

## Research Article

# Zooplankton abundance and diversity in the ballast water of ships, Shahid Rajaei Port, Persian Gulf, Hormozgan, Iran

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### Abstract

In the present study, the ballast waters from 24 ships arriving at Shahid Rajaei Port in Bandar Abbas, Hormozgan Province, Iran, were studied in two warm and cold seasons (summer 2017 and winter 2018). The zooplankton sampling was conducted under the MEPC 173(58) method, by bucket and using a 50-micron mesh to filter 300 liters of ballast water. In total, 57 zooplankton species, belonging to 8 phyla, 11 classes, 15 orders, and 35 families were identified in the sampled ballast water, among which the Arthropoda phylum with 16 families and 23 species recorded the highest number of species. The highest and lowest density among the studied samples belonged to the *Tintinnopsis gracilis* species from the Codonellidae family (11.4%) and *Calanopia elliptica* species from the Pontellidae family (0.04%), respectively. The results of the Pearson correlation test showed that there was a negative correlation between the measured pH parameter and the frequency of zooplankton ( $p < 0.01$ ). There was also a positive correlation between EC and Shannon diversity index ( $p < 0.05$ ). PCA test results showed that EC, temperature, salinity, and TDS factors had the most impact in stations with the highest number of species and abundance of zooplankton. The results of the cluster diagram showed the highest similarity between winter at the Western Indian Ocean (WW) and summer at the Western Indian Ocean (WS) at the level of 54%, and the lowest similarity between the winters at the Northwestern Indian Ocean (NW) at the level of 24% with other regions. All the studied species in the ballast waters of the Persian Gulf had been identified and reported by previous studies but certainly requires continuous monitoring management of the ballast water, and supervising their discharge into the marine environment.

**Keywords:** Zooplankton, Ballast water, Western Indian Ocean, Shahid Rajaei Port, Persian Gulf

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## Introduction

The transportation of more than 90% of the world's goods in terms of volume and about 70% in terms of value is done by ships at sea with the increase of trade and the expansion of shipping routes (Raaymakers, 2002; David and Gollasch, 2015; Hess-Erga *et al.*, 2019). Ships need to carry ballast water to navigate safely, maintain stability during loading and balance their weight during the discharge of cargos (Grob and Pollet, 2016; Demirel *et al.*, 2019). The diversity in ship types, increase in the volume of ship tanks, variety of ballast water uptake points, route, and remaining time of water in tanks leave a significant impact on the survival probability of aquatic species in the ballast tanks when transferred from one point to another (David and Gollasch, 2015; Desai *et al.*, 2018; Lakshmi *et al.*, 2021). Ships transfer 10 billion tons of ballast water around the world every year (Gollasch, 1997; Wang *et al.*, 2020), a cubic meter of which can contain up to 50 thousand zooplanktons and 10 million phytoplanktons (Mohammadkhanloo and Ghassem, 2017; Desai *et al.*, 2018). This means that if an aquatic species can survive the adverse conditions inside a ballast tank, survive, settle and reproduce in the new ecosystem, and succeed in replacing the indigenous species, it will be identified as an invasive species (Steichen and Quigg, 2015; Grob and Pollet, 2016), and a threat to ecological, natural environments,

human health and hygiene, and economy of the destination region (David *et al.*, 2007; Alim, 2021), with detrimental impacts for the aquaculture, fishing, and tourism (Hallegraeff *et al.*, 1993). The United Nations has also identified Harmful Aquatic Organisms and Pathogens (HAOP) as one of the four major threats against the oceans (David and Gollasch, 2015; Hess-Erga *et al.*, 2019).

In the early 1980s, the numerous aquatic species observed outside their natural habitats attracted the attention of international forums (Gollasch *et al.*, 2007), the International Maritime Organization (IMO) thus proceeded in 2004 to approve the International Convention on the Management of Ballast Water and Sediments, through Resolution A.868 (Diasamidze *et al.*, 2019), and was ratified by the Islamic Republic of Iran on 6 April 2011. (Zaideen, 2019). According to the Convention, all ships with a gross tonnage above 400 tons on international voyages to manage their ballast water and sediments up to a certain standard, comprising of Regulation D1 for Ballast Water Exchange at a distance of at least 200 or 50 nautical miles from the nearest land, and depth of at least 200 meters in open seas and oceans, and Regulation D2 for Ballast Water Performance, about the installation of Ballast Water Treatment Systems on ships up to the year 2024 (Rahman, 2017; Saglam and Duzgunes, 2018). The ballast water needs to be sampled

and analyzed to ensure compliance of ships with ballast water management requirements (Diasamidze and Shotadze, 2019). The ballast water main contains different aquatic micro-organisms and pathogens, and zooplanktons have been identified as the second producers of organic resources, effective in energy transfer and food chain in aquatic species (Al-Yamani *et al.*, 2009; Mustapha, 2009), while their distribution depends on physical parameters, such as temperature, salinity, and turbidity (Sun *et al.*, 2007).

Different studies conducted around the world have identified and reported the transfer of invasive and aquatic organisms by ships (Zaideen, 2019). As for the diversity and density of zooplankton in ballast water in the Persian Gulf, there are reports available, such as the identification of zooplankton in the ballast water of ships entering Imam Khomeini Port (Salami and Savari, 2017), which did not identify any invasive species among those observed. Another study considered the population diversity of zooplanktons on the northern coasts of the Persian Gulf, and reported *Centropages* spp. and fish larvae to be of the highest and lowest density among the zooplankton species, respectively (Izadi *et al.*, 2018). Similar studies on zooplankton have been conducted around the world, such as Japan and Australia (Williams *et al.*, 1988), Hong Kong Port (Chu *et al.*, 1997), the Atlantic Ocean (Wonham *et al.*, 2001), North Sea

(Chandrasekera *et al.*, 2009), Colombo Port, Sri Lanka (David *et al.*, 2007), Koper Port, Slovenia (Desai *et al.*, 2018), Argentina Ports (Boltovskoy *et al.*, 2011), Chinese Ports (Wu *et al.*, 2017), Chesapeake Bay (Carney *et al.*, 2017), Malaysian Ports (Siang *et al.*, 2018), and California, US (Choi *et al.*, 2005).

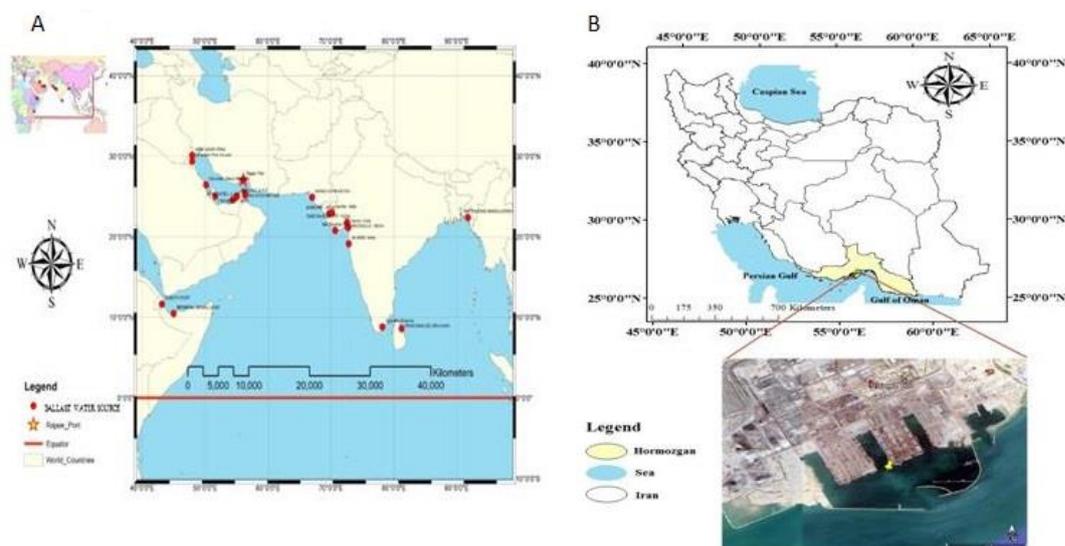
The Persian Gulf is a semi-enclosed sea with an average depth of 36 meters, and high salinity and temperature (Naser, 2013; Nosrati-Ghods *et al.*, 2017; Pazira *et al.*, 2019), which is of ecological, political, natural, and economic significance, and hosts over 40 percent of world tankers every year (Rashtchi *et al.*, 2013). After oil and petrochemical activities, fisheries are the most important economic activity conducted in this area (Mokhayer *et al.*, 2017). Shahid Rajaei Port is located at the entrance of the Persian Gulf, Strait of Hormuz, and the gateway to international waters, with a cargo loading/discharge capacity of 100 million tons, and shouldering 55 percent of Iranian trades and 70 percent of transits (Mahpour *et al.*, 2021). The high traffic of vessels at this port, however, also results in the entry of different non-indigenous and invasive species through the ballast water of ships. It is thus necessary to identify the threatening parameters for the marine ecosystems in this manner, intending to minimize such species invasions transferred by ballast water (Tjahjono *et al.*, 2017). For this purpose, the present study

investigated the diversity and density of zooplanktons as two parameters for predicting aquatic invasions through ballast water in Shahid Rajaei Port.

### Materials and methods

Shahid Rajaei Port is located in the Persian Gulf, Hormozgan province which was selected for the sampling of entered cargo ships (N: 27° 07'; E:

56° 04'). Sampling was done during two seasons, cold (winter 2017) and warm (summer 2018). The research period was selected to include warm and cold seasons, which could affect the type and number of incoming species. 24 ships from four regions including the Persian Gulf, East Indian Ocean, West Indian Ocean, and Northwest Indian Ocean were investigated in the Shahid Rajaei Port (Fig. 1).



**Figure 1:** A) Geographical location of origin ports of entered ships to Shahid Rajaei Port; B) Geographical location of Shahid Rajaei Port in Hormozgan Province, Iran (Jafari *et al.*, 2016)

The specifications of the surveyed ships are shown in Table 1. Desired parameters including temperature, salinity, pH, Total dissolved solids (TDS), and electrical conductivity (EC) were measured by the HQ40d multi-parameter device at the sampling site on ships. A sampling of zooplankton was performed according to the standard of MEPC 173 (58) method by filtering 300 liters of water by plankton net with a mesh size of 50

microns (MEPC, 2008; Boltovskoy *et al.*, 2011; Siang *et al.*, 2018). The samples were then fixed with 4% formalin and transferred to the laboratory. Identification and enumeration of zooplankton were performed using Bogorov chamber, Nikon ECLIPSE 80i inverted microscope, SMZ1500 Stereoscopic Microscope, and valid identification keys (Harris *et al.*, 2000; Al-Yamani *et al.*, 2011).

**Table 1: Details of the studied ships at Shahid Rajaei Port, Hormozgan province, Iran.**

No	Vessel Type	Ballast Water Source	Sample Point	Ballast Water Onboard (m <sup>3</sup> )	Region-Season-No
1	General Cargo	UMM QASR -IRAQ	Sample point	12511	PGW1
2	Bulk Carrier	SHUWAIKH -KUWAIT	Over flow (Air pipe)	11905	PGW2
3	Bulk Carrier	HAMAD - QATAR	Over flow (Air pipe)	15048	PGW3
4	OIL Tanker	JEBEL ALI - U.A.E	Over flow (Air pipe)	20923	PGS1
5	Bulk Carrier	MINA ZAYED - U.A.E	Over flow (Air pipe)	8511.26	PGS2
6	Bulk Carrier	UMM QASR - IRAQ	Over flow (Air pipe)	7923.62	PG-S-3
7	Container	MOMBASA - KENIA	Discharge valve in engine room	3337	EW1
8	General Cargo	MOMBASA -KENIA	Over flow (Air pipe)	6827	EW2
9	General Cargo	MOMBASA - KENIA	Over flow (Air pipe)	3279.26	EW3
10	Bulk Carrier	BERBERA - SOMALILAND	Over flow (Air pipe)	5528.84	ES1
11	Bulk Carrier	DJIBOTI PORT	Over flow (Air pipe)	13640	E-S-2
12	Bulk Carrier	DJIBOTI PORT	Over flow (Air pipe)	5165.4	E-S-3
13	Bulk Carrier	PORT PASIR GUNAYNG - MALAYSIA	Over flow (Air pipe)	11461.6	WW1
14	General Cargo	KENDAWANGAN - ENDONEZIA	Over flow (Air pipe)	11037	WW2
15	General Cargo	SINGAPORE PORT	Over flow (Air pipe)	14053.5	WW3
16	Bulk Carrier	SINGAPORE PORT	Over flow (Air pipe)	14393	WS1
17	General Cargo	SUNGAI PAKNING- ENDONEZIA	Over flow (Air pipe)	5520	WS2
18	OIL Tanker	SINGAPORE PORT	Over flow (Air pipe)	17576	WS3
19	OIL Tanker	TRINCOMALEE- SRILANKA	Over flow (Air pipe)	6172	NW1
20	OIL Tanker	MAGDALLA INDIA	Sample point	12780	NW2
21	Bulk Carrier	MUNDRA - INDIA	Sample point	11440	NW3
22	Bulk Carrier	CHITTAGONG- BANGLADESH	Over flow (Air pipe)	16138	NS1
23	Bulk Carrier	KARACHI-PAKISTAN	Over flow (Air pipe)	12947	NS2
24	Bulk Carrier	GMB DAHEJ PORT- INDIA	Over flow (Air pipe)	13502	NS3

\* PG: Persian Gulf; W: West Indian Ocean; E: East Indian Ocean; N: North West Indian Ocean  
W: Winter 2017; S: Summer 2018

### Biodiversity indices

Shannon-Wiener (H') and Simpson indices (D) were used for calculation of seasonal diversity as follow (Washington, 1984),

$$H' = \sum_{i=1}^s P_i \ln P_i$$

$$D = \sum_{i=1}^s P_i^2$$

Where  $P_i$  is the relative abundance of the  $i^{\text{th}}$  taxon,  $S$  is the total number of

taxa, Jaccard ( $S_j$ ) and Sørensen indices ( $S_s$ ) were used for calculation of seasonal similarity as follow (Washington, 1984):

$$S_j = \frac{a}{(a + b + c)}$$

Where  $S_j$  is the Jaccard similarity coefficient; a is the number of species common to (shared by) quadrats; b is the number of species unique to the first quadrat, and; c is the number of species unique to the second quadrat.

$$S_s = \frac{2a}{(2a + b + c)}$$

Where  $S_s$  is the Sorensen similarity coefficient, a is the number of species common to both quadrats, b is the number of species unique to the first quadrat, and c is the number of species unique to the second quadrat.

#### Statistical analysis

The normality of the data was checked using the Kolmogorov-Smirnov test. Then, significant differences between treatments were considered by one-way analysis of variance (One-way ANOVA). Duncan's test was used at a significant level of 0.05 to compare means (Elliott and Woodward, 2007). Pearson correlation analysis multiple

range tests were used to compare the means. Shannon and Simpson diversity and Jaccard and Sorensen seasonal similarity indices of sampled zooplankton were calculated by PAST and Ecological Methodology software (Krebs and Charles, 2001). Correlation between species number, abundance, and diversity indices and factors was investigated by SPSS software version 21. Cluster diagram was drawn by PRIMER software version 5 and PCA test was done by PAST software (Anderson *et al.*, 2019).

#### Results

According to the results obtained, 57 zooplankton species, belonging to 8 phyla, 11 classes, 15 orders, and 35 families were identified, among which the Arthropoda phylum with 4 classes, 8 orders, and 25 families, 43 species, recorded the highest density during the research period (Table 2). Moreover, Acartiidae and Paracalanidae had the highest number of species (5 species) among the identified families.

**Table 2: Systematic classification of identified zooplankton at Shahid Rajaei Port during cold (2017) and warm (2018) seasons, Hormozgan province, Iran.**

Phylum	Class	Order	Family	Genus/species
Annelida	Polychaeta	Phyllodocida	Lopadorrhynchidae	<i>Lopadorhynchus henseni</i> Polychaeta larvae
Chaetognatha	Sagittoidea	Aphragmophora	Sagittidae	<i>Sagitta enflata</i> <i>Sagitta regularis</i>
Chordata	Appendicularia	Copelata	Oikopleuridae	<i>Oikopleura dioica</i>
-	-	-	Fritillariidae	<i>Appendicularia sicula</i>
Chordata	Actinopteri	perciform	-	Fish larvae

Table 2 (continued):

Phylum	Class	Order	Family	Genus/species			
Ciliophora	Oligotrichea	Choreotrichida	Codonellidae	<i>Tintinnopsis gracilis</i>			
			Rhabdonellidae	<i>Protorhabdonella simplex</i>			
			Tintinnidae	<i>Amphorides amphora</i> <i>Dadayiella cuspis</i>			
			Ptychocylididae	<i>Favella</i> sp.			
Cnidaria	Hydrozoa	Siphonophorae	Diphyidae	<i>Diphyes chamissonis</i>			
Ctenophora	Tentaculata	Cydippida	Pleurobrachiidae	<i>Pleurobrachia pileus</i>			
Arthropoda	Ostracoda	Myodocopida	Cypridinidae	<i>Cypridina</i> sp.			
			Branchiopoda	Ctenopoda	Sididae	<i>Penilia</i> sp.	
Arthropoda	Hexanauplia	Calanoida	Calanidae	<i>Canthocalanus pauper</i>			
				<i>Acrocalanus gibber</i>			
				<i>Paracalanus indicus</i>			
				<i>Paracalanus</i> sp.			
			Paracalanidae	<i>Parvocalanus crassirostris</i>			
				<i>Parvocalanus elegans</i>			
				<i>Pontella investigatoris</i>			
			Pontellidae	<i>Calanopia elliptica</i>			
			Arthropoda	Hexanauplia	Calanoida	Pontellidae	<i>Labidocera bengalensis</i>
							<i>Labidocera kroyeri</i>
<i>Pontellopsis herdmani</i>							
Clausocalanidae	<i>Clausocalanus minor</i>						
Eucalanidae	<i>Euchaeta concinna</i>						
Centropagidae	<i>Centropages furcatus</i>						
	<i>Centropages tenuiremis</i>						
Pseudodiaptomidae	<i>Pseudodiaptomus arabicus</i>						
Temoridae	<i>Temora turbinata</i>						
Candaciidae	<i>Candacia bradyi</i>						
Acartiidae	<i>Acartia fossae</i>						
	<i>Acartia amboinensis</i>						
	<i>Acartia ohtsukai</i>						
	<i>Acartiella faoensis</i>						
	<i>cf Acartia</i> sp.						
	Tortanidae	<i>Tortanus barbatus</i>					
	Cyclopoida	Oithonidae	<i>Oithona simplex</i>				

**Table 2 (continued):**

Phylum	Class	Order	Family	Genus/species
				<i>Oithona nana</i>
				<i>Oithona brevicornis</i>
			Sapphirinidae	<i>Sapphirina nigromaculata</i>
			Corycaeidae	<i>Corycaeus andrewsi</i>
		Harpacticoida	Ectinosomatidae	<i>cf Microsetella</i> sp.
			Miraciidae	<i>Macrosetella gracilis</i>
			Tachidiidae	<i>Euterpina acutifrons</i>
			Peltidiidae	<i>Clytemnestra scutellata</i>
	Malacostraca		Mysidae	<i>Rhopalophthalmus</i> sp.
		Decapoda	Luciferidae	<i>Lucifer hanseni</i>
			Parthenopidae	<i>Parthenope</i> sp.
			Porcellanidae	<i>Pachycheles</i> sp.
		Stomatopoda	-	Stomatopoda larva
			Cyclopidae	<i>Nauplius</i> sp.
Mollusca	Bivalva	-	-	Bivalvia larvae

All the 24 incoming ships to Shahid Rajaei Port (Persian Gulf) under study carried zooplanktons in their ballast water. The comparison of the observed zooplanktons based on ballast water uptake locations and seasons is in Table 3. The number of species present in ship tanks in the two cold and warm seasons was 30 species on ships arriving from the Persian Gulf, 32 species on ships arriving from the Western Indian Ocean, 34 species on ships arriving from the Eastern Indian Ocean, and 24 species on ships arriving from the Northwestern Indian Ocean. According to these results, the ballast water of ships from the Eastern Indian Ocean also had a higher number of species and abundances in the ballast

water reservoirs. Among the identified species, *Tintinnopsis gracilis*, *Protorhabdonella simplex*, *Paracalanus indicus*, *Parvocalanus crassirostris* and *Acartia amboinensis* were the most abundant. According to specific species in Table 3, *Appendicularia sicula*, *Paracalanus indicus*, *Tintinnopsis gracilis*, *Oithona simplex* in the Persian Gulf region, *Protorhabdonella simplex*, *Centropages furcatus*, *Acartia amboinensis* and *Tortanus barbatus* in the east, *Centropages furcatus*, *Euchaeta concinna*, *Euterpina acutifrons* and *Parvocalanus crassirostris* in the west, and *Favella* sp. and *Parvocalanus elegans* in the northwest region were identified in both warm and cold seasons.

*Tintinnopsis gracilis* was present in all regions except Northwest Indian Ocean Summer and *Parvocalanus*

*crassirostris* in all regions except Persian Gulf Summer in both warm and cold seasons.

**Table 3: Comparison of presence (+) and absence (-) of identified zooplanktons at Shahid Rajaei Port during cold and warm seasons, Hormozgan Province, Iran**

Zooplankton	Persian Gulf		Indian Ocean (West)		Indian Ocean (East)		Indian Ocean (North West)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
<i>Acartia amboinensis</i>	+	+		+	+	+		+
<i>Acartia fossae</i>				+		+		
<i>Acartia ohtsukai</i>					+	+	+	
<i>Acartiella faoensis</i>							+	
<i>Acrocalanus gibber</i>	+	+	+	+				+
<i>Amphorides amphora</i>	+		+	+		+		
<i>Appendicularia sicula</i>	+	+						
Bivalvia larvae							+	+
<i>Calanopia elliptica</i>					+			
<i>Candacia bradyi</i>	+						+	
<i>Canthocalanus pauper</i>			+			+		
<i>Centropages furcatus</i>	+		+	+		+		
<i>Centropages tenuiremis</i>				+		+		
cf <i>Acartia</i> sp.	+							+
cf <i>Microsetella</i> sp.			+			+		
<i>Clausocalanus minor</i>			+		+	+		
<i>Clytemnestra scutellata</i>							+	
<i>Corycaeus andrewsi</i>	+					+		
<i>Cypridina</i> sp.	+		+		+	+		
<i>Dadayiella cuspis</i>		+						
<i>Diphyes chamissonis</i>			+	+				
<i>Euchaeta concinna</i>			+	+		+		+
<i>Euchaeta rimana</i>		+		+				+
<i>Euterpina acutifrons</i>	+	+	+	+		+		
<i>Favella</i> sp.	+		+				+	+
Fish larvae.	+							
<i>Labidocera bengalensis</i>	+					+		
<i>Labidocera kroyeri</i>	+					+		+
<i>Lopadorhynchus henseni</i>			+			-		+
<i>Lucifer hanseni</i>			+	+		+		
<i>Macrosetella gracilis</i>	+		-					
<i>Nauplius</i> sp.		+	+			+	+	
<i>Oikopleura dioica</i>					+	+		
<i>Oithona brevicornis</i>						+		
<i>Oithona nana</i>		+				+		
<i>Oithona simplex</i>	+		+	+				
<i>Pachycheles</i> sp.			+	+		+	+	
<i>Paracalanus indicus</i>		+				+		
<i>Paracalanus</i> sp.			+					

**Table 3 (continued):**

Zooplankton	Persian Gulf		Indian Ocean (West)		Indian Ocean (East)		Indian Ocean (North West)	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
<i>Parthenope</i> sp.				+				
<i>Parvocalanus crassirostris</i>	+		+	+	+	+	+	+
<i>Parvocalanus elegans</i>		+				+		+
<i>Penilia</i> sp.	+	+						
<i>Pleurobrachia pileus</i>			+					
Polychaeta larvae	+				+			
<i>Pontella investigatoris</i>		+					+	
<i>Pontellopsis herdmani</i>			+	+		+		
<i>Protorhabdonella simplex</i>	+	+	+		+	+		
<i>Pseudodiaptomus ardjuna</i>				+				
<i>Rhopalophthalmus</i> sp.					+	+		
<i>Sagitta enflata</i>	+	+				+		
<i>Sagitta regularis</i>		+	+		+		+	+
<i>Sapphirina nigromaculata</i>	+			+				
stomatopoda larva				+				
<i>Temora turbinata</i>			+	+		+		+
<i>Tintinnopsis gracilis</i>	+	+	+	+	+	+	+	
<i>Tortanus barbatus</i>					+	+		+

The highest abundance percentage of zooplankton during the study period was related to the Arthropoda (Fig. 2) and the highest number and abundance of zooplankton during the warm and cold seasons were related to Ciliophora and Arthropoda orders, respectively (Fig. 2; B: Warm Season; C: Cold Season).

According to Figure 3, Arthropoda and Ciliophora phyla had the highest density in the ballast water ships arriving from the Eastern and Western Indian oceans.

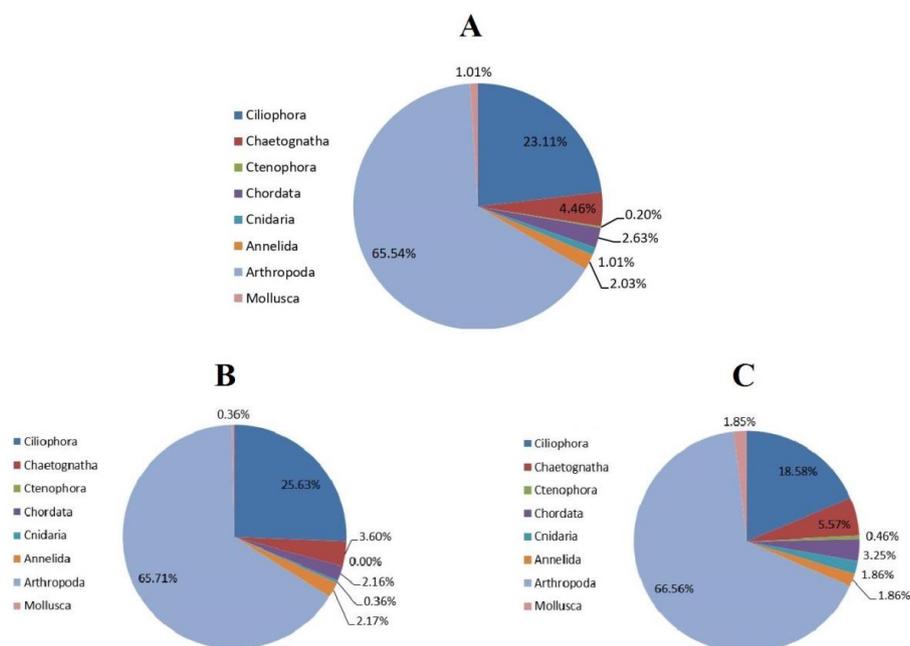
During the summer, ships arriving from the Eastern Indian Ocean carried the largest number of zooplanktons in their ballast water, and ships arriving from the Western Indian Ocean during the winter. The lowest numbers of

zooplankton were observed on ships arriving from the Northwestern Indian Ocean (Fig. 4).

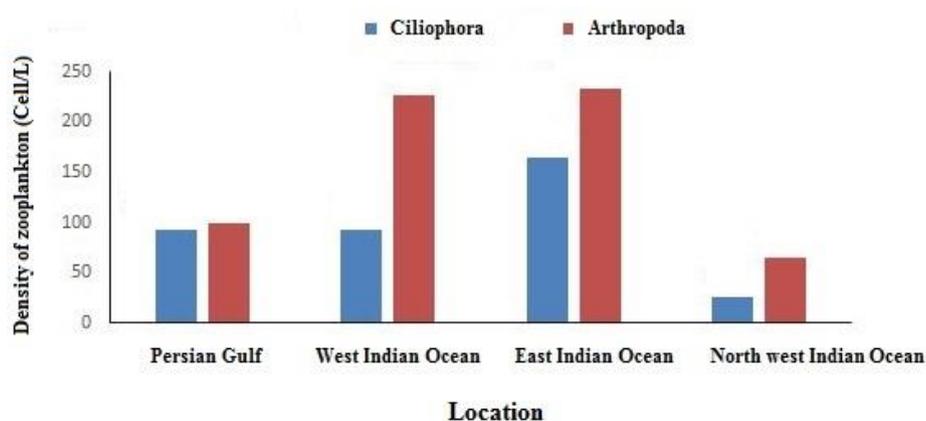
Based on the results of ANOVA, the temperature changes between different seasons and sampling ships were significantly different ( $p < 0.05$ ). The results of comparing the seasonal and average of environmental factors showed that there is a significant difference between the factors measured during the seasons and between sampling ships ( $P < 0.05$ ) (Table 4).

The comparison of the average number of species in seasons, the abundance of zooplankton, and diversity indices showed that there is a significant difference between these indices (except Shannon-Wiener

index) during the seasons and between sampling ships ( $p < 0.05$ ) (Table 5).



**Figure 2:** A) Abundance percentage of the identified zooplankton phyla in the ballast water of ships entering in Shahid Rajaei Port; B) Warm Season; C) Cold Season.



**Figure 3:** Main phyla of zooplankton sampled by origin in Shahid Rajaei Port, Hormozgan, Iran.

#### *Pearson correlation and PCA*

The results of Pearson correlation analysis between the measured factors with the number of species, the abundance of zooplankton, and diversity indices are shown in Table 6. The results of the Pearson correlation test showed that there was a negative correlation between the measured pH

parameter and the frequency of zooplankton ( $p < 0.01$ ). There was also a positive correlation between EC and Shannon diversity index ( $p < 0.05$ ) (Table 6). Also, PCA test results showed that EC, temperature, salinity, and TDS factors had the most impact in stations with the highest number of species and abundance of zooplankton

such as WW1, WW2, WW3, WS1, WS2, WS3, ES2, ES3, PGW2, PGW3, PGS2, NS1, and NS2 (Persian Gulf Winter (PGW), Persian Gulf Summer (PGS), West Indian Ocean Winter (WW), West Indian Ocean

Summer (WS), East Indian Ocean Winter (EW), East Indian Ocean Summer (ES), Northwest Indian Ocean Winter (NW), Northwest Indian Ocean Summer (NS) (Fig. 5).

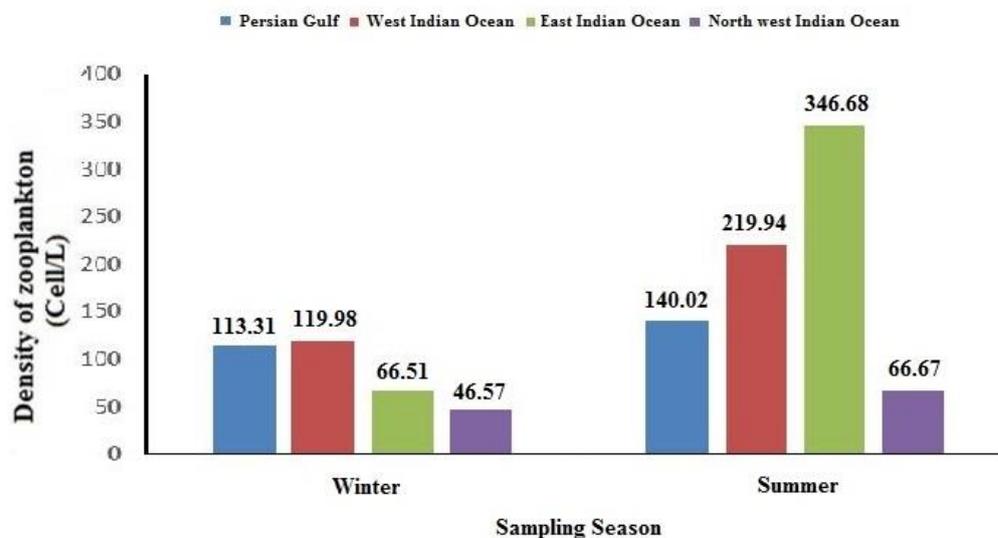


Figure 4: Effect of origin on the abundance of zooplankton (Cells per Liter) entering the ballast waters of Shahid Rajaei Port, Hormozgan, Iran.

Table 4: Comparison (Mean±SE) of measured factors at Shahid Rajaei Port during cold and warm seasons, Hormozgan province, Iran.

Location	Season	Temperature	Salinity	pH	*TDS	EC**
Persian Gulf	Winter	26.43±1.81 <sup>b</sup>	38.33±0.26 <sup>a</sup>	7.84±0.05 <sup>ab</sup>	37.16±0.63 <sup>a</sup>	55.66±1.48 <sup>ab</sup>
	Summer	37.8±0.36 <sup>a</sup>	38.16±0.98 <sup>a</sup>	7.81±0.05 <sup>b</sup>	37.6±1.02 <sup>a</sup>	58.5±1.38 <sup>a</sup>
West Indian Ocean	Winter	28.36±0.16 <sup>b</sup>	35.46±0.4 <sup>ab</sup>	8.1±0.05 <sup>ab</sup>	34.06±0.42 <sup>a</sup>	58.56±0.34 <sup>a</sup>
	Summer	37.43±0.6 <sup>a</sup>	36.36±0.92 <sup>ab</sup>	7.76±0.23 <sup>b</sup>	33.73±1.11 <sup>a</sup>	56±2.1 <sup>ab</sup>
East Indian Ocean	Winter	28.7±0.57 <sup>b</sup>	33.33±0.36 <sup>b</sup>	7.87±0.02 <sup>ab</sup>	32.64±0.22 <sup>a</sup>	51.4±0.25 <sup>b</sup>
	Summer	40.06±0.57 <sup>a</sup>	35.46±0.35 <sup>ab</sup>	8.23±0.07 <sup>a</sup>	36.36±0.59 <sup>a</sup>	56.4±1.28 <sup>ab</sup>
North West Indian Ocean	Winter	28.06±1.66 <sup>b</sup>	34.76±1.61 <sup>ab</sup>	7.95±0.03 <sup>ab</sup>	35.7±2.43 <sup>a</sup>	52.43±2.86 <sup>ab</sup>
	Summer	39.13±1.09 <sup>a</sup>	33.5±1.84 <sup>b</sup>	8.04±0.05 <sup>ab</sup>	37.06±2.24 <sup>a</sup>	52.08±1.68 <sup>ab</sup>

The same letters mean no difference and different letters mean a significant difference at the 5% level between experimental regions. \* EC: Electrical conductivity \*\*TDS: Total dissolved solids.

### Similarity

The results of the similarity test by Jaccard and Sorenson similarity indices showed that the highest similarity between WW and ES

(Jaccard: 0.41 and Sorenson: 0.582) and the lowest similarity between WS and EW (Jaccard: 0.1 and Sorenson: 0.182) and WS and NW (Jaccard: 0.1 and Sorenson: 0.182) were observed

(Table 7). Also, the results of the cluster diagram showed that there was the highest similarity between WW and ES at the level of 54% and the

lowest similarity between NW at the level of 24% with other regions (Fig. 6).

**Table 5: Comparison (Mean±SE) of Taxa, Individual, Simpson and Shannon diversity indices at Shahid Rajaei Port during cold and warm seasons, Hormozgan province, Iran**

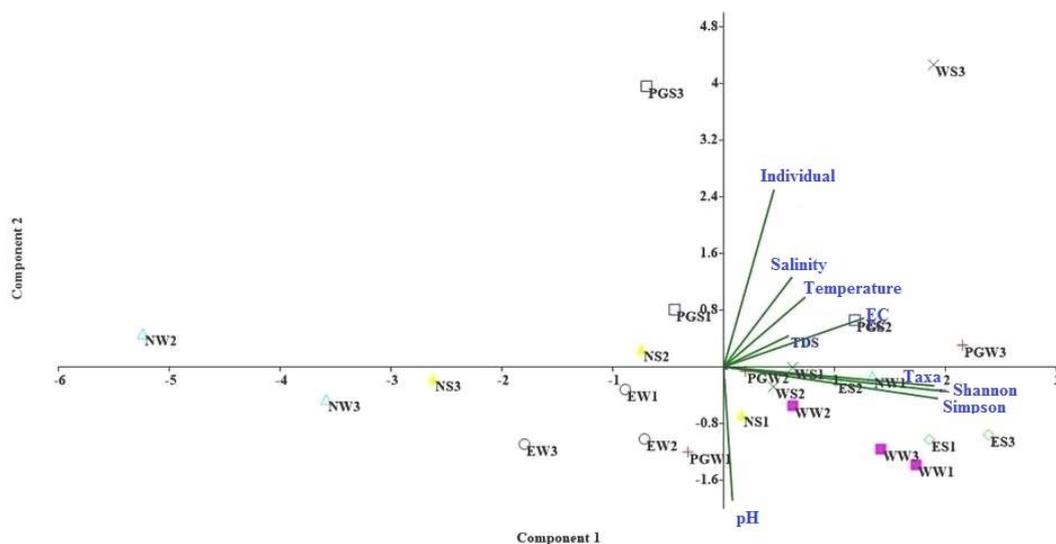
Location	Season	Taxa	Individual	Simpson	Shannon
Persian Gulf	Winter	8±2.64 <sup>abc</sup>	6.1±2.89 <sup>a</sup>	0.8±0.04 <sup>ab</sup>	1.82±0.28 <sup>a</sup>
	Summer	6±1.52 <sup>bc</sup>	35.66±25.65 <sup>ab</sup>	0.66±0.08 <sup>a</sup>	1.39±0.27 <sup>a</sup>
West Indian Ocean	Winter	10.66±1.45 <sup>ab</sup>	7.01±1.52 <sup>a</sup>	0.86±0.01 <sup>ab</sup>	2.16±0.14 <sup>a</sup>
	Summer	9.33±1.45 <sup>abc</sup>	48±39.35 <sup>a</sup>	0.85±0.02 <sup>a</sup>	2.06±0.17 <sup>a</sup>
East Indian Ocean	Winter	5.33±1.2 <sup>bc</sup>	6.77±4.48 <sup>ab</sup>	0.73±0.05 <sup>ab</sup>	1.46±0.21 <sup>a</sup>
	Summer	12.33±1.2 <sup>a</sup>	11.44±3.49 <sup>a</sup>	0.84±0.04 <sup>a</sup>	2.17±0.23 <sup>a</sup>
North West Indian Ocean	Winter	4±2.51 <sup>c</sup>	2.53±1.73 <sup>b</sup>	0.43±0.25 <sup>b</sup>	0.9±0.61 <sup>a</sup>
	Summer	4.66±1.76 <sup>bc</sup>	3.99±1.37 <sup>ab</sup>	0.67±0.09 <sup>ab</sup>	1.3±0.36 <sup>a</sup>

The same letters mean no difference and different letters mean a significant difference at the 5% level between experimental regions.

**Table 6: Pearson correlation between measured factors with Taxa, Individual, Simpson, and Shannon diversity indices at Shahid Rajaei Port during cold and warm seasons, Hormozgan province, Iran.**

Zooplankton Correlation		Taxa	Abundance	Simpson	Shannon
Temperature	Pearson Correlation	0.288	0.333	0.246	0.257
	Sig. (2-tailed)	0.172	0.112	0.246	0.225
	N	24	24	24	24
Salinity	Pearson Correlation	0.074	0.307	0.065	0.074
	Sig. (2-tailed)	0.730	0.144	0.764	0.730
	N	24	24	24	24
pH	Pearson Correlation	0.089	-0.665 <sup>**</sup>	0.026	0.019
	Sig. (2-tailed)	0.680	0.000	0.906	0.930
	N	24	24	24	24
TDS	Pearson Correlation	0.176	0.126	0.265	0.215
	Sig. (2-tailed)	0.412	0.558	0.210	0.312
	N	24	24	24	24
EC	Pearson Correlation	0.413 <sup>*</sup>	0.178	0.388	0.425 <sup>*</sup>
	Sig. (2-tailed)	0.045	0.405	0.061	0.038
	N	24	24	24	24

\*\* Correlation is significant at the 0.01 level (2-tailed); \* Correlation is significant at the 0.05 level (2-tailed).

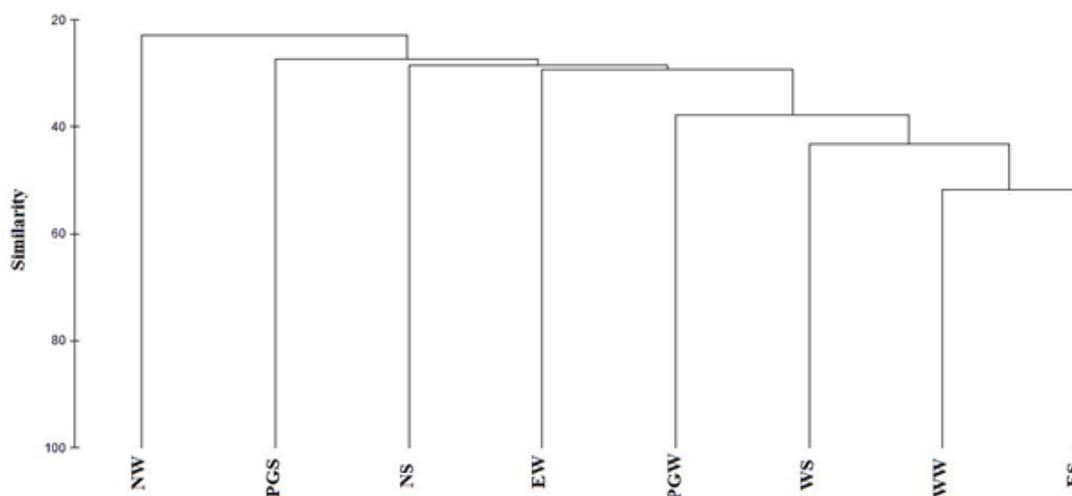


**Figure 5: Principle Component Analysis (PCA) based on the measured factors, Taxa, Individual, Simpson, and Shannon diversity indices at Shahid Rajaei Port during cold and warm seasons, Hormozgan province, Iran. Persian Gulf Winter (PGW), Persian Gulf Summer (PGS), West Indian Ocean Winter (WW), West Indian Ocean Summer (WS), East Indian Ocean Winter (EW), East Indian Ocean Summer (ES), Northwest Indian Ocean Winter (NW), Northwest Indian Ocean Summer (NS).**

**Table 7: Similarity comparison of sampled zooplankton at Shahid Rajaei Port during cold and warm seasons, Hormozgan Province, Iran**

Region and Season	Index	PGW	PGS	WW	WS	EW	ES	NW	NS
PGW	Jaccard	1	0.267	0.278	0.265	0.214	0.3	0.133	0.207
	Sorenson	1	0.421	0.435	0.419	0.353	0.462	0.235	0.343
PGS	Jaccard		1	0.182	0.156	0.16	0.237	0.174	0.2
	Sorenson		1	0.308	0.27	0.276	0.383	0.296	0.333
WW	Jaccard			1	0.136	0.194	<b>0.41</b>	0.2	0.226
	Sorenson			1	0.24	0.324	<b>0.582</b>	0.333	0.368
WS	Jaccard				1	<u>0.1</u>	0.333	<u>0.1</u>	0.207
	Sorenson				1	<u>0.182</u>	0.5	<u>0.182</u>	0.343
EW	Jaccard					1	0.303	0.19	0.174
	Sorenson					1	0.465	0.32	0.296
ES	Jaccard						1	0.132	0.184
	Sorenson						1	0.233	0.311
NW	Jaccard							1	0.182
	Sorenson							1	0.308
NS	Jaccard								1
	Sorenson								1

Persian Gulf Winter (PGW), Persian Gulf Summer (PGS), West Indian Ocean Winter (WW), West Indian Ocean Summer (WS), East Indian Ocean Winter (EW), East Indian Ocean Summer (ES), Northwest Indian Ocean Winter (NW), Northwest Indian Ocean Summer (NS).



**Figure 6:** Seasonal similarity comparison of sampled zooplankton at Shahid Rajaei Port during cold and warm seasons, Hormozgan province, Iran. Persian Gulf Winter (PGW), Persian Gulf Summer (PGS), West Indian Ocean Winter (WW), West Indian Ocean Summer (WS), East Indian Ocean Winter (EW), East Indian Ocean Summer (ES), Northwest Indian Ocean Winter (NW), Northwest Indian Ocean Summer (NS).

## Discussion

The results of some studies have shown that non-biological parameters such as transparency, pH, and the temperature in proportion to seasonal fluctuations affect the diversity and abundance of planktonic communities (Gabyshev and Gabyshevav, 2019). The measured temperatures of the tanks were in the temperature range of the Persian Gulf and were close to previous studies in this field (Nosrati-Ghods *et al.*, 2017). In total, the salinity of the measured water samples of the reservoirs was close to the salinity reported in the waters of the Persian Gulf and the Sea of Oman (Gwarmbeddin and Mosavi Dehmordi, 2019). The changes observed in the pH of the reservoir water ballast are usually due to the water nature of the oceans, which is currently alkaline (Taneez *et al.*, 2015). Due to the acidification of the seas due to human activities and the

fact that the Persian Gulf is a semi-closed sea and has a high potential for acidification, this range of pH does not pose a problem in the marine ecosystem (Kuffner *et al.*, 2008).

During this study, 8 phyla, 11 classes, 15 orders, 35 families, and 57 species of zooplanktonic communities were identified that arthropod had the highest species diversity, which conformed to the results obtained for waters of Bandar Abbas (Sadeghi *et al.*, 2018), and those on Saudi Coasts (Aziz *et al.*, 2003). Also, Acartiidae and Paracalanidae with 5 species had the highest number of species. In the study of Rezaei *et al.* (2010) on the zooplankton transmitted through the water ballast of ships in Shahid Rajaei Port in Bandar Abbas, 25 genera and species from 9 families including Oncaeidae, Oithonidae, Tachidiidae, Sagittidae, Eucalanidae, Temoridae, Centropagidae, Paracalanidae, and Acartiidae were identified. In the

study Salami and Savari (2017) on the identification of zooplankton groups transferred by water discharge of incoming ships to Imam Khomeini Port, a total of 21 families and 81 genera were reported, including 11 families Oithonidae, Oithonidae, Paracalanidae, Ectinosomatidae, Pontellididae, Sapphirinidae, Codonellidae, Oikopleuridae, Diphyidae, Oncaeidae, and Acartiidae which had the highest frequency. Zooplankton populations in ship ballast tanks may be affected by factors such as lack of light, adverse effects of non-living changes, water movements in the tank, food shortages, travel time, and vibrations caused by the ship's engine (Gollasch *et al.*, 2000). The diversity of species within ballast tanks can affect the environment and the region where the ballast water change operation has been performed (Chandy *et al.*, 1991; Carter *et al.*, 2005; McCollin *et al.*, 2007; Kumar *et al.*, 2020).

Zooplankton was found in all 24 ships entering the port of Shahid Rajaei (Persian Gulf). The number of species in the ballast water of ships originating in the Persian Gulf included 30 species, the Western Indian Ocean included 31 species, the Eastern Indian Ocean included 34 species and the Northwestern Indian Ocean included 21 species during the two cold and warm seasons. Some families of copepods (such as *Oithonidae simplex*) are common species in the Persian Gulf and the Gulf of Oman. The highest similarity

was observed between the identified species in the ballast water of ships originating in the Eastern Indian Ocean in the warm season and the Western Indian Ocean in winter due to the flow of water during the summer monsoon to the east and west (Gollasch *et al.*, 2000). The least similarity was observed between the identified species in the northwest. The reason for the low number of species can be due to the ballast water treatment in ships No. 20 and 21 that entered the Shahid Rajaei Port from India. In this study, the 57 zooplankton species observed in the ballast water of ships entering Shahid Rajaei Port were considered as indigenous species, previously reported by Al-Yamani *et al.* (2011) in a similar study. Liu *et al.* (2019) reported that the risk of species transfers by different types of ships entering the port of Kaohsiung in Taiwan is the same. Due to the lack of invasive species among the studied samples, the implementation of the reservoir ballast water exchange method can be appropriate to moderate the risk of transmission of invasive species, and Ship ballast water management should always be monitored because cysts may be present in ship floor sediments and may grow up to six months later (Hallegraeff, 1998; Tolian *et al.*, 2021). However, due to the tolerance of a wide range of different physicochemical environmental factors by different microorganisms and also the possibility of their

flourishing even in small numbers, it is recommended to use mechanical (such as filtration and separation), physical (such as sterilization by ozone, heat, electric currents and ultra-violet light) and chemical (such as adding biocides to ballast water, or combination of several treatment systems (with accurate and complete implementation of reservoir water change method (Chen *et al.*, 2021). Some researchers also consider the use of physical and chemical water treatment methods of ship ballast to be effective in eliminating invasive aquatic species (Tokus, 2019). However, the type of treatment system used depends on factors such as installation and maintenance costs, type of vessel, age of the vessel, and the region (Vorkapić *et al.* 2018). This is especially necessary for ships operating outside the Persian Gulf to change their ballast water. Also, the number of plankton species found in the ballast tanks due to sampling problems such as the large volume of water balance, shape, size, and the number of ballast tanks, uneven distribution, and their specific behavior in the tanks do not indicate the actual number of transferred species (Tolian *et al.*, 2021). These limitations can be considered a challenge for future studies.

Therefore, due to the existing limitations and the lack of seasons effect on the density of species transported by the water ballast of ships ( $p > 0.05$ ), the results of this study point to the effective

performance of the present methods for ballast water management in reducing the risk of micro-organism invasions, and their compatibility with the special conditions of the Persian Gulf with high salinity and temperature. There should be monitoring programs on the water quality of water ballast tanks to prevent the entry of invasive species in all seasons. Considering the potential growth of cysts and larvae, this can be construed as a threat against ecosystems. This information can be used as valuable information to assess the early risks of invasive species in the future and be an important tool for performing assessment and measurement of water ballast management.

According to the results obtained, Ship ballast water is one of the factors transferring different zooplankton species from the origin of the ship to the Persian Gulf. The results showed that the transferred species from the waters of the East, West, and Northeast of the Indian Ocean and other parts of the Persian Gulf to the waters of Rajai Port are similar to the species of the Persian Gulf and the invasive species or Non-natives were not identified among them. It is noteworthy that the Persian Gulf has a more acidic pH and higher salinity than the Indian Ocean, which reduces the survival of zooplankton. However, there is always a risk that invasive or resistant species will enter the ballast water tanks and become a major threat

to the ecosystem by adapting to the environment. Therefore, continuous management and monitoring of ballast water is an important issue.

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