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Research article

Microbial hitchhikers: dynamics of bacterial populations in ballast water during a trans-Pacific voyage of a bulk carrier

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Abstract

Bacterial abundance, population dynamics and related environmental parameters were determined in ballast water during a trans-Pacific voyage from Japan to the west coast of Canada. Water samples were collected from four ballast tanks, two of which underwent mid-ocean exchange (MOE) and two that remained unexchanged (control). Bacterial abundances in the unexchanged tanks increased from ~6.4×10^8 cells/L on Day 0 to 1.8×10^9 cells/L on Day 7, whereas in MOE tanks, abundances increased from approximately 8.6×10^8 cells/L (Day 0) to 2.2×10^9 cells/L (Day 10) before MOE (Day 14). After Day 10, bacterial abundances in all ballast tanks declined. Despite the greater decline in MOE tanks, the final abundances in the MOE and unexchanged tanks were not significantly different, which suggests MOE alone is not effective in reducing the risk of transferring the number of bacteria. Bacterial abundance was assessed with respect to changes in environmental parameters within the tanks and we found a significant increase with increased temperatures and a decrease with increased dissolved oxygen concentrations ($p<0.001$). If the relationship between bacterial abundance and both temperature and dissolved oxygen concentrations is shown to be a general characteristic for ballast water, new control protocols could be developed to minimize the number of bacteria being deballasted into coastal harbors.

Key words: invasive species, heterotrophic bacteria, ballast water, ballast water exchange, mid-ocean exchange

Introduction

The establishment of non-indigenous species in marine and freshwater environments is a worldwide issue and a concern to human and environmental health and aquatic biodiversity (IMO 2008; Transport Canada 2007). Oceanic shipping and transport has provided a global connection for species dispersal, and this has greatly increased the potential for and the rate of invasions (Joachimsthal et al. 2004; MacIsaac et al. 2002). The main vectors for species introductions by ships are ballast water, sediments in ballast tanks, and on a ship's hull (IMO 2008).

Invasive aquatic organisms are potentially transported from region-to-region via ballast water that is taken on-board ships at ports and stored in tanks to enhance the ship's stability and maneuverability during voyage (Transport Canada 2007). At the destination port, ballast water is typically discharged into the environment as cargo is taken on. It is estimated that 3 to 5 billion m^3 of ballast water is transported globally each year and that ballast water transports over 7000 different marine species worldwide (IMO 2008). The generally accepted method to control the introduction of aquatic invasive species is the mid-ocean exchange (MOE) of ballast water. This requires ships to exchange ballast water at least 200 nautical miles away from shore and where ocean depths are at least 200 m (IMO 2008; Transport Canada 2007), and the justification for this protocol is that coastal organisms are flushed out and replaced with oceanic organisms that are not likely to survive in low salinity or coastal systems. Moreover, the higher salinity of the mid-ocean water could be lethal to freshwater and perhaps coastal organisms adapted to lower salinity conditions (Santagata et al. 2008). When MOE is conducted in compliance with IMO guidelines, it can be highly effective reducing, by up to 80% to 95%, the initial concentration of planktonic organisms (Ruiz and Reid 2007). However, since there is invariably some residual water left in the tank, this results in the retention of low abundances of coastal organisms in the tanks, some of which may be euryhaline and able to survive the dilution with high salinity water

(Wonham et al. 2001; Taylor et al. 2007; Transport Canada 2007).

Most of the studies on ballast-mediated introductions of invasive organisms have generally been on eukaryotes, such as the zebra mussel *Dreissena polymorpha* (Hebert et al. 1989), or protozoa, such as dinoflagellate vegetative cells and cysts (Gregg and Hallegraeff 2007; Hallegraeff 1998). Several recent studies have reported on the microbial component prokaryotes and viruses (abundance and composition) in ballast water (Ruiz et al. 2000; Drake et al. 2002; Burkholder et al. 2007; Ma et al. 2009). This has lead to the recognition that prokaryotes numerically dominate ballast water communities, and there is potential for environmental and human health risks (Burkholder et al. 2007; Rigby et al. 1999). Most of the studies have addressed the abundances of microbial organisms in ballast water at the location of deballasting and relatively few studies have monitored the changes that occur en-route (Olenin et al. 2000; Gollasch et al. 2000a, 2000b; Drake et al. 2002; Mimura et al. 2005). To assess the risk associated with transporting micro-organisms in ballast water, it is essential to understand their dynamics (i.e., changes in abundances and composition) that occur during transit.

Some marine heterotrophic bacteria are pathogenic such as *Vibrio cholera* and *Escherichia coli* and can represent a potentially serious risk when transported in ballast water. The IMO has thus added pathogenic bacteria as a component to its guidelines for ballast water management in that there are limits on the number of viable *E. coli* and *V. cholera* cells allowed to be present in discharged ballast water (IMO 2008). Bacteria are small (0.2 to 1 μm), ubiquitous and abundant and are capable of enduring the conditions in ballast tanks in the form of spores or other resting stages (Gregg and Hallegraeff 2007; Ruiz et al. 2000). Hence, bacteria possess the characteristics that favor the introduction and successful establishment in new environments. Moreover, even when MOE is successfully conducted, open ocean water still contains a large number of viable organisms, including heterotrophic bacteria. For example in the open ocean, there are 0.5 to 1.0×10^9 cells/L of free living bacteria (Ducklow 2000), hence, MOE may not be effective in reducing the total number of bacteria in ballast water.

Given the potentially large propagule pressure from the ballast water transport and release of microorganisms, such as bacteria, there are surprisingly few studies characterizing both their content in ballast water as well as their in-transit abundance and population dynamics (Ruiz et al. 2000; Drake et al. 2002). Two of the fundamental questions that remain largely unexplored are (i) what changes occur to bacteria populations present in ballast water during transit from one port to another and (ii) what factors control these changes. Here, we assessed the temporal pattern of change in heterotrophic bacteria in ballast tanks that undergo MOE and those that remain unexchanged during a trans-Pacific voyage and assessed the environmental factors that regulate these changes.

Materials and methods

Vessel

A 22-day study was conducted onboard the 'M/V *Skaugran*' (bulk carrier of 182.5 m long; 41 900 gross tonnage). The ballast water was sampled daily for physico-chemical parameters and every three days for bacterial abundances from its point of departure in Hakata, Japan, on 26 July 2007 until its arrival at the west coast of Canada in New Westminster, British Columbia, on 16 August 2007 (Figure 1).

Experimental design

Four ballast tanks (all top side upper wing tanks) were sampled; two of the tanks underwent MOE and two tanks remained unexchanged. The unexchanged tanks were designated the control tanks. The volume of the MOE tanks and unexchanged tanks was approximately 445 m^3. The two sets of tanks were used to compare the efficacy of MOE in reducing bacterial numbers. The exchanged tanks underwent MOE on Day 14 at 44°30.501'N, 171°16.837'E (Figure 1) and the exchange lasted between 1 to 2 h. For each ballast tank, samples for bacterial enumeration were collected every third day as well as immediately before and after MOE. Two samples were collected from both the source port (Inchon, Japan) and destination port (New Westminster, BC).

Physico-chemical parameters

Temperature, dissolved oxygen concentrations (mg/L) and salinity (psu) were measured daily in the ballast tank water and once in each of the ports using a handheld YSI Model 85 meter (YSI

Incorporated, Yellow Springs, OH, USA). Measurements were made with the probe at three different depths (surface (0 m), middle (2 m) and mid-bottom (4 m)) prior to water sampling. Averages were taken from the three depths for each environmental parameter daily as the water samples were conducted from three depths and pooled into one sample (see below). In the source and receiving ports, these measurements were made over the side of the ship at depths of 0, 4, and 8 m and 0, 2, and 4 m, respectively. In addition, the ambient sea water temperatures were measured daily and were obtained from the ship's log.

Water sample collection

At one to three day intervals, water samples were collected using a 5-L Niskin bottle (deployed into the ballast tank through a deck hatch or over the side of the ship for port samples) at the 3 depths the physico-chemical parameters were measured. Water was pooled into 15-L carboys that were rinsed in 2% HCL and distilled water. This integrated ballast water sample was considered to be representative of the water column and was subsampled for bacterial analysis. At the source and receiving port duplicate water samples were collected following the same procedure.

Analytical methods

Samples for bacterial enumeration were fixed with ~2% (final concentration) formaldehyde and kept in the dark at 4°C for the duration of the voyage. Slides were prepared within three days of their shipment to the laboratory. To determine bacterial counts, bacterial slides were prepared by filtering 10 mL of preserved ballast and port water onto 25 mm diameter, 0.22 µm black polycarbonate filters (GE Osmonics, Inc.), staining with 200 µL of 4',6-diamido-2-phenylindole (DAPI; 5 µg/mL), and mounting on slides in Cargille Type A immersion oil (Porter and Feig 1980). Slides were stored at -20°C until analysis.

Epifluorescence direct counts were made using an Olympus BH2-RFCA equipped with a wide band blue filter (120× oil immersion lens; total magnification 1250×). For each filter, bacteria were counted in 10 to 20 fields of view with at least 600 cells/filter counted. The average Coefficient of Variation of bacterial counting during this study was 17%.

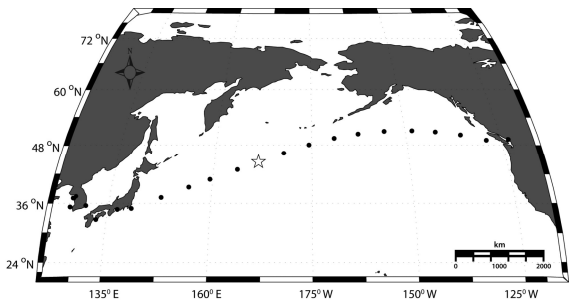

Figure 1. Shipping route of the 'M/V *Skaugran*' from its point of departure in Hakata, Japan, to its arrival at the west coast of Canada in New Westminster, British Columbia. The exchanged tanks underwent mid-ocean exchange on Day 14 at 44°30.501'N, 171°16.837'E (noted with a star).

Data analyses

To determine relationships between bacterial abundance and the environmental parameters temperature, dissolved oxygen concentrations and salinity, linear regression was used. Multiple regression analysis was also performed to determine the combined effects of temperature and dissolved oxygen on bacterial abundance. Differences in bacterial abundances in the MOE tanks and unexchanged control tanks and port water samples were determined using the General Linear Model one-way Analysis of Variance (ANOVA). All statistical analyses were conducted using Minitab v. 14.

Results and discussion

Physico-chemical parameters

Initial (i.e. Day 0) ballast water temperatures ranged from 21.7 to 22.5°C, and the temperature of all tanks followed the same general temperature trend for the voyage (Figure 2A). Temperatures increased and peaked to ~29.1°C on Day 9 for all tanks (range 28.6 to 29.6°C), after which they all declined with a more pronounced decline in MOE tanks on Day 14 after exchange occurred. The temperatures at the end of the voyage ranged from 15.7 to 17.6°C (Day 22). The changes in the ballast tanks paralleled the ambient ocean water temperatures through which the vessel travelled, and the ballast water temperatures matched the seawater temperatures with an approximate 2 to 3 day lag (Figure 2A).

Dissolved oxygen concentrations in all tanks ranged from 2.9 to 4.5 mg/L at the start of the voyage on Day 0 and it declined to below detectable levels (~0 mg/L) on Day 6 (Figure 2B). The rate of decrease in the unexchanged and MOE tanks averaged -0.55 $mg \cdot L^{-1} day^{-1}$ and -0.57 $mg \cdot L^{-1} day^{-1}$, respectively. After Day 6, the ballast water in the unexchanged tanks was near-anoxic for the remainder of the voyage. In contrast, in the MOE tanks, the introduction of oxygenated oceanic water increased dissolved oxygen concentrations on Day 14, with an increase to 5.5 mg/L (both tanks) after exchange, and this was followed by a decrease in O_2 concentrations at an average rate of -0.31 $mgL^{-1} day^{-1}$. Drake et al. (2002) reported a decline in DO concentrations but not to the same magnitude as reported here (Figure 2B). Differences may be due to a number of factors including ballast tank configuration and size, sea state, ambient temperature, source water composition and characteristics. The relatively small size of the ballast tanks (445 m^3) on the 'M/V *Skaugran*' and the source of the ballast water may have combined to enhance the observed rate of oxygen depletion.

Salinity ranged from 30.5 to 31.7 psu in the unexchanged tanks and 30.6 to 31.2 psu, in the exchanged tanks prior to exchange and ~32.7 psu afterwards (data not shown).

Bacterial abundances in port water

Water samples were collected from both the source and receiving port waters. Bacterial abundances in source port and receiving port waters were 6.7×10^8 cells/L and 1.3×10^9 cells/L, respectively. The difference between source and receiving port waters is approximately 1.9-fold, and bacterial abundances were significantly (p=0.04) higher in receiving port waters.

Bacterial abundances and growth rates during transit

We monitored ballast water bacterial abundances during the ship's transit from one ocean region to another and compared the changes occurring in tanks that undergo MOE with tanks that remain unexchanged. In the unexchanged ballast tanks, bacterial abundances significantly (p=0.01) increased (by 2.8-fold) from ~6.4×10^8 cells/L on Day 0 to 1.8×10^9 cells/L on Day 7 before declining significantly (p=0.03; 1.8-fold) to 9 to 11×10^8 cells/L by Day 22 (Figure 2C). The abundance on Day 22 was ~50% higher than on Day 0. In the MOE tanks, bacterial abundance significantly (p=0.02) increased (by 2.6-fold) from approximately 8.6×10^8 cells/L on Day 0 to 2.2×10^9 cells/L on Day 10 before declining significantly (p=0.02; ~3.7-fold) to 4 to 8×10^8 cells/L on Day 22 (Figure 2D).

The average rates of bacterial growth and losses in unexchanged tanks were computed for two distinct growth phases (Figure 2C and 2D): Phase 1 where abundances increased (i.e., growth phase; Days 0 to 7) and Phase 2 where abundances decreased (i.e. loss phase; Days 7 to 22). Using an exponential growth equation, the rates of growth were computed for each phase for each tank from the slope of the regression of the relationship between time and the logarithm of bacterial abundance; an initial increase in the bacterial abundance had a positive slope and the decrease had a negative slope. The average rates of growth and loss in the MOE tanks were determined following the same procedure except the growth phase was between Day 0 and 10 and the loss phase between Day 10 and 22. In the un-exchanged ballast tanks, the average growth rate for the two unexchanged tanks during the first 7 days was 0.24 d^{-1} (range = 0.20 to 0.28 d^{-1}) before the abundances declined at an average rate of -0.04 d^{-1} (range = -0.03 to -0.05 d^{-1}) between Days 7 and 22 (Figure 2C). In the MOE tanks, the average growth rate for both MOE tanks during the first 10 days was 0.15 d^{-1} (range = 0.14 to 0.16 d^{-1}). After MOE that took place on Day 14, bacterial abundances declined at an average rate of -0.07 d^{-1} (range = -0.06 to -0.08 d^{-1}) between Days 10 and 22. This decline started before the MOE on Day 14 and hence the reduction of bacterial abundance was only in part due to dilution with oceanic waters. In general, bacterial population dynamics followed the same general growth trends in all tanks. There was a significant increase in abundance until Days 7 and 10 in unexchanged and MOE tanks, respectively, after which populations declined, albeit with a greater rate of decline in MOE tanks (Figure 2). For the unexchanged tanks, the bacterial abundances were higher at the end of the voyage than at the beginning but not significantly. Drake et al. (2002) reported a gradual decline in bacterial abundances during a 19-day voyage across the Atlantic Ocean from Hadera, Israel, to Baltimore, USA, with abundances lower at the end of than at the start of the voyage. There were also no significant differences in bacterial abundance between unexchanged and MOE tanks on the last day of

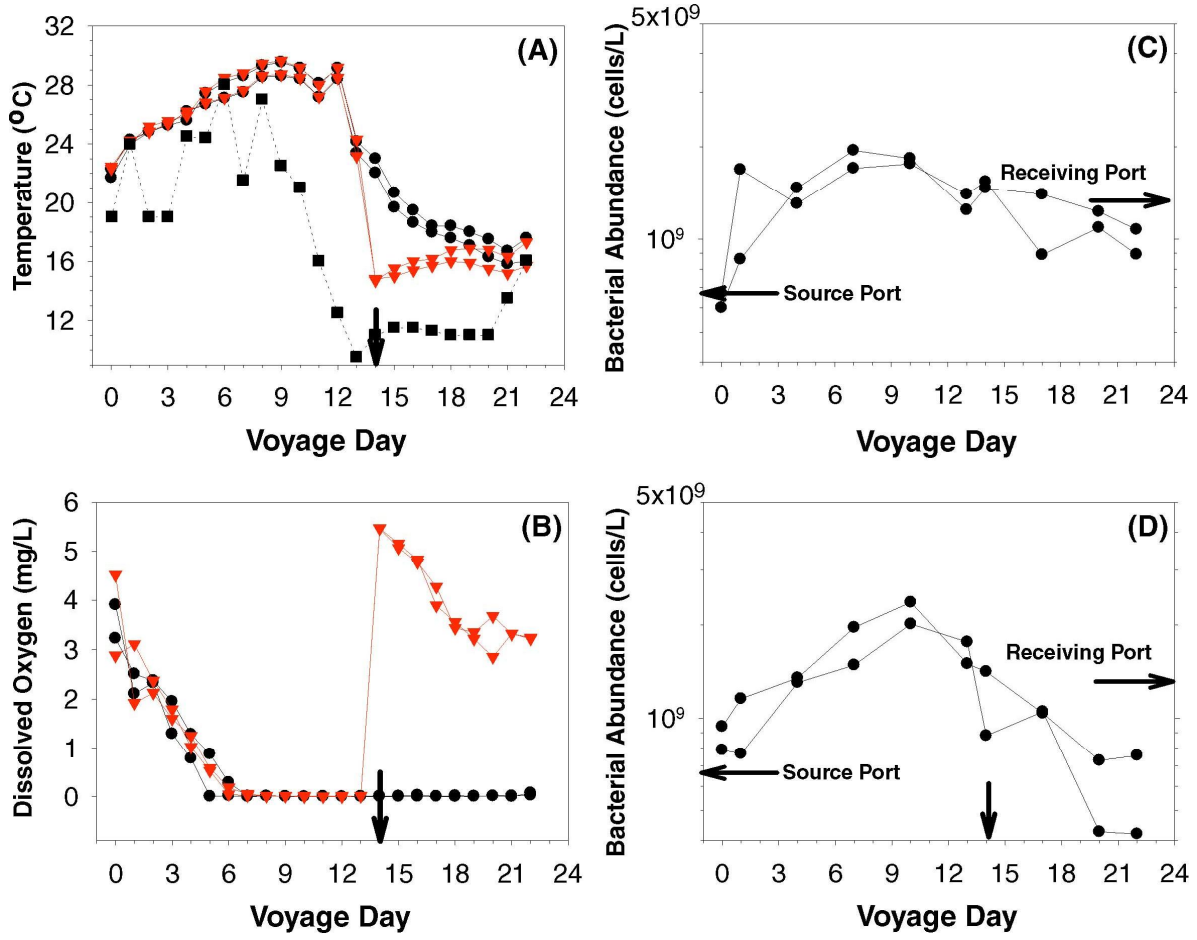

Figure 2. Changes in environmental parameters and bacterial abundance of the ballast water in the two tanks that underwent mid-ocean exchange (MOE; inverted triangles) and the two tanks that remained unexchanged (black closed circles) during the voyage. (A) Temperature of the ballast water in the two sets of tanks and ambient ocean water (black closed squares) temperatures through which the vessel traveled. (B) Dissolved oxygen concentrations (mg/L) of the ballast water in the 2 sets of tanks. (C) Bacterial abundance in the two unexchanged tanks. (D) Bacterial abundance in the two MOE tanks. The standard error bars in bacterial counts cannot be seen as they are smaller than the size of the symbols. Downward arrows indicate the day exchange took place (Day 14).

the voyage. The different patterns of bacterial dynamics reported here and by Drake et al. (2002) could be due to several reasons. The ships followed different tracts (Atlantic vs. Pacific) with distinct temperature regimes and the origin of the source water differed and likely contained different bacterial assemblages. Burkholder et al. (2007) found bacterial abundances in ballast water to be significantly lower in source water from the Atlantic than Pacific Ocean based on assessing bacterial concentrations in ballast water of ships arriving at ports.

Bacterial abundances and relationships with environmental parameters

Under natural conditions, the dynamics of bacterial populations are a balance of both bottom up and top down control, i.e., community structure and function depends on nutrient availability and physical conditions of the water (temperature, dissolved oxygen, and salinity) as well as grazing pressure by microzooplankton and viral lysis (Ducklow and Carlson 1992; Ducklow 2000). In this study, we show that

bacterial abundance was correlated with temperature and dissolved oxygen concentrations. Concurrently measured data (i.e. bacterial abundances, O_2, and temperature) from all tanks were pooled (n=40) and show a significant positive relationship (p<0.001; r^2=0.45; n=40) between bacterial abundance and temperature (Figure 3A) and a significant inverse relationship between bacterial abundance and dissolved oxygen concentrations (p<0.001; r^2=0.37; n=40; Figure 3B). From Figure 2, the trend in bacterial abundance parallels the trend in temperature changes. This is consistent with studies that have found a significant correlation between bacterial abundance and temperature in coastal and estuarine environments (White et al. 1991; Hoch and Kirchman 1993; Granéli et al. 2004). To our knowledge, similar relationships between bacterial abundance and dissolved oxygen concentrations have not previously been reported. When both temperature and dissolved oxygen are included in a multiple regression, the explained variance increases to 54%

$$BA=3.213\times10^8+0.484\times10^8(T)-0.923\times10^8(DO),$$

adj-r^2=0.54; p<0.001; n=40, where BA is bacterial abundance (cells/L), T is temperature, and DO is dissolved oxygen concentration (mg/L).

Thus considering both variables together can lead to a more robust prediction of bacterial abundance than each variable separately. The larger decline in abundance in the MOE tanks compared with the unexchanged tanks may be the result of the freshly oxygenated waters after exchange. Re-analyses of Figure 2 and 3A in Drake et al. (2002) shows a qualitatively similar relationship between bacterial abundance and temperature in ballast water; for individual tanks the relationship was significant, whereas when the data for all tanks are considered, as in Figure 3A of the present paper, there was no significant relations between these variables. In our study, no significant correlation was observed between bacterial abundance and salinity.

The results of this study suggest that when ballast tanks are maintained at lower temperatures and higher dissolved oxygen concentrations, bacterial abundances will be reduced. Bacterial mortality due to grazing and viral lysis cannot be separated from the physical factors in this study. It is possible that the observed effects of low dissolved oxygen concentrations and high temperatures on bacterial abundances were indirect and were mediated (at least in part) by changes in the sources and magnitude of their mortality (i.e. temperature and O_2 affected viruses and grazers). If our results on the relationship between bacterial abundance and temperature and dissolved oxygen show to be general patterns, it may be possible to control bacteria populations in ballast water by altering the temperature and dissolved oxygen concentrations of ballast water.

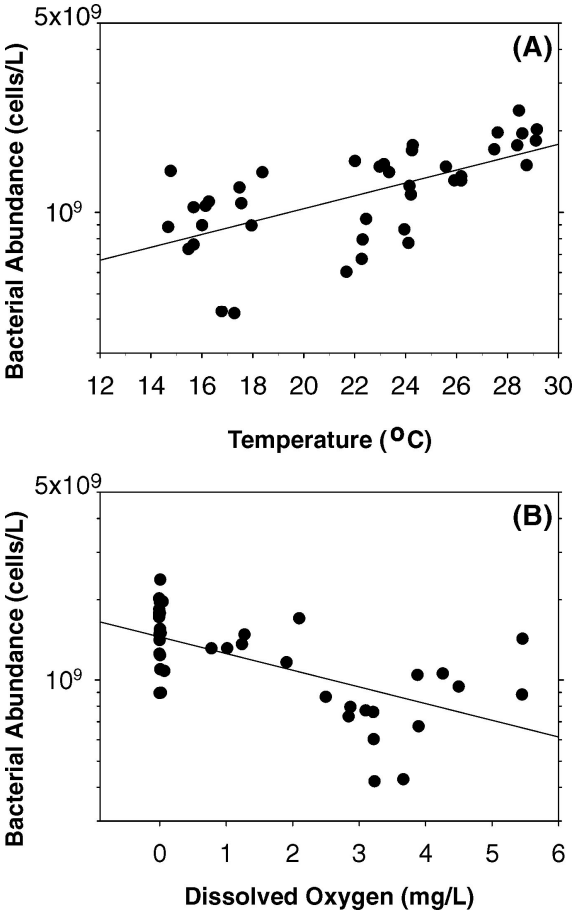

Figure 3. Regression relationship between bacterial abundance and (A) temperature ($BA=-2.23\times10^8+0.665\times10^8[T]$), where BA is bacterial abundance and T is temperature; p<0.001; r^2=0.45; n=40) and (B) dissolved oxygen concentrations ($BA=1.50\times10^9-0.159\times10^9[DO]$), where DO is dissolved oxygen concentrations; p<0.001; r^2=0.37; n=40) in all ballast tanks.

Final bacterial abundances in tanks

The end-of-voyage bacterial abundances in the MOE tanks were lower than those of the unexchanged tanks; however, this difference was

not significant (p=0.176) and the final abundances in the tanks were not significantly different from the receiving port waters (p=0.213). These results are consistent with those of Drake et al. (2002), and together, our results suggest that MOE does not significantly reduce bacterial numbers in ballast water. Given the estimates that open-ocean water still contains an average of 5 to 10×10^8 cells/L (Sherr and Sherr 2000; Whitman et al. 1998), it is not surprising that MOE does not reduce bacterial numbers. However, this alone does not invalidate MOE as being effective in controlling bacteria since the threat from bacterial invaders arise from both the total number of propagules introduced as well as the composition propagules. Mid-ocean exchange could and likely does cause a shift in the assemblages present as coastal waters are greatly affected by land-based pollution such as sewage effluent and agricultural run-off and could therefore be especially important in reducing the introduction of pathogenic bacteria (EPA 2006). For example, *Vibrio cholera* (e.g., *V. cholera* O1 and O139) and *Escherichia coli* have been found in ballast water (Joachimsthal et al. 2004; Ruiz et al. 2000; Burkholder et al. 2007; Brazilian Sanitary Surveillance Agency 2003) and it is thus necessary to study further the efficacy of MOE in reducing pathogenic species. The extent to which bacteria will be transported in ballast water is likely influenced by global bacterial biogeography (Martiny et al. 2006; Ramette and Tiedje 2007) and (or) the annual cycle of bacterial phylotypes (Fuhrman et al. 2006) in coastal and oceanic regions but is largely unexplored.

Although there have been a number of studies of the transport of potential invasive species in ballast water (Carlton 1985; Ruiz and Reid 2007), there have been very few on the heterotrophic bacteria in ballast water (Ruiz et al. 2000; Joachimsthal et al. 2004; Burkholder et al. 2007; Drake et al. 2002; Ma et al. 2009; David et al. 2007; Mimura et al. 2005) and these have generally focused on the microbial community present in ballast water upon arrival at receiving ports (however, see Drake et al. 2002; Mimura et al. 2005). The majority of these studies show that pathogenic and non-pathogenic bacteria are present in ballast water at the end of voyages. One of these authors (Burkholder et al. 2007) have reported relationships between the end-of-voyage bacterial abundance and concurrently measured environmental parameters (e.g., temperature, salinity, dissolved oxygen concentrations and pH), but found no significant relationships. Since the ballast tank environment is physically and chemically dynamic, there will be concurrent, but not necessarily directional changes in the biotic and abiotic characteristics within the tanks. It is not surprising that no relationships have been found between ballast water bacterial abundances and environmental characteristics at the end of voyages. To determine whether relationships exist between the bacterial community and the environmental parameters of the ballast water, it is necessary to study these parameters en route. Only one other published study has tracked changes in bacterial population size, as well as virus-like particles, during the voyage (Drake et al. 2002), and one other study examined the number of colony forming units of marine microorganisms in source port water, before and after exchange, and at the time of discharge during six voyages from Japan to Qatar (Mimura et al. 2005) but the microbial measures were not related to the changes in the environmental parameters of the tank over time. In addition to Drake et al. (2002) and Mimura et al (2005), several other studies have been conducted on planktonic organisms in ballast water during transit (Olenin et al. 2000; Gollasch et al. 2000a, 2000b; Klein et al. 2009; McCollin et al. 2007, 2008; Taylor et al. 2007). Four of these studies examined changes in phytoplankton and zooplankton abundance and assemblages during transit (Olenin et al. 2000; Gollasch et al. 2000a, 2000b; Wonham et al. 2001), one study reported changes in diatom cell densities and species richness (Klein et al. 2009), and three studies document changes in phytoplankton and zooplankton abundance and assemblages before and after exchange (McCollin et al. 2007, 2008; Taylor et al. 2007).

Table 1 summarizes the results of the studies documenting changes in overall abundances of microorganisms throughout transit that are comparable to the study reported. The time dependent patterns were complex and taxon specific. Generally however, there was decrease in both biomass and biodiversity within the tanks with time. The one exception was the harpacticoid copepod *Tisbe graciloides*, which increased by a factor of 100 in only a few days during the last week of the voyage (Gollasch et al. 2000a). Here, we for the first time relate patterns in temporal bacterial abundance to the changes in the environmental parameters of the tanks experienced during transit.

Table 1. Summary of representative studies monitoring changes in the overall abundance of planktonic organisms in ballast water during transit.

Source port	Receiving port	Tank treatment	Taxa monitored	Observed change in taxa	Voyage duration	Study
Strait of Malacca, Singapore	Bremerhaven, Germany	Unexchanged	Zooplankton	Decrease	25 days	Gollasch et al. (2000a)
			Phytoplankton	Decrease		
Colombo, Sri Lanka		Unexchanged	Zooplankton	Decrease for all except for the harpacticoid copepod *Tisbe graciloides*; increase during last week of voyage		
			Phytoplankton	Decrease		
Eastern Gulf of Finland	Continuous voyage exchanged at each of these areas. Final exchange Southern Baltic Sea	Exchanged	Phytoplankton	No change	4 days	Olenin et al. (2000)
			Zooplankton	Variable; general decrease after Day 2		
Northern Baltic Sea		Exchanged	Phytoplankton	Decrease	11 days	
			Zooplankton	Variable; general decrease after Day 5		
Straight of Dover		Exchanged	Phytoplankton	Increase until Day 4, then decrease	15 days	
			Zooplankton	Variable; general decrease after Day 5		
Strait of Zund		Exchanged	Phytoplankton	Increase then decrease	4 days	
			Zooplankton	Decrease		
St. Petersburg	Lisbon	Unexchanged	Zooplankton	Decrease	13 days	Gollasch et al. (2000b)
Cork	Sture	Unexchanged	Zooplankton	Decrease	2.7 days	
Kaohsiung	Hamburg (exchanged in middle of Indian Ocean)	Exchanged	Zooplankton	Decrease until MOE, after which there was an increase in taxa but not abundance, followed by a decrease in both abundance and taxa	26 days	
Odessa	Constanta to Varna and back to Odessa	Unexchanged	Zooplankton	Overall decrease	4 days	
Hadera, Israel	Baltimore, USA	Unexchanged	Phytoplankton	Decrease	16 days	Wonham et al. (2001)
			Zooplankton	Decrease		
		Exchanged	Phytoplankton	Increase following MOE, after which there was a decline but remained significantly higher than in unexchanged tanks at end of voyage		
			Zooplankton			
Hadera, Israel	Baltimore, USA	2 unexchanged tanks and 2 MOE tanks	Bacteria	Decrease	19 days	Drake et al. (2002)
			Virus like particles	Decrease		
Hakata, Japan	Westminster, B.C.	2 unexchanged tanks and 2 MOE tanks	Bacteria	Increase until Day 7 and Day 10 in unexchanged and MOE tanks, respectively, and then decrease	21 days	This study

Conclusions

The findings of our study have several important implications. (1) The relationship found between bacterial abundance, temperature and dissolved oxygen concentrations has potential to be used in predicting patterns of bacterial abundance during oceanic transit and hence propagule pressure. (2) MOE does not significantly reduce bacterial numbers but it is necessary to examine changes in community structure to fully determine the efficacy of MOE. (3) The increase in bacterial abundance in the first 7 to 10 days of our voyage and general decline in the voyage of Drake et al. (2002) show that the length of the voyage plays a critical role in determining the number of bacteria that will be deballasted. From this study, it can be inferred that under similar conditions of temperature, dissolved oxygen concentrations and salinity, if the voyage was <10 days, significantly higher bacterial abundances (1.8×10^9 cells/L and 2.2×10^9 cells/L in the un-exchanged and MOE tanks, respectively) would

have been transferred upon deballasting compared with the number at the end of the 22-day voyage (~9.8×10^8 cells/L in the unexchanged tanks and ~5.9×10^8 cells/L in the MOE tanks). Similar patterns are seen in the study of Drake et al. (2002). (4) The shipping tract will influence bacterial dynamics and are linked to the following: different source waters will represent different microbial assemblages, temperature regimes experienced during transit will be varied and in turn will affect growth patterns, and the duration of the voyage will be varied depending on the route and will have consequences on the number of bacteria transferred. The large uncertainty in patterns of bacterial population dynamics and transport of different phylotypes, especially those of ecological and health concerns, highlights the need to document and quantify the factors controlling the responses of microbial communities in ballast water during voyages.

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