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Research article

Redistribution of heterotrophic prokaryotes through ballast water: A case study from the west coast of Canada

Bei Sun*, Rebecca Mouland, Candice Way and Richard B. Rivkin

Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, A1C 5S7, Canada
E-mail: bsun@mun.ca (BS), rmouland@mun.ca (RM), cjway@mun.ca (CW), rrivkin@mun.ca (RBR)

**Corresponding author*

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Abstract

Oceangoing ships contribute to the introduction of invasive, benign and pathogenic bacteria via ballast water discharge. Here we report the bacterial abundance and cell size in ballast and receiving port waters in Vancouver, British Columbia (Canada) ports during 2007 and 2008. Bacterial abundance in port water (7.5×10^8 to 3.4×10^9 cells L^{-1}) was significantly ($P < 0.001$) higher than those in ballast water (2.5×10^8 to 2.1×10^9 cells L^{-1}) and was higher in unexchanged than ballast water that was exchanged at-sea. There was no significant difference in bacterial abundances between 2007 and 2008 for each sample type. Bacterial cell volume showed a different pattern, with no significant difference among sample types and a two-fold larger average cell volume during 2007 than 2008. Bacterial abundance and cell volume in ballast water were not correlated with ballast water age, end-of-voyage temperature, salinity or pH. The absence of predictive relationships between measured physiochemical and bacterial variables in ballast water highlights the difficulty of predicting bacterial abundance or cell volume from the physiochemical factors alone. Future studies should focus on the bacterial community structure in ballast and port waters, the fate of bacteria in the new environment, and regional susceptibility to invasion by the introduced bacteria.

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

Introduction

Oceanic transport accounts for more than 80% of total global shipments of commercial cargo. In addition to "intended" cargo, over 12 billion tonnes of ballast water are moved across vast coastal and oceanic domains annually (Anil et al. 2002). Ballast water is one of the primary vectors responsible for the global transport of vegetative- and resting-stages of aquatic microorganisms as well as for potentially pathogenic bacteria, such as *Vibrio cholerae O1* and *O139* (Carlton and Geller 1993; Ruiz et al. 2000). Successful establishment of non-indigenous organisms can cause unwanted economic (Pimentel et al. 2005), ecological (Frenot et al. 2005) and human health impacts (Juliano and Lounibos 2005). To attenuate the risk of ballast water-mediated invasions, the International Maritime Organization established mid-ocean ballast water exchange (MOE) guidelines in 1997 (International Maritime Organization 1997). MOE is an interim management option until the permanent performance standard comes into effect. The rationale for MOE is that most coastal organisms will be flushed out during exchange (being replaced by oceanic species) and that the different physicochemical characteristics of coastal and oceanic waters will impair the survival of the coastal organisms that are released from ballast water into the open ocean. Conversely, oceanic species released into ports with coastal environmental conditions will unlikely survive, or become established (Smith et al. 1999). However, this justification may not apply to resistant and euryhaline microorganisms with wide environmental tolerances.

Canada initiated mandatory mid-ocean exchange (MOE) for ocean-going ships entering all its ports as of June 8th, 2006 (Transport Canada 2006). However, voyages from nearby US ports traveling to Canada (e.g., north of Cape Blanco on the west coast; north of Cape Cod on the east coast) do not require MOE because, presumably, nearby ports would have similar community compositions. Commercial ships that carry out MOE are divided into two categories: 1) transoceanic, which exchange their ballast water greater than 200 nautical miles from shore where the water depth is at least 2000 m, and 2) intra-coastal, which are required to exchange

ballast water at least 50 nautical miles from shore and at water depth of at least 500 m (Transport Canada 2006). Therefore, ballast water from ships arriving from international destinations can be divided into trans-oceanic exchanged (TOE); intra-coastal exchanged (ICE); without mid-ocean exchange (NonMOE).

To evaluate if different ballast water operations (i.e., TOE, ICE and NonMOE) have predictable and systematic effects on the bacteria in ballast waters, we characterized bacterial abundances and cell volumes in the TOE, ICE, NonMOE ballast water categories, and in receiving port water, and assessed the relationships between bacterial variables and physiochemical factors (i.e., temperature, salinity, pH, and ballast water age). The results of this study contribute to our knowledge of the bacterial community, specifically, abundances and cell volumes in ballast water tanks on large spatial and temporal scales. The study will also illuminate the effects of ballast water management applications in Canada to the bacterial communities in ballast water.

Materials and methods

Ballast water and port water sampling

As part of the Canadian Aquatic Invasive Species Network (CAISN), sampling was conducted from March to November 2007, and May to October 2008 from Vancouver (British Columbia, Canada) ports. Commercial vessels that had ballast tanks with TOE, ICE, NonMOE were sampled, in addition, port water samples were collected 13 times during the sampling seasons. For each ballast tank, samples were collected from four depths in the tank (surface, mid surface, mid bottom, and near bottom) through a deck hatch, using a Niskin bottle. For each ballast tank, equal volumes of water from each of the sampling depths were combined. Associated environmental data (temperature, salinity, and pH) was also recorded from each sampling depth using a handheld YSI Model 85 meter (YSI Incorporated, Yellow Springs, OH, USA). A 500 ml sub-sample of the combined ballast water from the tank was preserved with formaldehyde (final concentration 3.7%) and shipped on ice to the Ocean Sciences Centre (St. John's, NL, Canada) for analyses within five days of collection. A total of 82 ballast and port water samples were collected during the two sampling seasons: 28 ballast and 3 port water samples during 2007 and 41 ballast and 10 port water samples during 2008 (Table 1).

Slide preparation

Slides for enumerating bacteria were prepared by filtering 10 ml of sample onto a 25 mm diameter, 0.22 μm black polycarbonate filter (GE Osmonics, Inc., Minnetonka, MN, USA). Filters were stained with Acridine Orange (AO; final concentration 1.872×10^{-5}g L^{-1}), and mounted on a glass slide in Cargille Type A immersion oil (Hobbie et al. 1977; Kirchman et al. 1982). 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 per filter counted.

Image analysis

Cell dimensions were determined with Image-Pro Plus V6.2 Image Analysis System which was configured to capture and store images, and size-size distributions. The images were individually examined, and the cell dimensions were recorded for at least 1000 cells per filter. Detrital particles or specific cells (i.e., clumped or aggregated) were removed from the analysis either through the direct removal from the working image, or by constraints assigned to acceptable cell dimension parameters. Cells with an aspect ratio <1.5 were calculated as sphere whereas cells with an aspect ratio >1.5 were calculated, respectively using the formula for cylinders. Cell volumes were calculated using shape-appropriate formulas.

Data analyses

Analyses of Variance (ANOVA) were carried out to assess the significance of the relationship observed for bacterial parameters (bacterial abundances and cell volumes) among sample categories (ICE, TOE, NonMOE and port water samples), and between sampling years (2007 and 2008). If a statistically significant result was found in an omnibus F-test for a one-way ANOVA, a post-hoc analysis using the Tukey test was conducted (Seber and Lee 2003). Multiple regression analyses were run to determine the relationships between bacterial abundances and environmental variables (i.e., temperature, pH, salinity, ballast water age), and relationships between cell volumes and these

environmental variables. All statistical analyses were conducted using Minitab Release 14.0 and SPSS V.16.0. The judgement criterion for statistics in this study is α=0.05.

Results and discussion

Ballast water management

Ballast water age was computed as the number of days between the date of MOE exchange (for ICE and TOE) or take-up in the port (for NonMOE) and the date when the ballast water was collected in a Canadian port. Preliminary experiments (data not shown) showed that the bacterial abundance did not change within five days for formaldehyde-preserved (final concentration 3.7%) samples (Sun 2009). Therefore, the time between sample collection and analysis (up to five days) was not considered in our analyses. The NonMOE treatment (mean ±SD = 8.4±19.1, median = 4) had the longest ballast water age range from 1 to 93 days, compared to ICE from 1 to 27 days (mean ±SD = 7.9±5.7, median =7) and TOE from 3 to 21 days (mean ±SD = 11.8±4.4, median = 11).

Bacterial abundance

Propagule pressure (i.e., the number of non-native individuals introduced into a given environment) shows a positive correlation with species establishment success (Lockwood et al. 2005) and the abundance of microorganisms in ballast waters is considered a proxy for propagule pressure (Drake and Lodge 2007). There have been very few studies on the heterotrophic bacteria in ballast water, and the results of representative studies are summarized in Table 2. Bacterial abundances in ballast water can be high and variable (e.g. 10^7 to 10^{10} cell L^{-1}; Drake et al. 2002; Joachimsthal et al. 2004), which are similar to the naturally occurring bacterial abundances in aquatic environments (10^7 to 10^{10} cell L^{-1}; Whitman et al. 1998). In this study, bacterial abundances ranged from 7.5×10^8 to 3.4×10^9 cells L^{-1} in port and 2.5×10^8 to 2.1×10^9 cells L^{-1} in ballast water samples. Figure 1 shows the average abundances for all Vancouver port and ballast water samples collected during 2007 and 2008. Bacterial abundances were significantly higher in port than in ballast water samples for both years (2007: $F_{1,29} = 28.59$, $P < 0.001$; 2008: $F_{1,49} = 40.15$, $P < 0.001$).

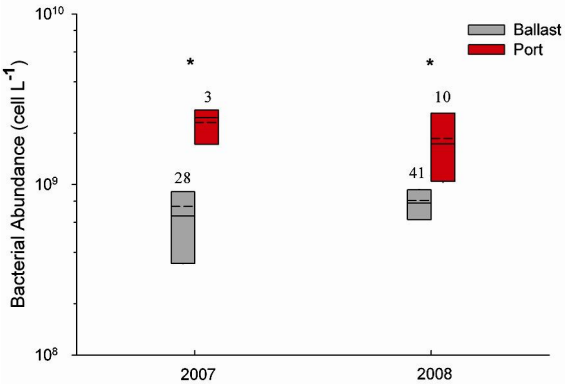

Figure 1. Bacterial abundance of ballast (grey) and port (red) samples during 2007 and 2008. The number on top of each box indicates the number of replicates for each of the sample categories. The lower and upper boundaries of each box are the 25th and 75th percentile, respectively. The dashed and solid lines within the boxes are the mean and the median, respectively. "*" indicates that the bacterial abundances were significantly higher in port than in ballast water samples (one-way ANOVA, 2007: $F_{1,29}$=28.59, P <0.001; 2008: $F_{1,49}$=40.15, P <0.001).

These ballast water samples were collected at the end of the voyage. This means that the selection pressure exerted by the ballast water environment in-transit, and the coastal vs. oceanic source for bacterial populations (i.e. lower abundances in oceanic than coastal environment; Carlson and Ducklow 1992; Culley and Welschmeyer 2002) could account for, or contribute to the observed patterns in abundance. Lower bacterial abundance in ballast than receiving port water was also reported in Singapore (Joachimsthal et al. 2003; Joachimsthal et al. 2004), and in Chesapeake Bay (Drake et al. 2001). The bacterial abundances among ballast water categories (TOE, ICE and NonMOE) during 2007 and 2008 are reported in Table 1. A two-way ANOVA results showed that there were no significant interaction between year and ballast water categories ($F_{2,63}$= 1.54, P = 0.222) nor significant year-to-year differences in bacterial abundances for any of the ballast water categories ($F_{1,63}$ = 0.09, P = 0.768), but there was a significant difference in bacterial abundance among the ballast water categories of TOE, ICE and NonMOE ($F_{2,63}$ = 7.97, P = 0.001, Table 1). Post-hoc comparisons using the Tukey test indicated that bacterial abundance was signifi-cantly higher in NonMOE than TOE samples, but there was no significant difference between NonMOE and ICE, or between TOE and ICE samples.

Table 1. Summary of bacterial abundances (10^9 cells L^{-1}) and average cell volume (μm^3) per cell in ballast water in 2007 and 2008. Analyses of Variances were used to determine overall differences in bacterial abundances and cell volumes among ballast water types each year. Tukey 95% Simultaneous Confidence Intervals (CI) were used to determine the differences among categories.

Year	Ballast Water Types[1]	N	Bacterial Abundance					Cell Volume				
			Minimum	Maximum	Mean	95% CI LL	95% CI UL	Minimum	Maximum	Mean	95% CI LL	95% CI UL
2007	TOE	14	0.25	1.88	0.61^{b}	0.32	0.89	0.064	0.102	0.079^{b}	0.072	0.087
	ICE	8	0.28	1.19	0.67^{ab}	0.46	0.88	0.069	0.120	0.097^{a}	0.084	0.110
	NonMOE	6	0.74	2.09	1.15^{a}	0.47	1.82	0.061	0.106	0.077^{ab}	0.053	0.101
	Port	3	1.72	2.73	2.31	1.00	3.62	0.078	0.093	0.087	0.068	0.106
2008	TOE	11	0.37	0.80	0.60^{b}	0.51	0.68	0.051	0.074	0.059^{a}	0.054	0.064
	ICE	14	0.34	1.62	0.84^{ab}	0.68	1.00	0.041	0.066	0.054^{ab}	0.050	0.059
	NonMOE	16	0.55	1.61	0.92^{a}	0.77	1.07	0.039	0.063	0.050^{b}	0.045	0.055
	Port	10	0.75	3.35	1.86	1.20	2.52	0.052	0.069	0.058	0.054	0.062

Notes:
[1]Trans-oceanic exchanged ballast water (TOE), intra-coastal exchanged ballast water (ICE), and without mid-ocean exchange ballast water (Non-MOE);
"a", "b", and "ab": Significant differences among mean values of ballast water types each year are indicated by superscript codes: mean with superscript "a" has significantly larger value than that with superscipt "b"; mean with superscript "ab" indicates there is no significant difference between "a" and "ab", or between "b" and "ab" superscript codes; means with same superscript are not significantly different;
"LL" and "UL" are lower limit and upper limit of 95% Confidence Intervals (95% CI).

Table 2. Representative summary reviews of published studies about bacterial abundance in ballast water.

Sampling	Bacterial Abundance	Influential factors	Notes	References
End of voyage: 62 samples from 28 ships along US coasts	mean ± S.D.: 3.1 ± 0.5×10^8 cells L^{-1}	Bacterial abundance was unrelated to vessel type, exchange status, age of water, environmental conditions measured (pH, DO, turbidity, nutrients), or phytoplankton abundance.	Bacterial abundance was significantly lower in ballast tanks with Atlantic than Pacific Ocean source water	Burkholder et al. 2007
End of voyage: vessels arriving to Chesapeake Bay from foreign ports.	mean ± S.D.: 8.3 ± 1.7×10^8 cells L^{-1}			Ruiz et al. 2000
End of voyage: 25 bulk carriers originating in foreign ports and arriving in Chesapeake Bay	5.7×10^7 – 2.0×10^9 cells L^{-1}, except for one sample with abundance 15×10^9 cells L^{-1}	Bacterial abundance was uncorrelated with temperature, and water age, but negatively correlated with salinity		Drake et al. 2001
During voyage: vessel from Hadera, Israel to Baltimore USA on a 19 - day voyage.	9.2 - 22×10^7 cells L^{-1}	Bacterial abundance decreased by a factor of 2.3 (unexchanged tanks) and 1.6 (exchange tanks) throughout the voyage. There was no difference in bacterial abundance between exchanged tanks and unexchanged tank at the end of voyage		Drake et al. 2002
End of voyage: ships arrived at Singapore Harbor	2.35×10^9 - 5.87×10^{10} cells L^{-1}		Bacterial abundance in ballast water was lower than that in local seawater	Joachimsthal et al. 2004
End of voyage: 69 vessels arriving at lower Chesapeake Bay from foreign and domestic ports	No concentration reported	No bacterial abundance difference between exchanged and unexchanged tanks	Bacteria discharged from vessels and surviving in the Port is 3.9×10^{18} cells per year.	Drake et al. 2007

Cell volume

Cell size and biovolume are used to estimate the contribution of microbes to total biomass and biogeochemical cycling. It is also useful as a proxy for the availability of nutrients in the environment (i.e., nutrient-replete bacteria are typically larger than nutrient-limited cells; Ducklow and Carlson 1992). Moreover, the size distribution of bacterial communities is also impacted by microzooplankton grazing (Hahn and Höfle 2001). In this study, the average bacterial cell volume in all ballast waters did not differ from those of receiving port water during either 2007 ($F_{1,29}$ = 0.09, P = 0.763) or 2008 ($F_{1,49}$ = 1.74, P = 0.193). However for all ballast water types, and for port water, bacterial cell volumes were significantly ($p<0.001$) larger during 2007 (0.060 to 0.120 μm^3, mean = 0.088) than 2008 (0.039 to 0.074 μm^3, mean = 0.055) (Table 1). There were also differences among ballast water types (Table 1). During 2007, bacterial cell volume was significantly larger in ICE than TOE ballast water ($F_{1,20}$ = 8.36, P = 0.009), but not between TOE and NonMOE, or between ICE and NonMOE sample categories. During 2008, bacterial cell volumes were significantly larger in TOE than NonMOE ballast water samples ($F_{1,25}$ = 8.48, P = 0.007), but there was no significant difference between the TOE and ICE or between the ICE and NonMOE categories. Since the cell volume measurements were made by the same analyst and the operations were semi-automated, the observed differences were not likely due to observer bias or analytical variability. In this study, the smaller average cell volume of the 2008 samples suggests that bacterial communities in the ballast tanks could have experienced adverse conditions (i.e. nutrient stress) or intensive grazing pressure in transit. In contrast, the larger cell-size of the bacteria during 2007 samples suggests conditions that supported higher cell activity due to replete nutrient supply and less grazing pressure.

Physiochemical factors, ballast water age and bacterial variables

Physiochemical factors (i.e., pH, salinity, and temperature; data not shown) were uniform with depth within the ballast tank; therefore, we present a ballast tank average value for each parameter. The ballast water pH ranged from 6.0 to 8.0. Salinity showed distinct characteristics for oceanic (TOE and ICE) and coastal (port and NonMOE) samples. Ballast water of oceanic origin had higher and narrower range of salinities compared with coastal sources samples (Table 3). Temperature of port, TOE, ICE, NonMOE samples all followed the same monthly patterns during the two sampling seasons (April to November in 2007; May to October in 2008). The average temperature started to increase from 10.4 °C in April and reached a plateau between June and August with average 17.6°C before decreasing.

Table 3. Salinity (psu) of each sample type.

Year	Sample Types	Salinity		
		Minimum	Maximum	Mean
2007	TOE	24.7	35.7	32.5
	ICE	31.2	35.2	33.1
	NonMOE	0.0	29.6	15.7
	Port	22.0	29.0	24.3
2008	TOE	32.2	33.6	32.7
	ICE	26.8	33.0	31.4
	NonMOE	0.0	29.6	9.2
	Port	18.7	26.9	22.4

The relationships among biological variables (bacterial abundance and cell volume), physiochemical factors and ballast water age were assessed by multiple regression analyses. Predictor variables (i.e., ballast water age, salinity, pH and temperature) were continuous, except for the salinity of NonMOE samples (NonMOE salanity showed a two-peak frequency distribution and was categorized into brackish (<20 psu) and saline (≥20 psu)). If there was significant difference between years, the bacterial abundance of samples from both years was analyzed together whereas cell volume was analyzed separately for 2007 and 2008. There were no significant relationships between biological variables and salinity, pH, temperature and ballast water age, except that cell volume was significantly larger in saline than brackish NonMOE samples for both years (2007: $F_{1,4}$ = 18.30, P = 0.013; 2008: $F_{1,14}$ = 12.52, P = 0.003).

In both this and previous studies, the end-of-voyage bacterial abundance did not show a significant relationship with ballast water age (Burkholder et al. 2007; Drake et al. 2001). However, during a 19-day voyage bacterial abundance decreased with increasing ballast

water age for both exchanged and unexchanged ballast water (Drake et al. 2002). Inverse relationships between ballast water age and species richness and biomass have been reported for fish (Wonham et al. 2000), zooplankton (Gollasch et al. 2000), and phytoplankton (Burkholder et al. 2007). The end-of-voyage bacterial communities are influenced by ballast water sources, the ballast water operations, and a suite of abiotic and biotic factors in ballast water tanks. The lack of relationship between measured biological parameters and physiochemical variables precludes predicting the end-of-voyage bacterial abundance (or cell volume) from simple abiotic predictor variables. In-transit studies are required to assess response patterns and quantify the factors controlling bacterial community dynamics in ballast water. In a recent study, bacterial dynamics and related environmental parameters in ballast water were characterized during a 24-day trans-Pacific voyage (Seiden et al. 2010). That study reported that for tanks that were unexchanged as well as exchanged in mid-ocean, bacterial abundance increased during the 7 to 10 days of voyage and this followed by a decline to near initial abundances. In addition, that study also reported a positive relationship between bacterial abundance and temperatures, and an inverse relationship between abundance and dissolved oxygen concentrations during voyage.

Conclusions

Although lower bacterial abundance in ballast than in port was observed in this study, there is insufficient information to assess the impact on biodiversity or ecosystem function or services. By extrapolating the measured average bacterial abundance in ballast water and the volume of ballast water introduced annually to Canada (Lo 2009), we can calculate that 3.1×10^{19} prokaryotic cells are transported into Canadian ports annually. Moreover, ballast water that is not exchanged in offshore areas can introduce more potentially invasive bacteria, proportionally greater propagule pressure, than ballast water that was exchanged in oceanic regions at least 200 nautical miles offshore. The lack of relationships between measured physiochemical and bacterial variables in all three ballast water types suggests that there is a complex control mechanism making it difficult to predict the bacterial abundance or cell volume from the physiochemical factors alone.

Before a species can successfully invade a new environment and become established, it must first overcome biogeographic, physiological and biotic barriers. (MacIsaac et al. 2007). Our study described how bacteria may survive the biogeographical barrier by "hitching a ride" in ballast water. Further studies concerning the adaptation of introduced bacterial communities to new environmental conditions in receiving waters, as well as the interactions between the introduced bacterial community and native community are needed to understand how bacteria are capable of surviving these barriers. This analysis, in turn, may allow the development of predictive and intention tools.

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References

Anil AC, Venkat K, Sawant SS, DileepKumar M, Dhargalkar VK, Ramaiah N, Harkantra SN, Ansari ZA (2002) Marine bioinvasion: Concern for ecology and shipping. *Current Science* 83: 214-218

Burkholder JM, Hallegraeff GM, Melia G, Cohen A, Bowers HA, Oldach DW, Parrow MW, Sullivan MJ, Zimba PV, Allen EH, Kinder CA, Mallin MA (2007) Phytoplankton and bacterial assemblages in ballast water of US military ships as a function of port of origin, voyage time, and ocean exchange practices. *Harmful Algae* 6: 486-518, doi:10.1016/j.hal.2006.11.006

Carlton JT, Geller JB (1993) Ecological roulette: The global transport of nonindigenous marine organisms. *Science* 261: 78-82, doi:10.1126/science.261.5117.78

Culley AI, Welschmeyer NA (2002) The abundance, distribution, and correlation of viruses, phytoplankton, and prokaryotes along a Pacific Ocean transect. *Limnology and Oceanography* 47: 1508-1513

Drake LA, Choi KH, Ruiz GM, Dobbs FC (2001) Global redistribution of bacterioplankton and virioplankton communities. *Biological Invasions* 3: 193-199, doi:10.1023/A:1014561102724

Drake JM, Lodge DM (2007) Rate of species introductions in the Great Lakes via ships' ballast water and sediments. *Canadian Journal of Fisheries and Aquatic Sciences* 64: 530-538, doi:10.1139/F07-029

Drake LA, Ruiz GM, Galil BS, Mullady TL, Friedmann DO, Dobbs FC (2002) Microbial ecology of ballast water during a transoceanic voyage and the effects of open-ocean exchange. *Marine Ecology Progress Series* 233: 13-20, doi:10.3354/meps233013

Ducklow HW, Carlson CA (1992) Oceanic bacterial productivity. *Advances in Microbial Ecology* 12: 113-181

Frenot Y, Chown SL, Whinam J, Selkirk PM, Convey P, Skotnicki M, Bergstrom D (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biological Reviews* 80: 45-72, doi:10.1017/S1464793104006542

Gollasch S, Lenz J, Dammer M, Andres HG (2000) Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. *Journal of Plankton Research* 22: 923-937, doi:10.1093/plankt/22.5.923

Hahn MW, Höfle MG (2001) Grazing of protozoa and its effect on populations of aquatic bactria. *FEMS Microbiology Ecology* 35: 113-121, doi:10.1111/j.1574-6941.2001.tb00794.x

Hobbie JE, Daley RJ, Jasper S (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology* 33: 1225-1228

International Maritime Organization (1997) Resolution A. 868 (20). http://globallast.imo.org/868%20english.pdf (Accessed 13 January 2010)

Joachimsthal EL, Ivanov V, Tay J, Tay ST (2003) Flow cytometry and conventional enumeration of microorganisms in ships' ballast water and marine samples. *Marine Pollution Bulletin* 46: 308-313, doi:10.1016/S0025-326X(02)00401-0

Joachimsthal EL, Ivanov V, Tay J, Tay ST (2004) Bacteriological examination of ballast water in Singapore Harbour by flow cytometry with FISH. *Marine Pollution Bulletin* 49: 334-343, doi:10.1016/j.marpolbul.2004.02.036

Juliano SA, Lounibos LP (2005) Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology Letters* 8: 558-574, doi:10.1111/j.1461-0248.2005.00755.x

Kirchman D, Sigda J, Kapuscinski R, Mitchell R (1982) Statistical analysis of the direct count method for enumerating bacteria. *Applied and Environmental Microbiology* 44: 376-382

Lo V (2009) Underwater aliens: Quantifying propagule pressure of aquatic invasive species in Canadian shipping ports. M.Sc. Thesis, University of British Columbia, British Columbia, Canada. https://circle.ubc.ca/bitstream/handle/2429/13696/ubc_2009_fall_lo_veronica.pdf?sequence=1 (Accessed 13 January 2010)

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution* 20: 223-228, doi:10.1016/j.tree.2005.02.004

Maclsaac HJ, Herborg LM, Muirhead J (2007) Modeling biological invasions of lakes. In: Gherardi F (ed), Freshwater Bioinvaders: Profiles, Distribution and Threats. Springer, Berlin. pp 347-368, doi:10.1007/978-1-4020-6029-8_18

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52: 273-288

Ruiz GM, Rawlings TK, Dobbs FC, Drake LA, Mullady T, Huq A, Colwell RR (2000) Global spread of microorganisms by ships. *Nature* 408: 49-50, doi:10.1038/35040695

Seiden JM, Way C, Rivkin RB (2010) Microbial hitchhikers: dynamics of bacterial populations in ballast water during a trans-Pacific voyage of a bulk carrier. *Aquatic Invasions* 5: 13-22, doi: 10.3391/ai.2010.5.1.3

Seber GAF, Lee AJ (2003) Linear regression analysis. John Wiley & Sons, New York.

Smith LD, Wonham MJ, McCann LD, Ruiz GM, Hine AH, Carlton JT (1999) Invasion pressure to a ballast-flooded estuary and an assessment of inoculant survival. *Biological Invasions* 1: 67-87, doi:10.1023/A:1010094527218

Sun B (2009) Abundance and composition of heterotrophic prokaryotes in ballast water discharged into Canadian Harbors. M.Sc. Thesis, Memorial University of Newfoundland, Newfoundland and Labrador, Canada.

Transport Canada (2006) Canadian Shipping Act. Section 657.1. http://laws.justice.gc.ca/en/SOR-2006-129/ (Accessed 03 August 2009)

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences* 95: 6578, doi:10.1073/pnas.95.12.6578

Wonham MJ, Carlton JT, Ruiz GM, Smith LD (2000) Fish and ships: Relating dispersal frequency to success in biological invasions. *Marine Biology* 136: 1111-1121, doi:10.1007/s002270000303