Science Diliman (January-June 2019) 31:1, 69-78

Viability of Phytoplankton from Ballast Waters of International Vessels Berthing at Ports of Cebu and Subic Bay, Philippines

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ABSTRACT

The viability of ballast water phytoplankton was assessed through incubation experiment. *Leptocylindrus* sp. and *Thalassionema* spp. were found to be viable when incubated in port water and ballast water media showing some increase in cell numbers. Bloom-forming diatom taxa, such as *Chaetoceros* spp. and *Coscinodiscus* spp., potentially harmful diatom species *Pseudo-nitzschia* spp., and dinoflagellates, *Gambierdiscus* spp. and *Prorocentrum* spp. were also identified in ballast waters from international vessels. These results further suggest possible successful transport of these organisms via shipping, which could facilitate the introduction and lead to bioinvasion in the local aquatic environment.

Keywords: Ballast water, diatoms, dinoflagellates, phytoplankton, ports

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ISSN 0115-7809 Print / ISSN 2012-0818 Online

INTRODUCTION

Ballast water essentially provides stability and maneuverability to ships, thereby assuring safe operating conditions (David 2015). In ports, ballast water operations are usually conducted either via gravity or pumps to take in or discharge ballast water (David 2015), which may contain potentially harmful algal bloom species that will be transported from one port to another. Ballast water has been identified as one of the major vectors for the global dispersal of alien invasive species, toxic harmful algal bloom species and pathogens causing ecological, economic, and health problems (Hallegraeff and Bolch 1991; Bolch and de Salas 2007; Drake et al. 2007; Eames et al. 2008; Simkanin et al. 2009; Klein et al. 2010).

The present study was aimed at assessing the viability of organisms in ballast water tanks of international vessels berthed at Cebu and Subic ports in Philippines. Attempts to identify common organisms found in ballast waters have also been made.

MATERIALS AND METHODS

Collection of ballast water samples

Four international vessels were boarded for onboard collection of ballast water samples in the months of May and July 2015 in an opportunistic sampling method only. Vessel 1 (V1) is a 4,718-ton general cargo vessel registered from Panama. Its last port of call was in Sumbawa, Indonesia. Previously visited ports include: (1) Makassar, Indonesia, (2) Singapore, (3) Chiba and Yokohama, Japan, (4) Busan, Korea, and (5) Qingdao, China. In addition, vessel 2 (V2) is a general cargo vessel with 6,150 tons gross tonnage under the flagship of Panama. Prior to berthing in Cebu International Port (CIP), its last port of call was in Zhanjiang, China. Its previously visited ports include: (1) Haiku and Hongkong, China, (2) Chiba and Funabashi, Japan, (3) Jingtang, China, (4) Incheon, Korea, (5) Hon Gai, Vietnam, (6) Bangkok, Thailand, (7) Kuantan, Malaysia, and (8) Kaohsiung and Guang Zhou, China. Moreover, vessel 3 (V3) is a 33,990-ton bulk carrier vessel registered from Singapore. Its last port of call was in Taboneo, Indonesia. Its previous visited ports include: (1) Makassar, Indonesia, (2) Singapore, (3) Port Klang, Vietnam, (4) Cai Mep, Vietnam, (5) Washington, USA, (6) Okkye, S. Korea, (7) Vostochny, Russia, (8) Qingdao, China, (9) Busan and Kunsan, S. Korea, and (10) Tamatave, Madagascar. Lastly, vessel 4 (V4) is a 31,753-ton bulk carrier vessel under the flagship of Panama. Its last port of call was in Balboa, Panama. Its previously visited ports include: (1) Cristobal, Panama, (2) New Orleans, USA, (3) Bayovar, Peru, (4) Buenaventura, Colombia, (5) Houston, Texas, USA, (6) Altamira, Mexico, (7) Yeosu, S. Korea, (8) Shanghai, China, (9) Kaohsiung, Taiwan, (10) Susaki, Japan and (11) Port Luis, Mauritius. Ballast water sample access points were via the (1) main engine pump for V1, (2) manholes for V2 and V4, and (3) sounding pipes for V3 based on the availability (Table 1).

Table 1. List of international vessels sampled in Cebu International Port (CIP), Cebu, Central Visayas and Naval Supply Depot (NSD) in Subic Bay, Zambales, Philippines in May and July 2015

Vessel	Type	Access Point	Sampling Gears	Sample Volume*	Sampling Date	Sampling Port	Last Port of
1	Gen. Cargo	Main Engine	Buckets	3	5.2015	CIP	Indonesia
2	Gen. Cargo	Manhole	Buckets	5	7.2015	CIP	China
3	Bulk Carrier	Sounding pipe	Pipe sampler	1	5.2015	NSD	Indonesia
4	Bulk Carrier	Manhole	Plankton Net	5	7.2015	NSD	Panama

** Sample volume is per bucket (1 bucket = ~ 2.5-3 Liters of Ballast Water)*

Sample collection was peformed using buckets, plankton nets, and pipe sampler. In the present study, the main emphasis was on phytoplankton only; hence, the samples were fixed with Lugol's solution and brought to the shore laboratory for further analysis. These samples were then analyzed for phytoplankton cell abundance and identified to genus or species level using identification keys (Yamaji 1984; Tomas 1997). In addition, samples were also collected and brought to the shore laboratory without preservative and used for incubation experiments. The unpreserved samples were pooled accordingly and passed through a 60-μm sieve to remove larger organisms, and the filtrate, containing smaller cells, were cultured in various media.

Media preparation and incubation experiment

Three types of culture media were used for this experiment: (1) Diluted F/2Media-prepared following the methods of Guillard and Ryther (1962), Guillard (1975), Corrales et al. (1995), and Azanza (1997); (2) port water; and (3) ballast water media; which were prepared by filtering through a 0.45-μm Whatman Filter paper following Kang et al. (2010) and were then sterilized by autoclaving.

Approximately 10-mL filtered ballast water samples were inoculated into each 100-mL media in triplicate and were incubated for 6 days. Three 1-mL aliquots were drawn from each flask on alternate days for phytoplankton analysis and identification. Cultures were maintained at 30°C and 33±1psu in a 12:12 light/dark cycle with 143.88±27.62-μmol photon $m^{-2}s^{-1}$.

RESULTS AND DISCUSSION

In this study, a total of 16 diatoms, three dinoflagellates and four zooplankton taxa were identified from the ballast water samples. Highest diatom count was observed in V4, moderate in V2, and minimal in V1 and V3. The presence of potentially toxic diatom *Pseudo-nitzschia* sp. (Figure 1a), and dino-flagellates *Gambierdiscus* sp. (Figure 1b), *Prorocentrum* sp. (Figure 1c), and *Protoperidinium* sp. (Figure 1d) in ballast water confirms that ships can harbor these species in ballast water (Hallegraeff and Bolch 1991; Gollasch et al. 2000; Drake et al. 2007; Burkholder et al. 2007; Klein et al. 2010). These species may have been carried into vessel tanks during the process of ballast water exchange. The presence of potentially toxic *Gambierdiscus* sp. in ballast water, on the other hand, supports the theory of Hallegraeff (1992) and Bomber et al. (1988) that, in spite of being not known to produce resistant cysts, these species are well capable of surviving and getting dispersed as epiphytes attached to drifting macro-algae.

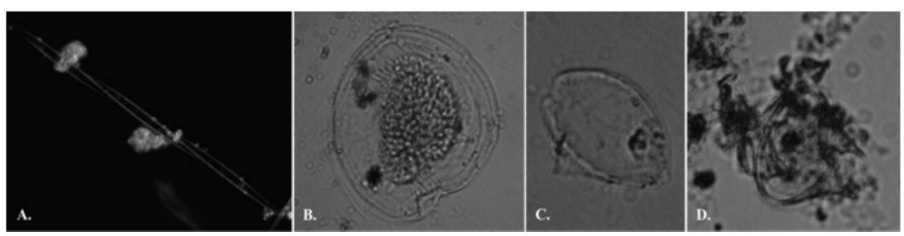
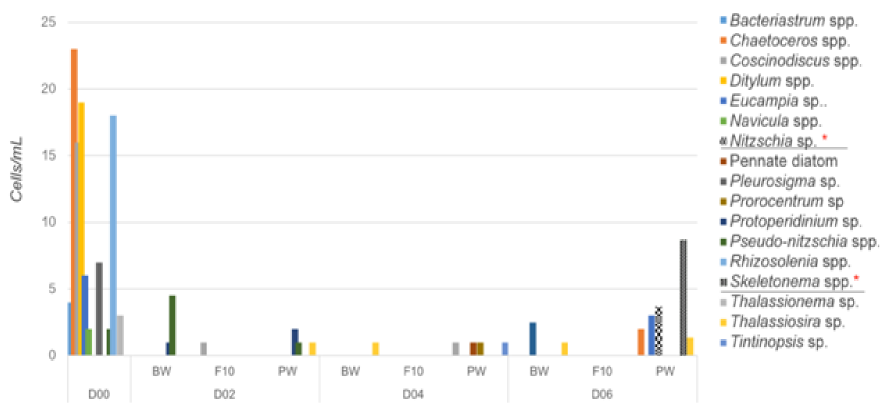


Figure 1. Potentially harmful phytoplankton taxa in ballast tanks sampled as light micrographs (LMs): (a) *Pseudo-nitzschia* sp., (b) *Gambierdiscus* sp., (c) *Prorocentrum* sp., and (d) *Protoperidinium* sp.

The main findings of the incubation experiment show that, among the vessels sampled, only V2 and V4 recorded viable cells (Figures 2a and 2b). -No *Skeletonema* spp. and *Nitzschia* sp. cells were recorded from V2; however, they appeared as viable cells after the 6th day of incubation in port water media (Figure 2a), which suggests that these cells were present in the ballast water, but were absent in the subsample taken for counting. This was also true in the case of *Leptocylindrus* sp., which was not found in ballast water sample, but exhibited considerable increase in cell number after six days in port water media. On the contrary, *Thalassiosira* sp., which is a bloom-forming diatom, was found to grow in all three types of media with maximum growth in port water media after six days (32 times). These results could be attributed to the capability of diatoms to produce viable resting spores that possess the ability to germinate even after a long period of time and that can withstand adverse ballast tank conditions (Doucette and Fryxell 1985; McQuoid et al. 2002; Harnstrom et al. 2011; Montresor et al. 2013). Experiments of Carney et al. (2011), for instance, also revealed the survival of phytoplankton cells and the successful germination of macro-alga *E. flexuosa* spores (Kolwalkar et al. 2007) even after prolonged exposure in the dark.

A.



B.

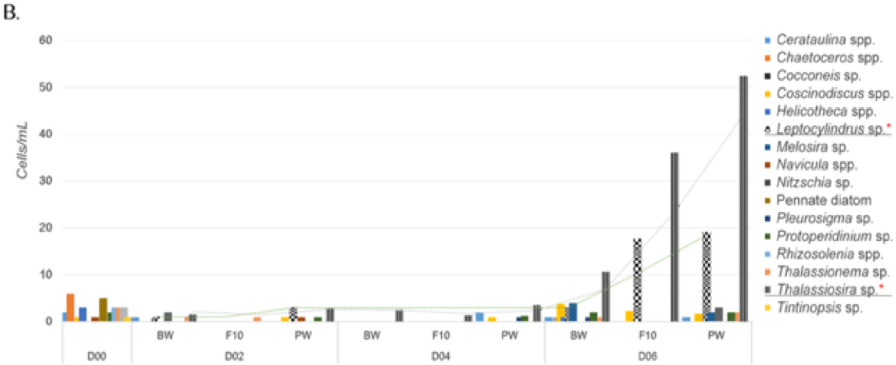


Figure 2. Phytoplankton composition in ballast water samples from (A) V2 and (B) V4, and viable diatom cells (*) following six days of incubation period. (Note: BW – ballast water, PW – port water, F10 – diluted F media, D00 – day zero, D02 – day 2, D04 – day 4, D06 – day 6)

These results suggest that these diatoms can possibly survive if discharged into new environments, leading to possible introduction of new species and/or invasion. Studies of Steichen and Quigg (2015) and Kang et al. (2010) have also reported that ballast water-borne diatoms exhibit viability and growth after exposure to changing salinity and nutrient regimes. Studies have also shown that even the use of modern technologies and techniques implemented in most ocean-going vessels as part of their ballast water management systems do not guarantee 100% efficacy (Grob and Pollet 2015). Certain photosynthetic organisms were able to grow within 4 to 20 days when released back into favorable conditions even after treatment (Stehouwer et al. 2010; van der Star et al. 2011; Liebich et al. 2012; Martínez et al. 2013).

On the other hand, analysis of phytoplankton composition and abundance in ambient waters of Cebu International Port (CIP) and Naval Supply Depot (NSD) terminal identified at least 53 phytoplankton taxa with 36 diatoms and 16 dinoflagellates. Ballast water-borne diatoms, such as *Skeletonema* spp., *Nitzschia* sp., *Leptocylindrus* sp., and *Thalassiosira* sp., were among the identified taxa from the ambient waters of Cebu and Subic Bay ports. These organisms were also identified in the waters of Panama, China, and Indonesia (D'Croz et al. 1991; Liu et al. 2005; Serihollo et al. 2015; Effendi et al. 2016). These findings imply that these species could be cosmopolitan and may have been traveling between these ports. The use of modern and new approaches could be very helpful in inferring these species' geographical sources in future studies.

This preliminary investigation suggests that the foreign vessels involved in trading with the Philippines, especially those which arrive for picking up of cargo, could pose potential risk of transport of harmful or bloom-forming organisms. Additional studies, if conducted in the future, would provide a database useful for assessing ballast water risk for Philippine ports by using a suitable model or tool, which in turn, will provide a Decision Support System (DSS) to the concerned authority in line with the International Maritime Organization – Ballast Water Management Convention - 2004.

ACKNOWLEDGEMENTS

We would like to thank the Department of Science and Technology (DOST) – Philippine Council for Agriculture, Aquatic Resources Research and Development (PCAARRD) for the funding support received in line with the project "Invasive

Marine Organisms transferable by ships in selected areas in the Philippines". We would also like to extend our appreciation and acknowledgement to the Philippine Coast Guard - Ports State Control Manila and Coast Guard District Central Visayas - Cebu Station Command who extended their support and assistance during the conduct of this study. We would also like to thank the Subic Bay Metropolitan Authority (SBMA) Ecology Center and Seaport Department for their field assistance. UPMSI PIMO Project also received assistance and support from the following people: Jayson Orpeza, Estrelita Flores, and Julius Mendoza.

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