

## Research Article

## Discharge compliance at Shanghai port – a case study on discharged ballast water from vessels with Ballast Water Treatment System

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### Abstract

A study was conducted on the biological compliance of discharged ballast water at Shanghai port from vessels with ballast water management systems (BWMS) installed. Samples were taken from 17 ships covering three ship types (cargo, tankers and containers) from 2019 to 2020. In addition, abiotic parameters including temperature, salinity, DO, pH and biotic parameters were measured. It was observed that over 80% of analyzed samples exceeded the D-2 standard. For  $\geq 50 \mu\text{m}$  organisms, the average density of living organisms ranged from 0 to  $2.3 \times 10^4 \text{ ind.} \cdot \text{m}^{-3}$ , including 6 phyla and 13 genera of which the dominant species was *Arthropoda*. The species number of living  $\geq 50 \mu\text{m}$  organisms found in ballast water samples was as high as 6. For 10–50  $\mu\text{m}$  organisms, the density of living organisms ranged from 0 to 156.5  $\text{cells} \cdot \text{mL}^{-1}$ , including 5 phyla and 38 species of which the dominant species were *Gymnodinium* sp., *Prorocentrum donghaiense*, *Cyclotella* sp., *Prorocentrum* sp. and *Platymonas* sp. The species number of living 10–50  $\mu\text{m}$  organisms found in ballast water samples was as high as 11. Another noteworthy result is that bacterial indicators detected in more than 40% of the ballast water samples were higher than the D-2 standard limit. Management decisions need to be made to prevent and control biosecurity issues caused by ship routes and reduce intestinal infections among residents in coastal port areas. The high percentage of non-compliant ballast water discharge after treatment at Shanghai port would significantly increase the risk of biological invasion to the local water, and preventive/mitigating measures shall be taken jointly by all involved parties.

**Key words:** ballast water management, biological invasion, non-compliant discharge, living organisms, shipping industry

### Introduction

Ballast water, usually taken in to maintain the stability and balance of the vessels during transit, has been associated with the transoceanic migration of living organisms (David et al. 2007; Li et al. 2013). Over 7,000 species are transported globally in ballast water carried by internationally sailing vessels every day (IMO 2003). These organisms may cause irreversible damages to the port and coastal ecology of the receiving waters (Cheniti et al.

**Table 1.** D-2 standard of Ballast Water Management Convention.

Organisms	≥ 50 μm organisms	10–50 μm organisms	Microbes		
			<i>Vibrio cholerae</i> O1 and O139	<i>Escherichia coli</i>	<i>Enterococci</i>
Limitation	< 10 ind per m <sup>3</sup>	< 10 cells per mL	< 1 CFU per 100 mL	< 250 CFU per 100 mL	< 100 CFU per 100 mL

CFU: colonial formation unit

2018; Ge et al. 2013; Mitchell et al. 2014; Park et al. 2017; Steichen et al. 2012). To mitigate the risk of the of Harmful Aquatic Organisms and Pathogens (HAOPs) transferred by ballast water, the International Maritime Organization (IMO) promulgated in 2004 the *International Convention for the Control and Management of Ships' Ballast Water and Sediments* (BWM Convention) (IMO 2004). The ballast water performance standard (D-2 standard) of the BWM Convention sets a standard on ballast water treatment and the maximum allowed density of living organisms in discharged ballast water are outlined in the Table 1. The BWM Convention entered into force in September 8<sup>th</sup> 2017, all vessels sailing internationally shall treat their ballast water to meet the D-2 standard for their ballast water discharge latest by September 8<sup>th</sup> 2024 (IMO 2018a).

*Prototheca* is often found in the discharged ballast water and it is also one common red-tide algae in China (Chen et al. 2019; Meng et al. 2016; Yang et al. 2018; Ye et al. 2018). Shanghai Port is adjacent to the high-risk area of red-tide occurrence in the East China Sea. In recent years, the incidence of red-tide in this area has been increasing (Li et al. 2016). Under certain conditions, the red-tide algae transported by ballast water may increase the risk of red-tide occurrence, and consequent ecological safety challenge shall also be paid sufficient attention (Wang et al. 2004). As per recent studies, Trochophore larva and Veliger larva, which are both marine shellfish larvae, may be transported by ballast water and lead to biofouling problems or become host of certain pathogens, after being discharged to recipient waters (David and Gollasch 2015; Motes et al. 1994).

Though most of the installed BWMSs have been type approved subject to type approval testing with very challenging testing water, the water conditions in real shipboard operations may be equally challenging, and the treatment efficiency of the BWMS during shipboard application is to be verified (IMO 2018c). It was found in some studies that the density of ≥ 50 μm organisms (mostly zooplankton) in ballast water without treatment could reach up to 10<sup>6</sup> ind·m<sup>-3</sup>, the density of 10–50 μm organisms (mostly phytoplankton) could reach up to 10<sup>4</sup> cells·mL<sup>-1</sup>, and *E. coli* could reach 5200 CFU/100 mL (David et al. 2007; Gollasch and David 2021; Li et al. 2013; Shao et al. 2018; Xue et al. 2011). These represented more strangest biological conditions than in the BWMS type approval testing and implied high challenges to the BWMS performance in shipboard operation. At the other hand, studies on BWMS performance onboard vessels during real operation under diverse and challenging conditions are still to be supplemented.

These can provide the shipowners and manufactures with more comprehensive practical proposals for improving compliance to the D-2 standard, as well as which valuable experience to the authorities and flag states (IMO 2019, 2020).

The ballast water exchange method, i.e. the D-1 method, is at this stage still valid for some vessels and is normally applied as contingency method when the treated ballast water cannot meet the D-2 standard. However, the intention of D-1 method is to exchange the stored ballast water with high sea water, of which the organisms are hard to survive in the local coastal water while being discharged. The D-1 method cannot replace the ballast water treatment, and furthermore, the D-1 method is phasing out and is not allowed for all vessels latest by September 8<sup>th</sup> 2024. Numerous internationally sailing vessels have installed the BWMS and apply ballast water treatment, however, the compliance of treated ballast water to the D-2 standard do not seem optimistic as per the latest studies (Bailey 2022). As China ratified the BWM Convention on January 22<sup>th</sup> 2019, treating the ballast water before discharge and meeting the D-2 standard are becoming a compulsory requirement for vessels calling at Chinese ports. For the Shanghai port, which is one of the world's largest container ports, it also becomes an urgent task to investigate and ensure compliance of ballast water discharged in the port, and thus reduce the ecological risk introduced by ballast water discharge.

The purpose of this study was to identify the type of living organisms (phytoplankton, zooplankton and microbial species and communities) in treated ballast water from vessels arriving at Shanghai port. The study is focusing on compliance to the D-2 standard among different ship types and BWMS technologies.

## **Materials and methods**

### *Basic Information of Ships*

The ballast water samples were taken from 17 vessels calling at Shanghai port from May 2019 to December 2020 for analysis of abiotic and biological parameters. Vessel information including vessel type and age, BWMS technology, holding time of ballast water before discharge were recorded by the ship crews, and shown in Table 2.

### *Sampling method*

The samples of discharged ballast water were collected from ships' ballast sampling ports. Before de-ballasting, one sampling pipe with a flowmeter was connected to the sampling port after the BWMS. In the first 5 minutes of de-ballasting, the sampling flow rate was adjusted to reach 50 L·min<sup>-1</sup>, no samples were collected during this period (IMO 2014, 2020). When sampling started after 5 minutes, the discharged water was sampled going

**Table 2.** Information of the sampling vessels.

Sample No.	Sampling date	Vessel type	Age of vessel (years)	BWMS technology	In-tank holding time before the de-ballast of ballast water	Water source
S1	May.22 2019	Tanker	12	Filtration + UV	5 days	Ulsan (Korea)
S2	May.27 2019	Container	1	Filtration + UV	15 days	Singapore
S3	May.31 2019	Tanker	12	Filtration + UV	0 hour	Shanghai (China)
S4	Jun.4 2019	Cargo	1	Filtration + UV	7 hours	Ulsan (Korea)
S5	Jun.17 2019	Container	2	Filtration + UV	52 days	Costal water of South China Sea (China)
S6	Jun.19 2019	Container	4	Filtration + UV	15 days	Bay of Bengal (Open Sea)
S7	Jul.3 2019	Cargo	9	Filtration + UV	1 day	Zhoushan (China)
S8	Jul.15 2019	Container	4	Filtration + UV	*	North Sea (Germany)
S9	Jul.15 2019	Container	4	Filtration + UV	13 hours	Qingdao (China), Japan
S10	Jul.16 2019	Container	8	Electrolysis + Neutralize	4 days	Busan (Korea)
S11	Jul.30 2020	Cargo	2	Filtration + UV	400 days	Shanghai
S12	Aug.4 2020	Cargo	32	Filtration + Electrolysis + Neutralize	10 days	*
S13	Aug.11 2020	Cargo	18	Filtration + Electrolysis + Neutralize	74 days	Northwest Pacific Ocean (Open Sea)
S14	Aug.19 2020	Cargo	34	Filtration + Electrolysis + Neutralize	*	*
S15	Sep.10 2020	Tanker	7	Filtration + UV	15 days	Nantong, Jiangyin and Zhangjiagang (China)
S16	Oct.20 2020	Cargo	5	Filtration + UV	5 days	Zhangzhou (China)
S17	Dec.8 2020	Cargo	7	Filtration + UV	5 days	Yancheng (China)

\* Data was not provided from the ships.

through a plankton net with a mesh size of 50  $\mu\text{m}$  in diagonal dimension, the net bottom soaked in the sampled water during the whole sampling process. Flow rate of 50  $\text{L}\cdot\text{min}^{-1}$  and water volume were recorded from then. The sampling finished when the sampling volume reached more than 1000 L. The plankton net was washed with the filtered sample water for 2–3 times and the concentrated  $\geq 50 \mu\text{m}$  organism samples were transferred into a 1 L HDPE bottle for further analysis in the laboratory (David 2013; David and Gollasch 2015; IMO 2015).

Samples for 10–50  $\mu\text{m}$  organisms, microbes and abiotic parameters were collected simultaneously during the  $\geq 50 \mu\text{m}$  organism sampling. A continuous drip sample was collected during the sampling process into a 20 L sterile capped plastic bucket. The total sampling volume was 10–20 L (David 2013; David and Gollasch 2015). The abiotic parameters including temperature, salinity, dissolved oxygen (DO) and pH were measured onboard by a portable multiparameter water analysis device (WTW-Multi 3430). From the bucket, two 500 mL and one 1 L sub-samples were collected with sterilized glass bottles for microbial analysis including *Vibrio cholerae*, *E. coli* and *Enterococci*, one 1 L sub-sample was collected with a brown polyethylene plastic bottle for analysis of 10–50  $\mu\text{m}$  organisms. All biotic samples were transferred to laboratory in 2–6  $^{\circ}\text{C}$  temperature.

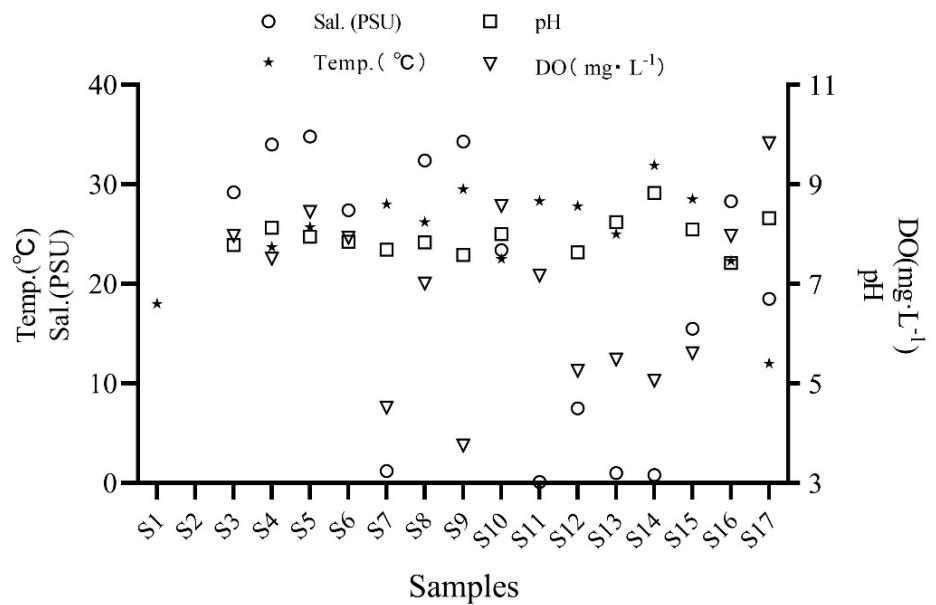
### Sample analysis

All samples were sent back to the laboratory within 2 hours after sampling. Analysis of  $\geq 50 \mu\text{m}$  and 10–50  $\mu\text{m}$  organisms were completed within 6 hours after sampling, and the incubation of microbial samples was started within 24 hours after sampling.

The 10–50  $\mu\text{m}$  organisms were counted with fluorescence method. The 1 L brown bottle was shaken at least 10 times, and water samples were taken into a 1 mL plastic tube to mix with fluorescein diacetate (FDA) and 5-chloromethylfluorescein diacetate (CMFDA) stains. The tube was shaken for well-mixing before being placed in the dark for 10 minutes for staining. The stained samples were put into the 1mL counting chamber and counted under a fluorescence microscope (Olympus DP73, Japan). Six 1 mL replicate slides were counted to minimize counting error. The counting were completed within 20 minutes after staining (EPA 2010; Steinberg et al. 2011). After the counting, the species were identified by sedimentation method after the samples were fixed in Lugol's solution. The whole of the counting chamber was analyzed with a Leica optical microscope at magnifications of 400 (David et al. 2007; Wu et al. 2019). The organism density was calculated as cells·mL<sup>-1</sup>.

The  $\geq 50 \mu\text{m}$  organisms were counted with microscope (Olympus SZX16, Japan). The concentrated samples were taken from the 1 L HDPE bottle into a 5 mL counting chamber. Counting for each sample stopped when over 100 living organisms were identified. If the living organisms were less than 100 in one 5 mL sample, the counts from the next samples were accumulated to the first sample until the organisms reached 100. Then the density of  $\geq 50 \mu\text{m}$  organisms was calculated with the counts and counted sample volume (EPA 2010).

For microbial samples, *E. coli*, *Enterococci* and *Vibrio cholerae* were analyzed. *Escherichia coli* and *Enterococci* samples were analyzed with IDEXX enzyme substrate method in Generic Protocol for the Verification (EPA 2010). With this method, the results of *E. coli* and *Enterococci* analysis were presented by the Most Probable Number (MPN), which was equal to CFU number in the D-2 standard. The detection limit of IDEXX method to *E. coli* and *Enterococci* was 1 to 2419.6 MPN/100 mL in freshwater samples of salinity < 1 PSU and 10 to 24196 MPN/100 mL in brackish or marine water samples of salinity  $\geq 1$  PSU. For *Vibrio cholerae* samples, 100 mL water were filtered by a 0.2  $\mu\text{m}$  sterilized membrane, the membrane was then folded and put in a 10 mL tube with liquid neutralize alga. After being incubated for 12 hours under 36 °C, liquid in the tube was transferred on a TCBS alga plate. The plate was incubated for another 12 hours under 36 °C, the incubated *Vibrio cholerae* was selected and testing using the DNA method in Generic Protocol for the Verification (EPA 2010). Three parallel samples were analyzed for each microbial sample.



**Figure 1.** Water quality parameters of ballast water samples. Sal.: salinity; Temp.: temperature; DO: dissolved oxygen.

### *The analysis of dominant species*

The species taxonomy and density of organisms  $\geq 50 \mu\text{m}$  and  $10\text{--}50 \mu\text{m}$  in every ballast sample were listed. Calculate species dominance using formula

$$Y = f_i \times p_i$$

where  $Y$  is the dominance of species, taking  $Y > 0.02$  as the sign of dominant species;  $f_i$  is the frequency of occurrence of the  $i$ -th species, that is, the proportion of the number of samples that this kind of organism appears in to the number of all sample points where organisms appear;  $p_i$  is the appearing probability of organisms, that is, the proportion of the total density of this kind of organism to the total density of all organisms.

## **Results**

### *Vessel information and water quality parameters*

The 17 vessels involved in this study covered 3 vessel types, i.e., cargo vessel, container vessel and tanker (Table 2). The ships in which the samples were collected in this study include those that have been sailing for one year and those that have been sailing for more than 30 years. The BWMS installed onboard included 13 Filtration + UV systems, 3 Filtration + Electrolysis + Neutralization systems and 1 Electrolysis + Neutralization system.

The water quality parameters of the ballast water samples were shown in Figure 1. From 2019 to 2020, samples of treated ballast water were collected from ships arriving at the Shanghai port in spring, summer, autumn and winter. The temperatures of treated ballast water varied from  $12^\circ\text{C}$  to  $31.9^\circ\text{C}$ . From the variation range of salinity (0.1 PSU to 34.3 PSU), the treated

ballast water samples collected in this study came from various sources. pH value of treated ballast water ranged from 7.4 to 8.8 and dissolved oxygen (DO) ranged from 4.5 mg·L<sup>-1</sup> to 9.8 mg·L<sup>-1</sup>.

#### *Results of $\geq 50 \mu\text{m}$ organisms*

In all samples, the density of  $\geq 50 \mu\text{m}$  living organisms in treated ballast water ranged from 0 to  $2.3 \times 10^4$  ind·m<sup>-3</sup> (Supplementary material Table S1). The density of  $\geq 50 \mu\text{m}$  living organisms in the ballast water samples that meet the D-2 discharge standard is all 0 ind·m<sup>-3</sup>. 8 out of 17 samples exceeded the D-2 standard for  $\geq 50 \mu\text{m}$  organisms, distributed to 4 cargo vessels, 3 container vessels and 1 tanker. The compliance rate for  $\geq 50 \mu\text{m}$  organisms specified to each vessel type was calculated to be 42.9% for cargo vessels, 57.1% for container vessels and 66.7% for tankers. The compliance rate for  $\geq 50 \mu\text{m}$  organisms specified to each BWMS type was calculated to be 46.2% for UV systems (6 systems compliant) and 75% for Electrolysis systems (3 systems compliant).

Nauplius larva, planktonic larvae and small copepods were more abundant in ballast water (Table S1). In all samples, 6 phyla and 13 genera of  $\geq 50 \mu\text{m}$  organisms were identified, including Arthropoda (dominant species), Annelida, Protozoa, Mollusa, Rotifera and Chaetognatha (Table S1). The number of species phyla in samples S1, S8 and S9 is relatively large, all of which are 3 phyla. Samples of S1, S8 and S9 have the largest number of species phyla, all of which are 3 phyla. The species number of living  $\geq 50 \mu\text{m}$  organisms in sample S1 was found as high as 6, and the density of this sample was also the highest.

#### *Results of 10–50 $\mu\text{m}$ organisms*

In all samples, the density of 10–50  $\mu\text{m}$  living organisms in treat ballast water ranged from 0 to 156.5 cells·mL<sup>-1</sup> (Table S2). 9 out of 17 samples exceeded the D-2 standard for 10–50  $\mu\text{m}$  organisms, distributed to 5 cargo vessels, 3 container vessels and 1 tanker. The compliance rate for 10–50  $\mu\text{m}$  organisms specified to each vessel type was calculated to be 28.6% for cargo vessels, 57.1% for container vessels and 66.7% for tankers. The compliance rate for 10–50  $\mu\text{m}$  organisms specified to each BWMS type was calculated to be 46.2% for UV systems (6 systems compliant) and 50% for Electrolysis systems (2 systems compliant).

The number of living species of 10–50  $\mu\text{m}$  organisms found in ballast water samples was higher than that of  $\geq 50 \mu\text{m}$  living organisms. In all samples, 5 phyla and 38 genera of 10–50  $\mu\text{m}$  organisms were identified, with *Gymnodinium* sp., *Prorocentrum donghaiense*, *Cyclotella* sp., *Prorocentrum* sp., and *Platymonas* sp. as dominant species (Table S2). Samples S11 has the largest number of species phyla (5 phyla). The highest species number (11) was found in Samples S3, but the density of living organism in S3 was much lower than that in S12 sample.

**Table 3.** Most Probable Number (MPN) of *E. coli* and *Enterococci* in treated ballast water samples.

Sample No.	<i>E. coli</i> (MPN per 100 mL)	<i>Enterococci</i> (MPN per 100 mL)
S1	3.1	23.1
S2	1	459
S3	1	1
S4	1	831.9
S5	1	200.5
S6	1	9.37
S7	49.5	836.1
S8	127.8	45.9
S9	22	289.7
S10	18.1	1011.2
S11	994.3	891
S12	< 1	< 1
S13	< 1	< 1
S14	14.2	9.3
S15	< 10	< 10
S16	23.3	< 10
S17	> 2419.6	41

### Results of microbes

In all treated ballast water samples, the density of *E. coli* ranged from < 1 MPN/100 mL to > 2419.6 MPN/100 mL, and the density of *Enterococci* ranged from < 1 MPN/100 mL to > 1011.2 MPN/100 mL (Table 3). *Vibrio cholerae* was absent in all samples. For *E. coli*, 2 out of 17 samples exceeded the D-2 standard, both were from cargo vessels with UV systems, which indicated vessel type specific compliance rate of 84.6% for cargo vessels, 100% for container vessels and tankers. For *Enterococci*, 7 out of 17 samples exceeded the D-2 standard, distributed to 2 cargo vessels and 5 container vessels. The compliance rate specified to each vessel type was calculated to be 71.4% for cargo vessels, 28.6% for container vessels and 100% for tankers, and the compliance rate specified to each BWMS type was 53.8% for UV systems (7 systems compliant) and 75% for Electrolysis systems (3 system compliant).

### Discussion

After the ratification of the BWM Convention in China from 2019, this is the first time to carry out a study on the D-2 compliance of the biological effectiveness of treated ballast water discharged from international ships arriving at Shanghai port. In this study, samples of ballast water treated by BWMS and discharged from 17 vessels arriving at Shanghai port were analyzed. The results revealed that the compliance to the D-2 standard was still a challenging issue for many of the investigated vessels. Due to the pandemic situation and lack of BWMS application on vessels calling at Shanghai port, the study might not have covered a significant range. However, the results indicated that the D-2 compliance of discharged ballast water at Shanghai port from vessels with BWMS installed could be a concern and should be brought to attention.



**Table 4.** The D-2 compliance of 17 ballast water samples.

Sample No.	$\geq 50 \mu\text{m}$ organisms (ind.·m <sup>-3</sup> )	10–50 $\mu\text{m}$ organisms (cells·mL <sup>-1</sup> )	<i>E. coli</i> (MPN per 100 mL)	<i>Enterococci</i> (MPN per 100 mL)	<i>Vibrio cholerae</i> O1 and O139 (CFU per 100mL)	D-2 compliance
S1	2.3×10 <sup>4</sup>	4.3	3.1	23.1	Absent	NO
S2	531	8.8	1	459	Absent	NO
S3	0	11.3	1	1	Absent	NO
S4	0	70.5	1	831.9	Absent	NO
S5	0	2	1	200.5	Absent	NO
S6	0	1.2	1	9.37	Absent	YES
S7	5.8×10 <sup>3</sup>	38.5	49.5	836.1	Absent	NO
S8	666	50.7	127.8	45.9	Absent	NO
S9	7.1×10 <sup>3</sup>	23.5	22	289.7	Absent	NO
S10	0	9.7	18.1	1011.2	Absent	NO
S11	100	75.3	994.3	891	Absent	NO
S12	0	156.5	< 1	< 1	Absent	NO
S13	0	3	< 1	< 1	Absent	YES
S14	100	18	14.2	9.3	Absent	NO
S15	0	0	< 10	< 10	Absent	YES
S16	7.9×10 <sup>3</sup>	14	23.3	< 10	Absent	NO
S17	0	5.3	> 2419.6	41	Absent	NO

Note: The gray shows that the value is exceeded D-2 standard.

#### *Discharge compliance of ships' ballast water received by Shanghai port*

In order to meet the D-2 standard, BWMS is normally chosen to treat the ballast water before discharge. Currently, there are various technologies of ballast water treatment systems available in the market, including but not limiting to filtration + UV, filtration + electrolysis + neutralization, filtration + deoxidation, filtration + advance oxidation, etc. (Bai et al. 2015; ClassNK 2021; Gerhard and Gunsch 2018). Though the species number and average density of both 10–50  $\mu\text{m}$  and  $\geq 50 \mu\text{m}$  organisms were lower than untreated ballast water, the discharge compliance of ballast water to the D-2 standard is still a challenging issue, even for those vessels with BWMS installed (David et al. 2007; Gollasch and David 2021; Li et al. 2013; Xue et al. 2011). BWMS as a ubiquitous prevention measure for reducing the introduction of nonnative species, it appears to be effective (Table 4). However, it is worrying that the discharge compliance rate is not as high as expected, which is probably because discharge compliance of ship ballast water is not only related to the treatment effectiveness of BWMS, but also closely related to many factors.

Ballasting and de-ballast of ballast water is especially necessary as ships load and unload cargo in departure port and destination port to compensate for changes in weight distribution (Miller et al. 2011). Therefore, the ballast and discharge capacity of each tank of a ship at different ports varies with the different demand of cargo transportation. It is inevitable that the ballast water carried in one tank may be mixed water from many different sources. As a result, the route information including the last port of call, the geographic source locations of ballast water, the ballast water volumes and dates; the ballast tank volume, the corresponding locations, volumes, and

dates of discharge for all ballast water dispersed to the destination port of the ship is also crucial to assess the discharge compliance of ships' ballast water. Combined with BWMS operation and ships' route information can help identify the root causes or key influencing factors of ballast water discharges. The effectiveness of BWMS also depends on the shipping routes (Komathy 2020). Combined with the operation of BWMS onboard and the ship's routes is helpful to find the key factors of the non-compliance of discharge ballast water. At present, further studies on the D-2 compliance of vessels with BWMS installed are needed, focusing on the effect of the vessel type and age.

When ships sail through different climatic regions, the environmental difference between ballast water uptake and discharge is the greatest, which also leads to the change of water quality of ballast water. It is well known that the changes of the abiotic factors can significantly influence the survival of the aquatic organisms, and in the present study it was observed that the abiotic parameters of water quality fluctuated widely among 17 samples. In addition, the species composition and density of living organisms  $\geq 50 \mu\text{m}$  in minimum dimension, and organisms  $10\sim 50 \mu\text{m}$  in minimum dimension and bacteria in 17 ballast water samples were different. It is indicated that the living organisms in ballast water have a high tolerance to abiotic conditions during ship's voyage (Cheniti et al. 2018; Wang et al. 2020a).

The density of viable phytoplankton and zooplankton organisms observed is similar as documented in previous studies (Gollasch and David 2021). It is observed that many small size and larvae organisms survive (Tables S1, S2), which seems to be the evidence of the in-tank reproduction. The harmful foreign organism is known to be highly diverse in ballast water of commercial vessels arrived at Shanghai (Wang et al. 2022). In this study, toxic bloom forming species *P. micans* was also found in sample S3. The findings suggested that for organisms in size class  $\geq 10\sim 50 \mu\text{m}$ , focus should not only be put on the organism amount in discharged ballast water, but also on the species of harmful bloom forming algae. This implicates that determining which non-indigenous organisms entered Shanghai through ballast water is as important as their total number. *Vibrio cholerae* was also absent in our findings, similarly with another related study (Salleh et al. 2021). *Vibrio cholerae* was also absent in our findings, similarly with another related study (Salleh et al. 2021; Wang et al. 2020b). It is suggested that there is a lower probability of *V. cholerae* introduced by ballast water of ships arrived at Shanghai port. However, it is important to note that the higher concentrations of water pathogens are found in several ballast water samples are bacteria that can cause gastrointestinal infections such as: *E. coli* and *Enterococci* (Table 4). The organism density exceeded the D-2 standard in 7 samples for *Enterococci* and 2 samples for *E. coli*. The highest *Enterococci* and *E. coli* values were approximately ten times of the acceptable limit of D-2 standard. To manage risks of the gastrointestinal pathogens

transmitted by vessels, stringent enforcement should be conducted to ensure that the discharge ballast water meets D-2 standards prior to discharge.

*The recommendation of commissioning testing of ballast water treatment systems after the installation*

All commercially BWMSs have been undergo type approval tests (including land-based testing, shipboard testing, environmental testing) to ensure their acceptable treatment performance. All the samples collected in this study have been treated by BWMS with type approval certificate, but the discharge ballast water still fails to meet the D-2 requirements (Table 4). Though a model BWMS receives type approval for general installation, this type approval certificate cannot account for the variation across ships. Commissioning tests are pending for specific ships to determine the suitability of onboard BWMS. Another thing should be noted that BWMS is a complex system includes equipment for ballast water treatment, control, monitoring and sampling. Installation of BWMS is a comprehensive work that requires modifications to the vessel's ballast system (piping, ballast tanks, valves, etc.), operation, control and monitoring (IMO 2018b). Prior to using a newly installed BWMS on board, this complex equipment is necessary to be commissioning tested in line with IMO rules (IMO 2018c) to validate the operational and biological performance after installation correctly in each unique shipboard environment.

This is required by IMO that commissioning testing will become mandatory for all BWMS-installations globally, as of June 1<sup>st</sup> 2022 (IMO 2018c). Notably, a small number of administrations have recommended or even mandated commissioning testing prior to this date (e.g., Australia, Canada, Croatia, Cyprus, France, Greece, India, Singapore, and Tuvalu) (Erma 2019). This suggests that the commissioning test is important to ensure the compliance of the BWMS onboard. As this was not yet a compulsory requirement by all flag states at the time period of this study (Table 2), it was likely that the investigated vessels did not conduct BWMS commissioning test and thus did not have the opportunity to confirm if the installation and operation of their BWMS might have any potential risk leading to non-compliance.

Looking to the future, periodic and ongoing compliance testing of discharged ballast water from vessel is necessary to confirm that BWMS continue to be environmentally protective and achieve the requirements of the BWM Convention.

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## Authors' contribution

Jieyou Xiang and Qiong Wang investigated, analyzed data, writing, reviewed and edited; Wenjun Wu reviewed and edited; Huifang Wang investigated and provided the funding, Huixian Wu designed the experiment and provided funding.

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#### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Taxonomy of  $\geq 50 \mu\text{m}$  living organisms ( $\text{ind} \cdot \text{m}^{-3}$ ) in treated ballast water samples.

**Table S2.** Taxonomy of  $10\text{--}50 \mu\text{m}$  living organisms ( $\text{cells} \cdot \text{mL}^{-1}$ ) in treated ballast water samples.

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