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Research Article

Phytoplankton assemblages in the ballast water of the navigated ships from the Indian Ocean to Shahid Rajaee Port, Hormozgan, Persian Gulf, Iran

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Abstract

The present study has been conducted to identify and enumerate ballast water phytoplankton on 24 ships entering Shahid Rajaee Port (13 bulk carriers, 4 oil tankers, 6 general cargo and 1 container), in two cold and warm seasons in winter 2017 and summer 2018, from the four origins as the name the Persian Gulf, western, eastern, and northwestern Indian Ocean.The sampling of phytoplankton was conducted by passing 100 liters of sampling waters through a 20-micron plankton net, in compliance with the G2 standard method approved by MEPC.173 (58). In this manner, 108 phytoplankton species were identified in the ballast water tanks of the sampled ships, belonging to 4 phylum, 5 classes, 23 orders, and 38 families. In the warm season, Dinophyceae (54%) and in the cold season, Bacillariophyceae (60.96%) were prevalent among the samples. The identified species from the Persian Gulf had the highest similarity with the species from the western Indian Ocean, and the least similarity with the eastern Indian Ocean. Apart from *Odontella longicruris*, all other species had been reported in the waters of the Persian Gulf in previous studies. This result shows that the phytoplankton assemblages between Shahid Rajaee Port and other parts of the Indian Ocean have a high similarity and does not impose a real threat to the environment from the ballast water transfer. However, from the identified species, 28 species (25.9%) are known to cause red tide and death of other aquatic species in the region, which highlights the importance of continuous monitoring and discharge control in the marine environment of the region.

Keywords: Phytoplankton, Invasive species, Ballast water, Shahid Rajaee port, Persian Gulf, Iran

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Introduction

One of the four major threats against oceans, and the main cause of transfer for 40 percent of non-indigenous and invasive species to the ports around the world, is the unintentional transfer of these species through ballast water (Hallegraeff and Bolch, 1992; Ruiz *et al*., 1997; Dickman and Zhang, 1999; Endresen *et al*., 2004; David *et al*., 2007; Pereira *et al*., 2014; Steichen *et al*., 2014; Soler-Figueroa *et al*., 2020). The growth of commerce, expansion of shipping routes, increase in speed, traffic, capacity, and the number of ships on the one hand, and reduced length of voyages, lower number of maritime incidents, promoted safety of navigation, and carriage of 3 to 5 billion tons of ballast water around the world per year (Drake *et al*., 2007; Tsolaki and Diamadopoulos**,** 2010; Satir, 2014; Cordell *et al*., 2015) on the otherhand has resulted in the transfer of 7 to 10 thousand living species from one location to another (Drake and Lodge, 2004; Lakshmi *et al.,* 2021), in a manner that every 9 weeks, an invasive species is relocated to a new environment (Dickman and Zhang, 1999; Steichen *et al*., 2014). The entry of invasive species into an ecosystem, with the assumption of their stability and persistence in the new environment and threat toward the indigenous species, imposes two major issues on the region: 1) impact on the economic and fisheries species, which would, in turn, affect the revenues generated from fishing activities; and 2) jeopardized biodiversity of the region, through threatening one or more indigenous species, which will leave a long-term impact, as elimination of a species from the life cycle will affect the related cycles before and after that species, and the whole ecosystem as a result (Burkholder *et al*., 2007; Balaji *et al*., 2014; de Castro *et al*., 2017).

To control the transfer and invasion by non-indigenous aquatic species through the ballast water of ships, the International Maritime Organization (IMO) responsible for developing international regulations for the safety of navigation and marine environment protection, has defined an approved ballast water management methods in the form of the International Convention for Control and Management of Ships’ Ballast Water and Sediments, for all ships above 400 GT (Gross tonnage**)** following their age and ballast water capacity (Gollasch *et al*., 2007; Tjahjono *et al*., 2017; Čampara *et al*., 2019). This convention entered into force on8 September 2017, the Islamic Republic of Iran ratified the convention as the 28th party (Balley and Munawar, 2015).

According to Regulation B-4 of the convention, all ships shall exchange their ballast water at least 200 nautical miles from the nearest land and in the water at least 200 meters in depth. If not possible for any reason, the exchange should be as far from the nearest land as possible, and in all cases at least 50 nautical miles from the nearest land and in the water at least 200 meters in depth, or within oceanic waters, due to the different conditions between coastal

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and oceanic areas (Cordell *et al*., 2015). As per Resolution MEPC.297 (72) regarding revision of Regulation B-3, all tankers from 150 to 400 GT, and other ships not required to have IOPP Certificates shall install ballast water management systems onboard complying with Regulation D-2 not later than 8 September 2024 (Gerhard *et al*., 2019; Van Slooten, 2021). By the same token, the Marine Environment Protection Committee (MEPC) identified the Persian Gulf as a special area in 2007 (MEPC.1/Circ.778/ Rev.3),which highlights the particular oceanic conditions, extensive traffic of ships, and the environmental sensitivity and vulnerability of the region to exert special measures over maritime activities, especially shipping (Wang and Liu, 2021).

The Persian Gulf is a semi-enclosed, secluded, and shallow sea (Al-Majed, 2000), and is considered among the most diverse marine ecosystems with its low depth (average 35m), high salinity (about 40ppt), and high temperature (average 26°C), and low precipitation (below100mL). It houses rich reserves of aquatic species and marine biodiversity of fish, and the majority is of native variety and low tolerance range against environmental changes due to severe stresses, which renders them highly vulnerable to the entry of pollutants and non-indigenous species to their ecosystem (Mirza *et al*., 2014). Several studies have been conducted on the changes of diversity and number of planktons in the ballast tank of ships in the Persian Gulf, such as plankton study of tanks on 2 oil tankers in Shahid Rajaee Port (Sangpour *et al*., 2010; Shapoori and Gholami, 2014), and plankton study in the ROPME Sea Area (Dorgham, 2013), as well as other sea areas, including Malaysia (Siang *et al*., 2018) and Cooper Port(Slovenia) (David *et al*., 2007) with a focus on studying the impact of ballast water exchange on plankton population changes.

Shahid Rajaee Port is located on the entrance of the Persian Gulf, Strait of Hormuz, and international waterways, where various ships, container carriers, and oil tankers navigate, load, and discharge their cargo. The high traffic of such ships to this port is thus accompanied by the introduction of different non-indigenous and invasive species into the region through ballast water on ships. The issue of identifying threatening factors that enter the marine environment in this manner and result in reduced or lost efficiency and health of the ecosystems is therefore crucial and inevitable as an asset to satisfactory management, as the restoration of the resulting changes to many of such ecosystems varies between severely difficult and practically impossible. In line with the above necessity, the present study was conducted to identify and study the diversity and density of phytoplankton, as well as the possibility for transfer of phytoplankton species through the ballast water of ships entering Shahid Rajaee Port (Sangpour

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et al., 2010; Shapoori and Gholami, 2014; Tolian *et al*.,2020)

Materials and methods

Shahid Rajaee Port is located in Hormozgan Province, Iran (N: 27° 07′; E: 56° 04′) (Shirowzhan *et al*., 2016). The sampling for the present study was conducted on 24 ships entering this port from origins located in four regions, including the Persian Gulf (Iraq, Kuwait, Qatar, and the United Arab Emirates), eastern Indian Ocean (Malaysia, Indonesia, and Singapore), western Indian Ocean (Kenya, Djibouti, and Somalia), and northwestern Indian Ocean (Sri Lanka, India, and Pakistan) (Fig. 1), in two seasons of winter 2017 and summer 2018. The particulars of the ships involved in the present study are presented in Table 1.

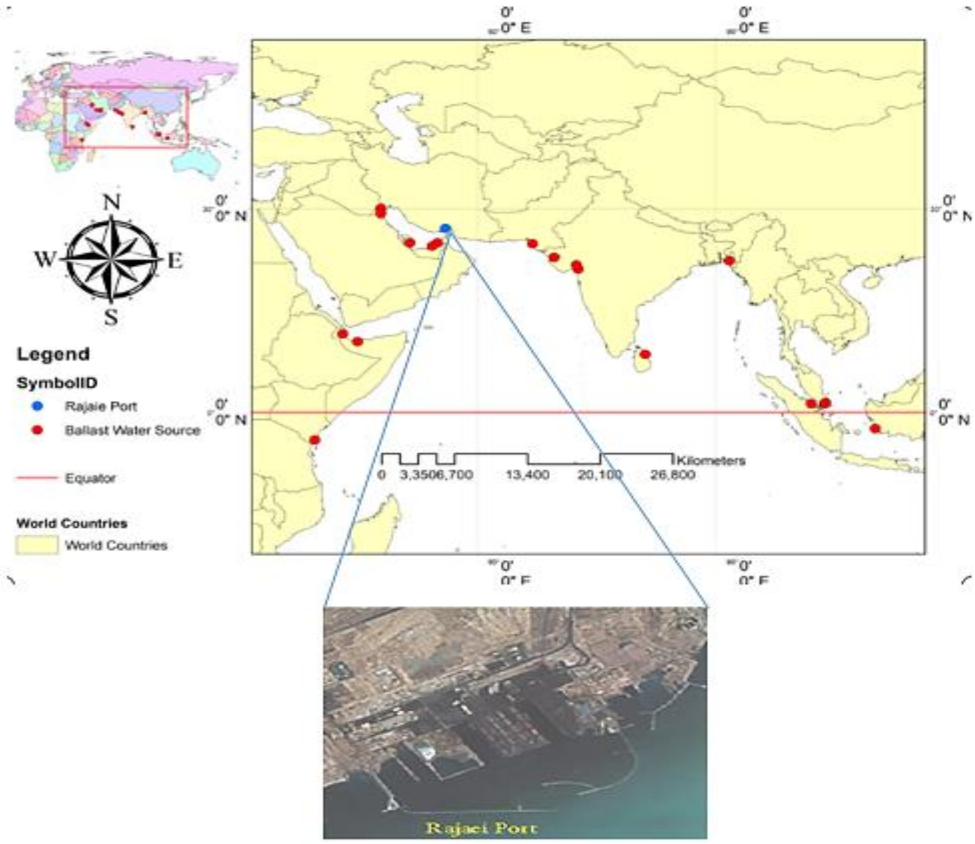

Figure 1: Geographical location of Shahid Rajaee Port, Hormozgan, Iran.

Sampling

The sampling was conducted in compliance with the G2 standard method approved by MEPC.173 (58) of the International Maritime Organization (IMO), and during the berthing of ships at the mentioned port (MEPC, 2008).

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Table1: Specifications of the 24 sampled ships in the Shahid Rajaee Port, Hormozgan, Iran.

Ships No	Ballast water Source	Ship Type	Gross Tonnage (t)	Season of sampling
S1	UMM QASR-IRAQ	General Cargo	34859	winter
S2	SHUWAIKH PORT- KUWAIT	Bulk Carrier	32983	winter
S3	HAMAD PORT- QATAR	Bulk Carrier	32945	winter
S4	JEBELALI-U.A.E	OIL Tanker	40037	summer
S5	MINA ZAYED- U.A.E	Bulk Carrier	17997	summer
S6	UMM QASR -IRAQ	Bulk Carrier	17979	summer
S7	MOMBASA- KENYA	Container	23389	winter
S8	MOMBASAKENYA	General Cargo	15670	winter
S9	MOMBASA- KENYA	General Cargo	16694	winter
S10	BERBERA- SOMALIA	Bulk Carrier	11743	summer
S11	DJIBOTI PORT	Bulk Carrier	33037	summer
S12	DJIBOTI PORT	Bulk Carrier	15609	summer
S13	PORT PASIR GUNAYNG - MALAYSIA	Bulk Carrier	33044	winter
S14	KENDAWANGAN- INDONESIA	General Cargo	25676	winter
S15	SINGAPORE PORT	General Cargo	36259	winter
S16	SINGAPORE PORT	Bulk Carrier	32305	summer
S17	SUNGAI PAKNING-INDONESIA	General Cargo	19354	summer
S18	SINGAPORE	OIL Tanker	29266	summer
S19	TRINCOMALEE- SRILANKA	OIL Tanker	32474	winter
S20	MAGDALLA- INDIA	OIL Tanker	32725	winter
S21	MUNDRA- INDIA	Bulk Carrier	32474	winter
S22	CHITTAGONG- BANGLADESH	Bulk Carrier	32305	summer
S23	KARACHI- PAKISTAN	Bulk Carrier	32945	summer
S24	GMB DAHEJ PORT- INDIA	Bulk Carrier	31117	summer

A total of 24 ships, including 13 bulk carriers, 4 oil tankers, 6 general cargo and 1 container, were sampled for phytoplankton existing in the ballast water. For 20 ships, the overflow method from the air pipe of the bottom tank was used, and on ships no. 1, 20, and 21 were sampled through the discharge valve near the discharge line. Considering the rapid loading and discharge of cargos ships, and in order not to delay the ships' operations, as well as safety considerations for the container carrier, the sampling on the container carrier was conducted by the crew through the discharge valve in the engine room.

For sampling purposes, 100 liters of ballast water was passed through a 20-micron plankton net, using a bucket, and the filtered samples were collected in a special container, fixed with 4-percent formalin, and transferred to the laboratory (David *et al*., 2007; Siang *et al*., 2018. Physical parameters inside ship tanks could affect the population of transferred species, and several such parameters, including temperature, salinity, and pH was thus

Phytoplankton Identification

To identify and study the density of phytoplankton at the species level, 3 takes (1 mL) of each sample were

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transferred onto Sedgewick Rafter counting chambers and studied by an inverted microscope (Axiovert S100), using standard identification keys (Hasle *et al*., 1996; Tomas, 1997; Al-Kandari *et al*., 2009).

Statistical analysis

The collected data was initially assessed through the Kolmogorov-Smirnov Test to ensure their normalcy, and then significant difference among parameters for sampling stations and seasons was considered through the One-Way ANOVA. Average values were also compared through Duncan multiple range tests, and Pearson Correlation Coefficient among the number of species, abundance, and the measured water parameters was calculated using the SPSS V. 21. The seasonal similarity of the sampled phytoplankton was determined through PAST and Ecological Methodology software packages, while the cluster analysis and PCA test were performed by PRIMER Version 5and PAST Version 2.13, respectively.

Results

Water parameters

The minimum and maximum ballast water temperature of 24 ships in winter 2017 and summer 2018 varied between 26.43° C and 40.06°C (average 33.24°C) in winter .In the cold season, salinity was between 34.76 and 38.33 ppt, the Persian Gulf had the highest registered level, while this parameter did not have any significant difference for the ballast water on ships loaded at the studied regions of the Indian Ocean ($p<0.05$).In the same season, the pH for ballast water taken from ships entering the port from origins in the western Indian Ocean registered the highest average of 8.10 ($p>0.05$). In the warm season, none of the measured parameters registered significant differences ($p<0.05$) between the Persian Gulf and the three studied regions of the Indian Ocean. The average value of ballast water physical parameters measured in the summer was 7.96 for pH, 36.73 ppt for salinity, and 38.60°C for temperature (Table 2).

Table 2: Measured physical parameters of 24 ship's ballast water in winter 2017 and summer 2018, Hormozgan, Iran.

Location	Season	Temperature (°C)	Salinity(ppt)	pH
Persian Gulf	Winter	26.43 ± 1.81^{b}	38.33 ± 0.26^{a}	7.84 ± 0.05^{ab}
	Summer	37.8 ± 0.36^{a}	38.16 ± 0.98^{a}	7.81 ± 0.05^{b}
West Indian Ocean	Winter	28.36 ± 0.16^{b}	35.46 ± 0.4^{ab}	8.1 ± 0.05^{ab}
	Summer	37.43 ± 0.6^{a}	36.36 ± 0.92^{ab}	7.76 ± 0.23^{b}
East Indian Ocean	Winter	28.7 ± 0.57^{b}	33.33 ± 0.36^{b}	7.87 ± 0.02^{ab}
	Summer	40.06 ± 0.57^{a}	35.46 ± 0.35^{ab}	8.23 ± 0.07^{a}
Northwest Indian Ocean	Winter	28.06 ± 1.66^{b}	34.76 ± 1.61^{ab}	7.95 ± 0.03^{ab}
	Summer	39.13 ± 1.09^{a}	33.5 ± 1.84^{b}	8.04 ± 0.05^{ab}

The same letters mean no difference and different letters mean a significant difference at the 5% level between experimental regions ($p<0.05$).

The values for ballast water temperature, salinity, and pH on ships entering Shahid Rajaee Port in winter 2017 and summer 2018 are depicted in Fig. 2. The values included limited fluctuation, and the only temperature registered a significant difference between summer and winter (p>0.05), meaning that on all ships understudy, the water temperature was higher in summer than winter (p<0.05).

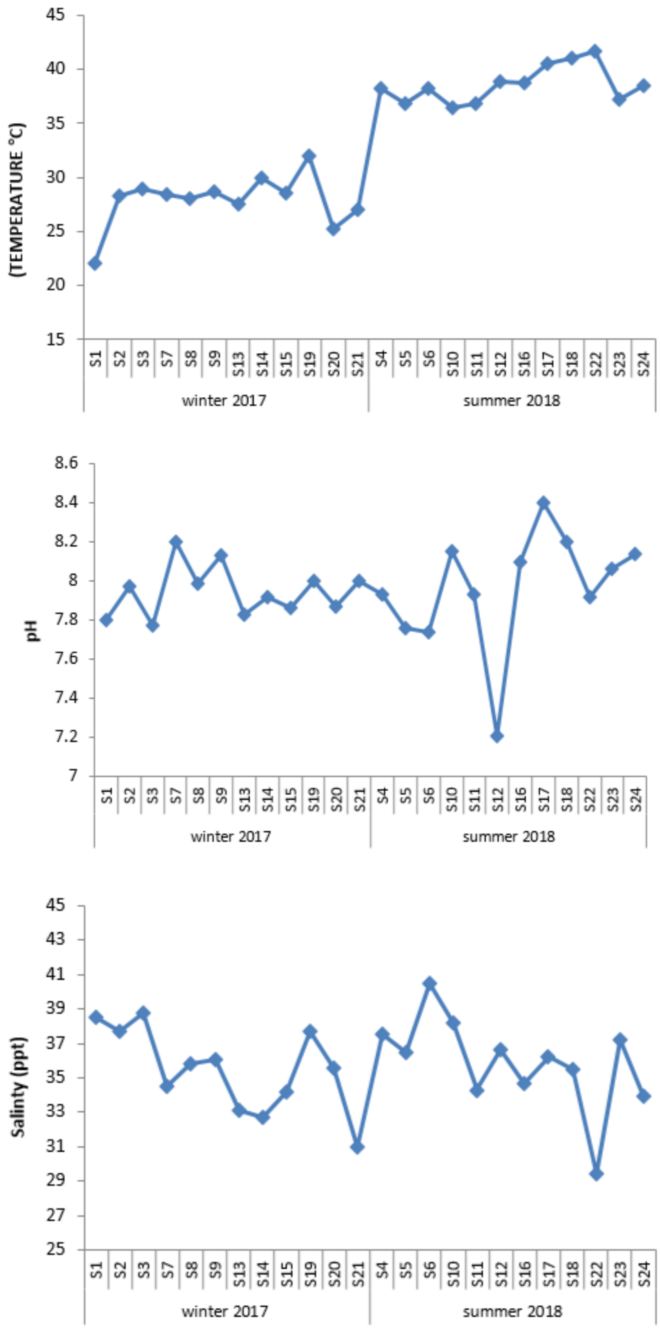

Figure 2: Measured physical parameters of 24 ship's ballast water entered to Shahid Rajaee Port in winter 2017 and summer 2018, Hormozgan, Iran.

Phytoplankton population composition

The results of sampling phytoplankton found in the ballast water of ships under study are presented in Table 3. The species identified in this study belonged to 4 phyla, 5 classes, 23 orders, 38 families, and 108 species. After studying the ballast tanks, it was determined that classes of Dinophyceae (with 6 orders, 14 families and 39 species) and Bacillariophyceae (with 13 orders, 18 families, and 57 species) accounted for 48 and 46 percent of the species respectively, while the classes of Raphidophyceae and Cyanophyceae (with 1 order, 1 family and 1 species) composed only 1 percent of the species identified. Moreover, in the warm season, class Dinophyceae (54 percent), and in the cold season, class Bacillariophyceae (60.96 percent) were prevalent among the samples (Figs. 3 and 4).

Table 3: Classification of phytoplankton observed in ballast water tanks on ships entering Shahid Rajaee Port (winter 2017 &summer 2018).

Phylum	Class	Order	Family	Genus/spices	HAB
Myzozoa	Dinophyceae	Gonyaulacales	Ceratiaceae	*Ceratium breve*	
				Ceratium macroceros	
				Ceratium dens	
				Ceratium furca	Al- Kandari *et al*., 2009
				Ceratium fusus	Al- Kandari *et al*., 2009
				Ceratium trichoceros	Al-Yamani *et al*., 2019(I)
				Ceratium lineatum	
				Ceratium massiliense	
				Ceratium tripos	
				Ceratium symmetricum	
			Ostreopsidaceae	*Alexandrium minutum*	Al-Yamani *et al*., 2019(I)
				Chaetoceros affinis	
				Chaetoceros curvisetus	Al-Yamani *et al*., 2019(I)
				Chaetoceros denticulatus	
				Chaetoceros socialis	Al-Yamani *et al*., 2019(I)
				Chaetoceros subtilis	
				Chaetoceros peruvianus	Al-Yamani *et al*., 2019(II) Al-Kandari *et al*., 2009

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Table 3 (Continued):

Phylum	**Class**	**Order**	**Family**	***Genus/spices***	**HAB**
			Gymnodiniaceae	*Cochlodinium polykrikoides*	
				Gonyaulax polygramma	SMR Fatemi *et al*., 2012; Matsuoka *et al*, 2010 Al-Yamani *et al*., 2019(I); Al- Kandari *et al*., 2009; Saraji, 2018
				Gyrodinium fusiforme	
				Gyrodinium spirale	
				Gymnodinium catenatum	Al-Yamani *et al*., 2019(I); Al- Kandari *et al*., 2009; Saraji, 2018
			Polykrikaceae	*Polykrikos kofoidii*	
			Pyrophacaceae	*Pyrophacus steinii*	
				Pyrophacus horroloogium	
			Peridiniaceae	*Peridinium quinquecorne*	Al-Yamani *et al.*, 2019(I)
				Scrippsiella trochoidea	Al-Yamani *et al*., 2019(I); Al- Kandari *et al.*, 2009
		Dinophysiales	Dinophysaceae	*Dinophysis rotundata*	Al-Yamani *et al*., 2019(I)
				Dinophysis acuta	Al-Kandari *et al.*, 2009
				Dinophysis miles Cleve	Al-Yamani *et al.*, 2019(I)
				Dinophysis caudata	Al-Yamani *et al.,* 2019(I); Al- Kandari *et al*., 2009
				Ornithocercusmagnificus	
		Peridiniales	Oxytoxaceae	*Oxytoxum scolopax Stein*	
			Protoperidiniaceae	*Protoperidiniumclaudicans*	
				Protoperidinium oceanicum	
				Protoperidinium pyriforme	
				Protoperidinium elegans	

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Table 3 (Continued):

Phylum	Class	Order	Family	Genus/spices	HAB
				Protoperidinium pellucidum	
			Brachidiniaceae	*karenia cf. brevis*	Al-Yamani *et al*., 2019(I); Al- Kandari *et al*., 2009
			Goniodomataceae	*Pyrodinium bahamense*	Al-Yamani *et al*., 2019(I); Al-Kandari *et al*., 2009 Saraji, 2018
		Prorocentrales	Prorocentraceae	*Prorocentrum gracile*	Al-Yamani *et al*., 2019(I); Al- Kandari *et al*., 2009
				Prorocentrum minimum	Al- yamani *et al*., 2019(I)
				Prorocentrummicans	Al-Yamani *et al*., 2019(I)
				Prorocentrum balticum	Al-Yamani *et al*., 2019(I) Al- Kandari *et al*., 2009
		Thoracosphaerales	Thoracosphaeraceae	*Goniodoma polyedricum*	
		Pyrocystales	Pyrophacaceae	*Pyrocystis noctiluca*	
Ochrophyta	Bacillariophyceae	Chaetocerotana eincertaesedis	Chaetocerotaceae	*Chaetoceros pseudocurvisetum*	Al-Yamani *et al*., 2019(I)
				Bacteriastrumfurcatum	Al-Yamani *et al*., 2019(II)
				Bacteriastrum hyalinum	
		Lithodesmiales	Lithodesmiaceae	*Helicotheca tamesis*	
				Ditylum brightwellii	
		Hemiaulales	Bellerocheaceae	*Bellerocheahorologicalis*	
				Bellerochea malleus	
				Climacodium frauenfeldianum	
			Hemiaulaceae	*Eucampia zoodiacus*	
				Hemidiscus cuneiformis	
				Eucampia sp.	
		Coscinodiscales	Coscinodiscaceae	*Coscinodiscus wailesii*	
				Coscinodiscus asteromphalus	

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Table 3 (Continued):

Phylum	Class	Order	Family	*nb*	HAB
				Coscinodiscus jonesianus	
				Coscinodiscus granii	
				Coscinodiscus marginatus	
				Coscinodiscus radiatus	
				Palmeria hardmaniana	
		Bacillariales	Bacillariaceae	*Bacillaria paxillifera*	
				Bacillaria socialis	
				Cylindrotheca closterium	Al-Yamani *et al.*, 2019(II) Al-Kandari *et al.*, 2009
				Nitzschia longissima	Al-Yamani *et al.*, 2019(I)
				Nitzschia behrei	
				Nitzschia sigma	
				Pseudonitzschia delicatissima group	
				Pseudonitzschia seriata group	
		Triceratiales	Triceratiaceae	*Odontella longicruris*	
				Odontella mobiliensis	
		Asterolamprales	Asterolampraceae	*Asteromphalus ehrenberg*	
				Asteromphalus flabellatus	
		Thalassiosirales	Stephanodiscaceae	*Cyclotella* sp.	
				Cyclotella stylorum Brightwell	
			Thalassiosiraceae	*Planktoniella sol*	
				Thalassiothrix frauenfeldii	
				Thalassiosira eccentrica	
			Lauderiaceae	*Lauderia annulata*	
		Naviculales	Diploneidaceae	*Diploneis splendida*	
		Leptocylindrales	Naviculaceae	*Navicula directa*	
				Navicula elegans	

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Table 3 (Continued):

Phylum	Class	Order	Family	Genus/spices	HAB
				Navicula sp.	
			Pleurosigmataceae	*Pleurosigma normanii*	
				Pleurosigma elongatum	
				Pleurosigma planctonicum	
				Pleurosigma diverse-striatum	
				Pleurosigma formosum	
				Pleurosigma angulatum	
			Pleurosigmataceae	*Pleurosigma strigosum*	
			Leptocylindraceae	*Leptocylindrus minimus*	
		Rhizosoleniaceae	Rhizosoleniaceae	*Dactyliosolen fragilissimus*	
				Guinardia flaccida	Al-Yamani *et al*., 2019(I)
				Guinardia striata	
				Proboscia alata	
				Rhizosolenia bergonii	
			Rhaphoneidaceae	*Diplomenora cocconeiforma*	
				Rhizosolenia robusta	
		Mastogloiales	Mastogloiaceae	*Mastogloia arabica*	
		Melosirales	Stephanopyxidaceae	*Stephano pyxispalmeriana*	
	Dictyochophyceae	Dictyochales	Dictyochaceae	*Dictyocha fibula*	Al- Kandari *et al*., 2009
		Surirellales	Surirellaceae	*Surirella splendida*	
				Surirella fastuosa	
Chromophyta	Raphidophyceae	Chattonellales	Chattonellaceae	*Heterosigma* sp.	
Cyanobacteria	Cyanophyceae	Oscillatoriales	Phormidiaceae	*Trichodesmium erythraeum*	Al-Yamani *et al*., 2019(I) Al- Kandari *et al*., 2009

According to Fig. 5, Bacillariophyceae was the most abundant class found on ballast waters from the Persian Gulf and western Indian Ocean, and Dinophyceae on ships from the eastern and northwestern Indian Ocean.

The density of phytoplankton also depicted that ships from the western Indian Ocean carried the highest density of phytoplankton in winter and ships from the eastern Indian Ocean in summer. The lowest density of ballast water phytoplankton was observed on ships from the northwestern Indian Ocean (Fig. 6).

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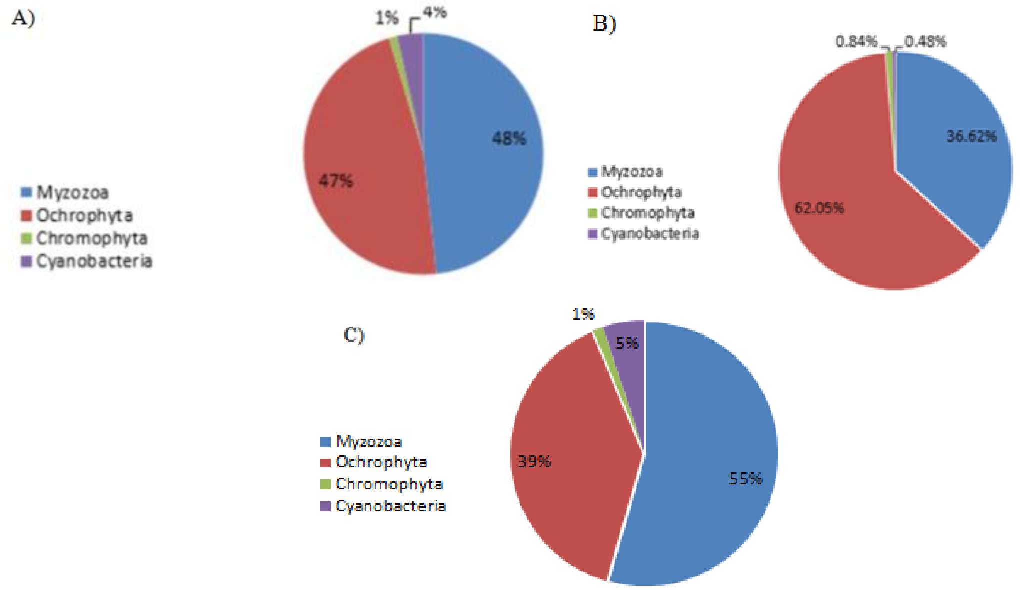

Figure 3: Abundance percentage (%) of identified prevalent phytoplankton; A: Total study period; B: winter 2017; C: summer 2018) in the ballast water of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

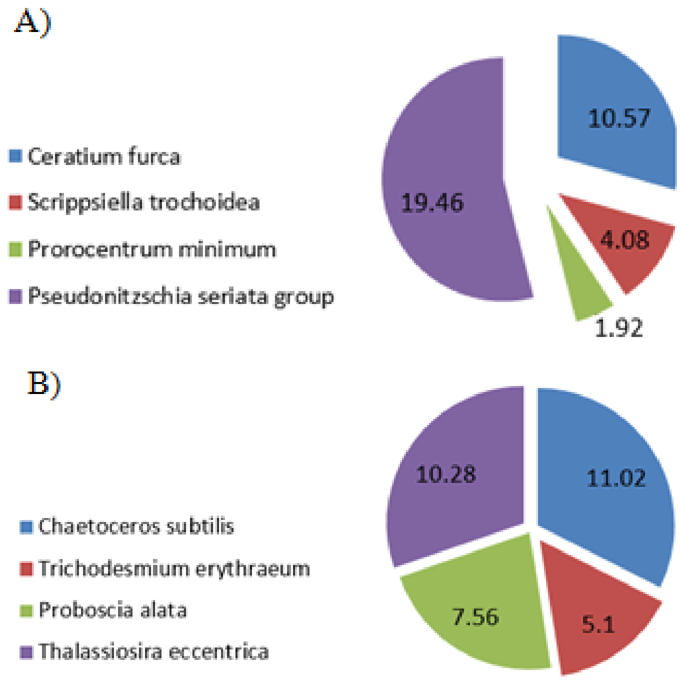

Figure 4: Abundance percentage (%) of identified prevalent phytoplankton species in the ballast water of entered ships (A: in winter 2017; B: in summer 2018) to Shahid Rajaee Port, Hormozgan, Iran.

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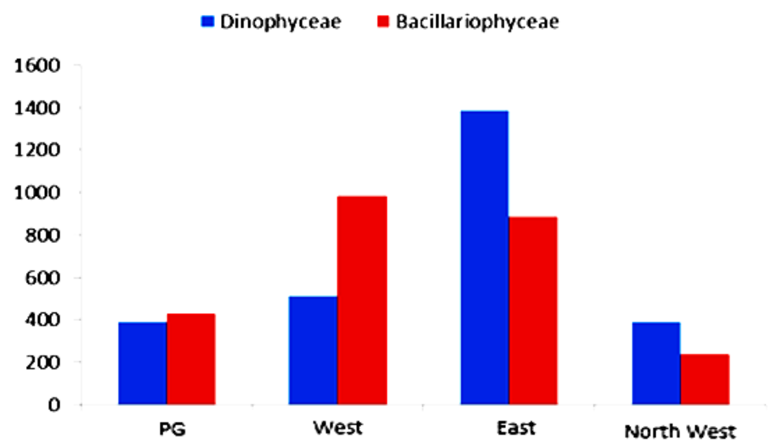

Figure 5: Main observed phytoplankton classes by regional origin in the ballast water of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

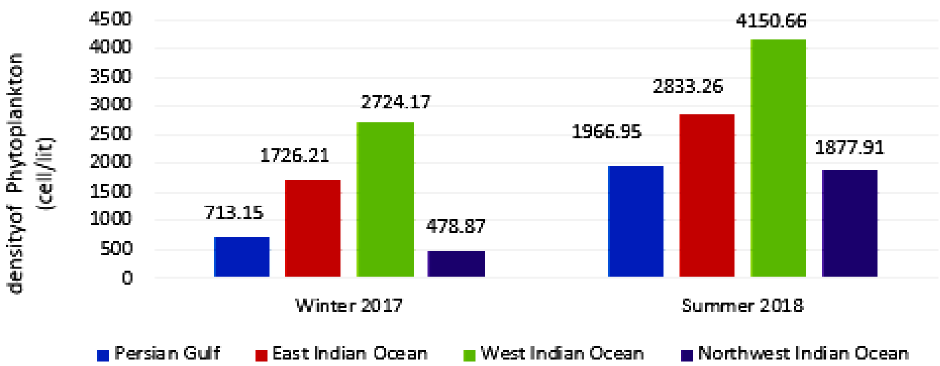

Figure 6: Impact of regional origin on the density of phytoplankton (Cells per Liter) in the ballast water of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

Ceratium breve and *Proboscia alata* were present in all regions in two seasons except on ships from the northwest Indian Ocean in winter. Different phytoplankton species are presented in Table 4 by season and region.

The results of the Pearson Correlation and PCA Test pointed to a positive correlation at 0.05 percent between phytoplankton abundance and temperature. In other words, the number of phytoplankton abundance increased with the increase in temperature of ballast water (Table 5 and Fig. 7).

Cluster analysis of similarity among regional origins of ships entering Shahid Rajaee Port

The results of the similarity test following Jaccard and Sorensen Indexes showed the highest similarity to exist between WW and WS (Jaccard: 0.563; Sorensen 0.72), and the lowest similarity between PGS and NW (Jaccard: 0.111; Sorensen 0.2), as presented in Table 6.

According to the above cluster analysis and identified phytoplankton, ships arriving from the western Indian Ocean had the highest similarity of phytoplankton density averages (64 percent) during winter and summer,

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while ships from the eastern and northwestern Indian Ocean registered the lowest similarity (44 and 4 percent, respectively) during winter and summer, with other regions under study (Fig. 8).

Table 4: Seasonal comparison of phytoplankton presence in ballast water tanks on ships entering Shahid Rajaee Port (winter 2017 & summer 2018), Hormozgan, Iran.

Species		Indian Ocean				Indian Ocean		
	Persian Gulf	West	East	North west	Persian Gulf	West	East	North west
	Winter				Summer			
Alexandrium minutum		+	+			+	+	
Asteromphalus ehrenberg		+					+	
Asteromphalus flabellatus		+				+		
Bacillaria paxillifera	+							
Bacillaria socialis							+	
Bacteriastrum furcatum		+	+					+
Bacteriastrum hyalinum			+				+	
Bellerochea horologicalis		+			+	+		
Bellerochea malleus		+	+				+	
Ceratium breve	+	+	+		+	+	+	+
Ceratium dens		+						
Ceratium furca	+	+	+	+		+	+	+
Ceratium fusus			+		+		+	+
Ceratiumlineatum		+	+			+	+	
Ceratium macroceros			+		+		+	
Ceratium massiliense		+	+			+	+	+
Ceratium symmetricum	+	+				+	+	
Ceratium trichoceros				+	+			+
Ceratium tripos					+	+		+
Chaetoceros affinis					+			
Chaetoceros curvisetus						+		
Chaetoceros peruvianus		+				+	+	+
Chaetoceros pseudocurvisetum		+			+			
Chaetoceros socialis			+			+		
Chaetoceros subtilis		+				+	+	+
Climacodiumfrauenfeldianum				+				
Cochlodiniumpolykrikoides							+	
Coscinodiscus asteromphalus		+					+	
Coscinodiscus granii	+			+	+	+	+	
Coscinodiscus jonesianus							+	
Coscinodiscus marginatus			+	+				
Coscinodiscus radiatus	+		+	+				
Coscinodiscus wailesii							+	+
Cyclotella sp.			+				+	+
Cyclotella stylorum Brightwell	+	+				+	+	
Cylindrotheca closterium			+			+	+	
Dactyliosolen fragilissimus							+	
Dictyocha fibula	+					+		+
Dinophysis acuta		+					+	+
Dinophysis caudata	+	+	+	+	+	+	+	+
Dinophysis cleve miles	+							
Dinoysis rotundata	+				+		+	

Table 4 (Continued):

Species	Persian Gulf	Indian Ocean			Persian Gulf	Indian Ocean		
		West	East	North west		West	East	North west
	Winter				Summer			
Diplomenora cocconeiforma			+				+	
Diploneis splendida				+				
Ditylum brightwellii						+	+	
Eucampia sp.						+		
Eucampia zoodiacus	+	+		+		+	+	
Goniodomapolyedricum							+	
Gonyaulax polygramma				+			+	
Guinardia flaccida	+	+		+				+
Guinardia striata	+						+	
Gymnodinium catenatum					+			
Gyrodinium fusiforme					+		+	
Gyrodinium spirale					+			
Helicotheca tamesis				+				
Hemidiscus cuneiformis								+
Heterosigma sp.			+		+		+	
karenia cf. brevis							+	+
Lauderia annulata					+		+	
Leptocylindrus minimus			+					
Mastogloia arabica		+				+	+	
Navicula directa			+	+				
Navicula elegans		+			+			+
Navicula sp.			+				+	
Nitzschia behrei							+	+
Nitzschia longissima			+					+
Nitzschia sigma		+			+	+		+
Odontella longicruris					+		+	
Odontella mobiliensis		+				+		
Ornithocercus magnificus	+	+				+		+
Oxytoxum scolopax Stein	+							
Palmeria hardmaniana	+							
Peridinium quinquecorne	+					+		+
Planktoniella sol	+	+				+		+
Pleurosigma angulatum			+					+
Pleurosigma diverse-striatum			+			+	+	
Pleurosigma elongatum	+	+				+		
Pleurosigma formosum			+					
Pleurosigma normanii				+			+	
Pleurosigma planctonicum							+	
Pleurosigma strigosum							+	
Polykrikos kofoidii					+	+		
Proboscia alata	+	+	+		+	+	+	+
Prorocentrum balticum			+				+	+
Prorocentrum gracile	+	+		+	+	+		+
Prorocentrummicans	+	+			+	+		+
prorocentrum minimum		+						+
Protoperidinium claudicans		+				+		
Protoperidinium oceanicum								+
Protoperidinium pellucidum		+					+	
Protoperidinium pyriforme		+				+		

Table 4 (Continued):

Species	Indian Ocean				Indian Ocean			
	Persian Gulf	West	East	North west	Persian Gulf	West	East	North west
	Winter				Summer			
Protopheridinium elegans								+
Pseudonitzschia delicatissima group	+	+				+	+	
Pseudonitzschia seriata group			+	+			+	
Pyrocystis noctiluca							+	
Pyrodinium bahamense		+	+			+	+	+
Pyrophacus horroloogium			+					+
Pyrophacus steinii								+
Rhizosolenia bergonii		+		+		+		
Rhizosolenia robusta				+				
Scrippsiella trochoidea		+				+		
Stephanopyxis palmeriana							+	
Surirella fastuosa				+				
Surirella splendida			+					+
Thalassiosira eccentrica	+	+			+	+		+
Thalassiothrix frauenfeldii			+		+		+	
Trichodesmium erythraeum	+		+		+	+	+	

Table 5: Correlation between measured parameters and phytoplankton density in the ballast water tanks on entered ships to Shahid Rajaee Port, Hormozgan, Iran.

Phytoplankton Correlation		Taxa	Abundance
Temperature	Pearson Correlation	.389	.483*
	Sig. (2-tailed)	.060	.017
	N	24	24
Salinity	Pearson Correlation	-.075	-.054
	Sig. (2-tailed)	.726	.801
	N	24	24
pH	Pearson Correlation	.080	-.029
	Sig. (2-tailed)	.712	.894
	N	24	24

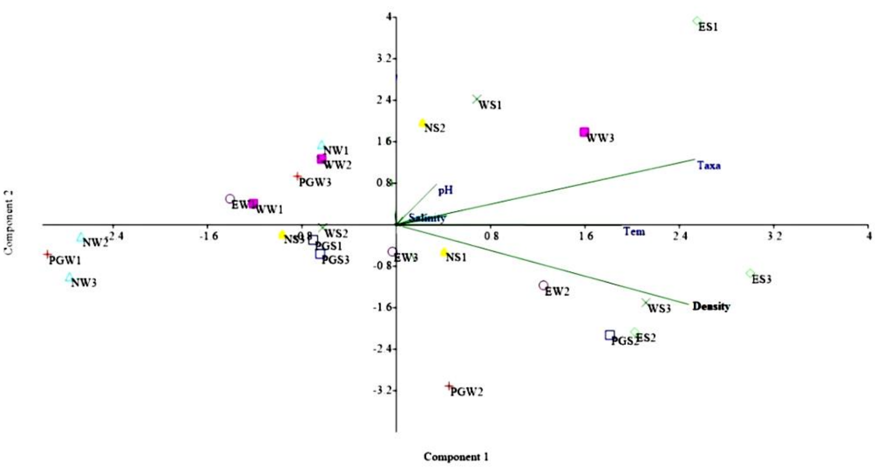

Figure 7: Principle Component Analysis (PCA) to consider the correlation between ballast water parameters and phytoplankton density in the ballast water tanks of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

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Table 6: Similarity among Regional Origins in the ballast water tanks of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

Region and Season	Index	PGW*	PGS*	WW*	WS*	EW*	ES*	NW*	NS*
PGW	Jaccard	1	0.178	0.28	0.37	0.113	0.196	0.195	0.326
	Sorenson	1	0.302	0.438	0.54	0.203	0.328	0.327	0.492
PGS	Jaccard		1	0.138	0.212	0.176	0.197	0.111	0.22
	Sorenson		1	0.242	0.349	0.3	0.329	0.2	0.361
WW	Jaccard			1	**0.563**	0.203	0.253	0.173	0.298
	Sorenson			1	**0.72**	0.338	0.404	0.295	0.459
WS	Jaccard				1	0.186	0.227	0.132	0.309
	Sorenson				1	0.314	0.37	0.233	0.472
EW	Jaccard					1	0.338	0.143	0.193
	Sorenson					1	0.506	0.25	0.324
ES	Jaccard						1	0.147	0.2
	Sorenson						1	0.256	0.333
NW	Jaccard							1	0.118
	Sorenson							1	0.211
NS	Jaccard								1
	Sorenson								1

*PG: Persian Gulf, W:west Indian Ocean, E:east Indian Ocean, N: North west Indian Ocean, W: Winter 2017, S: Summer 2018.

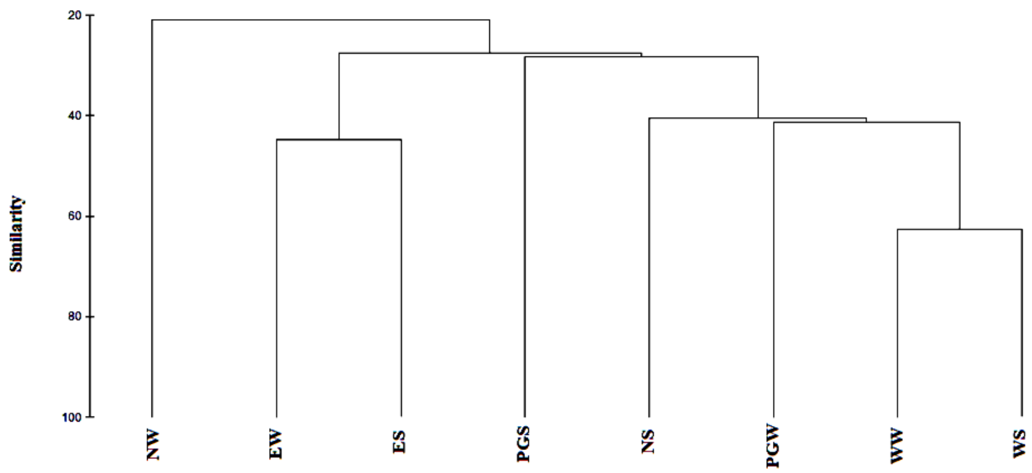

Figure 8: Similarity cluster analysis among regional origins in the ballast water tanks of entered ships to Shahid Rajaee Port, Hormozgan, Iran.
PG: Persian Gulf , W: West Indian Ocean, E: East Indian Ocean, N: North west Indian Ocean, W: Winter 2017, S: Summer 2018.

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Discussion

Ballast water carried on ships is a way which species are transferred from one aquatic environment to another and the consequences of such transfer exceed the mere introduction of pollutants such as oil, heavy metals, and persistent organic pollutants. Invasive and non-indigenous species either die under new environmental conditions or develop and spread, which leaves initially intangible and small impacts that aggravate and accelerate through time (Steichen *et al*., 2012; Vieira *et al*., 2018).

Uncontrolled exchange of ballast water and entry of invasive aquatic species is a growing concern that results in ecologic, economic, and health-related damages to the host environment (Werschkun *et al*., 2014; Wu *et al*., 2017). Hence, minimizing such invasion and transfer through ballast water carried on ships can be identified as a major priority for the maritime industries around the world (Balaji *et al*., 2014; de Castro *et al*., 2017).

Considering the importance of ballast water and the mandatory requirements to inspect it under articles6, 9 and 10of the convention focused on monitoring and promoting ballast water management in Iran (Bobka, 2018; Rey *et al*., 2018).The present study proceeded to analyze ballast water on board 24 ships, (including 13 bulk carriers, 4 oil tankers, 6 general cargo and 1 container) entering Shahid Rajaee Port in terms of phytoplankton species present, and the impact of ballast water physical parameters on their survival.

Changes in the physical parameters of the environment affect the survival of aquatic species to different degrees. In the present study, temperature as the only effective parameter on phytoplankton composition did not significantly differ among regional origins of ships (26.43°C to 28.70°C in winter, and 37.43°C to 40.06°C in summer), or with the temperature range in the Persian Gulf (32° C to 34°C) (Fazelpoor *et al*., 2016)(Table 5 and Fig. 7). With its impact on plankton metabolism, growth, enzyme activity, and cellular osmosis stress (D’ors, 2016), the temperature is considered among the most decisive parameters affecting micro-organic survival in ballast water tanks on ships.

The absence of significant temperature difference between origin and destination of ships thus confirms the similarity of phytoplankton species in such regions, and extensively increases the possibility of survival for species transferred from those regional origins. In a study by Siang *et al*. (2018), temperature and salinity were introduced as parameters affecting phytoplankton survival, and the impact of small temperature differences among ports of origin and destination on micro-organic survival after transfer into the new environment through ballast water discharge was highlighted, which corroborates the findings of the present study.

It was also observed that the number of phytoplankton in ballast water tanks was significantly higher in summer than winter ($p<0.05$), in a manner, that the average number of phytoplankton present in the ballast water of ships arriving from the Persian Gulf, western, eastern, and the northwestern Indian Ocean was measured as 655.65, 944.42, 1383.55 and 625.97 species in 100-liter water, respectively, in summer, and 237.72, 575.40, 908.05, and 159.62 species in 100-liter water, respectively,

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in winter. The reason can be attributed to the occurrence of stronger monsoons in summer than winter. The seasonal winds disrupt the water column and seabed structure, resulting in the higher dissolution of oxygen and other materials in water and the creation of more favorable living conditions for phytoplankton (Mitbavkar and Anil, 2006).

The ballast water tanks on all 24 ships entering Shahid Rajaee Port (the Persian Gulf) contained phytoplankton species: ships arriving from the Persian Gulf 43 species, western Indian Ocean 52 species, eastern Indian Ocean 65 species, and northwestern Indian Ocean 51 species. The results thus showed that incoming ships from the eastern Indian Ocean carried the highest number and density of phytoplankton in their tanks. Furthermore, the analysis of species and regional origins of the ships pointed to the similarity between species identified on ships from the Persian Gulf and western Indian Ocean (Fig. 8). The reason is the prevalent conditions in the Gulf of Oman at the connection of that Gulf to the Indian Ocean, the westbound currents in the northern parts, and eastbound currents on the southern coasts, which guide the water of the Indian Ocean (particularly in the west) toward the northern Gulf of Oman, and into the Persian Gulf through the Strait of Hormuz (Layeghi *et al*., 2019), and resulting in the highly similar biological conditions and plankton composition in the Persian Gulf and western Indian Ocean. The prevalence of class Bacillariophyceae in the ballast water further confirms such similarity, which can be attributed to the existence of silicate in ballast sediments as a nutrient for diatom growth and higher density and diversity of the mentioned class (Akbarzadeh *et al*., 2017).

The lowest density and number of samples and similarity between species was observed in the water coming from the Northwestern Indian Ocean, possibly due to the treatment system of the ballast water on ships no. 20 and 21 that arrived in Shahid Rajaee Port from India. The class of diatoms or Bacillariophyceae from the Ochrophyta phylum was frequent in the winter, because of the cryophilic behavior of the mentioned species (Hegedus *et al*., 2009). With the reduced frequency of this class under higher temperatures in summer, the class of Dinophyceae from the Myzozoa phylum replaced the Bacillariophyceae as the prevalent class.

Among the identified species, *Dinophysis clevemiles*, *Oxytoxum scolopax Stein*, *Palmeria hardmaniana*, and *Bacillaria paxillifera* were only observed in ballast water from the Persian Gulf, *Ceratium dens*, *Chaetoceros curvisetus*, *Scrippsiella trochoidea*, *Protoperidinium claudican*, *Protoperidinium pyriforme*, *Eucampia* sp., *Odontella mobiliensis*, and *Asteromphalus flabellatus* from the western Indian Ocean, *Cochlodinium polykrikoides*, *Goniodoma polyedricum*, *Pyrocystis noctiluca*, *Bacteriastrum*

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hyaline, *Coscinodiscus jonesianus*, *Bacillaria socialis*, *Navicula* sp., *Pleurosigma planctonicum*, *Pleurosigma formosum*, *Pleurosigma strigosum*, *Leptocylindrus minimus*, *Diplomenora cocconeiforma*, and *Stephanopyxi spalmeriana* only from the eastern Indian Ocean, and *Helicotheca tamesis*, *Climacodium frauenfeldianum*, *Diploneis splendida*, *Rhizosolenia robusta*, *Surirella fastuosa*, *Hemidiscus cuneiformis*, *Protopheridinium elegans*, and *Pyrophacus steinii* in the ballast water from the northwestern Indian Ocean. The species common between the Indian Ocean and the Persian Gulf were *Ceratium breve*, *Ceratium furca*, *Dinophysis caudate*, and *Proboscia alata*. Dinoflagellates are harmful species that result in red tide in the region. The class of Dinophysiales accounted for 3.88 percent of the identified species on average, with 4.05 percent in summer and 3.54 percent in winter. The harmful species identified in this study included *Dinophysis rotundata*, *Dinophysis acuta*, *Dinophysis miles Cleve*, *Dinophysis caudate*, *Peridinium quinquecorne*, and *Gonyaulax polygramma*, which not only pollutes marine species but also jeopardizes human life through intoxication. The presence of such invasive and dangerous species have been previously reported in studies by Lim *et al*. (2004; 2012) and Teen e*t al*.(2012) in the ballast water of ships entering in Kuantan Port of Malaysia, Roziawati *et al*. (2015) for Malacca, Tan *et al*. (2016) for Singapore, and Siang *et al*. (2018) for Malaysia. Out of the 108 species identified in the present research, 28 species were reported for causing red tide, such as *Prorocentrum gracile* in Qatar, *Prorocentrum baltīcum*, *Prorocentrum minimum*, *Peridinium quinquecorne*, *Guinardia flaccida*, *Prorocentrum balticum*, *Bacteriastrum furcatum*, *Gonyaulax polygramma* in Oman, *Trichodesmium erythraeum* in Oman and the Arabian Sea, *Cylindrotheca closterium* and *bahamense* in the United Arab Emirates. Others have caused death among animals and humans, such as *Alexandrium minutum*, *Ceratium furca*, *Ceratium fusus*, *prorocentrum minimum*, *Scrippsiella trochoidea*, and *Prorocentrum micans* (Table 3).

Thalassiosira and *Chaetoceros*, known for surviving extensive light levels (Liebich *et al*., 2012) and causing extensive and harmful red tide (Shen *et al*., 2012), were also observed among the Phytoplankton identified by the present study at Shahid Rajaee Port (Sidabutar *et al*., 2021). Among the 108 species identified by the present study in the ballast water of incoming ships, *Odontella longicruris* was the only species not previously identified and reported by relevant studies in the Persian Gulf (Al-Kandari *et al*., 2009; Saraji, 2018; Al-Yamani *et al*., 2019).

Based on the findings of the present study, although invasive or non-indigenous species were not observed among the identified species, and apart from the *Odontella longicruris* not

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previously reported in the region, all other species belonged to the phytoplankton composition of the host ecosystem, yet their unnatural presence in the new environment and survival capability due to negligible difference in temperature could be deemed as possible conditions for growing population and prevalence of a particular species, and the resulting adverse consequences, such as red tide and due to the high traffic of ships through the Strait of Hormuz and the possible transfer of invasive species to this port, however, the survival of even one non-indigenous species in the new environment could culminate in irreparable damages if not properly controlled and managed.

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