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Viewpoint

Biological controls on bacterial populations in ballast water during ocean transit

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ABSTRACT

Bacteria (and viruses) numerically dominate ballast water communities, but what controls their population dynamics during transit is largely unexplored. Here, bacterial abundance, net and intrinsic growth rates, and grazing mortality were determined during a trans-Atlantic voyage. The effects of grazing pressure by microzooplankton on heterotrophic bacteria during transit were determined for source port, mid-ocean exchange (MOE), and six-day-old source port ballast water. When the grazer component was removed, bacterial abundances significantly increased. Additionally, we determined that the grazer-mediated mortality for ballast water originating from ports was greater than MOE water and that mortality decreased over time for the source port ballast water. This study shows that bacterial populations in transit are controlled by microzooplankton grazing. If these findings are representative of ballast water environments, they suggest that if the grazing component is selectively removed by various treatment methods, bacterial populations may increase; this could have environmental and human health consequences.

1. Introduction

The discharge of ballast water into coastal areas and harbours is one of the main mechanisms for ship-mediated introductions of aquatic organisms (International Marine Organization (IMO), 2008). Ballast water is taken on-board at ports and stored in tanks to enhance the ship's stability and manoeuvrability during transit (Transport Canada, 2007). At the destination port, ballast water is discharged as cargo is taken on-board. Both oceanic shipping and transport have increased over the last several decades, and hence the global connection for species dispersal and the potential for aquatic species invasions has greatly increased (MacIsaac et al., 2002; Joachimsthal et al., 2004). Aquatic invasive species are a concern to human and environmental health and aquatic biodiversity (Ruiz and Reid, 2007; Transport Canada, 2007; IMO, 2008; Rilov and Crooks, 2009).

Mid-ocean exchange (MOE) of ballast water is used to control the introduction of aquatic invasive species. MOE is a process where ships exchange coastal ballast water for oceanic water at least 200 nautical miles offshore and where ocean depths are at least 2000 m (Transport Canada 2007; IMO, 2008). When MOE is conducted in compliance with IMO guidelines, it is supposed to be effective at reducing the initial concentration of some planktonic organisms by up to 80–95% (Ruiz and Reid, 2007).

Recent studies have reported the presence of heterotrophic microbes, such as bacteria and viruses, in ballast water (Ruiz et al., 2000; Drake et al., 2002; Burkholder et al., 2007; Ma et al., 2009; Seiden et al., 2010; Sun et al., 2010; Leichsenring and Lawrence, 2011; Altug et al., 2012). Heterotrophic prokaryotes numerically dominate ballast water biota and under some circumstances can be a potential environmental and human health risk (Rigby et al., 1999; Burkholder et al., 2007; Altug et al., 2012). For example, pathogenic bacteria such as *Vibrio cholerae* and *Escherichia coli* can be transported in ballast water and be introduced into previously uncontaminated areas. Additionally, bacteria have characteristics that optimize successful establishment in new environments. They are capable of enduring the harsh conditions in ballast tanks as spores or other resting stages, and because of their high intrinsic growth rates, bacteria can potentially establish high population levels (Ruiz et al., 2000; Gregg and Hallegraeff, 2007). Mid-ocean exchange may not be effective in reducing the total number of bacteria in ballast water since open-ocean bacterial abundances are $0.5–1.0 \times 10^9$ cells/L, which is quantitatively similar to that in coastal and other near shore regions (Ducklow, 2000). The propagule pressure of heterotrophic microbes, in particular bacteria and viruses, is orders of magnitude higher than other organisms (Ruiz et al., 2000; Drake et al., 2002, 2007; Quilez-Badia et al., 2007).

Studies of microorganisms in ballast water have generally determined their abundances or standing stocks at the deballasting locations (i.e., Burkholder et al., 2007; Sun et al., 2010; Altug et al.,

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2012). Relatively few studies have monitored the changes that occur en-route (Gollasch et al., 2000a, 2000b; Drake et al., 2002; Mimura et al., 2005; Klein et al., 2010; Seiden et al., 2010, 2011; Tomaru et al., 2010). The abundance (and community structure) at the time and place of deballasting reflects a balance between bottom up (i.e., bacterial growth depends on nutrient availability, temperature, dissolved oxygen, and salinity) and top down control (i.e., mortality; grazing pressure by microzooplankton and viral lysis) (Thingstad, 2000; Cuevas and Morales, 2006; Ram and Sime-Ngando, 2008) during transit. Thus, better predictions of the magnitude and nature of the ballast-water-caused propagule pressure will require a more complete understanding of the factors that influence the microbial population during transit.

Seiden et al. (2010, 2011) assessed the temporal pattern of change in heterotrophic bacterial abundances and the environmental factors that regulate these changes in ballast tanks that both underwent MOE and those that remain unexchanged during transit during four voyages in the Pacific and Atlantic. They found that there was a significant and positive relationship between bacterial abundances and temperature and a significant and inverse relationship between bacterial abundance and dissolved oxygen concentrations. However, during these studies, biological controls (i.e., bacteria mortality due to grazing and viral lysis) were not separated from the direct or indirect physical factors. Here, the biological control (i.e., microzooplankton grazing) of ballast water bacteria populations was assessed during controlled manipulation experiments for samples collected from ballast tanks during a trans-Atlantic voyage.

2. Materials and methods

2.1. Vessel

To assess bacterial grazing mortality due to grazing, dilution experiments were conducted on-board the "*M/V Eva N*" (bulk carrier; 305 m long; 107 512 gross tons) during a 10-day trans-Atlantic voyage (15–24 October 2008). The ballast water was sampled daily for bacterial abundances and related properties throughout the voyage from Rotterdam, The Netherlands, to Sept-Iles, Quebec, Canada (Fig. 1). Details about the ballast tank bacterial dynamics during transit are reported in Seiden et al. (2011).

2.2. Ballast water sample collection

Water for the dilution experiments to characterize microzooplankton-mediated bacterial mortality was collected from ballast tanks 4P, which was scheduled to undergo MOE (designated MOE tank; Table 1), and 5P, which was not scheduled to undergo MOE (designated unexchanged tank; Table 1). A 5-L Niskin bottle was deployed into the ballast tank through a deck hatch and used to collect water at three depths (0, 10, and 20 m) and the samples were then pooled into 15-L carboys that were pre-rinsed in 2% HCl and distilled water. This pooled sample was used as the source water for the dilution experiments (see experimental design) and for analyses of initial bacterial properties from the ballast tanks (reported in Seiden et al., 2011). For each ballast tank, samples for bacterial enumeration were normally collected daily (except for when severe weather conditions prevented sampling).

2.3. Experimental design

Grazing mortality of microbial prey, including bacteria, is typically quantified using dilution assays (Landry and Hassett, 1982; Rivkin et al., 1999). As described in Rivkin et al. (1999), a seawater sample is combined with particle-free seawater to obtain a diluted sample at a gradient of seawater: particle-free seawater ratios (from 100% seawater and 0% particle-free seawater to 10% seawater and 90% particle-free seawater). Each dilution provides an independent estimate of the apparent growth rate (AGR) of bacteria at different grazer densities. The dilution technique assumes that growth rate is density independent and grazer-mediated mortality is density dependant (Landry and Hassett, 1982) and grazing pressure is linearly proportional to the dilution factor. This assumption has been repeatedly tested for heterotrophic and autotrophic picoplanktonic prey and has been shown to be robust (Ducklow and Hill, 1985; Tremaine and Mills, 1987; Landry et al., 1995; Rivkin et al., 1999; Putland, 2000; Pearce et al., 2011 and reference cited therein). Apparent growth rate is calculated as follows:

$$\mathrm{AGR}\ (\mathrm{d}^{-1}) = \ln(P_t/P_o)/t \quad (1)$$

where t is the duration of the incubation and P_o and P_t are the initial and final bacterial abundances. The intrinsic rate of bacterial growth (μ, d^{-1}, growth in the absence of grazers) is the ordinal intercept of the regression of AGR vs. the dilution factor and microzooplankton-mediated grazing mortality of bacteria (g, d^{-1}) is the absolute value of the slope of this regression. Two 4-day dilution experiments were conducted to assess the effect of microzooplankton-mediated mortality on bacteria in the ballast tanks during the voyage. However, due to time and space constraints, the assay was performed using two dilutions as follows: an undiluted condition (representing the grazer-prey dynamic in the ballast tank; designated control); and a 5:1, a ratio of particle-free ballast water: undiluted ballast water (representing the grazer-free condition; designated grazer-free). Although some dilution assay experiments use several dilutions to approximate the rates of growth and grazer-mediated mortality of microbial prey, the experimental protocol used is substantially the same as that used to characterize grazer-free bacterial growth rates during seawater culture experiments to determine the relationship between bacterial growth and amino and nucleic acid uptake (Kirchman et al., 1982; Ducklow and Hill, 1985; Tremaine and Mills, 1987; Rivkin et al., 1996). We therefore reasonably assume that the 5:1 dilution of particle-free ballast water: undiluted ballast water was operationally grazer free and that the bacterial growth rates approximated the intrinsic growth rate (i.e., μ, d^{-1}: growth in the absence of grazing). The growth rate in the control incubation is the net growth rate (NGR; i.e., μ minus mortality; Eq. (2)) and the rate of grazing-mediated mortality (g, d^{-1}) can be determined by solving for g in Eq. (3):

$$\mathrm{NGR} = \mu - g. \quad (2)$$

Table 1
Tank designation and water used for experimental setup.

Tank	Designation	Water used for experimental setup	
		Experiment 1 (pre-MOE)	Experiment 2 (post-MOE)
4P	MOE tank (designated MOE1 in Seiden et al., 2011)	Source port ballast water	MOE ballast water
5P	Unexchanged tank (designated MOE2 in Seiden et al., 2011)	Source port ballast water	Six-day-old source port ballast water

Note: Experiment 1, 4 day incubations were carried out from 16 to 20 October 2008. Experiment 2, 4 day incubations were carried out from 20 to 24 October 2008. MOE, mid-ocean exchange.