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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 vear (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 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 of the International the form Convention Control for 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 on 8 September 2017, the Islamic Republic of Iran ratified the convention as the 28<sup>th</sup> 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

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 2007 (MEPC.1/Circ.778/ area in Rev.3), which highlights the particular oceanic conditions, extensive traffic of ships, and the environmental sensitivity and vulnerability of the region to exert measures over maritime special 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 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

*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.

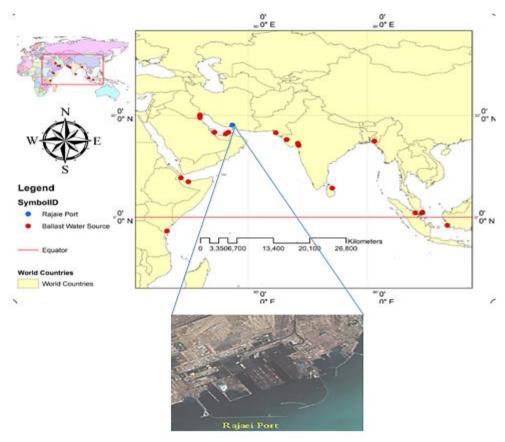


Figure 1: Geographical location of Shahid Rajaee Port, Hormozgan, Iran.

## 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).

Ships No	Ballast water Source	Ship Type	Gross Tonnage	Season of
NO			<b>(t)</b>	sampling
<b>S</b> 1	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
<b>S</b> 6	UMM QASR -IRAQ	Bulk Carrier	17979	summer
<b>S</b> 7	MOMBASA- KENYA	Container	23389	winter
<b>S</b> 8	MOMBASAKENYA	General Cargo	15670	winter
<b>S</b> 9	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 -	Bulk Carrier	33044	winter
	MALAYSIA	Buik Currier	33011	Willer
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

**Bulk Carrier** 

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.

GMB DAHEJ PORT- INDIA

S24

For sampling purposes, 100 liters of ballast water was passed through a 20micron plankton net, using a bucket, and the filtered samples were collected in a special container, fixed with 4percent 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 including parameters, temperature, salinity, and pH was thus

31117

summer

# Phytoplankton Identification

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

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 One-Way considered through the 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 5 and 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°  $\mathbf{C}$ 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. of the measured none registered significant parameters between differences (p < 0.05)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)	pН
Persian Gulf	Winter	$26.43\pm1.81^{b}$	$38.33\pm0.26^{a}$	$7.84\pm0.05^{ab}$
Fersian Gun	Summer	$37.8 \pm 0.36^{a}$	$38.16\pm0.98^a$	$7.81\pm0.05^{b}$
West Indian Ocean	Winter	$28.36\pm0.16^{b}$	$35.46\pm0.4^{ab}$	$8.1\pm0.05^{ab}$
west maran ocean	Summer	$37.43\pm0.6^{a}$	$36.36\pm0.92^{ab}$	$7.76\pm0.23^{b}$
East Indian Ocean	Winter	$28.7 \pm 0.57^{b}$	$33.33\pm0.36^{b}$	$7.87 \pm 0.02^{ab}$
East Indian Ocean	Summer	$40.06\pm0.57^{a}$	$35.46\pm0.35^{ab}$	$8.23\pm0.07^{a}$
Northwest Indian Ocean	Winter	$28.06\pm1.66^{b}$	$34.76\pm1.61^{ab}$	$7.95\pm0.03^{ab}$
Northwest Indian Ocean	Summer	$39.13\pm1.09^{a}$	$33.5\pm1.84^{b}$	$8.04\pm0.05^{ab}$

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).

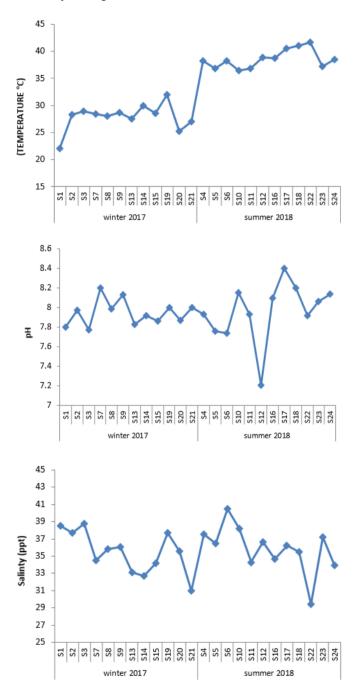


Figure 2: Measured physical parameters of 24 ship's ballast water entered to Shahid Rajaee Port in winter 2017 and summer 2018, Hormozgan, Iran.

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	Al-Yamani et
				peruvianus	al., 2019(II) Al-
					Kandari et al.,
					2009

**Table 3 (Continued):** 

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

**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	Chaetocerotaceae	Chaetoceros	Al-Yamani <i>et</i>
		eincertaesedis		pseudocurvisetum	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	

**Table 3 (Continued):** 

Phylum	Class	Order	Family	nb	HAB
				Coscinodiscus	
				jonesianus	
				Coscinodiscus granii	
				Coscinodiscus	
				marginatus	
				Coscinodiscus	
				radiatus	
				Palmeria	
				hardmaniana	
		Bacillariales	Bacillariaceae	Bacillaria paxillifera	
		Buelliariares	Buellialiaceae	Bacillaria socialis	
				Cylindrotheca	Al-Yaman
				closterium	
				Ciosierium	et al., 2019(II)
					2019(11) Al-
					Kandari et
				37. 7. 7	al., 2009
				Nitzschia longissima	Al-Yaman
					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.	
			1	Cyclotella stylorum	
				Brightwell	
			Thalassiosiraceae	Planktoniella sol	
			- Immosiosituode	Thalassiothrix	
				frauenfeldii	
				Thalassiosira	
				eccentrica	
			Tanada atau		
		NT. 1 1 1	Lauderiaceae	Lauderia annulata	
		Naviculales	Diploneidaceae	Diploneis splendida	
		Leptocylindrales	Naviculaceae	Navicula directa	
				Navicula elegans	

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 e
					al., 2009
		Surirellales	Surirellaceae	Surirella splendida	
				Surirella fastuosa	
Chromophyta	Raphidophyceae	Chattonellales	Chattonellaceae	Heterosigma sp.	
Cyanobacteria	Cyanophyceae	Oscillatoriales	Phormidiaceae	Trichodesmium	Al-Yamani et
				erythraeum	al., 2019(I)
					Al- Kandari e
					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).

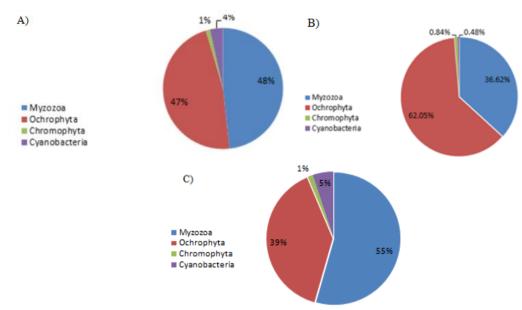


Figure 3: Abundance percentage (%) of identified prevalent phytoplankton; A: Total study period; B: winter 2017; C: summer 2018) in the ballast water of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

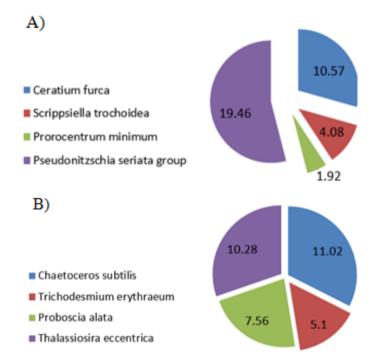


Figure 4: Abundance percentage (%) of identified prevalent phytoplankton species in the ballast water of entered ships (A: in winter 2017; B: in summer 2018) to Shahid Rajaee Port, Hormozgan, Iran.

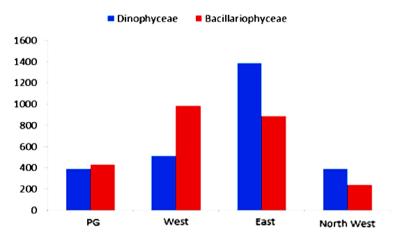


Figure 5: Main observed phytoplankton classes by regional origin in the ballast water of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

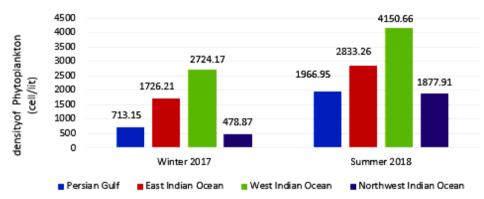


Figure 6: Impact of regional origin on the density of phytoplankton (Cells per Liter) in the ballast water of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

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,

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.

<u> </u>	•	In	dian Oc	ean		In	Indian Ocean		
Species	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	•		+	,	+		+	+	
Ceratium Jusus Ceratium lineatum		+	+		'	+	+	'	
Ceratium macroceros		ļ	+			ļ	+		
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	_	_1	+			.1		+	
Cylindrotheca closterium	+	+	.1			++	+		
•			+			+	+		
Dactyliosolen fragilissimus	,						+		
Dictyocha fibula	+					+		+	
Dinophysis acuta	,	+					+	+	
Dinophysis caudata	+	+	+	+	+	+	+	+	
Dinophysis cleve miles	+								
Dinoysis rotundata	+				+		+		

**Table 4 (Continued):** 

Table 4 (Continued):	Indian Ocean						Indian Ocean		
Species	Persian Gulf	West	East	North west	Persian Gulf	West	East	North west	
•		Win	ter			Sumr	ner		
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	+	ı				ı		'	
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):** 

		In	dian Oc	ean		Indian Ocean		
Species	Persian Gulf	West	East	North west	Persian Gulf	West	East	North west
		Win	ter			Sumr	ner	
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.

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

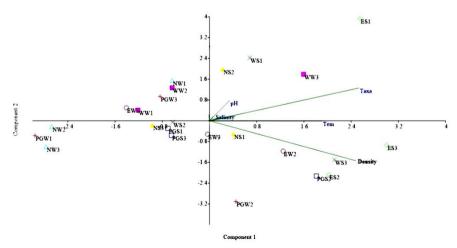


Figure 7: Principle Component Analysis (PCA) to consider the correlation between ballast water parameters and phytoplankton density in the ballast water tanks of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

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*	$\mathbf{EW}^*$	ES*	NW*	NS*
DCW	Jaccard	1	0.178	0.28	0.37	0.113	0.196	0.195	0.326
PGW	Sorenson	1	0.302	0.438	0.54	0.203	0.328	0.327	0.492
DCC	Jaccard		1	0.138	0.212	0.176	0.197	0.111	0.22
PGS	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
WW	Sorenson			1	0.72	0.338	0.404	0.295	0.459
WS	Jaccard				1	0.186	0.227	0.132	0.309
WS	Sorenson				1	0.314	0.37	0.233	0.472
EW	Jaccard					1	0.338	0.143	0.193
EW	Sorenson					1	0.506	0.25	0.324
ES	Jaccard						1	0.147	0.2
ES	Sorenson						1	0.256	0.333
NW	Jaccard							1	0.118
IN W	Sorenson							1	0.211
NC	Jaccard								1
NS	Sorenson								1

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

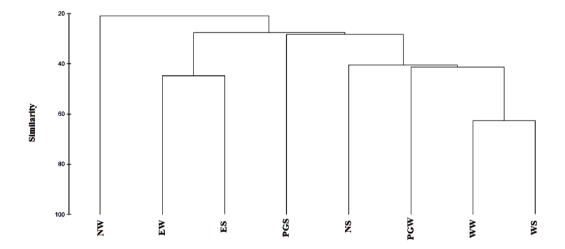


Figure 8: Similarity cluster analysis among regional origins in the ballast water tanks of entered ships to Shahid Rajaee Port, Hormozgan, Iran.

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

#### **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 nonindigenous 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 healthrelated damages the to 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 promoting ballast and management in Iran (Bobka, 2018; Rev 2018).The present 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 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 regions, and extensively such 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,

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. 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 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 Northwestern Indian Ocean. the possibly due to the treatment system of the ballast water on ships no. 20 and 21 that arrived in Shahid Rajaee Port from The class of diatoms 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.

identified Among the 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 mobiliensis, sp., **Odontella** and Asteromphalus flabellatus from the western Indian Ocean, Cochlodinium polykrikoides, Goniodoma polyedricum, **Pyrocystis** noctiluca, **Bacteriastrum**  hyaline, Coscinodiscus jonesianus, Navicula Bacillaria socialis, 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 Hemidiscus cuneiformis, fastuosa, Protopheridinium elegans, and Pyrophacus steinii in the ballast water from the northwestern Indian Ocean. species common between the Indian Ocean and the Persian Gulf were Ceratium breve. Ceratium Dinophysis caudate, and Proboscia Dinoflagellates are alata. 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 et 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 Oatar, Prorocentrum balticum. Prorocentrum minimum. Peridinium quinquecorne, Guinardia Prorocentrum balticum, flaccida, 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 nonindigenous species were not observed among the identified species, and apart from the Odontella longicruris not previously reported in the region, all belonged other species the phytoplankton composition of the host ecosystem, vet their unnatural presence in the new environment and survival capability due to negligible difference in temperature could be deemed as possible conditions for growing and prevalence population of 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 irreparable damages if not properly controlled and managed.

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