking for a significant storm track that we could predict mathematically and statistically. Therefore, we extract and analyze storm track information from climate simulation data and collect storm data at different stages of the storm's life cycle, such as cyclogenesis, development, maturity, cyclolysis, and landfall, using a reliable method k-mean clustering method.

From the collected data we determine the probable location of the probable storm. Since the storm data is random, we see variations in the trajectories. For example, the genesis region of storms is found somewhat closer to the equator at present. But, for future storms, the genesis region of the storm is slightly higher i.e., at a higher latitude Figure 6 shows the direction of change in the path of storms based on the results obtained by the k-means clustering method during the lifetime of current storms.

Similarly, from Figure 7 we can see the likely location of future storms. However, for current and future storms in the Developing and Mature stages, they are much more stable and the separation of their position changes is much less. However, the tendency of the storms to strike is more in the south-west, and in the future, it will move slightly to the south-east and hit the Sundarbans region of Bangladesh. Although these facts are not completely true, the probability of occurrence is very high. It is also worth noting that the possibility of the storms changing direction after hitting the land is also very high and it will move south-east after hitting south-west and hit Bangladesh, which we can see on the right-hand side in Figure 7 titled as Landfall Area. The synthetic storm track obtained in our study provides an approximate direction of current and future storms.

From the above explanation, we may successfully make a probabilistic cluster base synthetic track in the Bay of Bengal region. At the first stage, from the genesis area to developing area cyclone may shift the North-West part of the Bay of Bengal then stabilized and takes some energy from this area and shifted towards the cyclolysis area. At this time the cyclone moves slightly East side and goes into the fort quickly, after that it shifts to the landfall area. For the future climate condition, we have found that the track follows the definition of cyclone properly, but after landfall the behavior surprisingly changes dramatically due to the data pattern. But, from this study, we have found that the trend of cyclone movement from genesis to the development area is North-West side of the Bay of Bengal region.

Therefore, we believe that this research will play an important role in considering the storm track and sustainable development of coastal areas of Bangladesh. Besides, the disaster response and cyclone prevention and adaptation process will play a good role in shaping the development map. Although there are many limitations and weaknesses in the research, we think that the research will be useful for creating new research and improving research. The k-means clustering method of this research is the most accepted method and the results obtained from it are more acceptable. The authors of this study believe that this study will play an important role in considering the future storm trajectories and sustainable development of the coastal region of Bangladesh. Besides, as a part of the Cyclone Preparedness and Disaster Management Program of the Bangladesh Government. Cyclone prevention and adaptation processes will contribute well to map development.

Conclusion

Storm data derived from climate simulation data indicates that current storm tracks and future storm tracks are somewhat different. Storm data derived from climate simulation data indicates that current storm tracks and future storm tracks are somewhat different. Data obtained from K-means clustering of storm data at different latitudes have different central tendencies and are very stable over the inter-storm lifetime. In addition to the present time Cyclone impact location is different from the future cyclone impact location. Which, moving slightly eastwards from the south-west, tends to hit Bangladesh from the south-west direction.

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Compliance With Ethical Standards

Authors' Contributions

MAAM: Conceptualization, Methodology, Software and Writing- Original draft preparation
MT: Software, Data curation, Writing- Reviewing and Editing
AKJ: Software, Investigation and Validation
MSN: Visualization and Investigation
MRK: Visualization and Validation
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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RESEARCH ARTICLE

Mapping and recording of ancient shipwrecks by using marine remote sensing techniques: Case studies from Turkish coasts

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ABSTRACT

Within the context of the "Shipwreck Inventory Project of Turkey (SHIPT)," numerous acoustic remote sensing and photogrammetric surveys have been carried out along the coast of Turkey with the aim of detecting, mapping and documenting underwater cultural heritage. During the surveys, many ancient shipwreck sites have been discovered thanks to advanced technologies such as high-resolution side-scan sonar (SSS), sub-bottom profiler (SBP), and remotely operated vehicle (ROV). Once acoustic anomalies have been located, shipwreck sites have been verified by ROV and documented by a 3D photogrammetric survey. Analysis of the collected data confirmed that the survey design and data acquisition parameters are the most important criteria for obtaining the best quality image of the shipwrecks. Performing high-resolution remote sensing and photogrammetric survey for shipwrecks using optimal data collection techniques provides rapid results, decreasing time and budget and increasing the quantity of underwater cultural heritage. This paper discusses proper survey stages for the rapid, effective, and high quality detection and recording of shipwrecks using advanced technology. Acoustic images of shipwrecks comparing cargo, period, and form are also introduced in this paper, for the first time, which provides data on interpreting anomalies accurately for further exploration and monitoring surveys.

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Introduction

Underwater archaeological surveys carried out over the last 60 years demonstrate that the Turkish coasts are very rich in terms of underwater cultural heritage (Pulak, 1998; Ward & Ballard 2004; Bass, 2005; Royal, 2006; Brennan et al., 2012; Özdaş, 2010; Özdaş et al., 2012; Kızıldağ & Özdaş, 2021). Nevertheless, no systematic and long-term exploration had been performed until now, which covers Black Sea, Aegean Sea and Mediterranean Sea. The Shipwreck Inventory Project of Turkey (SHIPT) has been carrying out for years with a primary goal of documenting underwater cultural heritage off Turkish coasts.

Underwater cultural heritage (e.g., shipwrecks, settlement structures and artifacts) contains all traces of human existence having a cultural, historical or archaeological character which have been partially or totally under water (UNESCO, 2001, Article 1). Therefore, the documenting of shipwrecks of different ages and forms provides significant data on ancient shipbuilding, navigation, sea trade routes, sea battles, and transportation of materials. In addition to their cultural value, the wrecks also provide a habitat for the marine ecosystem as they form artificial reefs. Mapping of these resources provides great benefits for developing the Blue Economy.

A large number of shipwrecks, from the Bronze Age to the Ottoman Period, have been discovered and reported during several surveys carried out under SHIPT (Özdaş, 2009, 2010; 2022; Özdaş et al., 2012). Nevertheless, the shipwrecks found in shallow waters are mostly damaged by anchorage, illegal dives and fishing activities. After obtaining advanced remote sensing technology (e.g., SSS, SBP and ROV), SHIPT have focused on open-sea surveys, which allowed us to increase the quantity of the shipwreck sites; access the untouched shipwrecks in good-condition; and the rapid documentation of the sites before destroying.

Compared to conventional scuba diving methods, the use of remote sensing methods provides great advantages to underwater archaeological researches via the recording and monitoring of both shallow and deep water shipwreck sites in a rapid and cost-effective manner (Singh et al., 2000; Quinn et al., 2002; Ward & Ballard 2004; Delaporta et al., 2006; Bingham et al., 2010; Bates et al., 2011; Pacheco-Ruiz et al., 2019; Westley et al., 2019). However, during a marine survey, each manoeuvre of the survey vessel or visual inspection for verifying the acoustic anomalies increases the cost and time of the survey. Using optimum data acquisition techniques and interpreting data accurately are key factors to performing low-cost surveys and providing immediate and accurate results.

The image quality of side-scan sonar varies, depending on data acquisition parameters (frequency, range, etc.), ship velocity and limitations, towfish depth, equipment specifications and settings (resolution related to signal processing technology, transducer design, towfish stabilization, etc.), sea conditions (that affect pitch and roll motion of towfish and produce acoustic noise), features of the seafloor (e.g., sandy substrate, rocky outcrops, marine flora), orientation of the survey line relative to the target, and operator skills and experience (Bates et al., 2011). When detecting shipwrecks, setting survey operation configurations properly and obtaining positional information accurately are the key factors for side-scan sonar imaging. It is important to optimize the data collection parameters to avoid wasting time, causing extra effort, or increasing the costs.

The frequency and the range, as well as ship velocity, must be set according to possible dimensions of objects. Passing with slow speed over a feature provides better resolution in terms of getting more acoustic reflections. However, acquiring data with a high frequency and therefore narrow range (e.g., 50 m) increases the possibility of detecting the target. The sonar towfish altitude above the seafloor is also an important factor in recording a quality image. Getting the towfish closer to the seafloor increases the sweeping area and provides better resolution by reducing signal absorption and acoustic noise in the water column (Lurton, 2010). Since sea conditions affect the pitch and roll motion of sonar towfish, images of targets may be shown distorted. In windy weather, data often contains a great deal of noise, and this frequently causes the target to be hidden.

Once the shipwrecks are detected, the next step is to obtain a high quality optical record of the sites. Using photogrammetric techniques, a highly accurate site map can be built up and maximum information can be obtained (Drap et al., 2015). This allows to produce 1:1 scale photogrammetric models with 3D views of shipwreck sites and to identify the distribution area and quantity of the archaeological material. Another benefit of photogrammetry is to be able to monitor the wreck sites in terms of damage by making comparisons before and after conditions. In addition, underwater photogrammetry provides virtual access to the archaeological sites by both specialists and public (Drap et al., 2015).

This paper presents an effective procedure for underwater archaeological survey in order to (i) record and document the shipwreck sites in high quality, (ii) decrease the cost and time and (iii) increase the quantity of shipwreck. Proper survey stages were discussed for the rapid, effective, and high quality recording of the shipwrecks using advanced technology. Additionally, in this paper, for the first time, a comparison of acoustic images of shipwrecks, which have different cargo (e.g., amphora and stone) and are dated to different periods, were presented.

Material and Methods

As a part of SHIPT project, high-resolution remote sensing and photogrammetric data were collected along the coast of Çanakkale and Yalıkavak Peninsula in 2016 and 2018. In particular, Çanakkale has geologically strategic importance in terms of its location on ancient maritime route connecting the Mediterranean Sea with the Black Sea. Although many archaeological investigations have been carried out on land in Çanakkale during the last hundred years, underwater cultural heritage has remained undocumented. For this reason, the first systematic survey has been performed in Çanakkale's coastal waters by using the methods of (a) side-scan sonar (SSS) and (b) sub-bottom profiler (SBP) imaging for detection and documentation of shipwrecks; (c) remotely operated vehicle (ROV) and scuba diver inspection for visual verification and photogrammetric recording of wreck sites (Figure 1).

All data were integrated into the ArcGIS software, and a database was created which is called "Acoustic Database for Underwater Archaeological Sites of Turkey (ADAST)". It contains the sonar images, preliminary interpretation, depth, positional and operational information, underwater photos of targets, as well as marine environmental data (i.e., geological, geomorphological, biological and oceanographic data) of the archaeological sites. ADAST allows to us to classify, compare, and create maps with multiple layers of targets.

Side-Scan Sonar Imaging

During the side-scan sonar survey, multiple records of findings were acquired by using different configurations, which provided the opportunity to determine the best operational techniques for recording shipwrecks. A Klein 3000H model with a dual frequency of 445 kHz/900 kHz SSS was used for imaging seafloor. A low frequency of 445 kHz was used to explore a large-scale area, with the range varied between 100 m and 150 m per sides depending on the site, as the sonar was towed at an average speed of 3 knots. Line intervals were set at 160 m to provide overlap for the mosaic, while the sonar was run using a 100 m range with a swath of 200 m. After detecting and locating the targets, the frequency and the range were adjusted to 900 kHz and 50 m respectively and obtained high-quality images.

Different uses of SSS data acquisition parameters were compared in two sets of images for each wreck site in terms of frequency of the acoustic signal and range scale, to present their effect on the different wreck forms. The altitude of the towfish above the seafloor was approximately 10% of the range. Cable deployment was controlled with an Emce Clw model electrical winch equipped with 600 m armoured cable and a cable counter sheave. The positional data were provided by a JRC model differential global positioning system (DGPS) receiver.

Although target detection and recognition from side-scan data were mostly based on manual interpretation during data collection, all data were integrated into SonarWiz (Chesapeake Technology Inc) software for post-processing stage. Data were processed focusing on gain corrections to remove the variation in brightness. Automatic Gain Control (AGC) was applied to data in order to correct the differences in the amplitude of the signal and normalise the across-track gradient banding in the

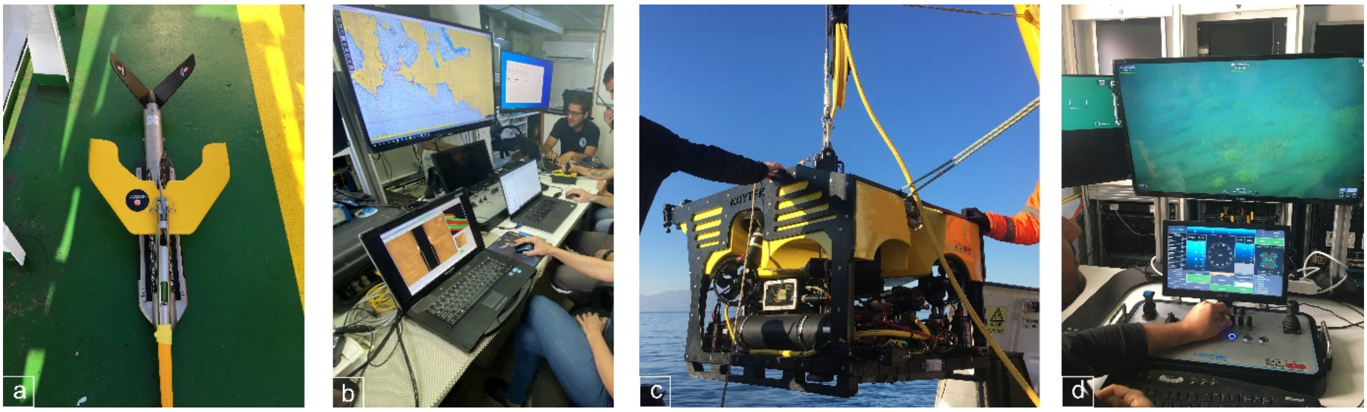

Figure 1. Marine remote sensing survey. (a) SSS towfish; (b) SSS control room; (c) ROV; (d) ROV control panel

imagery. Bottom-tracking corrections were applied and all lines were corrected for slant range to produce mosaic of the seafloor. Shadow lengths of the targets were measured in the target editor to estimate of object heights above the seafloor.

Sub-Bottom Profiler Imaging

Even though the primary goal was detecting and imaging the shipwrecks using side-scan sonar, the sub-bottom profiler imaging was integrated, both to eliminate non-artificial anomalies and to image the vertical profile of the subsurface beneath the shipwrecks. Integration of the sub-bottom profiler data can help in verifying the sonar targets. When a shipwreck is located among rocky outcrops, it can be difficult to distinguish whether the reflection derives from a wreck or a geological feature. On the basis of reflection geometry, the anomalies can be identified in the sub-bottom profiler data: while rocks extend into the deeper layers beneath the seafloor, wrecks generate a chaotic acoustic pattern and weak hyperbola-shaped reflections, forming an acoustically transparent zone underneath.

SBP systems were used to image below the seafloor. EdgeTech 3100-216 portable SBP with 2-16 kHz pulses and Bathy 2010 SyQwest Chirp SBP system with 9 transducers at a frequency of 3,5 kHz were operated. A transmit rate of 4 Hz was used during the survey. Wrecks, partially buried in sediment, require the use of high-resolution sub-bottom profilers to image the stratigraphy beneath the wrecks. Since high-resolution data is important for recognizing archaeological features, the recording speed was kept as low as possible, at a maximum of 3 knots.

Visual Inspection and Photogrammetry

Once targets were located and positions recorded, visual investigation was performed through ROV to identify acoustic anomalies. All targets were confirmed as shipwrecks on the first dive, thanks to the accuracy of the positional data and the software calculations of the sonar towfish location.

During the verification and photogrammetry operations, two ROVs were used. Hoytek 1000m model ROV equipped with BlueView Imaging Sonar (900 kHz) and Hoytek 200m model ROV equipped with Imagenex Imaging Sonar (900 kHz) were deployed. Applied Acoustics Easytrak Alpha model ultra-short baseline (USBL) was used for underwater acoustic positioning. Both ROVs were designed specifically for underwater archaeological surveys by equipping with manipulator, autopilot, HD and still cameras and lighting systems for photogrammetric imaging. The configuration of lights and cameras is crucial to obtain high-quality and clear images. In particular, the lighting is an important factor in photogrammetry. A well-designed lighting system allowed homogeneous exposure for each photo frame and consistency between images avoiding focusing on the particle. Four subsea LED lights (45,000 lumen) were mounted on our ROVs to ensure the optimum lighting.

ROV technology provided opportunity to generate photomosaics by taking thousands of photos on the wreck sites time and depth-independently, which improved quality of 3D models. The height of the ROV was kept at approximately 2 meters above the remains by using auto-altitude configuration. Minimum overlap of 50% between the images was provided for full coverage. The number of photos varied depending on the size of the site.

Using photogrammetric techniques, highly accurate site maps have been built up and maximum information has been obtained. 1:1 scale photogrammetric models with 3D views of shipwreck sites were produced and the distribution area and quantity of the archaeological material was identified. 3D models were generated using Agisoft Photoscan software. Thus, preliminary site maps of the shipwrecks were obtained using photogrammetry.

Results

Five shipwreck sites located on the western Turkish coast were presented in this paper (Table 1, Figure 2). The shipwreck sites were located at depths between 22 and 50 m, differing in terms of cargo, form, and age. The study area displays a mixed seafloor structure consisting of muddy and sandy sediments, various benthic habitats dominated by *Posidonia oceanica*.

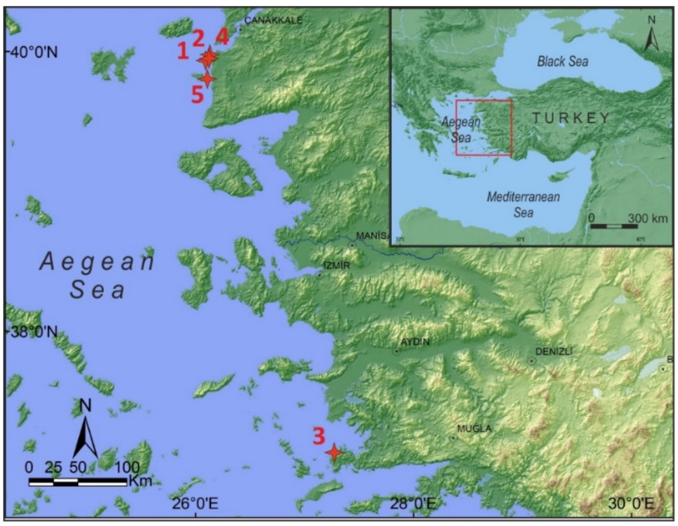

Figure 2. Location map of the shipwreck sites

Table 1. List of shipwreck sites located by acoustic remote sensing methods

Site Name	Cw	Date	Dm (m)	H (m)	Dp (m)	Ds (m)	Visible Artifacts	Condition
Wreck Site-1	Amphora cargo	4th-3rd c. BC	18 × 9	0.6	27	2300	100	Well-preserved
Wreck Site-2	Amphora cargo	5th-6th c. AD	14 × 9	0.6	25	1500	95	Well-preserved
Wreck Site-3	Amphora cargo	1st c. BC	20 × 8	1.4	50	620	350	Well-preserved
Wreck Site-4	Stone cargo	18th c. AD	19 × 8	1.5	22	1700	2000	Well-preserved
Wreck Site-5	Warship	18th-19th c. AD	38 × 20	1.6	25	2500		Heavily damaged

Note: Cw: Classification of wreck; Dm: Dimension; H: Height; Dp: Depth; Ds: Distance to shore. Artifact identification and dating by Harun Özdaş.

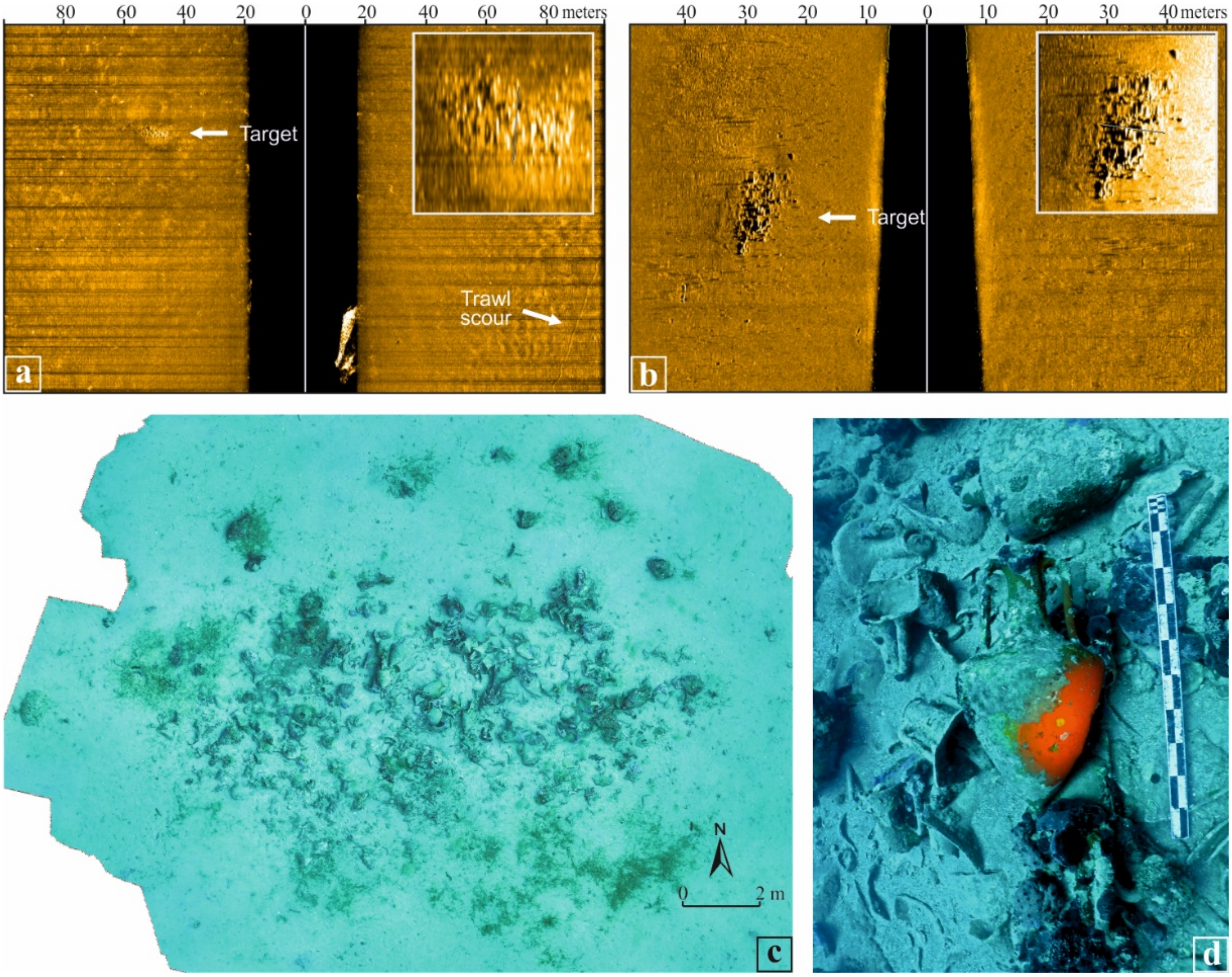

Figure 3. Wreck Site-1: (a) low frequency side-scan sonar image, (b) high frequency side-scan sonar image obtained using optimal data acquisition parameters, (c) photogrammetric image of wreck site showing the amphora pile, (d) an amphora among the cargo

Wreck Site-1

During the systematic side-scan sonar mapping off Çanakkale coasts, a target was detected at a depth of 27 m, at a fair distance from shore (2.3 km) on the flat and sandy seafloor, covering an area of 18 m × 9 m. (Figure 3, Table 1). An E-W-directed oval-shaped target was imaged transmitting a low frequency signal (445 kHz) at a range of 100 m (200 m swath range) and at a velocity of 3.5 knots (Figure 3a). A high-resolution image could be acquired at a high frequency of 900 kHz and 50 m range, at a velocity of 3 knots (Figure 3b). This remarkable target clearly corresponded to the shape of a shipwreck.

The visual inspection verified the presence of an amphora pile, which represents the cargo of a ship dated to the early Hellenistic period. The detailed study of the photogrammetry allowed to estimate the total number of amphorae visible on the seafloor. Over 100 amphorae were defined, largely in good condition, most of them on the surface and some partially buried in the sediment (Figure 3c, d).

Wreck Site-2

The second sonar target was detected 1.5 km off the coast of Çanakkale, at a depth of 25 m, with a frequency of 445 kHz and a range of 150 m (Figure 4a, Table 1). Setting the frequency at 900 kHz and the range at 50 m, a clear image of the target was obtained, even though the velocity of the ship was kept to a minimum 3.6 knots due to the strong current (Figure 4b). Based on the shape, dimension, and the acoustic signature, the sonar image was interpreted with each single reflection most likely deriving from a single amphora. In addition, bright reflections from the surrounding seafloor of the target may indicate a scour pit formed by the bottom currents around a possible shipwreck.

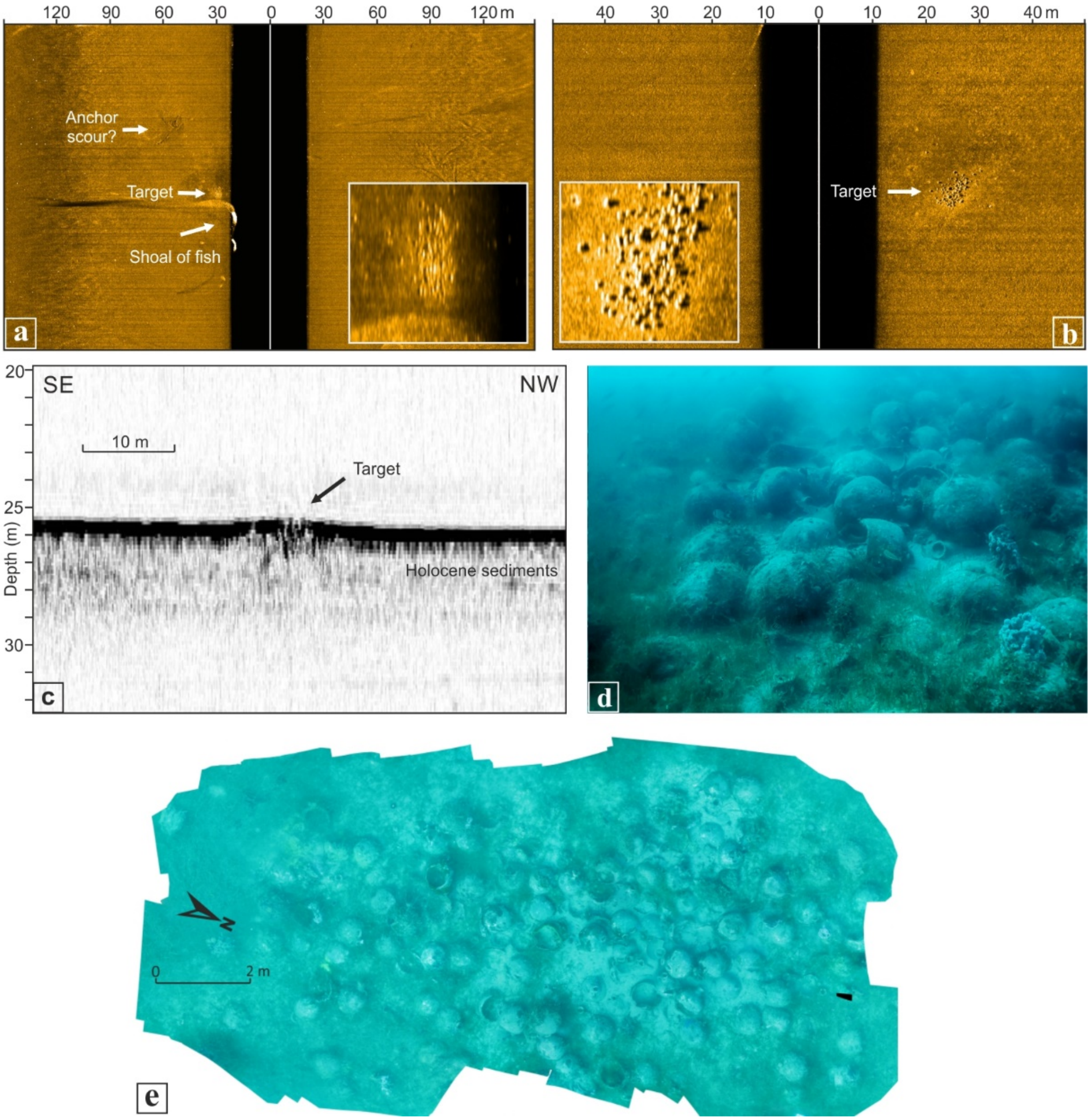

Figure 4. Wreck Site-2: (a) low frequency side-scan sonar image of the target that is partly covered by a shoal of fish, (b) high frequency side-scan sonar image, (c) chirp sub-bottom seismic profile over the shipwreck displaying the chaotic reflections underneath and general stratigraphical structure. The site is underlain by at least 3-4 m of Holocene sediments (d) amphora cargo, (e) photogrammetric image

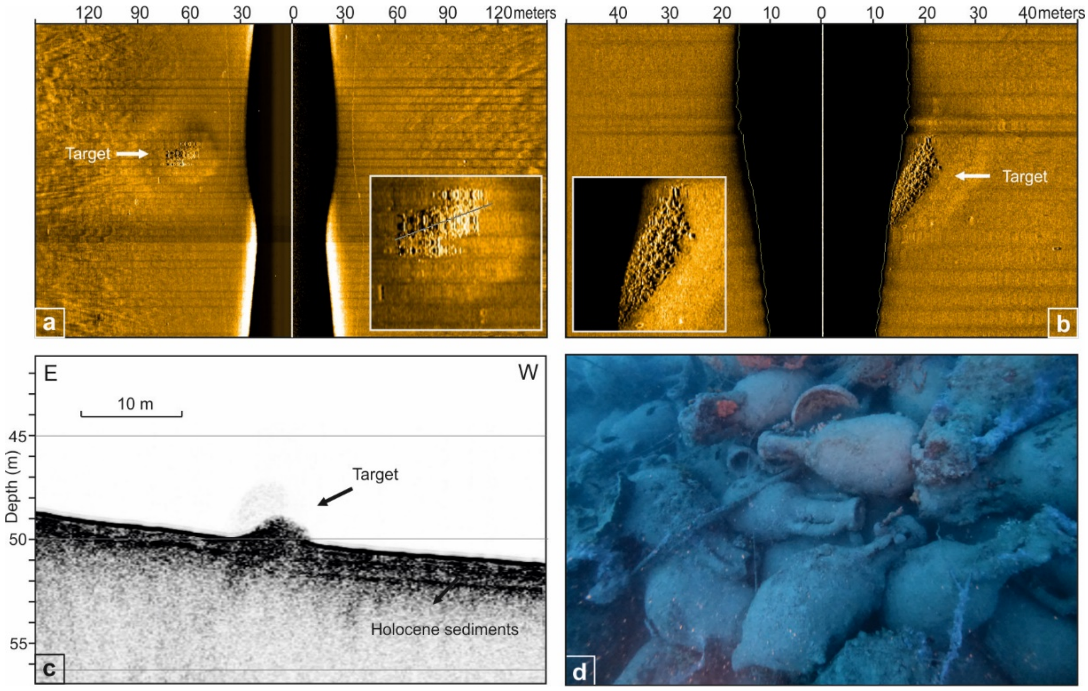

Figure 5. Wreck Site-3: (a) low frequency side-scan sonar image, (b) high frequency side-scan sonar image, (c) chirp sub-bottom seismic profile over the shipwreck displaying the height of the mound and general stratigraphical structure, (d) amphora cargo

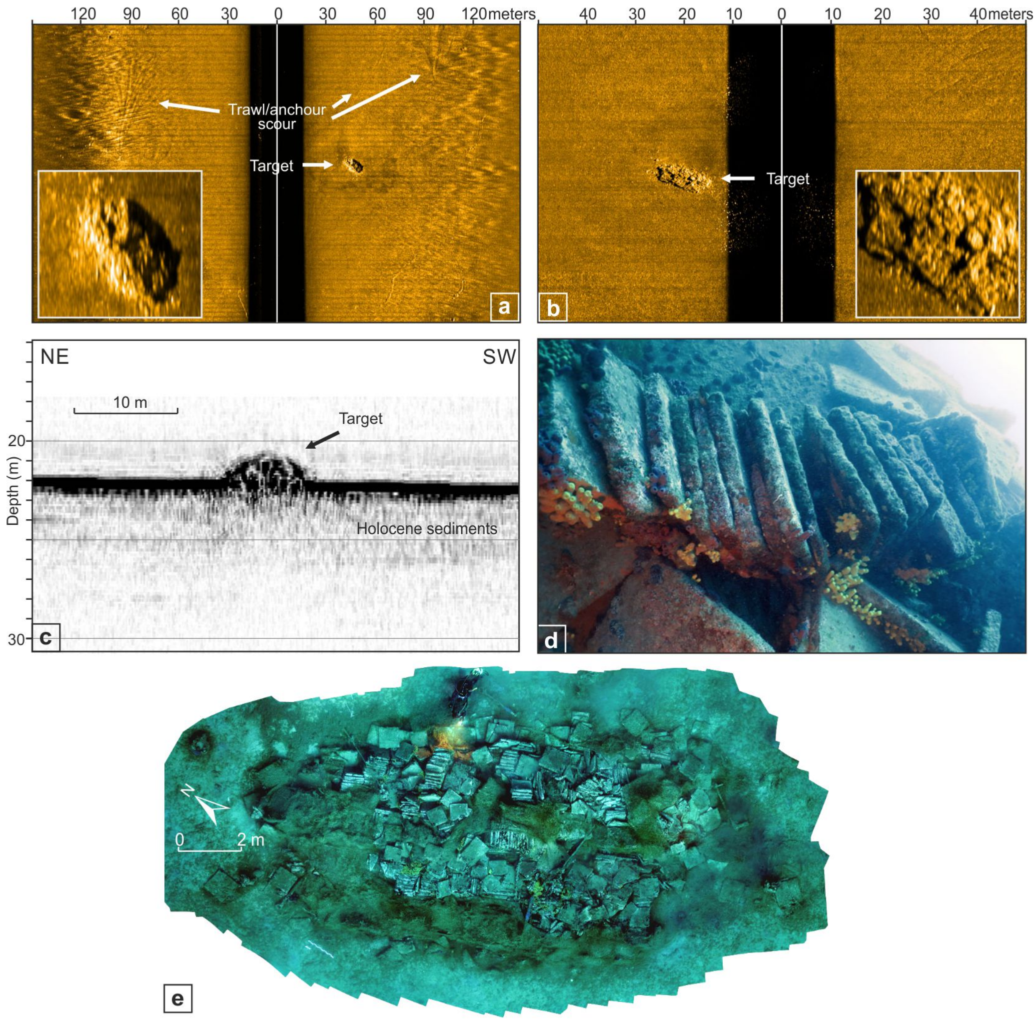

Figure 6. Wreck Site-4: (a) low frequency side-scan sonar image, (b) high frequency side-scan sonar image, (c) chirp sub-bottom seismic profile over the shipwreck displaying the height of the mound and general stratigraphical structure, (d) a stone pile from cargo, (e) photogrammetric image

Sub-bottom profiler data assisted with a more precise interpretation: the anomaly was compatible with an artificial feature (Figure 4c). A transparent zone appeared under the target and chaotic reflections were observed. Data indicated that the seafloor topography is generally flat. Throughout the profile, weak parallel acoustic reflectors were observed in the sub-surface underneath the Holocene sediments.

The visual inspection confirmed that the oval-shaped target is consistent with a shipwreck, which is dated to Late Roman period. The photogrammetric study provided information on the amphora distribution and condition, as well as the total number of visible artifacts (Figure 4e). The cargo is scattered in an area 14 m long and 9 m wide, consists of at least 100 Late Roman 2 (LR2) amphorae, which are mostly in good-condition (Figure 4e). Only one amphora layer with a height of the 0.6 m was visible on the seabed, however at least one more layer is buried in the sediment (Figure 4e). Marine flora growth can be observed over the remains, densely covering the amphorae.

Wreck Site-3

An amphora shipwreck close to Yalıkavak, Bodrum, was imaged by SSS, which was documented by Institute of Nautical Archaeology in 1990s (Figure 5a, b, Table 1). The NE-SW-directed shipwreck covers an area of 20 m × 8 m, at a depth of 50 m. The oval-shaped target lies on a flat and sandy seafloor.

SBP imaging helped us to determine the height of the wreck to be at least 1.4 m (Figure 5c). The stratigraphy under the wreck was interpreted as a 1-2 m thick layer of Holocene sediment overlying a relatively strong high-amplitude parallel reflector.

ROV inspection verified that well-preserved shipwreck consists of mostly intact more than 350 Lamboglia 2 type amphoras, dated to 1st century BC (Figure 5d). The absence of damage must be associated with its location in a modern harbour that protected from illegal trawling and diving activities.

Wreck Site-4

During the side-scan sonar survey another target was detected 1.7 km north of the Site 1 and 2.0 km offshore, at a depth of 22 m (Figure 6, Table 1). The target emerged as a rectangular bright reflection in the first image, at a frequency of 445 kHz and a range of 150 m (Figure 6a). A more detailed image was obtained by one more pass over the target at a high frequency and a 60 m range (Figure 6b). The acoustic signature was slightly different from the signatures produced by wreck sites 1-3. The minimum survey speed could be 3.5 knots due to strong wind and current that often prevented steering the boat on the survey line. The target covers an area 19 m long and 8 m wide.

The sub-bottom profiler record displayed a different shape and acoustic over the flat seafloor (Figure 6c). The target represents a distinctive mound shape with a height of at least 1.5 m, underlain by sediment that is several meters thick. Some continuous-to-discontinuous weak sub-bottom reflectors were observed.

Visual inspection confirmed that it was a well-preserved cargo consisting of a large pile of thin stones. Through the production of a photomosaic, more than 2000 concreted stones were visible (Figure 6d, e). The flat ashlar stones have a maximum length of 80 cm, width of 80 cm, and thickness of 5 cm. In addition to the main cargo, pottery fragments, metal rigging, and a number of iron nails were observed. Some wooden components of the hull can be seen but are mostly buried. A sample was taken from the planking for radiocarbon analysis. The results suggest with 95.4% probability that the ship was built at the end of the 18th century AD. A large portion of the wreck is covered by fishing-net with dense marine growth, which makes investigation difficult. Thanks to photogrammetric survey a 3D image could be obtained, which resulting from the alignment of more than 900 images (Figure 6e).

A number of trawling scars were recorded with side-scan sonar on the surrounding seafloor, which indicate intensive fishing activity in the region.

Wreck Site-5

Another shipwreck was imaged near Bozcaada, 2.5 km offshore at a depth of 25 m. This distinctive target appeared oval-shaped, corresponding to a ship form, lying in a flat, sandy seafloor scattering within an area of approximately 38 m x 20 m (Figure 7, Table 1). A deep scour trail was observed through the side-scan sonar records, passing over the target, which caused serious damage on the east side.

On the sub-bottom profiler record, an acoustic gap was observed in the underlying stratigraphic layer, which was likely caused by a reflected or absorbed acoustic signal due to a denser artificial feature (Figure 7c). The height of the target has been determined to be at least 1.6 m. The general stratigraphy inferred from the sub-bottom data was interpreted as a 3.5-4 m thick layer of Holocene sediment overlying a relatively strong high-amplitude parallel reflector.

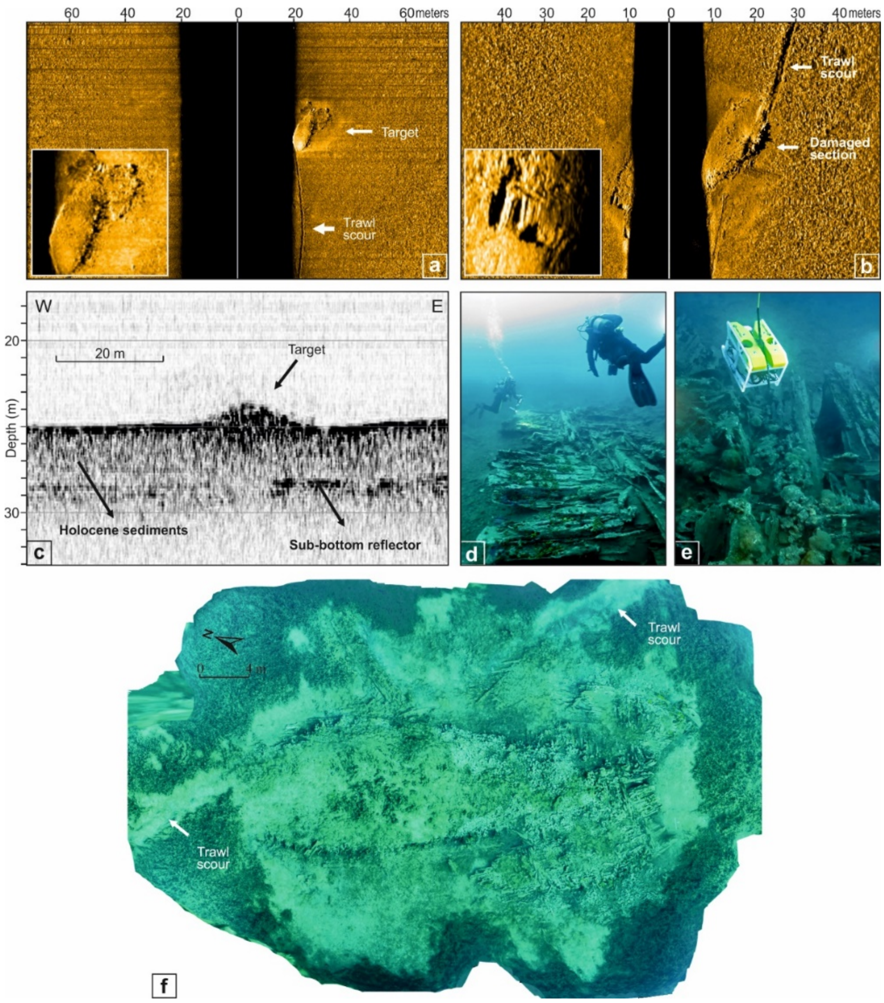

Figure 7. Wreck Site-5: (a) low frequency side-scan sonar image of target that lies on sandy seafloor surrounded by *Posidonia oceanica*, (b) high frequency side-scan sonar image displaying deep trawl scour in detail, (c) chirp sub-bottom seismic profile over the shipwreck displaying the acoustic gap and general stratigraphical structure, (d) underwater photo of the hull component, (e) ROV operation on the wreck site, (f) photogrammetric image

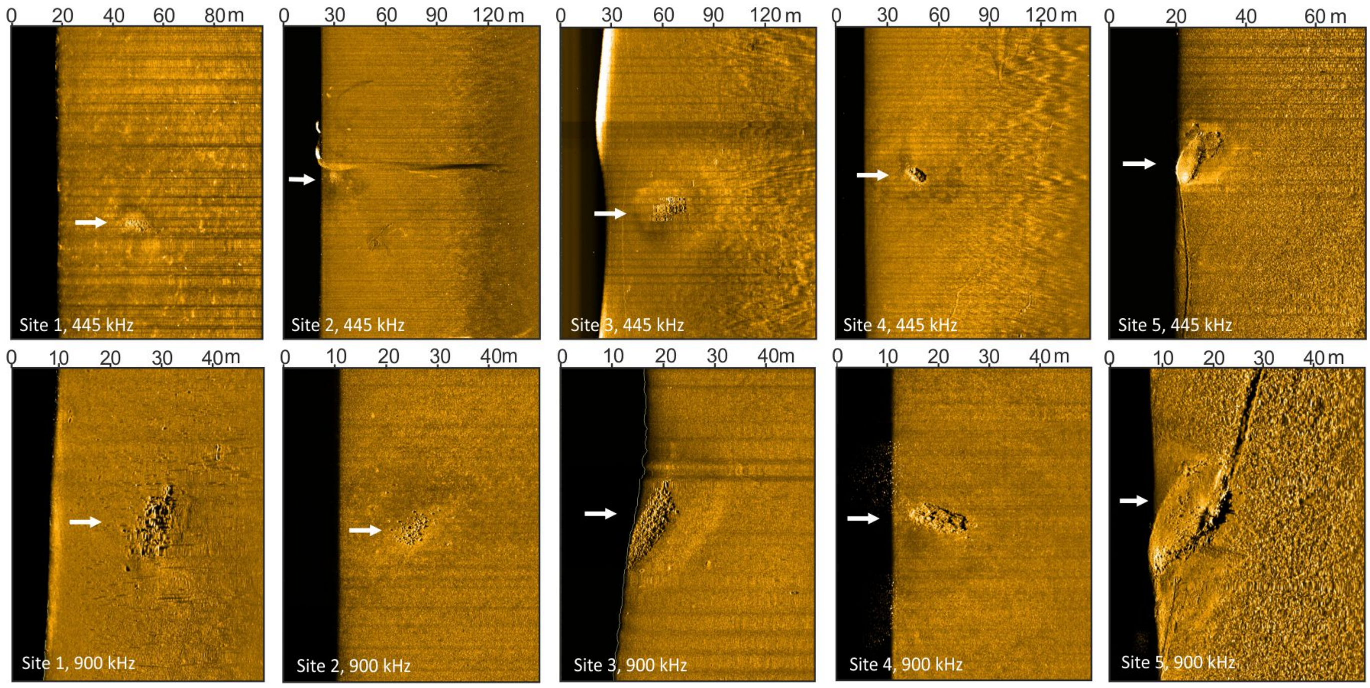

Figure 8. Comparative side-scan sonar images of shipwrecks (upper line displays low frequency images and lower line displays high frequency images)

The target was visually investigated by both divers and ROV and interpreted to be a warship from Ottoman period (Figure 7d, e). Partially-buried and heavily damaged hull timbers were observed. The wooden elements were in poor-condition and covered by marine growth, which made it difficult to identify their function. ROV survey provided a detailed photogrammetric model of the shipwreck, which consists of 1430 photographs (Figure 7f).

Discussion

In this paper, an effective procedure was presented that streamlines optimal remote sensing survey methods to detect underwater archaeological sites and obtain high-quality images of their current conditions. Remote sensing methods provide a lot of advantages that extend the survey area and time when compared with conventional scuba diving documentation. Determining the optimal survey design and defining the proper data acquisition parameters decreases the cost and time and increases the quantity of shipwreck that can be surveyed, at the same time providing high-quality data. Since deciding which targets require visual inspection is critical, as mistakes may result in extra effort, the capability and experience of the operator is also significant in the detection of shipwrecks and the acquisition of high-quality data.

Side-scan sonar surveys carried out along the coast of Turkish coasts have revealed some considerations when imaging the shipwreck sites. For instance, since biodiversity at the wreck sites is high, one must take into consideration that the remains may be covered by groups of fish. Moreover, the orientation of the survey line relative to the target also has an effect on obtaining a quality image. Furthermore, when an acoustic positioning system (ultra-short base line, USBL) is lacking, the velocity and the direction of the current should always be taken into consideration. In areas with currents, towfish may drift to the starboard or port side of the ship, not in line with the ship. In such areas, running parallel with the direction of the current makes it easier to accurately locate the position of the target, and therefore reduces the need for visual inspection. Setting the range scale and the run direction according to the target, velocity, and altitude of the towfish are important factors in acquiring maximum high-resolution data. In summary, data acquisition strategy and operator experience in the interpretation of anomalies are crucial components to obtaining optimal archaeological results rapidly and cost-effectively.

The integration of sub-bottom profiler and side scan sonar data verifies that a sub-bottom profiler can contribute to the elimination of non-artificial targets. This technique also provides information about geological structures and sedimentological data at the shipwreck site. In this study, sub-bottom records indicated that the region was affected primarily by a high amount of sediment accumulation, which would also affect the shipwreck sites.

ROV survey confirmed that photogrammetric imaging is rapid and accurate method for documenting of shipwreck sites when ROV is specially designed to generate photomosaics. A scuba diver can only work on the shipwreck less than 1 hour per day, while ROV can obtain images from the site without time constrains. Both recording hundreds of photos and using autopilot navigation of ROV instead of diver-operated record allowed to produce high-accuracy 3D models of the sites.

The results also verify the benefit of high-resolution side-scan sonar imaging, when properly performed, to identify and quantify damage to shipwreck sites. Side-scan sonar allowed us to image the trail of trawling nets both on the shipwreck and in its vicinity.

The comparative images of five ancient shipwrecks in terms of the variety of ships' cargo, age, and form are presented in this paper, which provide information for further sonar surveys aiming to detect shipwrecks. Four shipwrecks were cargo ships that carried amphorae and stone, dated to the early Hellenistic period (4th to early 3rd century BC, Site 1) (Prummel, 2003); to the Roman period (1st century BC, Site 3) (Boersma et al., 1986); to the late Roman period (5th-6th century AD, Site 2) (Opait, 2004); and to the Ottoman period (18th century AD, Site 4); while the other one was a warship from Ottoman period (18th-19th century AD, Site 5) (Bingeman et al., 2000; Zorlu 2008), which had wooden hull components relatively well preserved. Thus, we have the opportunity to compare the side-scan sonar images of the shipwrecks in terms of (i) cargo remains vs. wood remains; (ii) amphora cargo vs. stone cargo; (iii) early Hellenistic period vs. late Roman period. Sonar contacts of wrecks consisting of a one-layer amphora pile show similarities in terms of their shape and dimension, reflecting very similar acoustic characteristics (i.e., weak backscatter) (Figure 8).

The stone-carrying shipwreck reflects a different image from the others, due to its compact structure. Target 5 could easily be recognized as possible shipwreck, unlike the others, due to its relatively well-preserved shape, dimensions, and distinctive shadow. Varying sonar images compiled from different shipwrecks have the potential to provide data that will allow researchers to recognize anomalies in further surveys.

The sonar images and photomosaics of Site 1 and Site 2 demonstrate that only one amphora layer in each shipwreck remained visible on seafloor, even though Site 1 is a thousand years older than Site 2. This can be explained by oceanographic events and the geological environment. In general, a scour effect is observed in shipwreck sites, formed by bottom currents (Ballard et al., 2002; Quinn, 2006). This effect may give rise to the top layer being exposed instead of being completely buried.

Conclusion

The results have demonstrated that when proper survey strategy and equipment configuration are designed, high-resolution remote sensing survey is quite useful to rapidly and cost-effectively locate and record underwater cultural heritage. In particular, the critical techniques necessary for optimal side-scan sonar data acquisition in order to both explore potential archaeological sites and obtain high-quality images for the evaluation of the current conditions of the shipwrecks were described in detail in this study. This effective survey procedure and the comparative acoustic images can also provide a basis for further underwater archaeological investigations to be designed to locate, record, and monitor underwater cultural heritage.

This study emphasizes the necessity of rapid documentation and therefore the use of advanced technology, with effective survey design achieved by using proper equipment and data acquisition parameters, as well as careful interpretation. The sonar survey results demonstrate that the procedure for optimal data acquisition and analysis should be well understood by researchers, in order to discern potential shipwrecks from among other geological features on the seafloor. This increases the quantity of underwater cultural heritage that can be surveyed and reduces the time and budget of survey.

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Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

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

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RESEARCH ARTICLE

Investigation of the effects of whey powder on *Haematococcus pluvialis* cell growth kinetics

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ABSTRACT

This study was carried out to examine the effect on the growth of *Haematococcus pluvialis* using low-cost whey powder (WP). The *H. pluvialis* used in this study were from Çukurova University, Faculty of Fisheries, and 70% demineralized WP from Cici Dairy Industry Trade Inc. The experiment was conducted under laboratory conditions with 3 replications. During this experiment, cell numbers and biomass were analyzed every day. In addition, the specific growth rate of *H. pluvialis* was calculated according to the Monod Equivalence. The mean values of the cell number following the WP application were calculated according to the groups (C, W5, W10 and W15) as 763.34±419.62 cells/ml, 951.60±388.20 cells/ml, 1105.27±380.35 cells/ml and 978.63±411.07 cells/ml, respectively. The mean biomass value has been found the lowest in the control group (0.84±0.36 g/l), and the highest value in the W15 group (1.26±0.55 gl^{-1}). The mean specific growth rate was determined as 0.52±0.09 day^{-1} in the control group, 0.56±0.1 day^{-1} in the W5 group, 0.56±0.14 day^{-1} in the W10 group, and as 0.61±0,09 day^{-1} in the W15 group. In accordance with the data obtained, both the biomass and the specific growth rate of *H. pluvialis* were observed to increase in the W15 group. The use of WP as a nutrient medium is recommended due to its low-cost as well as increasing biomass.

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Introduction

Nowadays, as a result of the rapid population growth and the gradual decrease of limited natural resources, the danger of famine has become the biggest problem for countries. Food products of plant and animal origin are insufficient to meet the needs of people day by day. It also is used methods such as chemicals and/or genetic modification of the product to fill the food deficient. However, these methods adversely affect human health. For this reason, countries have turned to functional food research. One of the important researches of functional food is algae which meet the functional food demand. Algae are important in terms of their high nutritional content and contribution to human health. Sasa et al. (2020) reported that β-carotene, carotenoid and polyunsaturated fatty acids produced by algae have positive effects on human health and even reduce the risk of chronic diseases when used directly or added as food additives (Aslanbay Güler & İmamoğlu, 2021).

One of the main factors affecting the growth parameter of microalgae is the nutrient concentration in the medium. Nitrogen (N) and phosphorus (P) are two macronutrients of medium concentration, and they play an important role in cell metabolism as many biochemical processes. Nitrogen is mainly used to form proteins, amino acids, and nucleic acids, while phosphorus is mostly a component of nucleic acids and phospholipids (Bougaran et al., 2010).

The microalgae growth rate is limited by N or P. However, the N:P ratio for maximum growth may vary from species to species. In microalgae growth theory has been assumed that multiple nutrients limited growth, and it has been experimentally proven: (1) The relationship between external nutrient concentration and uptake rate is expressed by the Michaelis-Menten Kinetic Model. (2) The relationship between a single limiting nutrient and growth rate also achieves concentration saturation. Combining these relationships, including a growth model with a single limiting nutrient, was tested in both stable and variable conditions of light and temperature conditions. (3) Regarding multiple limiting nutrients, Liebig's law of minimum has been shown to hold. Microalgae's growth is dependent only on the intracellular concentration of the most limiting nutrient (Klausmeier et al., 2004).

Microalgal growth kinetics are expressed with Monod and Droop models only when nutrient concentration is taken into account (Aslanbay Güler & İmamoğlu, 2021). In batch culture systems, if only one of the nutrients is limited for growth, first of all, the nutrient ratio decreases rapidly and eventually microalgae growth stops, while in non-batch culture systems, growth is limited. Experimentally, the effect of limited substrate or nutrient is determined by the Monod equation. This model is mostly used in cases where the effect of a single nutrient in the culture medium or on conditions with a low concentration nutrient medium. Models depend on parameters, which are not always easily measurable or available, and as a result, mass flows, dynamics, and physiological variables often cannot be adequately detected. Traditional mass-based models (usually single nutrient, N or C) are generally relatively simple and operate using classical intake kinetic relationships. However, even these classical relationships are poorly characterized for many species or highly variable under different growing conditions (Aslanbay Güler & İmamoğlu, 2021).

Dairy products are included in the functional food group due to the scientific support for their positive effects on health. Whey powder is the wieldiest used commercially dairy product. Because drying the whey both extends the shelf life of the product and provides easy portability. Whey powder (WP) is defined as a substance containing high lactose, which is formed as a result of turning whey into powder form by subjecting it to a drying process in appropriate facilities. Today, WP is safely used as a food additive in baby foods, yogurts, candies, bakery products, meat products, soups, sauces and beverages (Yıldırım & Güzeler, 2013).

In general research with *H. pluvialis* has focused on biomass change and increasing astaxanthin pigment content depending on medium conditions (light, temperature, etc.) (Göksan, 2003; Eriştürk, 2005; Akın, 2005; İmamoğlu, 2005; Köksal, 2008; Çokcan, 2015; Giritli Yılmaz, 2019; Kalmaoğlu; 2020). This study aimed to examine the effect on the growth of *H. pluvialis* by using whey powder.

Material and Methods

Microalgae and Culture Conditions

The microalga *H. pluvialis* was provided by Çukurova University, Faculty of Fisheries Turkey. 70% demineralized WP (moisture 0.87%, lipid 1%, protein 6.6%, ash 5.5%-8.6%, lactose 82%, pH 6.5, salt 1.98%, color light yellow) from Cici Dairy Industry Trade Inc. The research has started to be carried out in the Basic Sciences Laboratory of Atatürk University, Faculty of Fisheries, Experimental Research Unit. Initially, microalgae were grown in 10 ml tubes. The microalgae that developed in the tubes were first taken into 100 ml and then 250 ml Erlenmeyer flasks, and were grown at 25°C, in a 43.15 μmolm^{-2}s^{-} lighting and 110 rpm shaking incubator (JRS Lab 32 brand) in a 16:8-hour daylight period. For intensive production of microalgae, they were taken into 3l glass containers with lids in the Algae Unit in the research unit.

In the experiment was used 3N-BBM+V nutrient medium modified as a nutrient medium. It is contained: 25 g/l $NaNO_3$, 2.5 g/l $CaCl_2.2H_2O$, 7.5 g/l K_2HPO_4, 7.5 g/l $MgSO_4.7H_2O$, 0.75 g/l Na_2EDTA, 17.5 g/l $KH_2PO_3.3H_2O$, 2.5 g/l NaCl and supply of essential micronutrients ($FeCl_3.6H_2O$, $MnCl_2.4H_2O$, $ZnCl_2$, $CoCl_2.6H_2O$ and $Na_2MoO_4.2H_2O$) (Anonymous, 2022).

Experimental Procedure

In the experiment, 3N-BBM+V + 5 g WP, 3N-BBM+V +10 g WP and the 3N-BBM+V +15g WP was added to medium per liter. After the sterilization application, predetermined algae prepared with 3N-BBM+V medium were inoculated into this medium. The control group (C) and the added groups (W5, W10 and W15) were formed with 3 replications each (Bold, 1949; Bischoff & Bold, 1963). No nutrient medium was added to any group, including the control group, during the trial period. pH was controlled by the on-demand injection of carbon dioxide into the air stream entering the culture. The room temperature of the experimental environment was adjusted to be 25°C. The experiment was completed on the 14th day, which is the transition phase.

Analytic Methods

Determination of microalgal growth

Cell numbers were made daily to examine the development period of *H. pluvialis*. 3 ml of homogeneous microalgae sample was taken and Lugol solution was dropped in the counting chamber and left for the night. Then, cell counts were made under the Zeiss Primo Vert model invert microscope (200X, 400X) (Utermöhl, 1958; Anonymous, 2003).

$$Phytoplankton\ count\ \left(\frac{cells}{ml}\right) = \frac{CxAt}{AsxSxV} \quad (1)$$

In this equality;

C: Number of organism (cell),
At: Count cell bottom area (mm^2),
As: Microscope field of view (mm^2),
S: Number of counted areas (cell),
V: Sample volume precipitated (ml).

The biomass of *H. pluvialis* was calculated every day during the production phase. Biomass measurement was done with a spectrophotometer to measure optical density. 50 ml of the homogeneous sample was taken and centrifuged at 13400 rpm and 4°C for 5 minutes. Then, A supernatant was discarded. The supernatants were dried at 80°C for 24 h. Readings were made at a wavelength of 680 nm on a Shimadzu UVmini-1240 brand spectrophotometer. Microalgae biomass was calculated from the following formula (Kang et al., 2005).

$$Dry\ cell\ weight\ (g/l) = 0.668 \times A_{680} \quad (2)$$

Kinetic Modeling

The Monod equivalence model is used when only one nutrient is limiting in the microalgae growth research. This equivalence assumed that the temperature and light intensity are constant throughout the production (Bougaran et al., 2010).

$$Monod\ equivalence: \mu = \frac{\mu_{max} \cdot C_s}{K_s + C_s} \quad (3)$$

where, μ, specific growth rate (day^{-1}); C_s, substrate concentration (mgl^{-1}); μ_{max}, maximum specific growth rate (day^{-1}); K_s, Monod saturation constant (mgl^{-1}).

Statistical Analyzes

The variation of *H. pluvialis* biomass, cell count, specific growth rate according to Monod equivalence, depending on groups and days was determined by One-Way (ANOVA) test using IBM SPSS 20. The significance of the differences was evaluated according to the DUNCAN test.

Results

Cells Number of H. pluvialis

The difference in the cell number of *H. pluvialis* according to the days and groups was found to be statistically significant ($p<0.05$) (Table 1). The mean values of the cell number according to the groups (C, W5, W10 and W15) were determined as 763.34±419.62 cells/ml, 951.60±388.20 cells/ml, 1105.27±380.35 cells/ml and 978.63±411.07 cells/ml, respectively. In this study, the number of cells started to increase from the 4th day, in all groups except the control group, the stationary phase started from the 6th day and a decrease was observed after the 12th day. In the control group, after reaching the highest cell count on the 8th and 9th days, a rapid decrease was detected (Figure 1).

Table 1. Change of *H. pluvialis* cell number (cells/ml) depend on groups and days (n=3; Mean±SD)

Day	C	W5	W10	W15
1	358.57 ±33.07^{Be}	391.86 ± 58.22^{ABe}	405.18 ±12.44^{Ae}	473.66 ±106.49^{ABe}
2	360.47 ±8.24^{Be}	499.34 ± 0.00^{ABe}	423.25 ±23.24^{Ae}	440.37 ±28.01^{ABe}
3	399.47 ±0.00^{Be}	391.86 ± 8.24^{ABe}	435.61±10.80^{Ae}	470.80 ±64.50^{ABe}
4	459.39 ±0.00^{Bd}	752.34 ± 38.53^{ABd}	959.68 ±79.96^{Ad}	786.58 ±51.07^{ABd}
5	1034.82 ±37.99^{Bb}	1293.52 ± 64.33^{ABb}	1314.45 ± 31.43^{Ab}	867.42 ±0.00^{ABb}
6	1078.57 ±0.00^{Bab}	1269.75 ±116.78^{ABab}	1372.47 ±30.06^{Aab}	1192.70 ±0.00^{ABab}
7	1065.25±42.74^{Bab}	1284.01±0.00^{ABab}	1284.01 ± 49.99^{Aab}	1308.74 ±70.84^{ABab}
8	1392.44 ±0.00^{Bab}	1315.40 ±0.00^{ABab}	1412.41 ±9.88^{Aab}	1406.71 ±0.00^{ABab}
9	1560.79 ±0.00^{Ba}	1520.84 ±12.44^{ABa}	1520.84 ±15.72^{Aa}	1412.41 ±0.00^{ABa}
10	1113.76±23.06^{Bab}	1209.82 ±34.59^{ABab}	1397.20 ±9.17^{Aab}	1426.68 ±2.85^{ABab}
11	771.36 ±65.71^{Bab}	1216.48 ±31.30^{ABab}	1216.48 ±13.18^{Aab}	1420.97 ±0.00^{ABab}
12	468.90 ±78.37^{Bc}	998.68 ±135.56^{ABc}	1386.73±29.65^{Ac}	1398.15 ±0.00^{ABc}
13	325.28 ±44.479^{Bd}	752.34 ±101.46^{ABd}	1158.46±25.36^{Ad}	696.22 ±0.00^{ABd}
14	297.70 ± 1.647^{Bd}	426.10 ± 116.58^{ABd}	1102.27±12.44^{Ad}	399.47 ±0.00^{ABd}

Note: *A, AB, B: Capital letters show the difference between groups on the same day and the difference between groups with different capital letters on the same line is statistically significant (p<0.05). a, ab, b, c, d, e: Lowercase letters indicate the difference between days in the same group, and the difference between days with different lowercase letters in the same column is statistically significant (p<0.05).

Table 2. Change of *H. pluvialis* biomass (gl^{-1}) depend on groups and days (n=3; Mean±SD)

Day	C	W5	W10	W15
1	0.57±0.015^{Bc*}	0.66± 0.044^{ABc}	0.46± 0.031^{ABc}	0.77± 0.017^{Ac}
2	0.56±0.051^{Bc}	0.64± 0.015^{ABc}	0.46± 0.000^{ABc}	0.83± 0.006^{Ac}
3	0.55±0.017^{Bc}	0.63± 0.010^{ABc}	0.47± 0.021^{ABc}	0.83± 0.010^{Ac}
4	0.53±0.000^{Bc}	0.63± 0.006^{ABc}	0.50± 0.030^{ABc}	0.83± 0.006^{Ac}
5	0.56±0.035^{Bc}	0.64± 0.012^{ABc}	0.64± 0.025^{ABc}	0.86± 0.023^{Ac}
6	0.56±0.026^{Bc}	0.64± 0.006^{ABc}	0.63± 0.023^{ABc}	0.86± 0.010^{Ac}
7	0.57±0.029^{Bc}	0.63± 0.029^{ABc}	0.65± 0.013^{ABc}	0.88± 0.012^{Ac}
8	0.57±0.058^{Bc}	0.67± 0.017^{ABc}	0.69± 0.035^{ABc}	0.87± 0.012^{Ac}
9	0.67±0.035^{Bb}	1.56± 0.196^{ABb}	1.93± 0.082^{ABb}	1.56± 0.297^{Ab}
10	1.27±0.058^{Bab}	1.47± 0.195^{ABab}	1.89± 0.553^{ABab}	1.98± 0.391^{Aab}
11	1.35±0.078^{Ba}	1.70± 0.088^{ABa}	2.01± 0.106^{ABa}	2.26± 0.067^{Aa}
12	1.43±0.006^{Ba}	1.66± 0.053^{ABa}	2.14± 0.134^{ABa}	2.32± 0.182^{Aa}
13	1.32±0.089^{Bb}	1.57± 0.553^{ABb}	1.45± 0.398^{ABb}	1.44± 0.349^{Ab}
14	1.26±0.076^{Bb}	1.37± 0.324^{ABb}	1.29± 0.159^{ABb}	1.34± 0.097^{Ab}

Note: *A, AB, B: Capital letters show the difference between groups on the same day and the difference between groups with different capital letters on the same line is statistically significant (p<0.05). a, ab, b, c, d, e: Lowercase letters indicate the difference between days in the same group, and the difference between days with different lowercase letters in the same column is statistically significant (p<0.05).

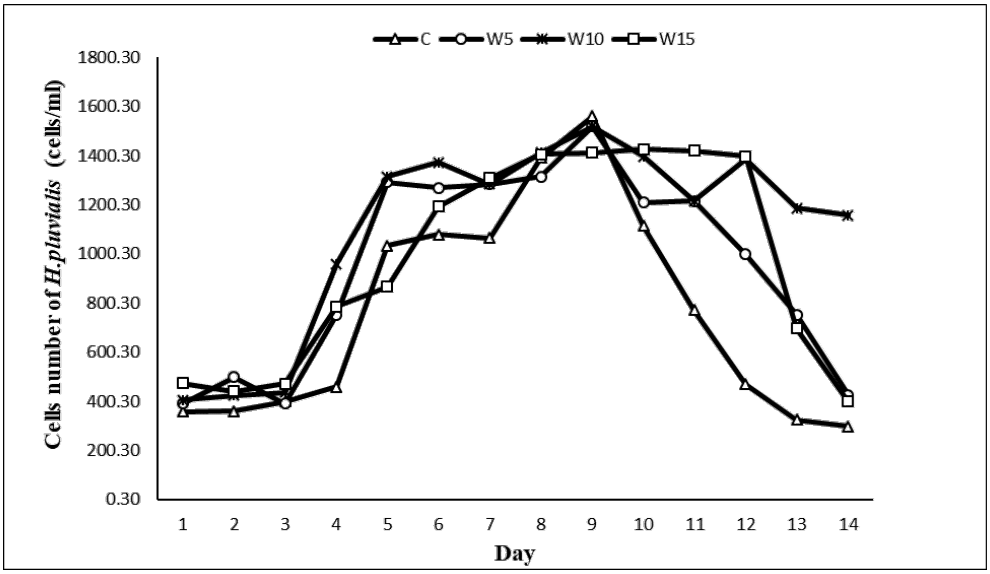

Figure 1. Change of *H. pluvialis* cell number (cells/ml) depend on days and groups

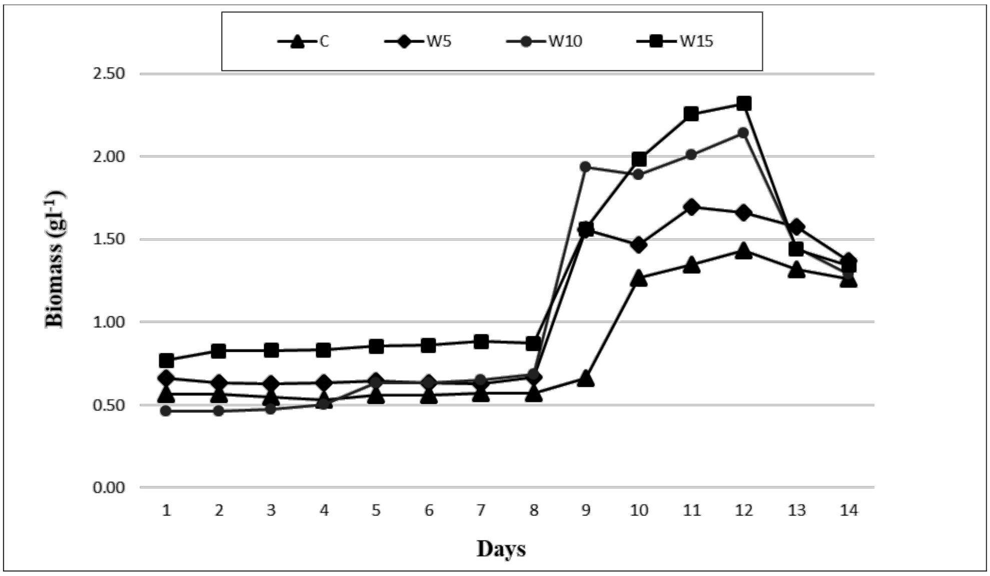

Figure 2. Change of *H. pluvialis* biomass (gl^{-1}) depend on days and groups

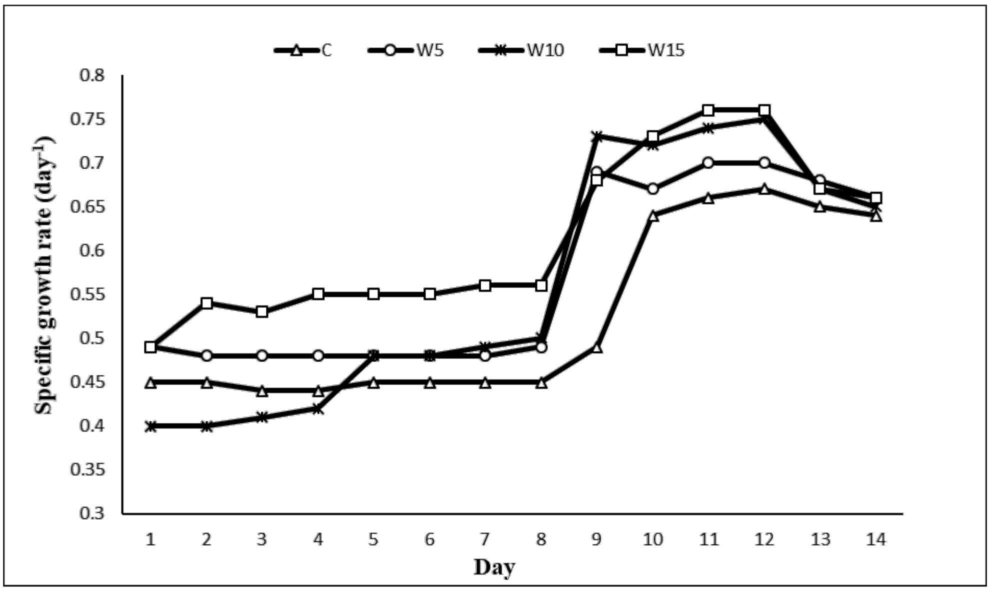

Figure 3. Change of *H. pluvialis* specific growth rate (day^{-1}) depend on days and groups

Table 3. Change of *H. pluvialis* specific growth rate (day^{-1}) depend on groups and days (n=3; Mean±SD)

Day	C	W5	W10	W15
1	0.45±0.00^{Bc*}	0.49±0.00^{ABc}	0.40±0.00^{ABc}	0.49±0.00^{Ac}
2	0.45±0.00^{Bc}	0.48±0.00^{ABc}	0.40±0.00^{ABc}	0.54±0.00^{Ac}
3	0.44±0.00^{Bc}	0.48±0.00^{ABc}	0.41±0.00^{ABc}	0.53±0.00^{Ac}
4	0.44±0.00^{Bc}	0.48±0.00^{ABc}	0.42±0.00^{ABc}	0.55±0.00^{Ac}
5	0.45±0.00^{Bc}	0.48±0.00^{ABc}	0.48±0.00^{ABc}	0.55±0.00^{Ac}
6	0.45±0.00^{Bc}	0.48±0.00^{ABc}	0.48±0.00^{ABc}	0.55±0.00^{Ac}
7	0.45±0.00^{Bc}	0.48±0.00^{ABc}	0.49±0.00^{ABc}	0.56±0.00^{Ac}
8	0.45±0.00^{Bc}	0.49±0.00^{ABc}	0.50±0.00^{ABc}	0.56±0.00^{Ac}
9	0.49±0.00^{Bb}	0.69±0.00^{ABb}	0.73±0.00^{ABb}	0.68±0.00^{Ab}
10	0.64±0.00^{Bab}	0.67±0.00^{ABab}	0.72±0.00^{ABab}	0.73±0.00^{Aab}
11	0.66±0.00^{Bab}	0.70±0.00^{ABab}	0.74±0.00^{ABab}	0.76±0.00^{Aab}
12	0.67±0.00^{Ba}	0.70±0.00^{ABa}	0.75±0.00^{ABa}	0.76±0.00^{Aa}
13	0.65±0.00^{Bab}	0.68±0.00^{ABab}	0.67±0.00^{ABab}	0.67±0.00^{Aab}
14	0.64±0.00^{Bb}	0.66±0.00^{ABb}	0.65±0.00^{ABb}	0.66±0.00^{Ab}

Note: *A, AB, B: Capital letters show the difference between groups on the same day and the difference between groups with different capital letters on the same line is statistically significant (p<0.05). a, ab, b, c, d, e: Lowercase letters indicate the difference between days in the same group, and the difference between days with different lowercase letters in the same column is statistically significant (p<0.05).

H. pluvialis Biomass

The difference in *H. pluvialis* biomass according to the day and groups was found to be statistically significant (p<0.05). The mean biomass value was determined the lowest in the control group (0.84±0.36 gl^{-1}), and the highest value in the W15 group (1.26±0.55 gl^{-1}) (Table 2). The highest biomass value was calculated on the 12th day in all groups except W5. Biomass increase started after the 4th day and reached the highest value on the 12th day (Figure 2).

Kinetic Modeling (Monod Equivalence)

Changes in specific growth rates were found to be significant (p<0.05) depending on between the groups and the days. It was calculated that the growth rate was the highest value (0.61 day^{-1}) in the W15 group. This group was followed by W5, W10 and control groups with an average of 0.57 days^{-1},0.56 days^{-1} and 0.52 days^{-1}, respectively (Table 3).

When the specific growth rate of the *H. pluvialis* was calculated by Monod equivalence, it was determined that the growth rate increased in all groups from the 4th day, similar to the change in biomass, and reached the maximum level on the 12th day (Figure 3).

During the experiment, the highest value of cell number in the W10 group has been found compared with the other groups. The lowest biomass has been calculated at 0.84±0.36 gl^{-1}in the control group, and the mean biomass value in the W5 and W10 groups was found 1.03±0.46 gl^{-1}and 1.09±0.64 gl^{-1}, respectively. The highest biomass value has been calculated as 1.26±0.55 gl^{-1} in the W15 group. The mean specific growth rate was determined as 0.52±0.09 day^{-1} in the control group, 0.56±0.1 day^{-1} in the W5 group, 0.56±0.14 day^{-1} in the W10 group, and 0.61±0.09 day^{-1} in the W15 group, respectively (Figure 4).

Discussion

Whey powder does not show the same effect in all microalgae species. According to Girard et al. (2014), *S. obliquus* reached the highest cellular concentrations (25±4×10^6 cells/ml); It has been reported that lactose supports the growth of *Scenedesmus* but does not change the cell number of *C. protothecoides*. In this study, it was determined that the cell number of *H. pluvialis* to which WP was added increased when compared to the control group.

The difference in cell numbers between the groups throughout the study was found to be statistically significant (p<0.05). The lowest cell number was calculated in the control group, and the highest cell number was calculated in the W10 group. In *Chlorella prenoidosa* culture, tofu whey was added to different concentrations (0, 20%, 40%, 60%, 80% and 100%) and its effect on cell growth was investigated. They found that the use of whey wastewater increased the number of cells more than only the BBM medium, however, the cell number was 83.5×10^6 cells/ml in the use of only whey wastewater. They reported that whey is a suitable and inexpensive nutrient medium to increase cell number in microalgae cultures (Wang et al., 2018).

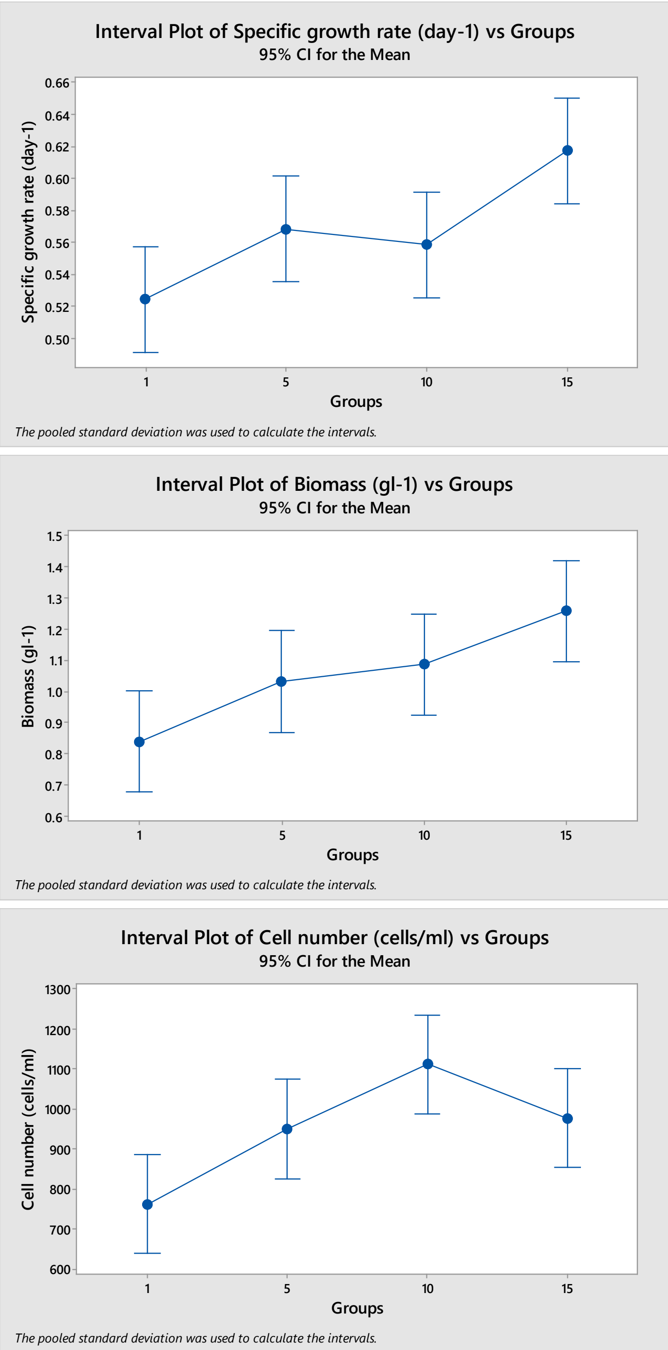

Figure 4. Change of *H. pluvialis* cells number (cells/ml), biomass (gl^{-1}) and specific growth rate (day^{-1}) depend on groups

It has been reported that in addition to the presence of nitrogen and phosphorus basic nutrients in microalgae biomass increase, the addition of inorganic and organic carbon increases photosynthesis (Girard et al., 2014). Studies conducted on some species of *Scenedesmus* and *Chlorella* in the Chlorophyta group have reported that the use of whey and/or powder increases the biomass more than using only the nutrient ratios suitable for the species (BBM, BG-11, etc.). However, in the same studies, the addition of glucose to the use of whey and/or whey powder increased the biomass 3-4 times, while the biomass of *C. prenoidosa* was 0.28 gl^{-1} only in BG-11 medium, its biomass was determined 4.76 gl^{-1} in the medium created by adding whey and glucose (Girard et al., 2014; Tsolcha et al., 2015; Wang et al., 2018).

As a carbon source, glucose and galactose were applied to the *Chlorella vulgaris* medium; They calculated the biomass value as 1.22 gl^{-1} only in WP application and as 2.24 gl^{-1} in only glucose + galactose application (Abreu et al., 2012). In this study, the highest biomass value was determined as 2.32± 0.18 gl^{-1} in the use of 15gl^{-1} whey powder. In the experiment we have done at different concentrations, it is predicted that the use of WP may increase biomass depending on the species.

The use of WP in microalgae media has a specific growth rate stimulating effect due to its high nutrient salts (N, P) content (Abreu et al., 2012). *Chlorella vulgaris*, which is also in the Chlorophyta group, was applied to the medium as a carbon source of whey powder, glucose and galactose. The specific growth rate was determined as 0.43 day^{-1} in the use of only whey powder and as 0.47 day^{-1} in the application of whey powder and glucose+galactose (Abreu et al., 2012). In this study, the highest specific growth rate was calculated as 0.76 day^{-1} in the use of 15 gl^{-1} whey powder. In studies with multiple limiting nutrients, the growth rate only depends on the microalgae intracellular concentration of the most limiting nutrient (Klausmeier et al., 2004).

Conclusion

Microalgae are used in many fields from the food industry to the pharmacy. The biggest problem in microalgae culture in these sectors is increasing the biomass of the selected species and supplying the lack of a cheap culture medium.

As a result, in this study, WP a by-product of the dairy industry was added to the culture medium for the development of *H. pluvialis* and at the end of the experiment, it was determined that WP had a positive effect on biomass. The addition of 15 gl^{-1} WP for *H. pluvialis* culture increased both the biomass and specific growth rate of *H. pluvialis*. The use of WP as a medium is recommended due to its low-cost as well as increasing biomass.

Acknowledgements

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Compliance With Ethical Standards

Authors' Contributions

Both authors have contributed equally to the paper. 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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RESEARCH ARTICLE

A discussion on alternative fuel criteria for maritime transport

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ABSTRACT

Alternative marine fuels are considered an important solution for reducing ship emissions from fossil fuels. These fuels have similar energy content with fossil fuels, but they create much less environmental burden during their use due to the elements they contain (or not), the ratio of elements to each other and different combustion characteristics. On the other hand, for these fuels to replace fossil fuels, they must meet a number of important criteria and conditions. These are divided under four main titles: Economic, technical, environmental, social and other. In addition, examining the environmental impacts of alternative fuels from a life-cycle perspective is also important for determining the holistic and cumulative impacts. In this study, the criteria determined for alternative marine fuels were evaluated from the life cycle perspective and it was investigated which criterion is the most important in terms of life cycle. Thus, it is aimed to summarize the assessments of the criteria for acceptance of the alternative fuels.

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Introduction

Ships are responsible for 6.8% of the world's total energy demand (IEA, 2020) and 2.89% of the total anthropogenic emissions generation, and moreover, if the current situation is not changed, the total ship-related emission amount will increase by 90-130% and 50% in 2050 compared to 2008 and 2018, respectively (Faber et al., 2020).

Of the ship-related emissions, the total number of which is thought to be around 450 (Kollamthodi et al., 2008), only a few are produced in quantities worth examining. They are also the pollutants with the greatest environmental risk. Emissions evaluated by the International Maritime Organization (IMO) within the scope of the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78) are ozone-depleting substances (ODSs), nitrogen oxides (NO_x),

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sulphur oxides (SO_x), particulate matter (PM) and volatile organic compounds (VOCs).

Among these emissions, which are evaluated within the scope of MARPOL Annex-VI, ODSs are completely prohibited on ships as of 1 January 2020 with the rules introduced by Regulation 12, and Regulation 15 imposes restrictions on the production of VOCs. In addition, various restrictions have been placed on NO_x and SO_x emissions through Regulation 13 and Regulation 14, respectively, in special regions called Emission Control Areas (ECA) covering the coasts of North America, the Caribbean waters of the USA, the Baltic Sea and the North Sea. The constraints are shown in Table 1 and Table 2 (IMO, n.d.-a, n.d.-c).

In addition, according to the rules brought by the European Union (EU), as of January 1, 2020, all ships sailing in EU waters other than ECA should use fuel containing up to 0.5% sulphur. In 2018, China declared ECA to cover the Yangtze and Xi-Jiang Rivers and all territorial waters, and as of January 1, 2020, the sulphur content of the fuel used by each ship sailing in these regions should not be higher than 0.1% (Zhao et al., 2021). Sulphur restrictions for regions currently in the world are presented in Figure 1.

With its Initial IMO Strategy on Reduction of GHG Emissions from Ships report published in 2018, IMO has set the chart for the next decade for the decarbonization of the maritime industry. The report has been prepared in line with the United Nations (UN) Sustainable Development Goals (SDGs) and reveals ways to reduce ship-related carbon dioxide (CO_2) emissions by 40% by 2030 and by 70% by 2050 (IMO, n.d.-b, 2018; United Nations, 2020). Short, medium and long-term methods are presented in Table 3.

According to Table 3, alternative fuels are introduced and accepted as an important solution in all short, medium and long-term measures. On the other hand, there are serious obstacles to the acceptability of these fuels, which have significant disadvantages as well as great advantages. While most of the studies on alternative fuels primarily focus on environmental impacts, economic impacts have recently been taken into consideration in a comprehensive manner. On the other hand, alternative fuels must meet various criteria in order to be accepted in all respects. In this study, alternative fuels were briefly introduced and an evaluation of the acceptance criteria was made, and this is the first study in which only the criteria were evaluated and interpreted from a life cycle perspective.

Assessment of Criteria for Alternative Marine Fuels

Although the use of alternative fuel is only one of the various methods used to reduce and zero ship-related emissions, it is the only method that can achieve 100% success in emission reduction, and its applicability and market acceptability is extremely high. On the other hand, it is a difficult process for conventional fossil fuels to be replaced by new fuels due to their strong infrastructure, popularity and widespread usage networks. Alternative fuels must meet some very important criteria in order to replace conventional fossil fuels. These criteria can be summarized under six titles as economic, technical, environmental, social, demand and other:

Alternative fuels can be classified as; ammonia, biofuels (especially biodiesel), dimethyl ether, ethanol, hydrogen, liquefied natural gas (LNG), liquefied petroleum gas (LPG), and methanol.

Table 1. The limits for NO_x (IMO, 2020a)

Tier	Ship Construction Date on or after	Total Weighted Cycle Emission Limit (g/kWh) n=Engine's Rated Speed (RPM)		
		n<130	*n=130-1999*	*n≥2000*
Tier I	1 January 2000	17.0	$45 \times n^{-0.2}$	9.8
Tier II	1 January 2011	14.4	$44 \times n^{-0.2}$	7.7
Tier III (ECA)	1 January 2016	3.4	$9 \times n^{-0.2}$	2.0

Table 2. The limits for SO_x (IMO, 2020b)

Outside an Emission Control Area	Inside an Emission Control Area
4.50% prior to 1 January 2012	1.50% prior to 1 July 2010
3.50% on and after 1 January 2012	1.00% on and after 1 July 2010
0.50% on and after 1 January 2020	0.10% on and after 1 January 2015

Table 3. IMO Initial Strategies (adopted from IMO (2018))

Period	Strategies
2018-2023	— Developing the existing energy efficiency framework with a focus on EEDI and SEEMP, — Developing technical and operational energy efficiency measures for new and existing ships, — Assessment of methane (CH_4) and VOCs emissions, — Developing national action plans and policies for the reduction of GHGs in accordance with IMO rules, — Using shore-side electricity from renewable sources, establishing infrastructure for alternative low and zero carbon fuels, — Establishing research and development units covering ship propulsion, alternative low and zero carbon fuels and innovative technologies to increase ship energy efficiency, — Increasing life-cycle GHG studies for alternative low and zero carbon fuels, — Further studies on emission reduction cost and alternative low and zero carbon fuels
2023-2030	— Developing an application program for the efficient use of alternative low and zero carbon fuels, — Implementing operational energy efficiency measures for new and existing ships, — Developing new and innovative emission reduction mechanisms
2030-	— Following the development of zero-carbon fuels to assess decarbonization in the second half of the 21st century, — Providing incentives for the development of other new and innovative emission reduction mechanisms

The economic criterion most basically includes a pricing policy that can compete with conventional fossil fuels. In addition, necessary machinery (and machinery equipment) modifications, specialist personnel (and training) costs, infrastructure costs, and life-cycle costs, including production, distribution, storage and bunkering processes, are also important factors (Brynolf, 2014; Thomson et al., 2015; Svanberg et al., 2018; Hansson et al., 2019, 2020; Andersson et al., 2020). What makes this criterion more important than the others is that the cost is placed first by the shipowners (Eise Fokkema et al., 2017). The most important advantage of conventional fossil fuels compared to alternative fuels is that they have a strong infrastructure based on centuries of experience. Conventional fossil fuels are not expected to incur additional costs, as their operational responses are well known. Personnel are trained on these fuels and do not require additional training costs. For all these reasons, the economic criterion is seen as the first hurdle to be overcome for the use of an alternative fuel in global scale. It is also the most difficult obstacle because it creates a vicious circle in which demand remains limited, as the costs in the supply system cannot be reduced without an increase in demand. For this reason, governmental incentive may be required in order to exceed the economic criterion at the desired level.

The technical criterion relates to the use of the fuel on board and includes machinery (and machinery) modifications, fuel storage conditions, supply and infrastructure constraints (Brynolf, 2014; Thomson et al., 2015; Svanberg et al., 2018; Hansson et al., 2019, 2020; Andersson et al., 2020). One study concluded that technical conditions are accepted as the most important criteria by shipowners (Mandić et al., 2021). Technical conditions of alternative fuels need to be at least close to conventional fossil fuels' level to overtake them. Conventional fossil fuels do not present unusual surprises as they are used under proven conditions and almost all existing ship systems have been developed for conventional fossil fuels. The engines have been designed, the storage conditions have been adjusted, and the other engine equipment has been arranged according to the characteristics of conventional fossil fuels. Moreover, infrastructure and supply facilities are designed to meet the needs of conventional fossil fuels. Since the technical infrastructure required by the majority of alternative fuels is very different from that of conventional fuels, any technical arrangement that needs to be made mean an additional cost and loss of time. Moreover, new regulations bring the risk of mistakes and may cause additional and unpredictable costs.

The environmental criterion is the reason for the emergence of alternative fuels and includes the regulation of total life cycle emissions, and in particular, emissions during operation. In addition, human health indirectly falls within the scope of this criterion (Brynolf, 2014; Thomson et al., 2015; Svanberg et al., 2018; Hansson et al., 2019, 2020; Andersson et al., 2020). The environmental criterion is most accepted among academics

(Mandić et al., 2021). This criterion constitutes a very important basis for the existence of alternative fuels, and the compliance of alternative fuels with existing and then potential environmental regulations has a primary priority. The existence of alternative fuels is very important for the reduction of carbon emissions (decarbonization), which is the main cause of global climate change, and also for the zeroing or reduction of other ship-related emissions (SO_x, NO_x, PM, etc.). Although these fuels are generally quite successful from an environmental point of view during the operation process, more rigorous studies are required on the emissions that occur throughout their life cycle.

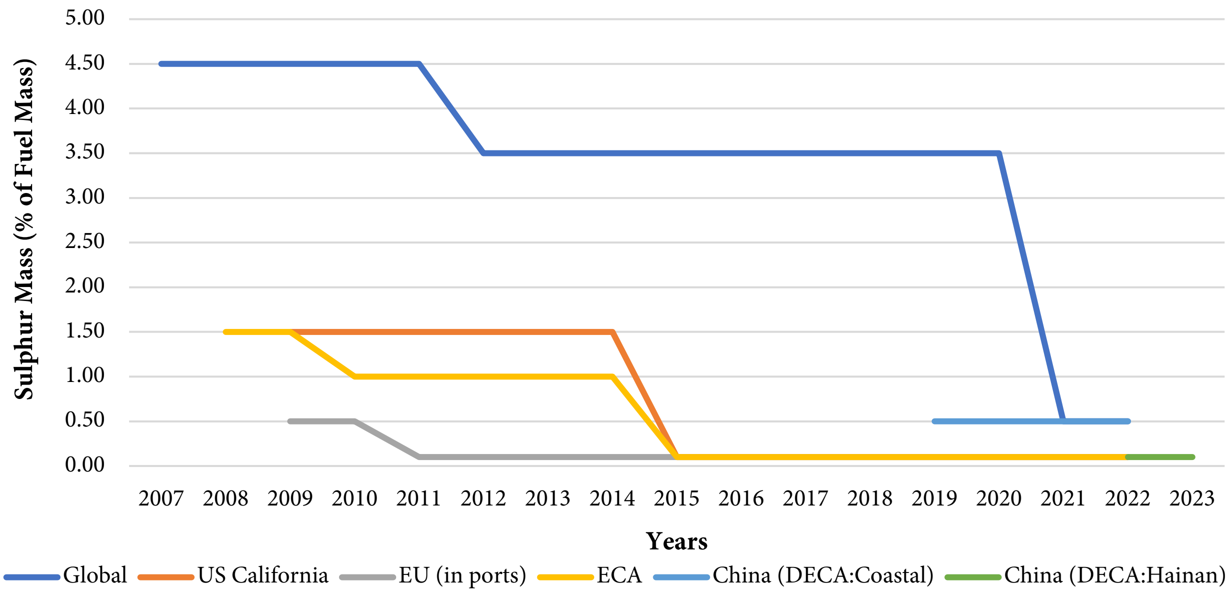

Figure 1. National and international sulphur content limits (adopted from Fan et al. (2021))

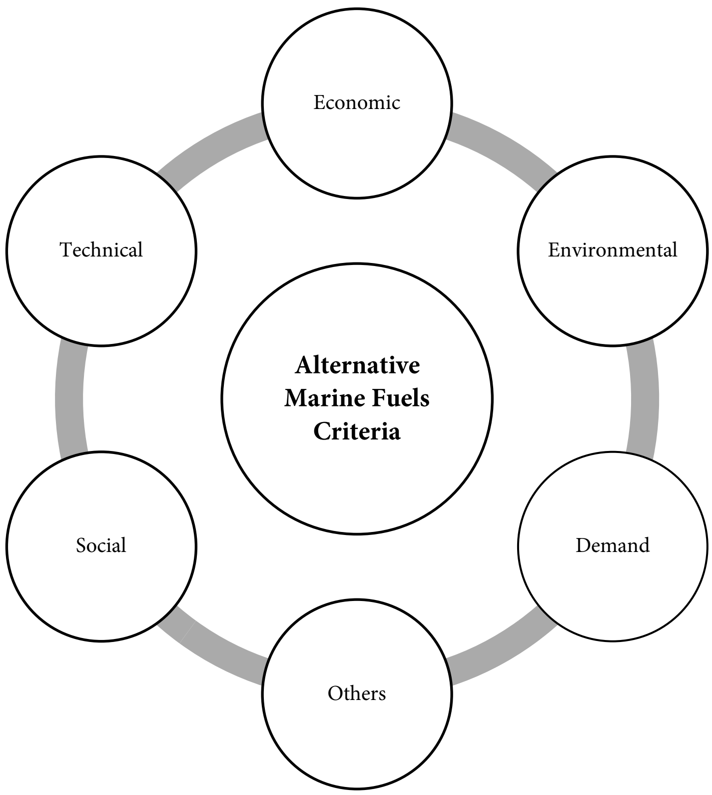

Figure 2. Alternative marine fuel criteria

Table 4. The summary of alternative fuel selection criteria

Title	Criteria	Reference
Economic	Initial investment Operational cost Fuel cost	Brynolf (2014), Thomson et al. (2015), Svanberg et al. (2018), Hansson et al. (2019, 2020), Andersson et al. (2020)
Technical	Infrastructure Supply chain Fuel and propulsion system	Brynolf (2014), Thomson et al. (2015), Svanberg et al. (2018), Hansson et al. (2019, 2020), Andersson et al. (2020)
Environmental	Climate change Human health Life cycle emissions	Brynolf (2014), Thomson et al. (2015), Svanberg et al. (2018), Hansson et al. (2019, 2020), Andersson et al. (2020)
Social	Safety	Hansson et al. (2019, 2020)
Demand	Price competition	Thomson et al. (2015)
Other	Logistics Ethics Politics	Brynolf (2014), Andersson et al. (2020)

Note: Fuels that successfully meet all these criteria are called drop-in Hsieh & Felby (2017).

The social criterion includes the risks that may occur especially during the operation (Hansson et al., 2019, 2020). Cargo and passenger transportation must, above all, ensure the safe transport of cargo and passengers (and crew). For this reason, it is necessary to determine the risks that may occur and take the necessary precautions sensitively. While the well-known characteristics of conventional fossil fuels and the extensive training process will minimize the risks that may arise, the elimination of new risks arising from the different characteristics of alternative fuels, some of which are quite difficult to overcome, will require some additional measures.

Other criteria include logistics, public opinion, ethics, and political and strategic perspective (Brynolf, 2014; Andersson et al., 2020). An alternative fuel accepted by the society is a fuel with high environmental performance and does not increase the product price due to its transportation cost, and public pressure is a significant force. The ethical approach focuses on whether it would be right to allocate the lands to be used for food production, especially for bio-based fuels, for fuel production. The political and strategic approach examines the effects of governments and companies on the creation of new jobs and the strengthening of local fuel producers with a long-term view. The summary of these criteria is presented in Table 4.

Results and Discussion

80-85% and 15-20% of the fuels currently used are residual fuels (Heavy Fuel Oil-HFO) and distillate fuels (Marine Diesel Oil-MDO and Marine Gas Oil-MGO) (Ammar, 2019). In a more recent study, these rates are given as 72% and 26%, respectively (DNV GL, 2021). Alternative fuels are expected to replace especially MDO and MGO, and the criteria listed above have emerged as a result of the comparisons made with these two fuels.

Ammonia can be used directly as fuel in internal combustion engines or alkaline fuel cells (Dimitriou & Javaid, 2020; Julia Hansson et al., 2020). Ammonia does not contain carbon and sulphur, thus, it is not a source of CO_2, CO, and unburned HC emissions, as well as SO_x and PM emissions (Hansson & Fridell, 2020). On the other hand, the greatest problem with ammonia is to be inhaled by the crew due to its high toxicity (Trivyza et al., 2020). Besides, a ship that uses ammonia as a fuel needs 1.6-2.3 times more volume and 1.4-1.6 times more weight (Kim et al., 2020). Also, the production cost of ammonia is higher than fossil-based fuels (IEA, 2019).

Hydrogen has a low flash point, high flame speed, a wide flammability range, and high diffusibility and can be used with other fuels (Pan et al., 2014; Kim et al., 2020). Hydrogen produces zero emissions when produced from renewable sources (Ryste, 2019). On the other hand, it is very reactive (Konnov, 2019; Elishav et al., 2020) and has major difficulties in handling and high transportation cost (Plana et al., 2010; Valera-Medina et al., 2017; Nemanič, 2019; Taccani et al., 2020).

LNG is a clean, colourless, odourless, non-toxic, and non-corrosive fuel and is compatible with IMO targets (Buhaug et al., 2009; Arteconi et al., 2010; EC, 2021). LNG usage lowers different types of emissions (Lehtoranta et al., 2021) but increase greenhouse gas emissions due the methane leakage during life-cycle (Schinas & Butler, 2016; Manouchehrinia et al., 2020; Ančić et al., 2020). Besides, LNG is competitively priced with conventional fuels but has a high initial investment cost (Brynolf et al., 2014).

Methanol can use the infrastructure of LNG (Andersson & Salazar, 2015; Verhelst et al., 2019; Ančić et al., 2020) but is toxic and corrosive (Ellis & Tanneberger, 2015; Ammar, 2019; Verhelst et al., 2019). Methanol produces low GHGs and other emissions (Tunér, 2015; Shahhosseini et al., 2018; Tunér et al., 2018) and has an acceptable initial cost (Ellis & Tanneberger, 2015).

While only 0.5% of the ships currently in operation are equipped with alternative energy systems, this rate has increased to 11.84% in the ships ordered (Lee et al., 2020). It is predicted that this rate will increase in the following years with the coming of new environmental regulations, but there are serious limitations that need to be overcome.

First of all, the ethical background encountered in the production process of bio-based fuels is a difficult problem to overcome. Farmers taking the route of producing fuel raw materials, which are more income-generating, can disrupt the local, regional and national food supply and this is an unacceptable problem in terms of its consequences. Despite their operational success, the impacts of alternative fuels (especially LNG) on life-cycle emissions are still not fully determined, and therefore it is likely to be frowned upon.

Considering life-cycle emissions should be a must-have approach to meet IMO's 2050 targets. Increasing the support for alternative fuels and breaking the vicious circle between supply and demand will be an important step in this direction. In this way, the necessary technical infrastructure will be established and a suitable environment will be created for the dissemination of alternative fuels.

Incentives to be applied to ships with alternative energy systems, project supports to be provided to institutions examining this issue, and various priorities to be provided to these ships will constitute a strong support point for the dissemination of alternative fuels.

More trained personnel would make the use of fuels safer, reduce unforeseen costs due to accidents and therefore make easier for shipowners to accept to use these fuels.

Informing the public is another important issue and it has been observed that South Koreans are ready to accept the additional costs caused by the environmental advantages of alternative fuels (Lee et al., 2020). Although it is not possible to expect this idea to be accepted by other societies, it seems possible to support the orientation of the public towards alternative fuels in cases where environmental and economic balance is achieved.

Conclusion

In today's world, where conventional fossil fuels are still in a very strong position, it is clear that effective measures must be taken in order to achieve environmental targets. Moreover, conventional fossil fuel prices are very sensitive to international crises, such as COVID-19 and Russo-Ukrainian War, whose effects are still not fully quantified. Developing alternatives that are just as powerful as conventional fossil fuels means that the world is not only making significant environmental gains, but also being stronger against potential energy crises.

This is the first study in which the criteria developed for the acceptance of alternative fuels were compiled holistically and examined from a life-cycle perspective. The most important obstacle for alternative fuels to replace fossil fuels is the lack of evaluation of whether these fuels are truly environmentally friendly, as the life cycle emissions of fuels have not yet been fully determined. According to the findings of the study, global acceptance of alternative fuels is possible within an optimization process of criteria with different dimensions. The criteria may come into conflict with each other. Therefore, the superiority of any criterion over the other is possible only from a subjective point of view, and there is no objective advantage. If the effects of the criteria are also examined from the life cycle perspective, it will be possible to get an idea of which one should be prioritized, and the evaluation errors within the criteria themselves can be eliminated. Recently, in addition to the economic dimension of environmentally beneficial practices, the social dimension has also been extensively included in scientific studies. Therefore, in the near future, more attention should be paid to the social dimension of an issue such as alternative fuels that may affect not only the maritime sector but also the lives of ordinary people.

In the light of the criteria evaluations, it is predicted that LNG will be preferred more in the near future due to environmental reasons as well as cost and infrastructure opportunities, but with the development of technology and decreasing costs in the medium term, hydrogen will play a more dominant role in the energy needs of the maritime sector.

Compliance With Ethical Standards

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

The study involves no human participant. For this type of study, formal consent is not required.

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RESEARCH ARTICLE

The seasonal fish diversity of Aliağa, a heavy industry zone on the Turkish coast of the Aegean Sea

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ABSTRACT

The stress on the marine environment caused by industrialization in developing economies is indisputable. The Aliağa region, which has unique features such as being heavily influenced by industrialization and having different types of marine habitats was preferred as a monitoring point. By determining the current status of fish density and diversity, the focus was on obtaining data that could allow future comparisons. Without the seasonal variability, 39 fish species representing 14 families were identified, with two abundant families: ten species in both Sparidae and Labridae. The greatest fish diversity was recorded respectively in the spring, summer and autumn. Abundant species were *Boops boops* with 19.3%, *Chromis chromis* (17.4%), *Spicara smaris* (15.0%) and *Atherina boyeri* (12.5%) in total abundance. A total of 1.89 individuals/m^2 that weighed 20.43 g/m^2 were identified in the study period.

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Introduction

Fishes are an essential component in marine ecosystems; however, at present, they are threatened by anthropogenic stressors (Pauly et al., 2002; Lotze et al., 2006). Fish biodiversity which represents an important indicator in determining ecosystem health (McField & Kramer, 2007), is an assessment of different fish species living in a particular area, and also provides numerous and valuable ecosystem goods and services (Worm et al., 2006; Beaumont et al., 2007; Halpern et al.,2012). The advantages of fish-based monitoring of the ecosystem health described in detail in Whitfield & Elliott (2002) are that; they are present in almost all aquatic ecosystems; have information about the extensive life history and environmental response; they are easy for species identification; harmless

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sampling is available; various trophic levels that provide a long-term record of environmental stress.

Industrial activities play a significant role in the economies of the countries. Aliağa is one of the most important heavy industry zones in Turkey and has the only shipbreaking industry among OECD countries in the Mediterranean region. The negative effects of marine traffic and port activities on marine habitats are ship pollution and emissions, collisions and noise, grounding and anchoring damage, and transportation of non-indigenous species (Abdulla & Linden, 2008; Brynolf et al., 2016). Increasing industrial activities in Aliağa and the environmental effects of these activities make the region riskier.

Long-term data is required to determine the changes in marine ecosystems directly affected by intense industrial activities. This study aimed to determine the current status of fish biodiversity in a marine industrial zone and provide a comparison source for future research.

Material and Methods

The study was conducted at Nemrut Bay (38°45′53″N-26°54′20″E), located southwest of the Aliağa Port complex on the Aegean Sea coast of Turkey (Figure 1).

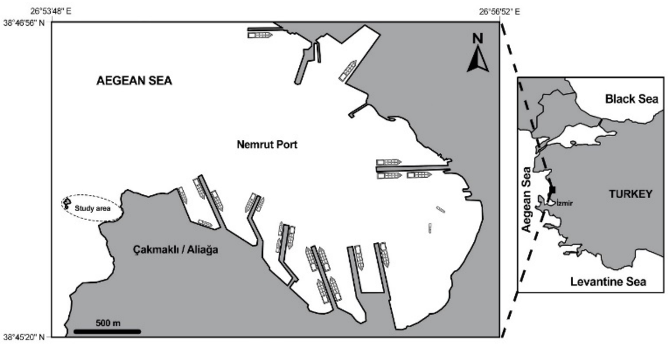

Figure 1. Study area

The non-destructive underwater visual census (UVCs) method (Harmelin-Vivien et al., 1985; Engin et al., 2018) was used following the 700 meters route marked on the map by two free divers both with experience in situ identification of fish species. UVCs were carried out in four seasons (December 2019-October 2020) and on a random date for each season between 9.00 a.m. and 11.00 a.m. The study was conducted on shallow waters between 0-10 meters in depth as they generally have high primary and secondary productivity levels maintaining the richest ichthyofauna (García-Rubies & Zabala, 1990). Two replications were performed (2 × 700 m in the same route) per season. Species, abundances, and size structure of fishes were recorded on a waterproof notepad. Divers simultaneously recorded their observations on the same route. The mean density data were used in the calculations to reduce the error rate that may arise from the divers.

Fish species richness and dominancy were evaluated using the Shannon-Weiner diversity index (H'), Margalef richness index (Dmg) and Pielou's evenness (J') index. Bray-Curtis similarity index, non-metric multidimensional scaling (nMDS), and similarity percentage (SIMPER) were performed using the Primer-E v7 package software to detect seasonal differences.

Results

A total of 10607 individuals (weighing 114.453 kg) of 39 species belonging to 14 families were observed in the study area. The most diverse families of the fish assemblage were Labridae and Sparidae, with 10 species each (Labridae 25.6%; Sparidae 25.6% of the total number of species). Blenniidae and Gobiidae were the other species-rich families, with 4 and 3 species (Table 1; Figure 2).

The highest fish density was found in spring (2.88 fish/m^2), and the highest biomass was found in autumn (28.2 g/m^2). The lowest density (0.5 fish/m^2) and biomass (11.75 g/m^2) were found in winter.

Based on the abundance, *Boops boops* (19.8%), *Spicara smaris* (16.1%) and *Atherina boyeri* (14.8%) were the most abundant species in the spring. In summer, *B. boops* (18.9%) and *Chromis chromis* (18.2%) were abundant fish species, followed by *A. boyeri* (14.6%) and *S. smaris* (13.7%). In autumn, similar to spring and summer, fish assemblage was dominated by *B. boops* (19.2%), *C. chromis* (17.3%), *S. smaris* (15.7%) and *A. boyeri* (13.4%). In winter, *C. chromis* (28%), *B. boops* (14.3%), *Oblada melanura* (12.9 %) and *S. smaris* (11.4%) were abundant fish species in the total number of the counted individual (Table 1).

Based on the seasonal variation of the biomass, *B. boops* (31.7%) was the most dominant fish species, followed by *O. melanura* (12.7%), *S. pilchardus* (7.3%) and *S. smaris* (6.9%) in spring (58.6% of the total biomass). During summer, *B. boops* (38.8%) was dominated the biomass and followed by *O. melanura* (17.2%), *S. pilchardus* (6.4%) and *S. smaris* (6.1%) which represented 68.5% of the total biomass. In autumn, similar to spring and summer fish assemblages dominated by *B. boops* (32.9%) and followed *O. melanura* (18.1%), *S. smaris* (7.0%) and *A. boyeri* (6.4%), both contributing with 64.4% of the total biomass. In winter, compatible with the other seasons, the fish biomass was mostly dominated by *B. boops* (29.7%) and *O. melanura* (14.4%), but differently, an increase in the dominance of *C. chromis* (9.2%) and *D. vulgaris* (8.7%) in biomass was detected (62% of the total biomass).

On the basis of the species richness, the highest number of species (38) was registered in spring, most of the species were belonging to Sparidae (10) and Labridae (9) families. In contrast, the lowest number of species (30) were registered in winter dominance with sparid fishes (9) and wrasses (8) again. A decrease was observed in the number of total recorded species after spring, and equal numbers were determined in summer (35) and autumn (35) (Table 1, Figure 2).

Table 1. The seasonal and annual abundance and biomass results of fish species

Family	Species	Spring		Summer		Autumn		Winter		Annual		IUCN Status
		Percentages (%) in										
		Abundance	*Biomass*	*Abundance*	*Biomass*	*Abundance*	*Biomass*	*Abundance*	*Biomass*	*Abundance*	*Biomass*	
Atherinidae	*A. boyeri*	12.40	6.42	14.63	5.26	13.48	6.44	0.00	0.00	12.54	5.13	LC
Blenniidae	*A. sphynx*	0.02	0.03	0.06	0.07	0.12	0.10	0.29	0.16	0.08	0.09	LC
	P. gattorugine	0.05	0.04	0.03	0.02	0.08	0.04	0.14	0.05	0.06	0.04	LC
	P. tentacularis	0.02	0.01	0.06	0.02	0.04	0.01	0.29	0.05	0.06	0.02	LC
	S. pavo	0.05	0.06	0.03	0.04	0.04	0.03	0.14	0.08	0.05	0.05	LC
Clupeidae	*S. pilchardus*	9.92	7.31	9.14	6.48	5.78	5.24	0.00	0.00	8.01	5.28	LC
Gobiidae	*G. geniporus*	0.12	0.07	0.12	0.03	0.12	0.02	0.43	0.05	0.14	0.04	LC
	G. niger	0.17	0.17	0.24	0.23	0.31	0.22	0.86	0.39	0.27	0.24	LC
	P. quagga	0.50	0.05	0.49	0.02	0.46	0.02	1.43	0.03	0.55	0.03	LC
Labridae	*C. julis*	1.12	1.91	1.62	1.80	1.85	1.97	4.02	2.76	1.64	2.02	LC
	L. viridis	0.10	0.06	0.21	0.10	0.31	0.15	0.14	0.05	0.19	0.10	VU
	S. cinereus	0.15	2.22	0.15	1.49	0.12	0.83	0.29	2.48	0.15	1.54	LC
	S. mediterraneus	0.02	0.01	0.09	0.02	0.15	0.03	0.00	0.00	0.08	0.02	LC
	S. melanocercus	0.30	0.40	0.34	0.22	0.23	0.21	0.86	0.50	0.33	0.29	LC
	S. ocellatus	0.02	0.01	0.06	0.03	0.12	0.09	0.14	0.07	0.07	0.06	LC
	S. roissali	0.12	0.04	0.18	0.08	0.15	0.05	0.00	0.00	0.14	0.05	LC
	S. rostratus	0.00	0.00	0.12	0.03	0.08	0.02	0.29	0.07	0.08	0.03	LC
	S. tinca	0.10	0.39	0.15	0.57	0.15	0.42	0.29	1.22	0.14	0.58	LC
	T. pavo	0.52	1.39	0.40	0.90	0.54	1.77	0.86	1.82	0.51	1.43	LC
Mugilidae	*C. labrosus*	0.30	2.16	0.00	0.00	0.15	0.99	0.00	0.00	0.15	0.76	LC
Mullidae	*M. surmuletus*	0.05	0.08	0.18	0.28	0.19	0.67	0.14	0.32	0.13	0.38	LC
Muraenidae	*M. helena*	0.05	6.07	0.00	0.00	0.00	0.00	0.00	0.00	0.02	1.16	LC
Pomacentridae	*C. chromis*	14.88	5.84	18.29	5.72	17.33	6.02	28.69	9.23	17.44	6.35	LC
Scaridae	*S. cretense*	0.30	0.76	0.24	0.53	0.35	0.55	0.86	2.55	0.33	0.87	LC
Serranidae	*E. costae*	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	DD
	S. scriba	0.22	0.52	0.30	0.61	0.31	0.90	0.86	1.63	0.31	0.84	LC
Sparidae	*B. boops*	19.84	31.77	19.81	38.88	19.25	32.92	14.35	29.77	19.33	34.15	LC
	D. annularis	0.45	1.35	0.70	1.38	1.00	1.89	2.87	5.63	0.82	2.16	LC
	D. puntazzo	0.05	0.15	0.12	0.29	0.12	0.31	0.14	0.25	0.09	0.27	LC
	D. sargus	0.30	1.04	0.40	1.16	0.65	1.79	2.15	5.79	0.54	2.02	LC
	D. vulgaris	0.94	4.02	1.37	4.43	1.96	4.65	5.74	8.77	1.64	5.05	LC
	L. mormyrus	0.22	0.44	0.18	0.17	0.19	0.13	0.00	0.00	0.19	0.19	LC
	O. melanura	10.42	12.71	9.14	17.29	10.40	18.10	12.91	14.50	10.18	16.29	LC
	S. salpa	9.92	4.37	7.16	5.18	8.09	6.29	8.61	4.32	8.53	5.29	LC
	S. aurata	0.07	0.60	0.12	0.48	0.00	0.00	0.14	0.27	0.08	0.31	LC
	S. smaris	16.12	6.89	13.72	6.15	15.79	7.10	11.48	7.16	14.99	6.76	LC
Torpedinidae	*T. marmorata*	0.02	0.62	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.12	LC
Tripterygiidae	*T. melanurus*	0.07	0.02	0.06	0.01	0.04	0.00	0.14	0.01	0.07	0.01	LC
	T. tripteronotum	0.02	0.01	0.06	0.01	0.08	0.01	0.43	0.03	0.08	0.01	LC
Number of species (n)		38		35		35		30		**39**		
TOTAL	**Abundance (n)**	4032		3281		2597		697		**10607**		
	Biomass (g)	21882.5		36595.9		39521.8		16452.8		**114453**		

Note: Based on IUCN status for Mediterranean Sea (2021) LC: Least Concern; NT: Near Threatened; VU: Vulnerable Population trends; DD: Data deficient.

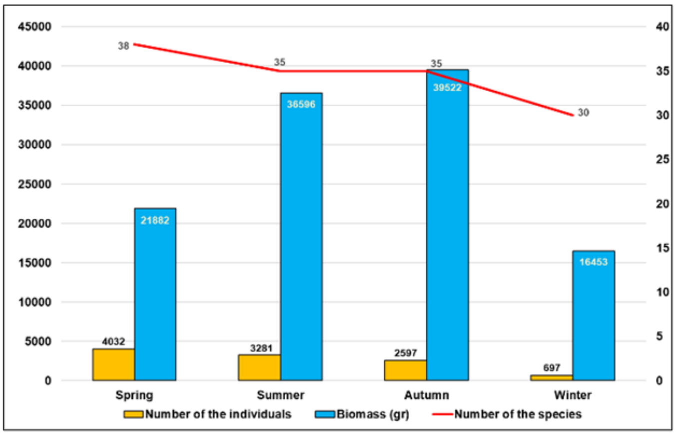

Figure 2. The number of species, individuals, and total biomass values are observed by season

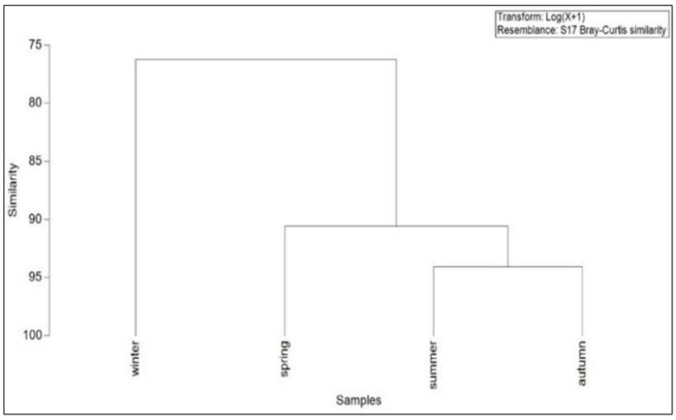

Figure 3. Bray-Curtis similarity dendrogram of the seasons

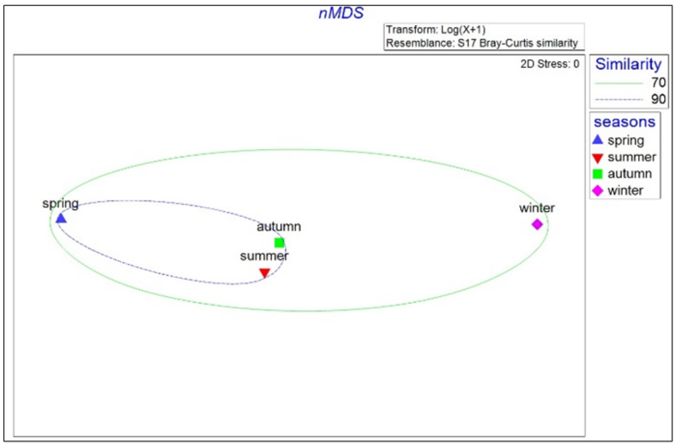

Figure 4. nMDS graph based on Bray-Curtis similarity

Based on Shannon-Wiener's diversity index (H'), the highest diversity was recorded in winter (2.31), and the lowest in spring (2.15). Species richness index Margalef's (d) had the highest value in spring (4.57) and the lowest in summer (4.2). The highest value of the Pielou's evenness (J') index was calculated in winter (0.68) and the lowest in spring (0.61) (Table 2). Comparisons of diversity indices did not reveal significant differences between seasons ($p<0.05$).

Bray-Curtis similarity analysis based on the counted number of individuals for the seasons were calculated ($p<0.05$), the maximum similarity between summer and autumn the minimum similarity between winter and summer-autumn (Figure 3). Non-metric multidimensional scaling (nMDS) based on Bray-Curtis similarities data showed approximately 90% similarity among the spring, summer, and autumn, however, the cold season (winter) was the least similar to others (70%) (Figure 4).

Discussion

The Mediterranean is the largest and deepest enclosed sea that occupies 0.8% of the surface area of the world's oceans (Bianchi & Morri, 2000; Psomadakis et al., 2012). Although it covers a small area, the Mediterranean Sea is a region of high biodiversity, with 7.5% marine fauna and 18% of the marine flora of the world's oceans (Fossi & Lauriano, 2008). However, the sensitive shallow and deep-sea habitats of the Mediterranean are under the influence of industrialization, such as a high volume of marine traffic and port activities (Abdulla & Linden, 2008).

Results of the Simper analysis based on seasonal abundance data of the species data showed that the maximum dissimilarity among the seasons was spring-winter (26.9%), and the minimum was summer-autumn (5.9%). The fish species that caused the difference between seasons are given in Table 3.

Table 2. Biodiversity indexes by seasons

Season	Total species (S)	Total individuals (N)	Margalef's index (d)	Pielou's evenness (J')	Shannon diversity index (H')
Spring	38	4032	4.57	0.61	2.15
Summer	35	3281	4.2	0.63	2.24
Autumn	35	2597	4.32	0.64	2.29
Winter	30	697	4.43	0.68	2.31

Table 3. Simper analysis results for fish species contributed to differences among the seasons

Spring vs. Summer		**Spring vs. Autumn**		**Summer vs. Autumn**	
Species	**DC%**	**Species**	**DC%**	**Species**	**DC%**
C. labrosus	15.2	*S. aurata*	7.08	*C. labrosus*	14.4
S. rostratus	9.54	*S. rostratus*	5.61	*S. aurata*	14.4
M. helena	6.51	*M. helena*	5.61	*S. pilchardus*	6.17
M. surmuletus	5.02	*S. pilchardus*	4.99	*S. mediterraneus*	4.82
S. cinereus	4.11	*C. labrosus*	4.88	*S. rostratus*	4.57
E. costae	4.11	*S. cinereus*	4.68	*P. tentacularis*	3.63
T. marmorata	4.11	*S. ocellatus*	4.36	*L. viridis*	3.63
Av. dissimilarity = 8.62		Av. dissimilarity = 10.21		Av. dissimilarity = 5.90	

Spring vs. Winter		**Summer vs. Winter**		**Autumn vs. Winter**	
Species	**DC%**	**Species**	**DC%**	**Species**	**DC%**
A. boyeri	14.34	*A. boyeri*	17.3	*A. boyeri*	17.42
S. pilchardus	13.83	*S. pilchardus*	15.99	*S. pilchardus*	14.91
C. labrosus	5.92	*S. roissali*	5.45	*L. mormyrus*	5.32
L. mormyrus	5.31	*L. mormyrus*	5.45	*S. smaris*	4.83
S. smaris	4.81	*B. boops*	5.22	*S. cinereus*	4.78
B. boops	4.78	*S. smaris*	4.81	*S. roissali*	4.78
S. salpa	4.34	*S. cinereus*	3.88	*C. labrosus*	4.78
Av. dissimilarity = 26.93		Av. dissimilarity = 22.54		Av. dissimilarity = 21.79	

Note: DC%= percentage contribution to total dissimilarity.

Because fishes are sensitive and mobile organisms, they respond more quickly to ecosystem changes than sessile organisms. Due to this reason, they are suitable indicators of ecosystem changes (Harrison & Whitfield, 2004; Breine et al., 2007; Martinho et al., 2015; Souza & Vianna, 2020). This study focused on fish diversity that was used as an indicator, and seasonal monitoring was carried out for one year using the UVC method.

Previous studies using the UVC method to investigate fish biodiversity on the eastern coast of the Aegean Sea were reviewed, focusing on a particular habitat (artificial habitats, reefs or islands) or fish group (cryptobenthics). In terms of fish biodiversity, in previous studies conducted on artificial habitats such as shipwrecks and sea-cage fish farms, and natural reefs, it has been stated that 27-40 fish species belong to 10-22 families (Gül et al., 2006, 2011; Lök et al., 2008; Akyol et al., 2019; Acarlı et al., 2020). In addition, in the other studies that focused on the cryptobenthic fishes, 19 species were stated by Dalyan et al. (2021), 23 species by Kesici & Dalyan (2020), and 33 gobiid species were stated from the northeastern Aegean by Engin et al. (2018). In the scope of this study, 39 species belonging to 14 families were observed in the natural habitat. In common with Lök et al. (2008), De Raedemaecker et al. (2010), Gül et al. (2011), Akyol et al. (2019) and Acarlı et al. (2020) the most diverse families were Sparidae and Labridae. This was interpreted as a usual case on the Mediterranean rocky shores (Harmelin, 1987; Ruitton et al., 2000). *B. boops* was the frequently dominant fish species from the family in this study (19.3% in total abundance; 34.1% in total biomass) which is similar to findings from previous studies focused on fish biodiversity in artificial habitats (Fernandez-Jover et al., 2008; Arechavala-Lopez et al., 2011; Šegvić Bubić et al., 2011; Acarlı et al., 2020). *B. boops* is abundant and widely distributed from the Eastern Atlantic to the Mediterranean and the Black Sea and inhabits all types of habitats (Bauchot & Hureau, 1986). In terms of total abundance (a) and biomass (b), the top species were *B. boops* (a:19.3%, b:34.1%), *C. chromis* (a:17.4%, b:6.3%), *S. smaris* (a:15%, b:6.7%), *A. boyeri* (a:12.5%, b:5.2%), *O. melanura* (a:10.2%, b:16.3%) and *S. salpa* (a:10.2%, b:5.3%). Gül et al. (2006), Ulaş et al. (2007), and Gül et al. (2011) stated similar results for artificial reefs in the Aegean Sea with this study.

The species richness and diversity indexes, Margalef's index (d) ranged from 4.20 (summer) to 4.57 (spring), and Shannon index from (H') 2.15 (spring) to 2.31 (winter). The highest Pielou's evenness (J') was calculated in winter (0.68) and the lowest in spring (0.61). The highest number of individuals and taxa counted in spring (4032;38), and the lowest was in winter (697;30) (Figure 2). Although the calculated biomass values for the summer and autumn seasons were close to each other, the highest biomass was in autumn with 35 species, and the lowest was in winter. In other studies, the seasons with the highest and lowest values were respectively stated as summer and autumn by Kalogirou et al. (2010), spring and summer by Acarlı et al. (2020), and also summer and autumn were stated as highest by Gül et al. (2006). Species distribution and abundance were associated with environmental factors in seasonal changes which typically increased during summer and decreased during winter (Jin & Tang, 1996). In spring and early summer, an increase in abundance with new additions to fish stocks, a decrease in abundance and species number in winter, but an increase in biomass were determined. Also, the simper analysis based on abundance calculated the highest average dissimilarity between spring and winter with 26.9%.

As a result of Simper analysis based on seasonal fish abundance data, the highest average uniqueness was calculated between spring and winter. Also, the simper analysis based on abundance show the maximum average dissimilarity between spring and winter with 26.9%.

The coastal marine area that conducted this study has a unique structure as it includes different habitats such as rocks, sand, seagrass, island slope and also being under the influence of marine traffic and port activities. It is known that the more heterogeneous habitats provide substrata for feeding, recruitment, and refuge from predators (Ruitton et al., 2000; Aburto-Oropeza & Balart, 2001; De Raedemaecker et al., 2010).

Conclusion

The importance of industrialization in the economies of countries is indisputable. However, determining the negative effects of industrialization on marine ecosystems is a global problem that needs long-term observations. Fish biodiversity represents an important indicator in determining ecosystem health. This study aimed to determine the current status of fish biodiversity in Aliağa which is an affected area by heavy industrial activities and marine traffic. Without the seasonal variability, a total of 10607 individuals (weighing 114.453 kg) of 39 species belonging to 14 families were observed in the study area. The most diverse families of the fish assemblage were Labridae and Sparidae, with 10 species each. The greatest fish diversity was recorded in the spring. In the fish-based monitoring studies on Turkish coasts, researchers have focused on artificial reefs or island ecosystems but coastal ecosystems especially those located in industrial areas have not been studied enough. Should be given due sensitivity to this type of marine ecosystem and more intensive, long-term periodical surveys should be carried out in these areas. Long-term data is required to determine anthropogenic activities' effects on marine ecosystems. For this purpose, the current status of a special region that needs continuous assessment of fish biodiversity was determined, and basic data were obtained to reveal the changes in the future.

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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RESEARCH ARTICLE

LNG bunkering dynamics: An exploratory study for Türkiye

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ABSTRACT

United Nations' decarbonization and greenhouse gas reduction targets are to be achieved through the utilization of alternative fuels globally. LNG is a viable alternative for mitigating maritime transport-related greenhouse gas emissions. This study investigates LNG bunkering supply chain development in Türkiye. Semi-structured interviews with bunkering supply chain representatives were conducted to explore phenomena. The results of the research provided a pathway to establish a new supply chain for alternative marine fuels. The research findings indicate that collaboration and setting a regulatory framework are vital for supply chain development.

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Introduction

More than 80% of world trade by volume is handled by shipping and maritime transportation is considered the most fuel-efficient mode of transport. However, ships are increasingly defined as a critical source of air and water pollution. According to GHG (Greenhouse Gas) research conducted by the International Maritime Organization (IMO), CO_2 emissions from the shipping industry might increase by 50% to 250%, depending on economic development and energy demand (IMO, 2015). Shipping-related pollutants such as nitrogen oxides (NO_x), particulate matter (PM), sulphur oxides (SO_x), and unburned hydrocarbons (UHC) can deplete the ozone layer, cause acid rain, contribute to greenhouse gas (GHG) emissions and cause significant impact on climate change. The initial target set by the International Maritime Organization (IMO) for decreasing GHG emissions from ships is to reduce total GHG emissions by at least 50% by 2050 compared to 2008 (IMO, 2020). The maritime sector must address these environmental challenges over time while using

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the most cost-effective fuel. Alternative fuels in shipping include LNG, ammonia, hydrogen, LPG, methanol, ethanol, fuel cells, and batteries. Compared to other clean fuel options, the LNG supply chain is already in place and ready for expansion at the bunkering stage. The LNG supply chain is extended to marine end-users through strategic collaborations between stakeholders such as ports and suppliers (Wang & Notteboom, 2015). In some context, LNG marine fuel requires to use existing supply chain in some context, and bunkering can develop where the LNG market already exists for industrial purposes or power generation (Sharples, 2019).

Türkiye already has a natural gas grid and LNG terminals serve for power generation, industrial use and domestic heating. However, as a marine fuel, it has not yet been activated commercially. Türkiye is listed in the top 20 of the world's largest economy by GDP and by port call (UNCTAD, 2019), taking advantage of being surrounded by the sea and controlling an important shipping route from the Black Sea to the Mediterranean. LNG as ship fuel has an increasing demand across the world and it's worth investigating LNG bunkering development in Türkiye. Erkmen (2018) only conducted research in the literature by studying small-scale LNG in Türkiye however this study was not focused on the supply chain development (Erkmen, 2018). This study explores different perspectives of bunkering supply chain stakeholders in the Türkiye context as the first research in this field. In this study, semi-structured interviews have been conducted with potential stakeholders of small-scale LNG supply chain and LNG bunkering. Ship owners, suppliers, port authorities, policymakers and technical service providers' opinions were explored to explore the LNG bunkering option in Türkiye.

This study aims to explore and contribute to LNG bunkering application in Türkiye based on different stakeholders' views in the supply chain. The following research questions have been formulated for this purpose:

RQ1- What are the bunkering dynamics in Türkiye? RQ1 is important to gain insight into Türkiye's present bunkering structure, supply locations and volumes.

RQ2- How is the current situation in the existing natural gas and LNG infrastructure in Türkiye? RQ2 is critical to determine potential LNG bunkering development at an optimum level.

RQ3- How can a small-scale LNG supply chain for LNG bunkering be developed in Türkiye? RQ3 is essential for gathering different stakeholders' view in the supply chain and proposing a model for LNG bunkering development.

The structure of the study is organised as follows. Section 2 explores bunkering activities in Türkiye, section 3 investigates natural gas and LNG in Türkiye. The research method and data collection have been described in section 4. Section 5 discusses empirical findings and section 6 includes the conclusion and future research.

Bunkering Activities in Türkiye

Ship supplies are critical for any shipping activity. Provision, crew change, spare parts and consumable deliveries have tremendous importance to operating a vessel effectively. Bunkering could be vital among all these supplies as vessels cannot proceed from A to B or cannot perform cargo operations without fuel on board or without convenient fuel onboard. Ship supply hubs are strategically located on busy maritime transportation routes. Straits, narrow seaways, and busy ports create demand for any type of supply, repair, or service as well as bunker (Lam et al., 2011).

Türkiye involves in significant international and domestic shipping activities. Istanbul and Çanakkale Straits are the densest nods since they connect Black Sea ports to international shipping activities. The Marmara Sea has substantial marine traffic which includes not only transit traffic from the Black Sea to the Mediterranean but also port calls such as Izmit, Gemlik and Tekirdağ. Nemrut, Aliağa and Izmir ports show high traffic density. Cabotage traffic around Türkiye's West Coast is also significant along with connection with islands although it represents seasonality. Mediterranean ports such as Antalya and Mersin have dense marine traffic activity. However, Iskenderun Gulf is observed as the busiest region as it has substantial port activities for dry bulk, containers, and oil and gas cargoes. As these ports usually handle large vessels, high traffic density not only explains the number of the vessels, but also substantial demand potential as larger vessels lift much more bunker quantity per operation.

There is important port traffic in Türkiye. Kocaeli handles the largest proportion as total GT and by the number of the vessels calling. Other Marmara Sea ports such as Ambarli, Gemlik, Tekirdağ, and Tuzla are in the top ten according to the total GT of the vessels calling these ports. On the other hand, two smaller ports Çeşme and Bodrum have significant touristic traffic between the Greek Islands and Türkiye. Çeşme also has regular Ro-Ro traffic which connects Çeşme to Italy and France. Iskenderun and BOTAŞ are under different port authorities in Iskenderun Gulf. Their total share takes the second largest part as GT (15.3%) after Kocaeli.

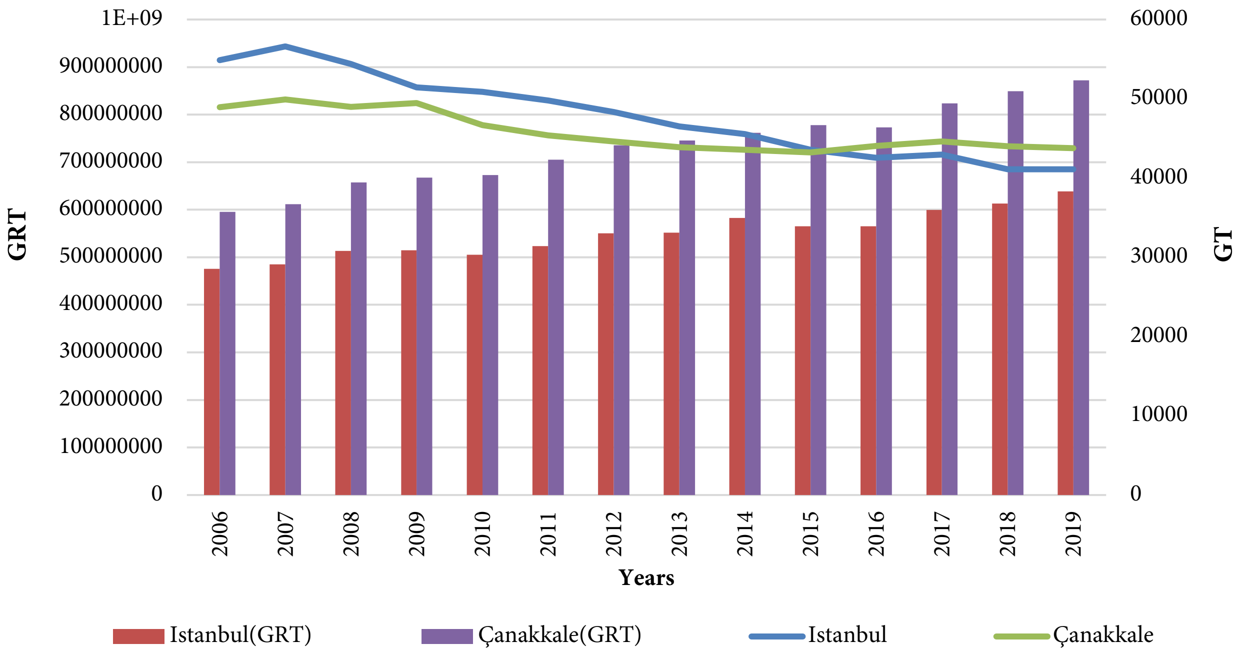

Figure 1. Istanbul and Çanakkale Straits' traffic data as number and GT (*Source*: Author, compiled from MOTI (2020b))

Table 1. Total Bunker Deliveries of Türkiye

Year	Delivery with SCT	Delivery without SCT	Delivery under Transit Regime	Total (ton)
2018	9548	34258	1999129	2036934
2017	5501	35720	1891362	1932583
2016	5629	38610	1434790	1479029
2015	234	0	324633	507332
2014	8687	36139	1279474	1324300
2013	4625	356008	688555	1049188
2012	25452	479368	1325632	1830452
2011	6879	278021	1331803	1616703
2010	11693	232343	1105909	1349945
2009	14343	266638	942064	1223045
2008	15049	266089	1109477	1390615
2007	11401	243541	1085211	1340153

Source: Author, Compiled from EMRA Annul Reports covering 2007 to 2018

To understand the shipping activities in Türkiye, Çanakkale and Istanbul Strait passages traffic provides an important indicator.

Figure 1 summarises the number of vessels passing through Istanbul Strait and Çanakkale Strait as number and GT by data obtained from the Ministry of Transport and Infrastructure database covering 2006 to 2019. The number of vessels passing through Çanakkale and Istanbul Straits is decreasing however, the total GT of the vessels are increasing in these straits -from 595,826,240GT to 872,314,222GT for Çanakkale and 475,796,880GT to 638,892,062GT for Istanbul. There are more than 40000 passages for each strait since 2006 as they are declining over the years.

Table 1 summarises Türkiye's total bunker deliveries with and without special consumption tax (SCT) and delivery under the transit regime. The table excludes 'export' figures as it does not distinguish 'cargo' and 'bunker' exports. Another reason is the explanation remark of the EMRA report which states that "Deliveries based on Cities\Türkiye have been included in transit regimes and export". However, once we calculate the data, it is observed that deliveries to the city table exclude exports and only take into account figures in Table 1.

Natural Gas and LNG in Türkiye

Türkiye's energy mix is dominated by fossil fuels and its share in the energy supply is approximately 85% (Kırlı & Fahrioğlu, 2019; Sigma, 2020). Due to lack of fossil fuel sources, Türkiye is a highly import-dependent country for energy which involves gas, oil, and coal 99.6%, 94.32%, 97.3%, respectively. This dependency has significant effect on country's trade deficit. Energy imports in 2019 was 41.18 billion dollars and it takes 20,3% of the country's total import expenditure (Sigma, 2020). Oil takes the first place as primary energy source among other alternatives with 29%. The second energy source is coal (26%) and natural gas's share is 27% and other energy sources, renewables and hydro-electric followed natural gas with 11% and 7%, respectively.

Natural gas takes a critical part in Türkiye's energy strategy. Its share in power generation is 24%, despite a significant decrease from 2018 to 2019. In addition to power generation; heating and industrial use of natural gas increased rapidly and now it accounted for more consumption than power generation (Topuz, 2019). Türkiye has LNG import terminals, Floating Storage and Regasification Unit (FSRU)s and numerous pipelines and interconnections between Asia and Europe as gas infrastructure (Topuz, 2019). However, Öge (2021) argues that Türkiye has not benefitted from its geopolitical position in terms of pipeline transit due to contractual obligations.

Natural gas takes third place as Türkiye's primary energy source following petroleum and coal. By the end of 2019, the natural gas market reached 45685.34 Sm3 total supply (production and import) and 46048.18 Sm3 total demand (domestic sales and exports) volume (EMRA, 2020). Türkiye carries out natural gas imports via pipelines and LNG import points. Pipeline imports are received from Russia, Iran and Azerbaijan with long-term purchase agreements, long term LNG agreements and LNG spot markets. Russia takes an important part in Türkiye's natural gas imports while taking over 50% share over the years, in 2019 it reduced to the lowest level of 33.61% (EMRA, 2020). These differences were compensated by increases in Azerbaijan gas imports from 14% to 21%. Total natural gas imports have decreased by 10% and lowered dependency on Russian natural gas. However, this reduction in Russian gas quantity is not reflected in the country's budget due to 'take or pay' obligations. The pipelines are the main natural gas sources of Türkiye, however, LNG investments, FSRUs are changing the pipeline gas/ LNG balance positively to the LNG side. LNG share increased from 12.92% to 28% from 2013 to 2019. In 2019, pipeline imports reached the lowest volume since 2010 (EMRA, 2020).

Natural gas takes an important part in Türkiye's energy sources and power generation. LNG is another alternative to reduce pipeline natural gas dependency and it's share in natural gas imports are gradually increasing in parallel to new FSRUs. Small-scale LNG and LNG as a marine fuel could create another market option for LNG imports and bunker suppliers.

Methodology

This study employs the interview method and the researchers can use the interviews to gather valid and reliable data that is relevant to the research questions (Saunders et al., 2009). The semi-structured interviews allow the researcher to have lists of themes and questions that may vary for different respondents. Some questions may vary depending on the interview's flow or be focused on certain topics based on the context, giving the researcher the freedom to investigate new phenomena. Researchers can perform an exploratory and explanatory study using this strategy in semi-structured interviews. Semi-structured interviews help to understand variables in the small-scale LNG supply chain and LNG bunkering in the Türkiye context. Therefore, a semi-structured interview method has been used as this study aims to explore a relatively new concept that has no operational applications in the sector in Türkiye.

By considering only data from a subgroup rather than all possible cases or components, sampling techniques allow you to minimize the amount of data you need to collect. Some research questions would necessitate sample data that will enable you to statistically generalize about all of the cases from which your sample was drawn. There are two types of sampling technic: probability and non-probability (Saunders et al., 2019). Probability sampling is usually associated with survey research strategies. The chance, or likelihood, of each case being chosen from the target population, is known with probability samples, and it is normally equal for all cases. This ensures that you can answer research questions and meet goals that include statistical estimation of the target population's characteristics from the sample. However, since the probability of each case being chosen from the target population is unknown in non-probability samples, it is difficult to answer the study questions or meet the goals that include statistical inferences about the population's characteristics (Saunders et al., 2019). Purposive sampling occurs when a researcher has a clear understanding of what sample units are needed based on the study's objectives, and then approaches possible sample participants to see if they meet the requirements. Those who comply are employed, while those who do not are dismissed (Easterby-Smith et al., 2015). In the small-scale supply chain literature, stakeholders have been categorised. Therefore, to fulfil these requirements of the research objectives, purposive sampling strategy has been used for semi-structured interviews. This research explores relatively new topic in the maritime industry and there are limited numbers of expert in this field.

The population of the study covers shipowners, suppliers, regulatory bodies and technical service providers. The purposive sampling strategy was used to maximise the variations in the phenomenon. Interviews were conducted with six shipowners, five suppliers, four regulatory bodies and four technical service provider representatives. The number of the interviews was not determined before, rather, the size of the samples was restricted in line with theoretical saturation, in other words, till data collection generates no insight.

The first contact with interviewees has been made through email or phone call and the means of the interview have been decided accordingly as online meeting, phone conversation or email. All interviews were conducted via online meetings and phone talks due to COVID-19 restrictions. Managers are less committed to doing remote interviews since they are not required to host the interviewer or see them at a specific time. Remote interviewing, however, does not always favour the researcher for these reasons. Face-to-face interviews provide instant contextualization, depth, and nonverbal communication that mediated interviews lack (Easterby-Smith et al., 2015). However, internet-based or telephone conversation could be particularly useful in real-time and process-based research.

The themes of the questions were determined as safety (Jeong et al., 2017; Park et al., 2018), technical (Choi & Park, 2019; Kim et al., 2019a), operational (Kim et al., 2018, 2019b), regulatory framework (Xu et al., 2015; Wan et al., 2018) and commercial components (Schinas & Butler, 2016; Eise et al., 2017; Yoo, 2017) following the findings obtained from the literature review. Interviews were conducted between 16th November 2020 and 15th January 2021.

Table 2. List of Interviewees with Experts in Türkiye

Code	Title	Function	Type
R1	Director	TSP	Mobile Phone
R2	Business Development Manager	TSP	Online
R3	Planning Manager	Shipowner	E-mail
R4	Country Manager	TSP	Online
R5	Country Manager	Regulatory Body	Online
R6	General Manager	Supplier	E-mail
R7	Energy Manager	Shipowner	Mobile Phone
R8	General Manager	Shipowner	Online
R9	Senior Technical Officer	Regulatory Body	E-mail
R10	Ass. Sec. Gen.	TSP	Online
R11	General Manager	Supplier	Online
R12	GM	Shipowner	E-mail
R13	Investment Manager	Supplier	E-mail
R14	Director	Supplier	Online
R15	General Manager	Regulatory Body	E-mail
R16	Energy Specialist	Regulatory Body	Online
R17	General Manager	Supplier	Online
R18	Operation Manager	Shipowner	E-mail
R19	Operation Manager	Shipowner	E-mail

The rigor of the study is established through Wallendorf & Belk (1989) protocol. In order to establish credibility, before each interview, the aim/scope of the research and the background of the interviewer were explained. A brief summary was made after each interview in order to confirm that the understanding of the interviewee is correct. The purpose sampling method was used to achieve transferability which is achieved through variation of participants in terms of title and segments. Dependability is ensured through not restricting sample size and the data collection process was completed once theoretical saturation was assured. In order to achieve confirmability; the responses of different participants were not shared with any other, and the findings were interpreted through quotes. The integrity of the research was assured through compliance with ethical principles. Names and company details were kept confidential. Table 2 summarises the details of the semi-structured interview participants.

Shipowners' representatives run bulk carriers, tankers and container vessels. One of the tanker owners has already LNG fuelled vessels on their order list. The technical service providers include the shipbuilding industry, shipyard, ship design and leading LNG technology provider in the world. The shipyard representative has already built LNG-fuelled vessels and other alternative fuelled vessels in their shipyard. The design/consultancy firm representative has delivered numerous projects fuelled with LNG. The suppliers in the list include port/terminal representatives and bunker suppliers. Regulatory body participants are policymakers and classification society representative.

Results and Discussion

This study aims to explore the views of the experts in Türkiye related to or potentially relating to LNG bunkering. The interviewees were classified according to their fields of expertise: namely; shipowners, suppliers, regulatory body (RB) members and technical service providers (TSP). The questions, in parallel with literature findings, were organized according to environmental, safety, technical, commercial and regulatory frameworks from a Turkish perspective. As Covid-19 restrictions were in place, the interviews were conducted through online applications, mobile phone networks or e-mail. 5 shipowners, 6 suppliers, 4 TSPs and 4 RBs representatives participated in the interviews. All general and specific expertise-related questions have been answered by the interviewees. Interviews were transcribed and analysed thoroughly.

Challenges of LNG Bunkering Development in Türkiye

The use of LNG as ship fuel is seen as a positive development because it is a cleaner energy source and is economical compared to other fuels. LNG not only complies with today's emission limits but also offers various advantages over other alternatives in terms of available infrastructure and capacity.

- For this reason, LNG bunkering was considered a promising alternative marine fuel for the future by 14 participants out of 19. Two shipowners and 1 technical service provider participant do not see any future in LNG marine fuel.

Türkiye's LNG bunkering potential was evaluated in terms of transit via the Turkish Straits, large ports, the domestic fleet, the proximity to some main maritime trade routes, the existing LNG terminals and the LNG market.

- Suppliers and Technical service providers point out that Türkiye has significant potential for LNG bunkering based on the aforementioned criteria thus highlighting Türkiye's advantage. On the other hand, 2 shipowners and 1 supplier underlined that Türkiye is not in an advantageous condition due to market structure and geographical location.

The challenges for LNG bunkering in Türkiye were defined in parallel to global challenges. Its environmental advantage compared to other fossil fuels is important, but it is not sufficient to meet global targets.

- Commercial factors, LNG demand, LNG price and the LNG market were some of the keywords underlined by 19 participants.
- High capital expenditure is not affordable for shipowners is another finding that is highlighted by 19 participants.
- Infrastructure is developing rapidly but global coverage has not yet been established.
- Crew competency along with safety considerations are other key subjects that were highlighted by 6 shipowner participants. 2 of the TSP also underlined the same issue.
- Fleet type, such as container vessels in liner services, Ro-Ro fleet, and large carriers (either tankers or container vessels) were emphasised by shipowners highlighting some potential in LNG fuelled vessel applications in different segments.

Türkiye's LNG bunkering legislation and regulatory framework are important items which were emphasised by the supplier and regulatory body participants.

- To date, there is no legislation in place for LNG bunkering. This is another factor that is slowing down the LNG bunkering infrastructure development process from the supplier side as they seek references and standards to comply with local regulations.

Technical service providers are the most optimistic stakeholders regarding LNG bunkering development. Well-known problems of LNG bunkering such as boil-off gas and methane slip can be minimised with technological improvements. The International Code of Safety for Ship Using Gases or Other Low-flashpoint Fuels (IGF Code) standard for boil-off gas (BOG) treatment in bunker barges has been set at 15 days and the industry is able to comply with these restrictions. The Turkish shipbuilding industry is already producing LNG fuelled vessels as well as other alternatively fuelled vessels. Commercial factors were again highlighted by the technical service providers, specifically that individual investment by a shipowner is unlikely due to the high capital expenditure costs.

Suppliers in Türkiye are closely monitoring developments in alternative fuels in shipping. There are investments in LNG bunkering facilities which were mentioned during the interviews.

- Collaboration was one of the keywords to advance LNG bunkering developments in Türkiye. It was emphasised by 4 suppliers.
- With regards to collaboration, 5 suppliers also highlighted government subsidies in order to encourage investment in infrastructure and develop the markets.
- Oil price, LNG price market structure and demand are the other keywords which were emphasised frequently by shipowners and suppliers. High capital expenditure and the pay-back time of the investment were defined as barriers to entering the market.

LNG Bunkering Case for Türkiye

Türkiye is an important ship-supply location in maritime traffic. The Istanbul area provides not only bunkering but also crew change, provisions, spare parts, water/oil supply and repair facilities which all contribute to Türkiye's economy. Traffic density in the Istanbul and Çanakkale Straits makes this place one of the busiest shipping routes and the total tonnage of vessels passing through has been increasing gradually over the years. In addition to transit passage through the straits, the growing shipping activities at ports are crucial to the measure of bunkering potential. By the end of 2018, Türkiye's total bunkering volume reached 3 million tonnes (Shipandbunker, 2020). It is provided by 55 bunker barges in service. However, it is argued that this is still below the potential of Türkiye as only about 1,200 ships per year receive bunker in Istanbul and this is only approximately 3% of the total transit passage of the Istanbul Strait. Another crucial item is the storage facilities which are located in the Izmit Gulf which affect the competitive price of bunker deliveries. In light of these bunkering facts in Türkiye, the LNG bunkering case was discussed.

Bunker supply chain stakeholders in Türkiye generally have a positive attitude towards utilising LNG as an alternative marine fuel. One of the regulatory body participants justified using LNG as a marine fuel as follows:

"…. LNG emerges as an important alternative fuel. Considering the operational costs, LNG is advantageous as a fuel compared to low sulphur fuel in terms of lower prices and higher energy efficiency. All these points show that LNG stands out as an alternative fuel for ships compared to other options." (R16).

The Marmara region is a highly populated area and there is significant marine traffic in the Marmara Sea. Ship-induced air pollutants such as SO_x, NO_x, and PM have a significant effect on human health and ecosystems. The positive contribution of LNG to air pollution should have great importance for the city of Istanbul, which is located around one of the busiest waterways in the world. These environmental benefits are supported by operational benefits by the supplier participants.

".. In addition, bunker LNG reduces the operational costs of ship machinery such as maintenance and creates less vibration, soot and odour in ships (especially for cruises ships), which provides important advantages" (R13).

On the other hand, one of the shipowner participants is not optimistic about LNG as a marine fuel. He argued against it as follows:

"I think it is difficult to use it as ship fuel in the short term due to current market conditions, economic crises, high costs, alternative fuels, alternative systems and challenging conditions, insufficient incentives, operational-operating costs and insufficient supply points" (R18).

These arguments are similar to other shipowners' perspectives not only in Türkiye but also in the world.

The Turkish perspective on LNG as a marine fuel seems slightly less promising than the global context. Although environmental benefits are emphasised by all participants; 2 out of 4 shipowner participants did not view LNG to be important alternative fuel, taking into account high capital expenditure. This is the primary reason why shipowners are reluctant to invest in alternative fuels. Unless forced by regulation, shipowners seek less risky and less costly alternatives in their operational and tactical decision-making processes.

Table 3. Comparative table of the LNG bunkering challenges based on experts in global and experts in Türkiye

No	LNG Bunkering Challenges in Global Context	LNG Bunkering Challenges in accordance with experts in Türkiye
1	Commercial Factors	Commercial Factors
2	Safety	Regulatory Framework
3	Sustainability/Environmental	Safety
4	Regulatory Framework	Operational Factors
5	Technical Factors	Crew Training
6	Infrastructure	Operating Region
7	Location/Operation Region	Infrastructure
8	Collaboration	Subsidies
9	Fleet type	Collaboration
10	Operational Factors	Environmental

Source: Author, compiled from (Doymus & Denktas Sakar, 2020)

Türkiye's LNG bunkering potential was usually evaluated via its geostrategic location and existing LNG handling facilities by the participants. This potential was underlined by one of the regulatory body participants as follows:

".. Considering this potential, it is important to be able to supply LNG as a bunker, which stands out as an alternative fuel in ships depending on the worldwide trend, environmental constraints and regulations, as well as the conventional bunker supply" (R15).

An existing LNG infrastructure, knowledge, and experience are the advantages for Türkiye in the East Mediterranean and the Black Sea region. This is pointed out by a supplier as follows:

"…Due to the existing LNG Terminals and an established / experienced market structure in the bunker fuel trade, it is possible to access bunker LNG from Turkish ports at competitive prices and under favourable conditions" (R18).

Türkiye has a very dynamic shipbuilding industry. LNG fuelled vessels have already been built in Türkiye. There is number of shipyards in Türkiye which have the knowledge and capability to build vessels in Green Ship concepts which are fuelled by alternative fuels such as Cemre Shipyard, Tersan Shipyard, and Sanmar Shipyard. This point was emphasised by several participants to illustrate the potential of Türkiye.

The challenges for LNG bunkering development in Türkiye have similar considerations as is the case on the global scale. Insufficient demand for LNG bunker is a major challenge for suppliers in taking the decision to invest in LNG. The long pay-back time prediction due to the infancy of the market was underlined by all supplier participants. On the shipowner side, parallel to global concerns, the high CAPEX requirement and lack of global coverage of infrastructure were defined as the main reasons to be reluctant to invest in LNG fuelled vessels. At the same time, shipowners will remain in favour of traditional solutions as long as the regulations allow, relying on economic facts. Another outstanding challenge highlighted by all shipowners is the lack of a qualified crew. It was argued that even if they made the decision to invest in LNG fuelled vessels, it would be difficult to find and employ qualified crew who are certified in LNG fuelled vessels. However, one technical service provider argued that:

"…Türkiye has had LNG handling experience with BOTAŞ and EGEGAZ over the years, crew training could be easily arranged with these companies together with the collaboration between universities and shipowners" (R4).

There are numerous training institutes in Türkiye for ship crew. These institutes are providing manpower for shipowners. On the other hand, there is not any shipowner running an LNG vessel, and LNG qualified crew requirements for the Turkish shipowners. FSRU units are equipped with foreign crew members. As it's argued by a TSP, Türkiye could develop training facilities for crew members while taking into account LNG handling experience over the years in LNG import terminals.

Technical standardisation and the regulatory framework at the global scale are almost ready. However, it is still attracting substantial criticism from the supplier participants in the Turkish case. One of the suppliers argued that:

"The technical standards are not yet ready. When making such a high-cost investment, it is necessary to know what the local rules, limitations and requirements are. Therefore, first of all, there should be legislation that we need to follow" (R17).

Another participant who represents a supplier also pointed out that:

"*…Even though we have invested in LNG bunkering, we do not have the chance to compete with foreign countries with the current customs legislation. Profit margins are very low due to the current legislation and the operational constraints it imposes*" (R6).

In addition, the RB participant points out that in terms of legislation, the Ports Regulation adopted in 2012 needs to be updated regarding LNG bunkering safety and other related issues. On the other hand, in 2019, the necessary arrangement was made in the Turkish Straits Maritime Traffic Regulation in order to regulate the passage of LNG fuelled vessels through the Turkish Straits (R15). Legislation for LNG bunkering is not yet ready in Türkiye. This has a significant effect on how stakeholders deal with uncertainties concerning high-cost investment. The legislation is crucial for defining the technical specifications and structuring limits of the investments. Moreover, as LNG transfer is not the same as conventional bunkering, current customs legislation also needs to be updated regarding operational standards and safe handling procedures for LNG. Doymus & Denktas-Sakar's (2020) study reveals LNG bunkering challenges in global context. Table 3 compares findings of LNG bunkering challenges in the global context and with experts in Türkiye.

The table includes frequently highlighted issues by the experts. It should be emphasised that there are almost two years of a time lag between two the studies. However, challenges show similarities in many contexts. Inevitably, commercial factors take the highest concern for all the experts. LNG bunkering price, LNG contracts, uncertainty, lack of transparency are important factors along with high CAPEX requirements. Participants from Türkiye pointed out regulatory concerns more than their counterparts in global. This is one of the important concerns in front of Türkiye side -missing clear legislation for LNG bunkering. Safety factors, operational consideration, collaboration, and infrastructure are the common elements almost equally emphasised in both studies. One important keyword for Türkiye side is the subsidies. This was also highlighted in a global context but for the Türkiye case, it was one of the top keywords stressed by all the participants. Environmental concerns were underlined and acknowledged as one of the most important drivers for LNG bunkering in a global context. However, in the Türkiye case, it took limited attention while justifying LNG as marine fuel but was not underlined as the main driver for LNG bunkering supply chain development. In parallel to this finding, public awareness was highlighted frequently as well. In Türkiye case, it was only mentioned by one TSP. Public awareness or public opinion is one of the strongest developments for the LNG bunkering case, when the existing small-scale LNG supply chain was investigated in NW Europe and the Baltic Sea. Public opinion also triggers green funds and government subsidies for high CAPEX investments. In the Türkiye case, public pressure on environmental issues is not strong or just parallel to daily politics. Reaching out subsidies and incentives is not easy as in developed countries.

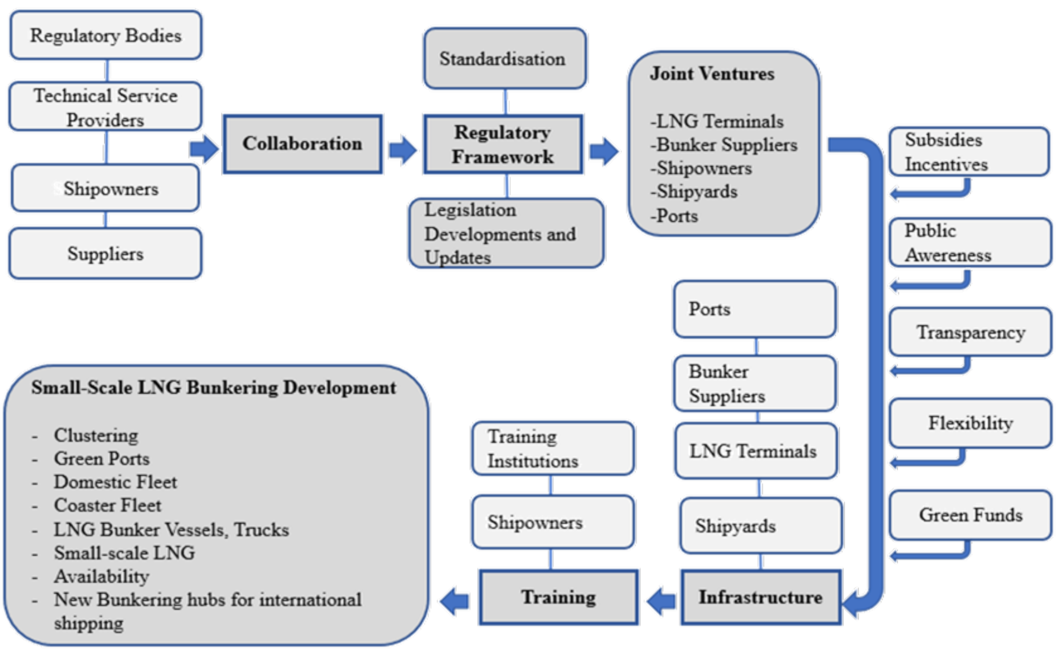

Figure 2. LNG bunkering development pathway in Türkiye (*Source*: Author)

Figure 2 summarises the LNG bunkering development pathway in Türkiye. Collaboration is a must between stakeholders. The regulatory framework needs to be updated and standards should be in place. Joint venture agreements are necessary for high CAPEX required new business. The risk of the new investment decision should be shared by the stakeholders. The organisation should be supported by subsidies and incentives. Infrastructure should be developed by joint venture participants. Crew members should be trained in cooperation with shipowners and training institutions. Small-scale LNG supply chain and LNG bunkering development should be supported by a clustering approach, renewing domestic fleet, renewing coaster fleet and green port concept. Trucks could be used firstly while the market is unmature and demand is low. Creating the demand at a small-scale scale and availability will lead to growing demand, larger ships and new hubs as it has been seen in other locations across the world.

The global coverage of the LNG bunker infrastructure on the main trade routes has been completed. Developments in the West Mediterranean region are also promising as Spain's LNG bunker deliveries increased 272% in 2020 compared to the previous year, including activities on the Atlantic Coast (Bunkerspot, 2021). Greece and South Cyprus have already invested in LNG projects and small-scale LNG not only for marine fuel but also for power production. LNG is not the ultimate marine fuel solution to meet UN decarbonization targets, but it is ready, and it has a fast-developing infrastructure across the world. Other important alternatives such as ammonia, methanol and hydrogen still need time to prepare infrastructure and overcome availability concerns. Arkas Bunker signed an agreement with Sumitomo Corporation for LNG bunkering (Arkas, 2021). One of the important Turkish shipowners has already ordered 10 LNG-ready vessels and is expecting to receive the first delivery in 2022 (R7). This is the only known Turkish shipowner who invested in LNG-fuelled ships on a large scale. Turkish shipyards have built numerous LNG fuelled, battery or hybrid driven vessels so far. For example, Tersan shipyard built 4 LNG-powered fishing vessels, 4 LNG-powered Coastal Passenger vessel, 5 battery-powered Ro/Ro Passenger vessels, 5 hybrid (gas and battery) powered Ro/Ro passenger & vehicle carrier for Norwegian shipowners so far (Tersan, 2021). Moreover, 3 battery powered passenger ships, 4 battery-driven fishing boats, 2 battery-driven renewable energy vessels were built in Cemre Shipyard. 2 fishing boats are LNG-battery driven under construction and are to be delivered in 2022 (Cemre, 2021). Other shipyards such as Sanmar, Kuzey Star, and Sefine shipyards have already built LNG-powered, battery-powered and hybrid ferryboats, tugs and fishing vessels (Portnews, 2020; Sefine, 2021; Sanmar, 2021a). Turkish shipyard built one of the largest batteries driven vessel (2000kwh) in the world as well. Türkiye is also the only country in the world that built hybrid-powered fishing boats (R2). Turkish shipbuilding industry is not only building vessels based on foreign designs; but also developing their own designs. Moreover, the Turkish shipbuilding industry is also exporting technology to Spain in electrical outfits to be used on the green concept ships (R1). Recently, Sanmar shipyard signed an agreement with Canada to build LNG fuelled 5 tug boats (Sanmar, 2021b). Despite these steps in LNG bunkering, it was observed that the LNG bunkering concept is not fully comprehended by the Turkish maritime industry. The high capital expenditure requirements for LNG fuelled vessels are the biggest barriers for shipowners. Safety concerns along with a lack of infrastructure and qualified crew requirements are also other issues to consider. Turkish bunker suppliers' demand concerns are not specific to the Turkish fleet as they provide service to all vessels conducting strait transits or calling on Turkish ports. The shipbuilding industry is able to build LNG fuelled vessels. However, they are not competitive compared to Far Eastern shipyards in this field as they still need to invest in research & development to develop tank designs and gain a competitive advantage. The big players of the world economy; Japan, Korea, China, Australia, Singapore, EU, the USA and Canada are improving their LNG bunkering infrastructure. Not only LNG but also other alternative fuels are gaining momentum globally parallel to the UN's decarbonization targets.

Conclusion

The study collects data through interviews with shipowners, bunker suppliers, ports, terminals, shipbuilders, policymakers, classification societies, and technical service providers were contacted. The key outcomes of this research have been highlighted as recommendations for the Turkish maritime industry:

- Collaboration is key for LNG bunkering development. Supply chain stakeholders; ports, terminals, shipowners, bunker suppliers, shipyards and policymakers should collaborate to overcome the current challenges of LNG bunkering development.
- Legislation to deliver LNG as a marine fuel and small/mid-scale LNG operations have to be developed, and customs requirements need to be updated to comply with safe LNG

handling operations while taking into account the competitiveness of Türkiye's bunker market. The regulatory framework, technical standards and legislation need to be ready before taking any high CAPEX required investment decision by all stakeholders.

• Global LNG bunkering supply chain development analysis indicates that high CAPEX investments have occurred via special subsidies, incentives and funds. Türkiye should seek international funds which prioritise the environmental benefits of LNG, air pollution mitigation strategies and contributions to reducing CO_2 emissions.

• The Turkish shipbuilding industry has experience in building vessels in the green concept as well as in LNG fuelled vessels. However, building LNG fuelled vessels at a competitive price is critical mainly due to LNG tank design know-how. Cryogenic container manufacturing is available in Türkiye (Aritas, 2021). Building the tanks for LNG fuelled vessels needs further research & development investment.

• Crew competency is an important barrier in LNG bunkering development not only in Türkiye but also in the world. The first LNG fuelled vessels were not even tanker and crew members conducted these operations after training in liaison with classification societies. IGF code set standards for LNG-fuelled vessels now. Türkiye already has LNG handling experience. The number of the LNG fuelled vessels or LNG bunker barge will be limited in the near future, therefore training of countable crew members problem could be solved with the cooperation of training institutes, shipowners and the LNG industry in Türkiye.

• The shipowner dimension is another critical point for small-scale LNG development. Türkiye has an ageing domestic fleet of ferries and Ro-Ro vessels (MOTI, 2021). Renewing the ageing coaster fleet is a long-lasting discussion in Türkiye, but it has not been achieved to date due to a lack of funds, collaboration or strategic level maritime policies. Supporting shipyards with new orders enables them to invest in R&D. Renewing the coaster fleet with green concept vessels enlarges the market for the Turkish coaster fleet, particularly in EU ports (Cogea, 2017).

• Bunker suppliers need to see demand in order to invest in LNG bunkering and calculate their pay-back time. Local bunker demand in small quantities plays important role in setting up a market and allows it to grow up gradually. The domestic fleet renewal project is a strategic decision and requires the collaboration of all stakeholders.

• Regulatory pressure is the main motivation for the shipping industry to take initiative and invest in new designs, new fuels or new vessels. Emission Control Areas (ECAs) are an important driver of technological improvement and investment decisions. IMO's plan to set new ECA in the Mediterranean and the Black Sea could be an important driver to shift to environmentally friendly marine fuels.

• Green incentives could support ports and shipowners in line with environmental policies and regulations. At the same time, Türkiye is an important country for tourism and the 'Green' concept is quite important for cruise shipping. Providing LNG bunkering facilities in Galataport, Istanbul, Izmir and Kusadasi could attract new-build, energy efficient and environmentally friendly ships to Turkish ports.

LNG is one of the alternative fuels for the maritime industry and it is gaining momentum across the world. Other alternative fuels will emerge in the near future as well. Türkiye should take strategic level decisions to be a leading country in shipping while using its know-how in the shipbuilding industry, manpower in shipping, fleet size and growing port activities. The findings and recommendations of this study are in the LNG bunkering concept but similar arguments could be adapted to other alternative fuel supply chain developments. Türkiye's ambition to become an energy hub, despite being a net importer, is subject to using the resources cleverly and creating new opportunities rather than being just a follower. LNG bunkering and small-scale supply chain literature is very limited as it is relatively new phenomena. The study firstly contributes to small-scale LNG supply chain literature by defining challenges and important consideration for future development. Some studies in the literature address this problem, however, researches are limited to one country, or with a one stakeholder perspective, with one segment of the shipping or limited to quantitative researches (Wang & Notteboom, 2014, 2015; Aymelek et al., 2015; Xu et al., 2015; Calderón et al., 2016; Ge & Wang, 2017; Jafarzadeh et al., 2017; Gucma et al., 2019; Doymus et al., 2022). This study encompasses different supply chain stakeholders through semi-structured interviews and bring a holistic insight to the phenomenon. Moreover, the study also contributes to knowledge by bringing real-time practitioners' experience in bunkering into this relatively new issue in shipping and provides a comprehensive framework for further research.

This study also contributes to investment decisions in LNG bunkering for shipowners and suppliers. The ports are a vital part of the small-scale LNG supply chain. Not only regulatory pressure but also public pressure takes an important role over port management. Providing service for alternative fuels is critical for the sustainability targets of the ports. Therefore, port managements should consider investing in 'green' concepts. LNG in trucks and ISO containers could be operational in small/mid-size ports. The large ports should take into account ship-to-ship operations. Simultaneous operations supported by detailed risk assessment are crucial for LNG bunkering developments at ports. Providing service for alternative fuels is essential for cruise terminals. In Türkiye case, Istanbul, Kusadasi, Izmir and Galataport should consider LNG bunker service in order to attract 'green' concept cruise vessels and gain a competitive advantage against other alternative ports in the region. LNG terminals play a pivotal role in infrastructure developments and being part of the joint ventures should be considered to reduce the risk and invest in high CAPEX required business.

Türkiye has a very well-developed shipbuilding industry capable of adopting new technologies but struggling with a lack of finance and sustainable order lists. Bunker suppliers are working within very limited profit margins and the high CAPEX required for investment is not realistic. Shipowners are on the edge of profit margins and can barely manage the running cost of the ageing fleet. Therefore, the industry needs strong support from the government that could orchestrate stakeholders with incentives, subsidies and guarantees as part of strategic maritime and energy policies. Ministry of Energy and Natural Resources, Ministry of Environment and Urbanisation, Ministry of Trade, Ministry of Transport and Infrastructure and Energy Market Regulatory Authority are crucial regulatory bodies and policymakers in that should take part to develop a convincing structure not only for LNG bunkering but also for other alternative fuels in shipping. The regulatory body should shape its strategic plans not only from a commercial perspective but also for public welfare as shipping-related activities have substantial negative effects on public health. Eliminating air pollution over the highly populated cities by incentives provided to the shipping industry could pay back in the long-term by reducing the pressure on the public health system.

The study has some limitations. There are 19 respondents for the semi-structured interviews in Türkiye. Ship-to ship LNG bunkering operations have not been performed in Türkiye, yet. Shipowners and supply experts in Türkiye are inexperienced in this field and they shared their views based on their theoretical knowledge.

This study employed semi-structured interviews as a qualitative research method. Small-scale LNG and LNG bunkering could be explored with the Delphi method by surveying a panel of experts as well. Another data collection method could be a focus group study which brings a group of experts into a room and provide feedback on the related phenomenon. A similar study could be conducted for other alternative marine fuels such as hydrogen, methanol, ammonia and LPG. A comparative study could provide another decision tool for shipowners, suppliers and other stakeholders in the maritime industry.

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Compliance With Ethical Standards

Authors' Contributions

MD: Conceptualization, Methodology, Visualization, Formal analysis, Investigation, Writing - Original Draft, Data Curation, Writing – Review and Editing.
GDS: Supervision, Methodology, Writing – Review and Editing

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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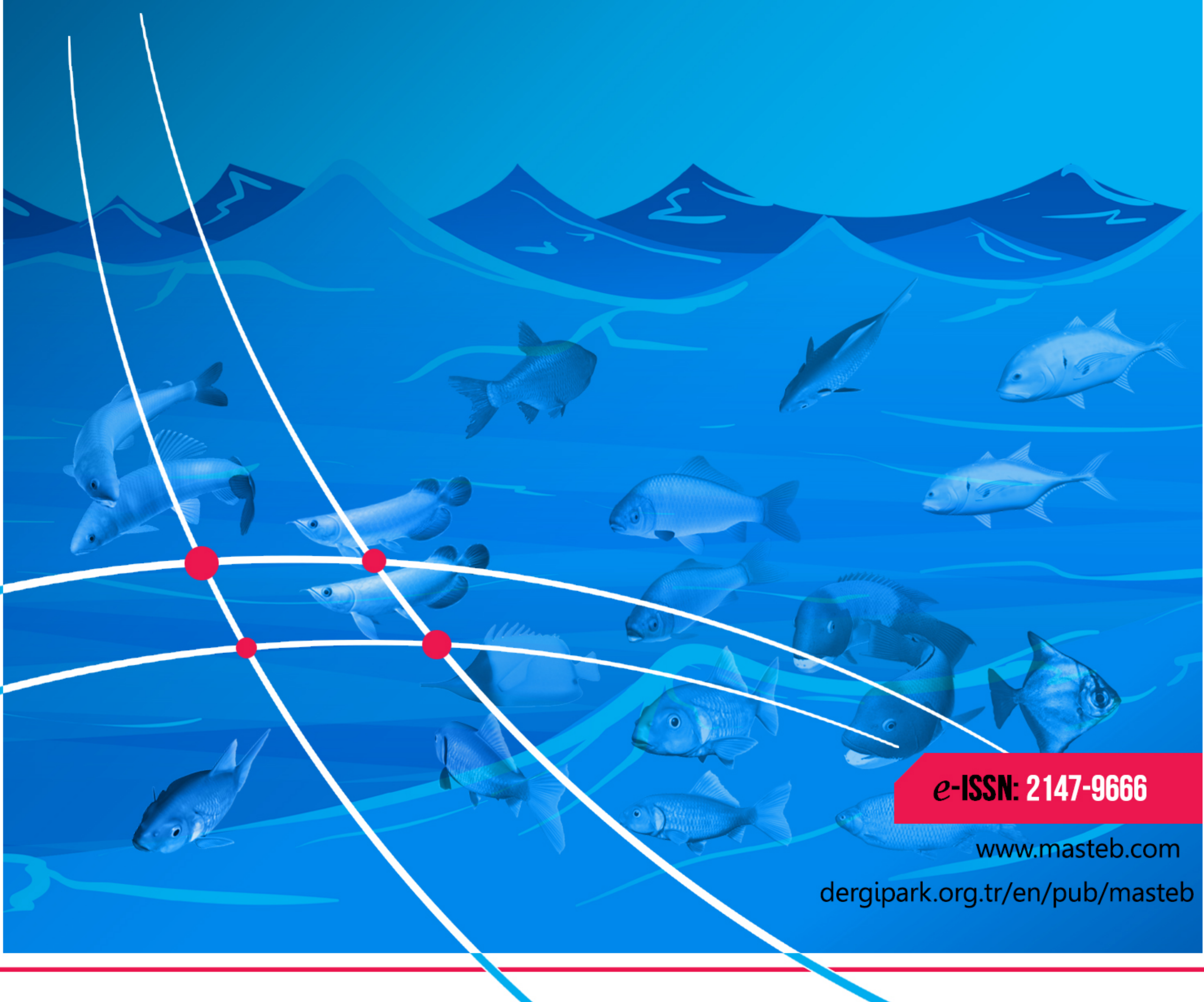

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