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BALLAST WATER FROM SHIPS BERTHED AT MAJOR PORTS OF MALAYSIA

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Abstract: With the adoption of the Ballast Water Management Convention in 2004, research attention has been conducted on the development of ballast water treatment technology, but few studies focuses on the contents of ballast water discharge at ports. In this study, we collected ballast water samples from ships berthed at major ports in Malaysia to determine the types of organisms present, mainly plankton and microbes. We also formulated a ballast water quantifying method using the "bucket" system. Throughout the study period from November 2012 to May 2014, ballast water samples were collected from 36 ships, of which 28 ships were container ships. The ballast water samples were mostly collected manually through manholes and by means of overflow through air-vents. The phytoplankton and zooplankton densities in ballast water were in the range of 25 -77600 cells/L and 0.26 – 450 individuals/L respectively. We identified a total of 119 phytoplankton taxa, of which 7 taxa belonged to the dinoflagellates group. This group of phytoplankton has the potential to form harmful algae bloom. For zooplankton, a total of 53 taxa were found in ballast water samples. This included a number of potentially invasive taxa that we were unable to identify to genus and species level such as zoea, fish eggs, ophiopluteus, larvae of polychaete, barnacle, actinula, starfish, bivalve and gastropod. As for *E. coli*, the colony count ranged from 0 – 256 CFU/100 mL, while both *Vibrio* spp. and Enterococcus had colony count ranging from 0 to TNTC (too numerous to count: >300 CFU/100mL). Ballast water sampling using the bucket method had proven to be able to quantify plankton and microbes present in ballast tank. Therefore, the proposed sampling method could serve as an alternative for authorities in ballast water compliance monitoring.

KEYWORDS: Ballast water sampling, marine invasive species, phytoplankton, zooplankton, microbes

Introduction

The issues with ballast water pollution have long been the attention of many countries (Ruiz *et al.,* 1997; Pereira *et al.,* 2014). Marine species are being transported from one country to another through the shipping industry. Their fate upon reaching the new destination depends on their ability to adapt themselves in new environmental conditions. Those exhibiting adaptation will be able to survive and expand their territory and may compete with local species for space and food (Molnar *et al.,* 2008; Steichen *et al.,* 2012; Leidenberger *et al.,* 2015). The type of species that were carried in the ballast water depended on the initial location where the water was taken into the ballast tank (Hallegraeff, 1998).

The guide for Ballast Water Sampling-G2 (IMO, 2008) provides general information with no details on the volume of ballast water sample needed, representative sampling of ballast water and type of sampling gears. Various ballast water sampling methods are used, which include the use of the water column sampler (David & Perkovič, 2004), buckets (Boltovskoy *et al.,* 2011), plankton nets (Burkholder *et al.,* 2007; Kang *et al.,* 2010; Baek *et al.,* 2012) and air pump (David & Perkovič, 2004; David *et al.,* 2007) through manhole or sounding pipe. All of these methods may yield different outcomes, depending on the distribution of the organisms in the ballast water tank and the methods used to retrieve these organisms (Costa *et al.,* 2015).

Malaysia has ratified the Ballast Water Convention on 27th September 2010. The Convention came into force on the 8th of September 2017 and currently, 73 states have ratified it, thus representing 75.35% of the

world's merchant vessel tonnage. With all vessels ultimately needing to comply with the D-2 ballast water performance standard, the focus of the research community has been on the development of ballast water treatment technology. There are very few studies relating to the contents of ballast water. We conducted ballast water sampling at selected major ports in Malaysia. Through this study, we formulated a ballast water sampling method using a "bucket" system. For this method, we adapted the published ballast water sampling guidelines according to Gollasch and David (2011) and IMO (2008); and took into consideration the type of vessels, situation on-board and ballast water tank access point. Apart from knowing the type of organisms present in the ballast water, we also linked the organisms' survivability to their residence time in ballast tanks. This study provides useful information on the sampling method's efficiency in obtaining the organisms present in ballast water. This method might be useful for enforcement agency (ies) in formulating procedures to monitor vessels' compliance to ballast water standards.

Materials and Methods

Ballast Water Sampling

Ballast water sampling was conducted at four major ports in Malaysia, namely Port Klang, Port of Tanjung Pelepas, Penang Port and Kuantan Port (Figure 1). Selection of ships for ballast water sampling was based on information obtained from the port operator, such as types of ships calling at the port, whether loading or unloading cargo, and ships' load port of origin. Ship arrival and departure timings were also equally important as vessel crews would be occupied with port clearance work if they had just docked or in preparation for departure. Tankers and bulkers were of interest for sampling as they carried substantial amount of ballast water. Container ships, on the other hand, could be loading and unloading cargo at the same port, which would require minimum uptake or discharge of ballast water. Some container ships had internal ballast water on-board (non-dischargeable) and it was redistributed internally between the ballast tanks for trimming purposes. This type of container ships would have zero discharge of ballast water. Our priority was to obtain ballast water samples from vessels plying between countries and across continents: cargo ships, tankers and bulk carrier ships, and also ships coming from high risk areas.

Most parts of our ballast water sampling work onboard selected ships were conducted with the help of the Port State Control Officers of the Marine Department Malaysia. With their assistance, we were granted access to the ships by the captain/master of ships. Meeting with the ship's captain/master was held onboard to explain our sampling method and suggest the preferred access point for ballast water sampling. However, the decisions of the ballast tank to be sampled and the ballast water access point were the captain's prerogative. The ship's crew would accompany our research team to the agreed access point and provide access. Our sampling gears included buckets (15 or 20 L capacity; pre-calibrated by marking the volume level on the bucket), heavy-duty ropes, blue-tack (to unbalance the bucket for ease of water collection), plankton nets (20 µm & 50 µm mesh sizes) and stand, sample bottles, sterilised sample bottles, ice-chest with ice-cubes, multi-parameter water quality probe (Hydrolab Quanta), headlamp, coverall, safety helmets, safety shoes, gloves, and life jackets.

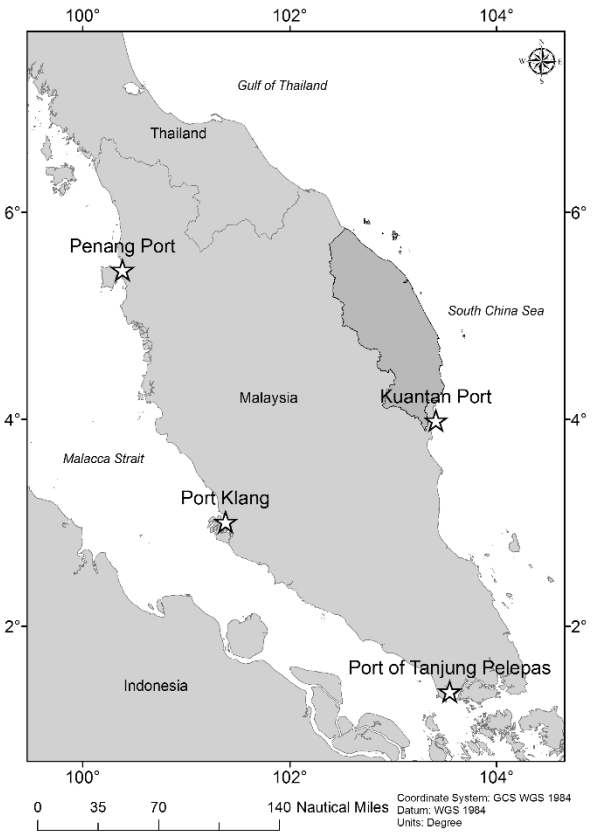


Figure 1: Ballast water sampling at Malaysia major ports: Port Klang (PK), Port of Tanjung Pelepas (PTP), Penang Port (PP) and Kuantan Port (KTN).

Sampling through the Manhole

Collection of ballast water from the manhole was preferred. Ease of access to the manhole depended on its location. Manholes located at after peak, forepeak and topside tanks were easy to access. Bottom tank manholes were hard to access as all the sampling gears and sample bottles needed to be carried from the main-deck to the lowest deck. Once the manhole was opened, Hydrolab Quanta was lowered into the tank to obtain the *in situ* temperature and salinity readings. The internal projections of walls of the ballast tank and the presence of vertical ladders could hinder the use of water sampler and plankton net, preventing them from reaching the bottom part of the ballast tank even though it was full of water. The vertical or slightly inclined ladder would increase the risk of sampling gear getting stuck between the steps of the ladder.

Another way to collect ballast water sample would be with a water pump but this would require a power source. The use of any portable power generating equipment on board is generally not allowed especially for vessels transporting chemicals, oil and liquefied petroleum gas. Considering the amount of water needed (100 L), the Niskin sampler (5 L capacity) would require a long sampling time. The use of buckets (15 or 20 L capacity) speed up the sampling process. Ballast water collected in buckets was filtered through plankton nets with mesh sizes of 20 µm and 50µm. Plankton nets were arranged in layers with the 50 µm mesh size as the top layer followed by the 20 µm mesh size. Approximately 100 L of ballast water was

poured through the net slowly and the plankton collected were concentrated and then separated according to size. We encountered difficulties when filtering of water was not allowed onsite. Under such circumstances, we had to transport the ballast water in buckets or water containers to the main deck or to another designated area to do the filtering. With experience, we re-strategised the sampling method by bringing along enough buckets to collect the total volume needed and the ballast water was then filtered through the plankton nets over the same manhole back into the same ballast water tank. For bacteria analysis, ballast water samples collected in bucket were transferred into a sterilized 1 L capacity Scott bottles and stored in ice-chest (temperature below 4 ^{o}C) before analysis was conducted.

Sampling Through the Air-Vent and Sounding Pipe

When the ballast tank was full of water, the manhole could not be opened. In this situation, a ship's captain suggested sampling by overflow through the air-vent as an alternative. The overflow process could be triggered by little intake of port water, which pushed the ballast water out from the air-vent. The location of air-vent was on the main/top deck, which was not near to the ballast water intake opening, hence the chances of the samples being contaminated with port water were minimal. Ballast water that flowed out from air-vent was then collected in buckets and measured for temperature and salinity.

For bacteria analyses, the water samples were collected from water overflow directly into the respective sample bottles. For plankton sampling, ballast water was collected in buckets before being filtered through the plankton nets. The advantage of this sampling method was that water sampling process could be completed in less than 30 minutes. The other alternative was to obtain ballast water samples via sounding pipe(s). This method is the least preferred because we could only use a 10 mL capacity cylinder (provided by the ship's crew) to sample ballast water. It was very time consuming considering the amount of water needed for the analysis (approximately 100 L). The amount of time that this might take could also affect the ship's routine operation.

Ballast Water Content Analysis

In the laboratory, quantitative analyses of phytoplankton and zooplankton samples were conducted using Lackey's Drop method and Sedgewick-Rafter counting chamber respectively (APHA, 2005). Plankton was identified to the lowest taxonomic level possible according to Hasle and Syvertsen (1996), Steidinger and Tangen (1996), Lim *et al.* (2012), and Richardson *et al.* (2013). For bacteria *Escherichia coli* and Enterococcus analyses, a total of 100 mL ballast water sample was filtered through sterile 0.45 µm membrane, transferred onto m-FC agar (for *E. coli*) and m-E agar (for Enterococcus). For *Vibrio* spp., water samples were filtered through 0.2 µm membrane and patched onto TCBS agar. All analysis was carried out aseptically in a laminar flow and in triplicates. The agar plates were incubated at 37 ^{o}C for 24 h in an incubator. The presence of bacterial colonies was recorded and expressed as colony-forming units in unit volume (CFU/mL) (USEPA, 2002).

Results and Discussion

We boarded 60 vessels in total, but collection of ballast water samples was done on 36 vessels (hence 36 ballast water samples) from 28 container ships, five bulker ships and three tanker ships. The remaining 24 vessels were not sampled because of these reasons: empty ballast tank, ballast water was taken directly from Malaysian waters or at designated ports, access to the ballast tank was denied by the captains, access was only allowed through the sounding pipe, access to manholes located at the lowest deck was difficult especially in container vessels, manhole was blocked by overlaying cargo, vessel was in preparation for departure,

and ballast water had been fully discharged. Taking the ships' last port of call as the source, we had samples of ballast water taken from Malaysian waters (seven ships), Southeast Asia waters (ten ships), China, India, Bangladesh, Hong Kong, Taiwan, Japan with the farthest being the Atlantic Ocean and Australia. There were three samples that had mixed origin or were of unknown source (Table 1). Twenty-six ballast water samples were collected through the manholes, nine samples were by overflow through the air-vent and one was through the sounding pipe.

Ballast Water Temperature and Salinity

Temperature and salinity are of great interest as they determine the survival of organisms in the ballast water tank and in port waters. Many studies had reported that temperature and salinity affected phytoplankton metabolisms, growth, enzyme activities and osmotic stress on cells (D'ors, 2016). The salinity of Malaysian port waters was lower than 33 psu and the world's oceans had salinity in the range of 34–37 psu (Table 2). Ballast water from Yangon (PK1 and PK6), Chittagong (PTP1 and PP4) and an unknown source (PKN4) had salinity lower than 5psu while ballast water from the Atlantic Ocean (PK9), Bengal Bay (PP1 and PP5) and the Indian Ocean (PTP4) had salinity higher than 35 psu. Salinity varied between 18.69–34.01 psu for ballast water originating from other Asian and South East Asian countries (Figure 2). Organisms present at lower salinity (<5 psu) are very unlikely to survive and thrive in Malaysian port waters with salinity higher than 26 psu (Paavola *et al.,* 2005; Kang *et al.,* 2010). If the ballast water had salinity close to port water salinity, this indicated that salinity may not be a factor hindering the survival of the organisms in Malaysian waters. By referring to Table 2, any ship which had conducted ballast water exchange at open ocean such as Indian, Pacific and Atlantic Oceans, would carry ballast water with salinity of 33 psu or higher. From the interview with ship's captain/master, none of the ship had conducted ballast water exchange because it was not a compulsory practise required by local enforcement authority.

Ballast water temperature ranged from 26.34–32.78 ºC, with the highest temperature from Port Klang (PKN2) Malaysia while the lowest was recorded for water which originated from Chittagong (PP4) (Figure 2). The biggest temperature difference between ballast and port water was +4.66 ºC which was between ballast water from Singapore (PKN3) and port water at Kuantan Port. This temperature differential between ballast water and port water might not pose serious threat to the survival of organisms discharged to port waters.

Table 1: Ballast water origin, vessel type, access point, sampling date, ballast water age, plankton and bacteria density in ballast water; TNTC: too numerous to count (>300 colonies). PK- Port Klang; PTP – Port of Tanjung Pelepas; PP – Penang Port; PKN – Kuantan Port

Sample ID	Ballast water sources	Vessel type	Access point	Sampling date	Days in ballast tank	Plankton Density				Bacteria Count (CFU/100 mL)		
						Phytoplankton (cells/L)		Zooplankton (Individuals/L)		*E. coli*	*Vibrio* spp.	Entero-coccus
						20 µm	50 µm	20 µm	50 µm			
PK1	Yangon	Container	Manhole	05-11-12	7	80	434	0.1	0.5	78	8	123
PK2	Singapore	Container	Manhole	05-11-12	3	52	286	0	13.7	40	98	TNTC
PK3	Kaohsiung	Container	Sounding pipe	05-11-12	14	-	-	-	-	37	97	114
PK4	Shekou	Container	Manhole	06-11-12	6	97	6214	0	25.1	73	139	TNTC
PK5	Hong Kong	Container	Manhole	06-11-12	33	357	24966	0.3	17.9	55	27	TNTC
PK6	Yangon	Tanker	Manhole	06-11-12	28	15	5	0.2	0.1	16	5	100
PK7	Chennai	Container	Overflow	06-11-12	5	23896	25175	0.1	3.6	80	29	TNTC
PK8	Ho Chi Minh	Container	Manhole	07-11-12	34	3626	73974	0.2	2.1	67	182	TNTC
PK9	Atlantic Ocean	Container	Manhole	07-11-12	71	1063	357	3.4	0.1	54	64	235
PTP1	Chittagong	Container	Overflow	18-03-13	9	83	30	0.3	2.7	115	2	34
PTP2	Shekou	Container	Manhole	18-03-13	4	30	7	0.6	1.2	2	216	187
PTP3	Chittagong	Container	Manhole	19-03-13	8	47	4	0.6	0.6	4	286	182
PTP4	Indian Ocean	Container	Manhole	19-03-13	7	102	14	0	1	0	174	67
PTP5	Nansha	Container	Manhole	19-03-13	23	21	4	0.1	0.4	0	11	TNTC
PTP6	Klang and Tanjung Pelepas	Container	Manhole	20-03-13	1	425	595	0.4	12.6	8	TNTC	210
PTP7	Jakarta and Kuantan	Container	Manhole	20-03-13	3	1209	11	0.5	2.3	0	159	TNTC
PTP8	Qingdao	Container	Manhole	20-03-13	22	177	7	0.2	0.3	0	63	9
PTP9	Melbourne	Container	Manhole	20-03-13	16	156	63	0.7	2.5	2	TNTC	61
PTP10	Laem Chabang	Container	Manhole	21-03-13	11	1161	502	0.6	38.3	1	TNTC	5
PTP11	South China Sea	Container	Manhole	21-03-13	73	25	5	0.2	0.6	0	120	0
PTP12	Ho Chi Minh	Container	Manhole	21-03-13	5	19289	2235	3.2	45	0	TNTC	0
PP1	Bengal Bay	Container	Manhole	19-08-13	7	1579	534	4.9	22.4	256	TNTC	149
PP2	Bintulu	Tanker	Manhole	19-08-13	5	2745	6124	43.7	406.3	10	58	100
PP3	Penang	Container	Overflow	20-08-13	19	440	139	0.4	1.1	15	TNTC	166
PP4	Chittagong	Container	Overflow	21-08-13	5	423	25	9.2	93.8	80	TNTC	69
PP5	Kakinada	Bulk Carrier	Manhole	20-08-13	10	720	124	0.5	1.8	12	TNTC	244
PP 6	Semarang	Container	Manhole	21-08-13	1	2065	587	16.3	51.5	88	TNTC	57
PP7	Klang	Bulk Carrier	Overflow	21-08-13	33	6817	695	0	2.5	5	TNTC	44
PP8	Hong Kong	Container	Manhole	21-08-13	20	1146	1492	0.5	19	12	TNTC	60
PP9	Klang	Bulk Carrier	Manhole	22-08-13	1	52	260	0	39.6	13	TNTC	170
PKN1	Songkla	Container	Overflow	06-05-14	4	206	64	0.8	10	145.3	183.0	0.0
PKN2	Klang	Container	Manhole	06-05-14	4	7671	5020	0.6	6.1	86.0	0.0	0.0
PKN3	Singapore	Bulk carrier	Overflow	08-05-14	5	16	11	0.2	1.6	122.0	0.0	0.0
PKN4	Unknown	Tanker	Manhole	08-05-14	-	28	11	0	7.6	110.7	22.7	0.0
PKN5	Singapore and North Pacific	Container	Overflow	08-05-14	1	117	3737	0	4.9	116.3	9.3	0.0
PKN6	Pasir Gudang	Bulk carrier	Overflow	08-05-14	5	10	25	0.2	2.8	81.3	0.0	10

Table 2: Salinity and temperature at ports of Malaysia (current study) and world oceans' salinity (NASA Aquarius and World Ocean Atlas, 2005)

Location	Salinity range (psu)	Temperature range (ºC)	Month and Year
Port Klang	26.46 ± 1.11	29.69 ± 0.16	November, 2012
Penang Port	29.18 ± 1.87	29.61 ± 0.31	August, 2013
Port of Tanjung Pelepas	32.75 ± 0.26	30.26 ± 0.71	March, 2013
Kuantan Port	32.18 ± 0.15	31.22 ± 0.13	May, 2014
Indian Ocean	34.0 - 36.0	-	Annual mean salinity
Pacific Ocean	33.0 - 36.0	-	Annual mean salinity
Atlantic Ocean	34.0 - 37.0	-	Annual mean salinity

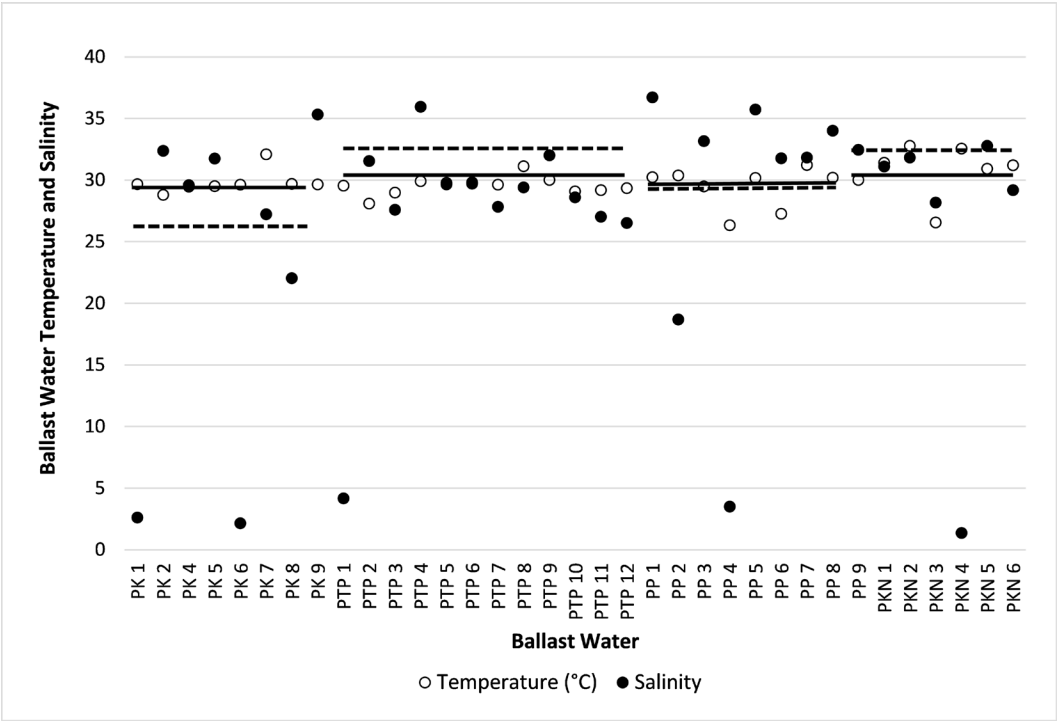


Figure 2: Temperature (ºC) and salinity (Practical Salinity Unit, psu) values in each ballast water sample. (- - - represents average salinity and ——— represents average temperature at respective ports). PK- Port Klang; PTP – Port of Tanjung Pelepas; PP – Penang Port; PKN – Kuantan Port

Plankton in Ballast Water

Our analysis showed that all ballast water samples contained plankton. For phytoplankton, we managed to identify 119 taxa (Table 3). PP7 (Port Klang), PTP10 (Leam Chabang) and PTP12 (Ho Chi Minh) were among the ballast water samples that contained a high number of taxa with 64, 62 and 56 taxa respectively. On the other hand, ballast water samples from the Indian Ocean (PTP4), Nansha (PTP5), Chittagong (PTP3) and Pasir Gudang (PKN6) had less than 15 taxa recorded. *Thalassiosira* sp., *Cyclotella* sp., *Pluerosigma* sp., *Skeletonema* sp., and *Thallasionema* sp. were present in 85% of the samples. Species like *Cladophxis* sp. (PK8, Ho Chi Minh), Gymnodinoid, (PP5, Kakinda), *Gyrosigma* sp. (PTP7, mixed source), *Fallacia* sp. (PK5, Hong Kong), *Licmophora* sp. (PP1, Bengal Bay), *Rhizosolenia bergonii* (PK1, Yangon; PTP10, Leam Chabang), *Rhizosolenia robusta* (PP9, Klang), *Chaetoceros* sp. (PP1, Bengal Bay), *Cheatoceros denticulatus* (PP7, Klang) were only present in a few samples. For zooplankton, a total of 53 taxa were identified with the majority belonging to the class of Maxillopoda, Oligotrichea, Bivalvia and Gastropoda (Table 3). The highest number of taxa was found in PTP10 (Leam Chabang) with 24 taxa. This was followed by ballast water sample from Klang (PP9) and mixed water from Malaysia (PTP6) with 23 taxa each. Ballast water from Ho Chi Minh (PK8), Chennai (PK7), and Penang (PP3) had fewer than 5 taxa. Crustacean nauplius which is the larval stage of copepods was present in all ballast water samples, with density ranging from 0.059 individuals/L (sample from Yangon, PK6) to 47.91 individuals/L (sample from Chittagong, PP4). Other zooplankton present in high density included *Tintinnopsis* sp., *Paracalanus* sp., *Othiona* sp., gastropod larvae and bivalve larvae.

In general, the total phytoplankton density in ballast water was in the range of 25-77600 cells/L (Table 1). Ballast water samples that contained

lesser than 100 cells/L of phytoplankton were from Yangon (PK6), Nansha (PTP5), Singapore (PKN3), the South China Sea (PTP11), Pasir Gudang (PKN6), unknown (PKN4), Shekou (PTP2) and Chittagong (PTP3). The highest phytoplankton density was from Ho Chi Minh (PK8), followed by Chennai (PK9) and Hong Kong (PK5) with 77600, 49071 and 25323 cells/L respectively. *Skeletonema* sp. which made up 80–97 % of the total phytoplankton was the dominant species in the samples. Our results also showed that the majority of the phytoplankton retained by the 50 µm plankton net was the chain forming phytoplankton such as *Skeletonema* sp., *Chaetoceros socialis, Chaetoceros teres, Chaetoceros curvisetus, Hemiaulus sinensis, Lauderia* sp, *Thalassionema* sp., *Thalassiothrix* sp., *Hemiaulus membranaceus, Thalassiosira* sp. and *Bacteriastrum hyalinum.*

Zooplankton composed less than 10 individuals/L in 61 % of the ballast water samples. Exceptional high zooplankton concentration (450 individuals/L) was found in ballast water sample from Bintulu Port (PP2). *Tintinnopsis* sp. made up 96 % of the count in this sample. More than 80 % of the total zooplankton was retained by plankton net mesh size of 50 µm in 25 ballast water samples. Therefore, the filtration/separation of plankton using 50 µm mesh size plankton net would reduce the number of plankton in ballast water effectively. Our study found large differences in plankton densities among ballast water samples and the densities did not show any correlation with ballast water age (Table 1) (r=0.1236, phytoplankton; r=-0.1667, zooplankton). This could be due to the productivity differentials between the sources of ballast water. Phytoplankton and zooplankton of up to 25323 cells/L and 18.2 individuals/L respectively were found in water that had been in the ballast tank for 33 days. This was in accordance with a study conducted by Carney *et al.* (2011), where phytoplankton were able to survive in prolonged darkness for up to 28 days. We do not have data on plankton density during the intake of ballast water from source port to draw convincing conclusion on the age of the ballast water in relation to plankton survival.

Potential Harmful Plankton in Ballast Water

We further classified plankton into potential harmful dinoflagellates and invasive species. 97% of the ballast water samples contained potentially harmful dinoflagellates or invasive species with densities of 0.1–214 cells/L for phytoplankton and 0.04–14.0 individuals/L for zooplankton. *Dinophysis* sp., which was found in sample from Ho Chi Minh (PTP12), was the most abundant potentially harmful dinoflagellate. Other potentially harmful dinoflagellates present in ballast water samples included *Alexandrium* sp., Gymnodinoid, *Dinophysis caudata, Dinophysis rotundata, Peridinium* sp., and *Gonyaulax* sp. A bloom of these dinoflagellates will cause contamination on shellfish and may result in intoxication if consumed by humans (Lim *et al.,* 2004; 2012; Roziawati *et al.,* 2015; Tan *et al.,* 2016). *Skeletonema, Thalassiosira* and *Chaetoceros* which were reported to have the ability to survive UV radiation (Liebich *et al.,* 2012) and form harmful algal blooms (Shen *et al.,* 2012) were also found in the samples with densities as high as 75000 cells/L (PK8, Ho Chi Minh). A number of meroplanktonic taxa such as zoea, fish eggs, ophiopluteus, larvae of polychaete, barnacle, actinula, starfish, bivalve and gastropod were found in ballast water samples. These meroplankton, even though not identified to genus/species level, were potential invasive organisms. Ballast water sample from Shekou (Guangdong province, China) contained the highest number of gastropod veliger. A number of invasive abalone was reported in the Guangdong (China) marine ecosystem (Xu, *et al.,* 2012). There was a possibility that these were veliger of the invasive abalone. In addition, larvae of polychaete, actinula and gastropod were found alive after 30 days in the ballast tank and this might pose risk to Malaysian waters if discharged from the ballast tank.

Bacteria in Ballast Water

We examined the density of bacteria *E. coli, Vibrio* spp. and Enterococcus in ballast water samples. *E. coli* count was in the range of 0–256CFU/100 mL. Only ballast water sample

from Bengal Bay (PP1) had *E. coli* count exceeding the D2-2 ballast water performing standard (250CFU/100 mL). *Vibrio* spp. was present in 32 ballast water samples. It had densities ranging from 5CFU/100 mL to TNTC (too numerous to count: >300CFU/100 mL). For ballast water samples PP1–PP9, we further identified the *Vibrio* spp. and found that none of the samples contained toxigenic *Vibrio cholerae.* No Enterococcus was detected in seven ballast water samples. The remaining samples had 5CFU/100 mL to TNTC of Enterococcus. Ten ballast water samples were in compliance with the D2-2 performing standard of less than 100CFU/100 mL (Table 1).

Ballast Water Sampling Method – Bucket System

The sampling of ballast water using buckets through manhole and by air-vent overflow were able to produce results comparable to other sampling methods (Burkholder *et al.,* 2007; David *et al.,* 2007; Kang *et al.,* 2010). The bucket sampling method was also used by Boltovskoy *et al.* (2011). However, detailed comparison was not possible due to the brevity of their report of the method. These methods collected species that were floating near the surface of the ballast water. With our method, 80% of the phytoplankton collected was long-chain phytoplankton. This somehow indicated that this long-chain phytoplankton had managed to stay afloat and was in contrast with other reports which stated that long-chain phytoplankton had a greater sinking rate (Guo *et al.,* 2016). This could be due to the presence of turbulent mixing caused by sloshing and waves in the ballast tank could result in the decreased sinking rate for phytoplankton (Ruiz *et al.,* 2004; Vieira, 2018). The total darkness in ballast water tanks might have mimicked night time in natural conditions in which the zooplankton would be actively migrating to the upper layer to prey on other plankton (Pearre, 2003). The dead zooplankton would unlikely remain near the surface and the carcasses would settle to the bottom of the tank (Elliott, 2010).

Table 3: The number of phytoplankton and zooplankton taxa in each ballast water sample. PK- Port Klang; PTP – Port of Tanjung Pelepas; PP – Penang Port; PKN – Kuantan Port.

	Phytoplankton												**Zooplankton**												
	Conjugatophyceae	Chlorophyceae	Pyramimonadophyceae	Cyanophyceae	Dinophyceae	Bacillariophyceae	Fragilariophyceae	Cosmodiscophyceae	Mediophyceae	Dictyochophyceae	Unidentified	**Total Taxa**	Acanthania	Polychaeta	Aphragmophora	Branchiopoda	Malacostraca	Maxillopoda	Bryozoa	Thaliaceae	Oligotrichea	Ophiuroidea	Foraminifera	Bivalvia	Gastropoda	Nematoda	Radiolarian	Unidentified	**Total Taxa**
PK1				1	4	9	3	10	15			**42**						6										**6**
PK2				1	6	11	3	11	17	1	1	**51**	1	1				8	1						1			**12**
PK4					3	5	3	6	14			**31**						9							1			**10**
PK5					3	7	3	7	11	1		**32**						5	1						1			**7**
PK6					2	3	1	2	7	1		**16**						5			1				1			**7**
PK7				1	2	2	1	8	16			**30**						2		1	1							**4**
PK8		1			4	3	1	5	9	1		**24**						2										**2**
PK9					7	2	2	6	11			**28**						2		1	1				1			**5**
PTP1	1	1		2	7	3	6	7	10	1	1	**39**		1				3	1		2		1		1			**9**
PTP2				1	5	10	6	7	9	2		**40**						4	1		3		1		1			**10**
PTP3					2	4	1	3	5			**15**		1				3			3				1			**8**
PTP4					2			4	4			**10**						7			1		1	1				**10**
PTP5			1		3	1	2	1	5			**13**						1	1		2		1		1		1	**7**
PTP6					5	9	4	12	20			**50**	1	1	1	1		13	1		3	1		1				**23**
PTP7					9	9	3	7	13	2		**43**		1	1		1	9			4			1	1			**18**
PTP8					2	3	2	7	7		1	**22**						3			2		1	1				**7**
PTP9			1	1	9	7	4	10	10	2		**44**		1				7			2			1	1		1	**13**
PTP10					9	9	3	14	27			**62**		1	1		1	14	1		4			1	1			**24**
PTP11					4	2	4	7	8			**25**		1			1	6			2		1					**11**
PTP12	1	2			7	10	4	13	19			**56**		1				9			2		1	1	1			**15**
PP1				1	9	10	5	12	22		1	**60**		1				9			8			1				**19**
PP2				1	7	5	2	5	14			**34**			1			5			1	1		1				**9**
PP3						7	3	10	20			**40**						2			2							**4**
PP4		2		1	5	4	4	2	10			**28**		1				7						1	1			**10**
PP5		2			9	10	5	9	17	1	1	**54**						3	1		2			1		1		**8**
PP6				1	4	10	5	7	14			**41**						9			1			1				**11**
PP7				1	9	8	3	13	30			**64**		1				8	1		1			1				**12**
PP8					7	10	3	11	19			**50**						8	1	1	1			1	1			**13**
PP9				1	3	6	2	11	17			**40**		1	1	2	1	13		1	3			1				**23**
PKN1				1	10	8	2	5	11		1	**38**						11	1		2			1			1	**16**
PKN2					5	6	4	9	14			**38**						4			4			1	1			**10**
PKN3					4	3	2	4	3			**16**						3	1		2			1	1			**8**
PKN4		1			4	4		4	7			**20**						4			1				1			**6**
PKN5					2	6	3	5	19			**35**						5			1			1	1			**8**
PKN6					2	3		3	7			**15**						4			4			1		1		**10**

Proposed Guidelines for National Ballast Water Sampling Strategy

With the implementation and enforcement of the Ballast Water Management (BWM) Convention, the agencies involved need to carry out inspection on the ship's compliance with the BWM Convention Standards. For compliance monitoring, the selection of ship for inspection purposes could be based on the following criteria:

A. The source port risk classification

High risk ports are ports that have the highest possibility of receiving and donating invasive species. These are normally ports with intense shipping activities or high trading volumes. Ships coming from areas with known invasive species problem should be given inspection priority, especially when the source port has similar environmental conditions with the receiving port. Countries located in the same geographical regions could be classified as having high similarity in environmental conditions as well as species composition. This is based on an ocean model study by Xu and Malanotte-Rizzol (2013), where they reported that free water movements within ocean basins in the South China Sea, the Straits of Malacca, Indonesian seas, the Gulf of Thailand and the Andaman Sea. In addition, the map showing marine ecoregion of coastal and shelf areas by Spalding *et al.* (2007) could be used as a guideline to identify areas that are in the same ecoregion. Ballast water taken from same risk area or same ecoregion could then be identified and given exemption or lower priority for inspection. However, if there was a case of reported invasive or harmful algae outbreak, inspection will be crucial even though the ballast water is from the same risk zone.

B. Types of vessel

Tankers and bulkers, especially for those coming in for cargo load, carry a large volume of ballast water. These should be selected for inspection. Container ships that are loading and unloading cargo at the same time require minimum uptake and discharge of ballast water, and therefore should receive least priority.

C. Ballast tank access point

i. Compliance monitoring for Ballast Water Exchange Standard (D-1)

The inspection for ballast water exchange standard could be done by measuring the salinity of ballast water. A few mililiters of ballast water is enough for salinity measurement using a handheld refractometer and the result could be obtained instantly. For this purpose, sounding pipe is a good option as it is the easiest access point to ballast water and therefore with shorter sampling operation time. Upon detecting that a ship's ballast water has salinity lesser than 33 psu, further inspection could then be carried out to prove the ballast water origin. To comprehend the mandate for oceanic ballast water exchange, one need to understand the natural behaviour of the organism and their distribution patterns in the marine environment. Firstly, the groups of organisms that have the highest possibility to become invasive species include fouling and benthic epifauna organisms such as bivalves (Johnson, 1996; Dittel & Epifanio, 2009). These groups of organisms are usually found on or near coastlines and on the seabed that have a variety of substrata and man-made structures for their planktonic larvae to attach to or settle once reaching the adult stage. They depend on the movement of the water current to transport their planktonic larvae to new settlement areas. The distance that they could travel horizontally towards the open ocean or vertically from deeper seafloor to the surface is somehow limited. Therefore, the presence of a very high percentage of this particular group of larvae (how high is high, and how have you determined this threshold) in the ballast water would indicate that the origin of water is most likely from coastal waters. Even though the presence of teleplanic larvae in oceanic waters is evident, their concentration is very low (less than $1.35x10^{-3}/m^3$) (Scheltema, 1988), thus might not be found in significant density in the ballast water. Secondly, most studies on zooplankton composition in oceans showed that free-living copepods (holo-planktonic organisms) made up the largest group of zooplankton at between 70–90 % (Zaleha *et al.,* 2006; Steinberg *et al.,*

2008). Therefore, a high percentage of holoplanktonic copepods in ballast water (>70 %) would indicate that the water originated from oceanic or offshore waters. The oceanic holoplankton rarely develops into invasive organisms compared to freshwater and brackish water species (Cordell & Morisson, 1996).

ii. Compliance Monitoring for Ballast Water Performance Standard (D-2)

The D-2-1 standard only requires quantitative data on the number of target organisms present in the ballast water (with respect to organism size). Therefore, there is no need to identify the type or group of organisms in the ballast water. However, the colony count for specific indicator microbes (*V. cholerea, E. coli* and Enterococci) as stated in D-2-2 standard must be conducted. The guideline for ballast water sampling (G2) stated that for D-2 standard compliance monitoring, the ballast water should be sampled through the discharge line.

However, in practice, sampling through the discharge line will be very difficult to carry out. This is because the person carrying out the sampling will need to be on a boat near shipside and attempting to obtain water sample from the very strong flow of the discharge. In the case where the discharge line is fully submerged, sampling would be impossible.

Conclusion

This study proposed suitable sampling protocols for ballast water which have been modified accordingly based on situations on board, accessibility and internal design of ballast tanks. The bucket sampling method applied in this study may raise concerns on the sample's representativeness because only organisms that remained within one meter below the water surface would be captured. However, in the current study, this method was able to quantify density of phytoplankton and zooplankton of up to 77600 cells/L and 450 individuals/L respectively. As a whole, only one ballast water sample (PP3, Penang) was free from potentially harmful dinoflagellates or invasive species. From our sampling experience, we would like to take this opportunity to propose a sampling protocol for compliance monitoring for the relevant enforcement agencies to enforce the Ballast Water Convention (2004). This proposed protocol for ballast water sampling could also be used by other researchers intending to carry out studies related to ballast water pollution and for real time verification of ballast water treatment efficiency on board ships.

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