REABIC

Review

Efficacy of open-ocean ballast water exchange: a review

Vanessa Molina[1],* and Lisa A. Drake[2]

[1]*Excet, Inc., Springfield, VA 2215, USA*

[2]*U.S. Naval Research Laboratory, Key West, FL 33040, USA*

Corresponding author

E-mail: vanessa.molina.ctr@nrl.navy.mil

Abstract

A literature search was conducted to summarize studies that quantified the efficacy of ballast water exchange. Here, a number of parameters were collated from 68 studies to provide a meta-analysis of the efficacy of exchange. The percent removal of ballast water, organisms, and various physical parameters was investigated to quantify exchange efficacy in terms of volumetric, biological, and water quality measurements, respectively. The two types of exchanges implemented—empty-refill and flow-through—demonstrated 66 to >99% efficiencies of volumetric exchange when this factor was measured using rhodamine dye as a tracer. Of the reports and papers reviewed, zooplankton were the most common biological parameter studied, and nearly all data showed a decrease in concentrations following exchange, although the changes in community structure varied. Additional biological parameters reported were the concentrations of protists, bacteria, and virus-like particles (VLPs). Results of protist studies were highly variable—ranging from a decrease in cell concentrations to an increase following exchange— while bacteria and VLPs concentrations showed no change in most cases. Of course, the diversity and physiological condition of organisms in ballast water can cause differences in responses that may lead to inconsistencies when determining if organisms were removed during exchange. Additionally, organisms present in sediment may also repopulate ballast water following exchange. In contrast to the biological results, some water quality parameters, such as chromophoric dissolved organic matter (CDOM) and trace elements (e.g., barium and manganese), were consistent indicators of exchange. In conclusion, although the volumetric efficacy of exchange showed, in most studies, that the majority of the water was removed from the tanks, biological and most water quality parameters did not show a consistent response to exchange.

Key words: invasive species, zooplankton, bacteria, protists, shipping, empty-refill

Introduction

The establishment of invasive species presumably transported in ships' ballast water has resulted in actions to minimize their transport and delivery. At a global scale, the International Maritime Organization (IMO) adopted the Ballast Water Management (BWM) Convention (IMO 2004). It includes the practice of conducting ballast water exchange with an efficiency of at least a 95% volumetric exchange; if flow-through exchange is performed, a 300% exchange is considered equivalent to the 95% standard. Exchange is to occur at least 200 nm from land and in water with a depth of at least 200 m (IMO 2004). Once the BWM Convention enters into force, however, the practice of exchange will be superseded by ships needing to meet a standard stipulating the number of living organisms allowable in discharged ballast water.

In the United States, several legislative and executive actions governing ballast water discharges were promulgated by the US Coast Guard (USCG) and the US Environmental Protection Agency (EPA) between 1990 and 2013 (e.g., US Coast Guard 2009; US Coast Guard 2012; EPA 2013), and they are codified in the Code of Federal Regulations (e.g., US National Archives and Records Administration 2012). Initially, open ocean exchange was required, but now, a discharge standard is in place (although some ships will need to address both requirements, as discussed below). Both the IMO and US actions aim to greatly limit the number of living organisms discharged in ballast water and have essentially the same limits (Table 1).

In response, an industry for commercial ballast water management systems (BWMS) has developed in the decade following the adoption of the IMO BWM Convention. In many cases, the BWMS currently

Table 1. Ballast water discharge standards. [a]Nominally zooplankton. [b]Nominally protists. [c]Serotypes O1 and O139. cfu = colony forming unit, IMO = International Maritime Organization, US = United States, and zoopl. = zooplankton.

Organization and standard	Living organisms ≥50 µm in minimum dimension[a]	Living organisms ≥10 µm and <50 µm in minimum dimension[b]	Toxigenic *Vibrio cholerae*[c] (Pacini, 1854)	*Escherichia coli* (Migula, 1895)	Intestinal enterococci
US Discharge Standard	<10 m^{-3}	<10 mL^{-1}	<1 cfu 100 mL^{-1}	<250 cfu 100 mL^{-1}	<100 cfu 100 mL^{-1}
IMO Regulation D-2 Ballast Water Performance Standard	<10 m^{-3}	<10 mL^{-1}	<1 cfu 100 mL^{-1} or <1 cfu g^{-1} (wet weight zoopl.)	<250 cfu 100 mL^{-1}	<100 cfu 100 mL^{-1}

installed on vessels, or those in development, employ technical approaches commonly used in the drinking water or wastewater treatment industries. In general, a combination of approaches is used: physical separation (e.g., filtration) followed by a disinfection step (e.g., electrochlorination or UV radiation).

In the US, both the USCG and EPA are currently responsible for regulating ballast water discharges. Compared to the USCG ballast water regulations, the EPA Vessel General Permit (VGP) includes additional requirements for vessels entering the Great Lakes through the St. Lawrence Seawater System if they have (1) operated outside the Exclusive Economic Zone (EEZ, nominally 200 nm offshore) and >200 nm from any shore, and (2) taken on ballast water with a salinity of <18 psu within the previous 30 days (EPA 2013). If both of these qualifications are met, once a vessel is required to meet the numeric discharge standard, likely by treating the ballast water with a BWMS, it must also conduct ballast water exchange or saltwater flushing.

"Ballast water exchange" is defined in the VGP as an exchange in the mid-ocean (also known as the open-ocean), which may be conducted either by emptying and refilling tanks with as close to 100% of the water as can safely be done or by overflowing tanks with a volume of water equivalent to three times the volume of the tanks. "Saltwater flushing" is conducted by adding mid-ocean water to empty ballast water tanks so the residual water in the tank has a salinity ≥30 psu or is equal to the salinity where the additional water was added (EPA 2013). The exchange or flushing for vessels entering the Great Lakes must occur >200 nm from shore. For clarity, the term "exchange" will be used in this paper to signify either type of exchange, whether it is defined as ballast water exchange or salt water flushing. Likewise, the terminology in the original reports (invasive, non-indigenous, etc.) will be retained.

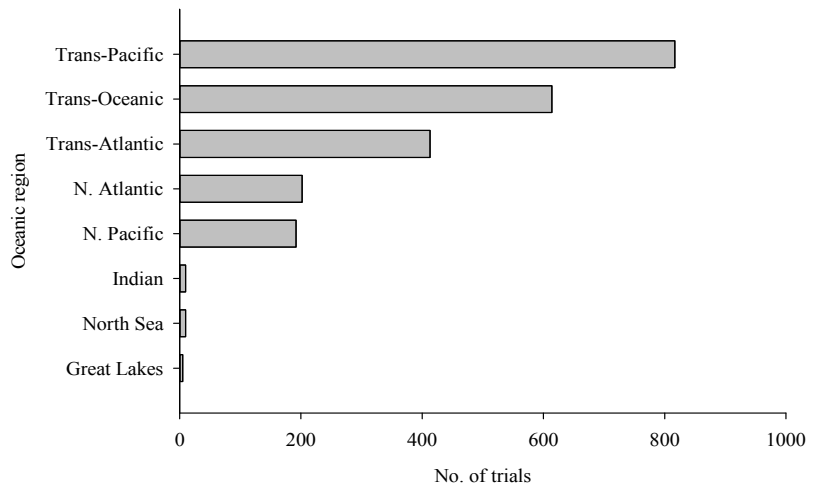The practice of combining exchange with a requirement to meet the discharge standard was codified in the VGP to provide an extra barrier to the arrival (and potential establishment) of invasive species into the Great Lakes. Given the effort this step will incur (in both labor and materials), as well as the potential safety risks of conducting exchange, the efficacy and practicability of conducting both exchange and ballast water treatment is being investigated. This paper reviews one of these practices—exchange—using published reports to develop a holistic understanding of the efficacy of exchange.

Types of exchange

Ships undergo exchange prior to arrival at a coastal or inland port to reduce the transfer of potentially invasive organisms between locations. Organisms, which tend to be found at lower densities in open-ocean waters than in coastal waters, are less likely to survive in lower salinity environments after discharge. Exchange not only physically removes organisms from ships, but the elevated salinity resulting from exchange can also cause a toxic "salinity shock" that can be fatal to organisms (e.g., Johengen et al. 2005; Santagata et al. 2008).

One type of ballast water exchange ships undergo is the empty-refill method, also referred to as the sequential method. This process requires the removal of all ballast water (except for the unpumpable residual water and sediment) followed by replacement with open-ocean water at sea. It can be 95 to 99% effective, as determined using rhodamine dye as a tracer (Ruiz et al. 2005; Murphy et al. 2006; Ruiz and Reid 2007). Although effectively exchanging the original water with open-ocean water, the empty-refill method can be hazardous, posing risks to the ship's stability, such as incurring hull shear forces, reducing visibility from the bridge, reducing propeller immersion, and weakening dynamic loads (e.g., Karaminas 2000; Endresen et al. 2004).

Another type of exchange is the flow-through method. This procedure requires a tank to remain full

Figure 1. Number of trials conducted within each oceanic region. The total number of trials is larger than the total studies reviewed (68) because some individual studies described multiple trials.

while ballast water is replaced, overflowing the tank on deck. Flow-through exchange is typically performed with 100% or 300% water replacement (i.e., the equivalent volume of one or three tanks, respectively). In the literature, the volumetric efficacy of this type of exchange, as determined using rhodamine, has been reported as 30 to 94% for 100% flow-through (Ruiz et al. 2005) and 75 to 99% for 300% flow-through (Ruiz et al. 2005; Murphy et al. 2006; Ruiz and Reid 2007).

Aside from the efficacy, it is important for individual ships to assess the risk factors associated with the type of exchange performed. The safety of exchange is paramount, as exchange may not only endanger the integrity and stability of a ship, but further may also be impractical during inclement weather and unsteady sea surface conditions. It is in each ship's best interest to perform the exchange method that is least likely to incur unnecessary risk, while obtaining a complete, efficient exchange. Although little information has been reported on the cost per ship for exchange, irrespective of the type of exchange implemented, exchange can incur economic costs and the possibility of requiring several days to execute (Hay and Tanis 1998; Endresen et al. 2004). The additional fuel and ballast system upkeep required to complete exchange can be a concern.

Methods

A review was conducted to investigate parameters used in previous studies to quantify the efficacy of exchange. It was carried out using a key word search, and from the results, a total of 68 studies were reviewed, which encompassed 62 peer-reviewed journal articles, 5 reports, and 1 memorandum. The studies were published over a period of 26 years, from 1989–2015.

Trials and regions

Of the studies that provided information on the number of independent trials conducted, 1 to 546 trials were reported per study (median = 6). Within this range, 60% of the studies reported data from 10 or less trials. The low replication is surely due to ship availability and the constraints of collecting samples from vessels of opportunity. Ship routes covered the globe, with the most vessels steaming in the Pacific, Atlantic, and Trans-Oceanic regions (Figure 1). The most common start- and end-points were the East and West Coasts of Canada, the northeast US, and Japan.

Range of analysis

Within the literature, numerous types of analyses were performed to evaluate the efficiency of exchange (Table 2), and they can be grouped into three types of tracers: chemical, biological, and water quality. The bulk of the analyses focused, by far, on biological metrics, particularly microscopy counts using white light or epifluorescence. Molecular techniques (e.g., polymerase chain reaction, PCR) tended to be used in recent studies.

Results and discussion

Voyage duration

Water age—the length of time that ballast water remains in ballast tanks—plays a pivotal role in the survival of organisms. Of the studies reviewed, organism concentrations and species richness were

Table 2. Types of tracers, analyses, and measurements performed within studies. [a]DGGE, Denaturing Gradient Gel Electrophoresis; [b]PCR, Polymerase Chain Reaction; and [c]qPCR, quantitative real-time Polymerase Chain Reaction.

Tracer	Analysis	Measurement	Authors
Chemical	Fluorescence	Rhodamine dye concentration	Taylor and Bruce 2000; Drake et al. 2002; Murphy et al. 2006; Taylor et al. 2007
Biological	Bulk lipid	Microbial biomass	Drake et al. 2002
	DGGE[a]	Organism characterization	Tomaru et al. 2010; 2014; Steichen et al. 2014
	DNA sequencing	Targeted organism genome presence	Doblin et al. 2004; Mimura et al. 2005; Burkholder et al. 2007; Briski et al. 2014; Steichen et al. 2014
	Epifluorescence microscopy counts	Organism concentration	Drake et al. 2002; Sun et al. 2010; Leichsenring and Lawrence 2011; Villac et al. 2013; Tomaru et al. 2014; Briski et al. 2014; Seiden and Rivkin 2014
	Flow cytometry	Organism concentration	Drake et al. 2002; Burkholder et al. 2007
	Fluorescence	DNA detection (with DNA-specific probes) Chl *a* and phaeopigment concentration	Drake et al. 2002; Johengen et al. 2005; Leichsenring and Lawrence 2011
	Grazing	Bacterial mortality	Seiden and Rivkin 2014
	Hatching experiments	Diapausing egg survivability	Bailey et al. 2004; Bailey et al. 2006; Gray et al. 2005, 2007; Briski et al. 2010; Gray and MacIsaac 2010
	Immunofluorescence	Bacterial identification and concentration	Anderson et al. 1989; Ruiz et al. 2000; Baier et al. 2014
	Live/dead scores	Visual enumeration	Beeton et al. 1998; Bruijs et al. 2001; Wonham et al. 2001
	PCR[b]	Targeted gene identification	Doblin et al. 2004; Mimura et al. 2005; Burkholder et al. 2007; Briski et al. 2012, 2014
	Plate counts	Microbial concentration	Mimura et al. 2005; Tomaru et al. 2014
	qPCR[c]	Organism concentration	Doblin et al. 2004; Burkholder et al. 2007
	Scanning electron microscopy	Species identification	Dickman and Zhang 1999; Zhang and Dickman 1999; Burkholder et al. 2007; Villac et al. 2013
	White light microscopy counts	Organism concentration	Locke et al. 1993; Smith et al. 1996; Zhang and Dickman 1999; Dickman and Zhang 1999; Olenin et al. 2000; Taylor and Bruce 2000; Gollasch et al. 2000a; Gollasch et al. 2000b; Bailey et al. 2004; Ruiz et al. 2005; Choi et al. 2005; Minton et al. 2005; Gray et al. 2007; Burkholder et al. 2007; McCollin et al. 2007, 2008; Taylor et al. 2007; Santagata et al. 2008; Simard et al. 2011; Dibacco et al. 2012; Villac et al. 2013; Briski et al. 2014; Briski et al. 2015; Cordell et al. 2015
WaterQuality	Excitation emission-excitation matrix spectrometry	Dissolved organic matter concentration	Taylor and Bruce 2000; Murphy et al. 2004; Murphy et al. 2006
	Gravimetric analysis	Total suspended solids concentration	Burkholder et al. 2007
	High temperature non-dispersive infrared-combustion techniques	Total organic carbon concentration	Burkholder et al. 2007
	ICP-MS	Trace element concentration	Murphy et al. 2004
	Salinity	Salinity effects	Anderson et al. 1989; Cosper et al. 1989; Smith et al. 1996; Beeton et al. 1998; Bailey et al. 2004; Ellis and MacIsaac 2009; Seiden et al. 2010; Sun et al. 2010
	Temperature	Temperature effects	Hoch and Kirchman 1993; Smith et al. 1996; Drake et al. 2002; Bailey et al. 2004; Seiden et al. 2010; Sun et al. 2010

negatively correlated with ballast water age (Smith et al. 1996; Johengen et al. 2005; Burkholder et al. 2007). Bacterial concentrations have also been reported to decrease with voyage duration (Drake et al. 2002), although it has also been reported that bacterial concentrations were unrelated to ballast water age (Burkholder et al. 2007). It has been estimated that a ballast water age of >30 days would likely meet the IMO and US discharge standard for living organisms ≥50 µm in minimum dimension (nominally zooplankton, <10 living organisms m^{-3}) (Cordell et al. 2015). Although increasing water age has been related to a decrease in concentrations of organisms and species richness, it is important to take into account the origin of ballast water and its concomitant community. Original organism densities, salinity and temperature changes throughout voyages, absence of light or predators in ballast water tanks, resilience (or not) of the species, and changes in food chain dynamics may affect organisms within aged ballast water (e.g., Drake et al. 2002; Burkholder et al. 2007; Cordell et al. 2015).

Volumetric ballast exchange efficacy

To determine the volumetric efficacy of ballast water exchange, a chemical tracer, commonly rhodamine dye, is often used. Using rhodamine, empty-refill and flow-through exchange have been reported to result in a final dilution of 95% of the original water, although a 300% flow-through exchange may be necessary to fulfill the standard using flow-through exchange (Murphy et al. 2004; Tsolaki and Diamadopoulos 2010; Simard et al. 2011). Of five studies using rhodamine dye, the efficacy of exchange had a range of 66 to >99% removal of original ballast tank water. Four reports within these studies indicated >95% removal of original water, and two reports indicated a ballast water exchange efficiency of 66% and 88% (Taylor and Bruce 2000; Ruiz et al. 2005; Murphy et al. 2006; Ruiz and Reid 2007; Taylor et al. 2007).

Although all ballast water exchange efficiencies remain relatively high for all ships tested, as indicated by Ruiz et al. 2007, lower efficiencies can occur, and they may be due to the size and shape of ballast tanks. As shown by the aforementioned studies, using either empty-refill exchange or flow-through exchange, an efficiency meeting the discharge standard of 95% is achievable.

Biological efficacy

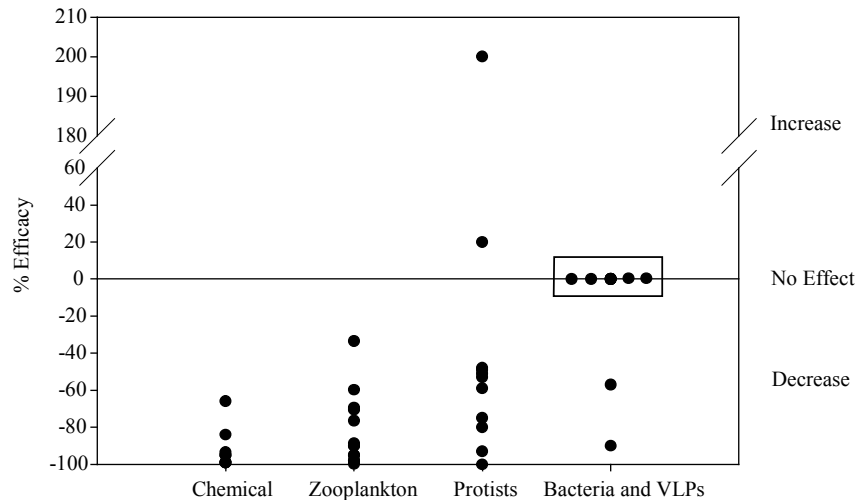Various measurements of biological organisms are used to measure open ocean exchange efficacy. For purposes of this review, biological profiling is divided among the three biological categories prescribed by the IMO Convention and US regulations (Table 1). While the category for the smallest organisms (<10 µm) pertains to three indicator and pathogenic bacteria by both the IMO and the US, for this review, it encompasses all bacteria and virus-like particles (VLPs; these particles are classified as such because they are virus-sized and contain nucleic acid, but they have not demonstrated the ability to infect a specific host [e.g., Hewson et al. 2001]).

It should be noted that sampling biases may lead to varying estimates of biota in ships' ballast water. Differences in sampling approaches, e.g., in equipment design, can enhance or weaken replicability over trials. Such approaches can include designs of relatively costly, semi-permanent sampling equipment to be installed and used over an extended period of time on the same ship (e.g., Drake et al. 2014) and also designs that are temporary, and less costly, which can be used for trials on various ships (Cangelosi et al. 2013). Different methods used to collect organisms will decrease consistency among studies, and they have been shown to result in different organism sampling efficacies (e.g., Gollasch 2003). Not only do varying collection practices introduce biases, but varying behavior of organisms can also contribute to the difficulties of sampling (Gollasch and Matej 2011). Thus, it is important to keep in mind that due to the lack of uniform sampling approaches for organisms in ballast water, it can be difficult to compare biological measurements across studies (Gollasch and Matej 2011). In parallel, the overall experimental design of studies may affect the evaluation of exchange efficiency. Because of this consideration, it is noteworthy that studies evaluating the efficacy of ballast water exchange within one vessel may have differing outcomes than studies in which comparisons are performed among various ships.

Zooplankton

Of the reports analyzed, total zooplankton concentrations were the most common biological parameter used to evaluate exchange efficacy. Within studies employing direct counts of zooplankters, 11 of 14 studies indicated a decrease in organism concentrations, with reductions ranging from 34 to 100% (Locke et al. 1993; Gollasch et al. 2000a; Taylor and Bruce 2000; Wonham et al. 2001; Ruiz et al. 2005; Minton et al. 2005; Gray et al. 2007; McCollin et al. 2008; Taylor et al. 2007; Simard et al. 2011; Briski et al. 2015). Although empty-refill exchange is thought to be a more effective method for reducing total zooplankton concentrations than flow-through

Figure 2. Percent efficacy (removal) of chemical and biological parameters using exchange; each symbol represents a study. For studies that indicated a range of removal, the median is shown. The black box encompasses five studies of bacteria and VLP concentrations with an efficacy of 0%. VLPs = virus-like particles.

exchange (Choi et al. 2005), an array of efficiencies has been reported for both types of exchange. While the range is notable, it is evident that at least to some degree, exchange reduces the delivery of zooplankton when the ballast water is eventually discharged (Figure 2).

Exchange efficiency has also been evaluated by examining zooplankton community structure, as substantial changes in taxonomic composition and diversity may be a useful indicator of exchange occurrence and efficiency (Hay and Tanis 1998). The community composition in ballast water arriving to the US West Coast illustrated this trend, with higher concentrations of non-indigenous zooplankton in ships that had not undergone exchange compared to ships that had undergone exchange (Dibacco et al. 2012). US East Coast arrivals also showed differences in community structure among ships that had and had not undergone exchange, but the differences were not attributed to the presence (or absence) of non-indigenous species (Dibacco et al. 2012). A decrease in total zooplankton taxa following exchange is noted in several studies: after an empty-refill exchange in the Indian Ocean on a voyage from Singapore to Germany (Gollasch et al. 2000a), in studies conducted by Olenin et al. (2000), and in freshwater zooplankton taxa, which showed a 67 to 86% reduction in concentrations (Locke et al. 1993).

Other studies have investigated the removal and changes in species diversity of targeted zooplankton indicator species. Varying, and sometimes contra-dicting, results have been reported between different types of exchange, and among ships, even within the same study. For example, zooplankton concentrations

in a container ship remained high in ballast tanks even after exchange, while in the same study, zooplankton taxa were reduced 90 to 100% in a chemical tanker (Taylor and Bruce 2000). Variability amongst species diversities and concentrations may be due to differing types of vessels, as well as tank configurations and size (Ruiz and Reid 2007; Seiden et al. 2010). In ballast tanks of an oil tanker, concentrations of zooplankton taxa decreased after exchange, and some taxa, including polychaete larvae and juvenile stages, were almost completely removed (Taylor et al. 2007). An additional study conducted with different tanks on the same ship reported concentrations of non-indigenous organisms were reduced an average of 70 to 98% for 300% flow-through and empty-refill methods, respectively (Ruiz et al. 2005). Zoo-plankton diversity on a collier decreased from 44 to 6 taxa following empty-refill exchange (Wonham et al. 2001). Finally, changes in zooplankton community composition can be attributed to differences in the salinity of the source port water in relation to the salinity following exchange, which is a function of the geographic location of the exchange site and the depth of water at the site (McCollin et al. 2008).

Residual sediment at the bottom of ballast tanks, which provides a habitat for organisms and their eggs to potentially persist, is an added complication in assessing the efficacy of organism removal following exchange. In addition to the possibility of adult organisms residing within these sediments, resilient cysts and eggs may persist between ballast events (Locke et al. 1993; Ruiz and Reid 2007; McCollin et al. 2007; Briski et al. 2010). Suspended

sediments in ballast water can settle to the bottom of ballast tanks and allow benthic organisms, including diapausing (resting) eggs of zooplankton, to accumulate (Bailey et al. 2004). These diapausing eggs represent a dormant life stage, allowing organisms to withstand adverse conditions and environmental changes.

Hatching responses of eggs can provide insight into the probability of organism transfer under unfavorable and highly changing environmental conditions, such as those encountered following exchange. Diapausing eggs isolated from ballast tank sediments and exposed to a range of salinities from 2 to 35 psu showed an inverse relationship between salinity and hatching, although hatching rates were species-dependent (Bailey et al. 2004; Bailey et al. 2006). Here, hatching rates *decreased* with increasing sediment pore water salinity for all species, most likely due to low salinity tolerance of freshwater species. On the other hand, hatching *increased* when eggs were introduced from a higher salinity to lower salinities, most effectively in 0 psu water (Bailey et al. 2004; Bailey et al. 2006). In accordance, diapausing eggs placed in incubation chambers within a ballast tank that underwent exchange had significantly lower hatching success than those placed in a control tank without exchange (Gray et al. 2007). This finding indicates that an increase in salinity from the initial freshwater port to the higher salinity seawater can reduce the hatching ability of zooplankton diapausing eggs. In contrast, egg viability was not affected when exposed to water with a salinity of 32 psu, and species richness was reduced in only 1 of 7 trials (Gray et al. 2005). Although these results present the possibility that exchange may decrease the likelihood of eggs hatching when they are released into high salinity open-ocean waters, eggs retained in sediment may remain viable until the sediment is released into lower-salinity locations where eggs can hatch, thus contributing to the spread of aquatic nuisance species.

Due to limitations inherent in shipboard studies, many reports compare zooplankton communities in ballast tanks before and after exchange without the use of a control tank. While results obtained from these studies provide useful information on the effect of exchange, comparisons of exchanged tanks to control tanks also provide pertinent information when evaluating changes in organism mortality and diversity as a function of exchange. Decreases in concentrations of organisms in tanks following exchange may not necessarily be attributed solely due to ballast exchange, but also to other parameters. That is, factors within the tanks themselves (e.g., temperature change, sediment accumulation, water age, or die-off of prey items) may contribute to changes in zooplankton concentrations and community structure (e.g., Taylor et al. 2007; Simard et al. 2011). High zooplankton survival rates were reported in tanks that did not undergo exchange and were filled with water from the Great Lakes (Gray et al. 2007). Conversely, another study found tanks not undergoing exchange showed a decline of zooplankton concentrations over the length of voyage (Gollasch et al. 2000b).

Protists

Another approach for measuring exchange efficiency is by measuring protist concentrations. In a trend previously described, higher concentrations of protists would be expected in ballast water originating from coastal sources, as opposed to open-ocean sources. This paradigm, in theory, would result in a decrease in protist concentrations following exchange. Such decreases have been reported in several studies, although increases in concentrations have also been noted (Figure 2). Mean concentrations of chlorophyll *a*, a bulk measurement of phytoplankton (which typically dominate the protist size class), decreased after exchange, yet 15 days after exchange, there was no significant difference in chlorophyll *a* concentrations between control and exchanged tanks during a voyage between Israel and the US (Drake et al. 2002). Chlorophyll *a* biomass was reported to have declined over the length of a voyage between the Netherlands and Canada (Simard et al. 2011), and also in exchanged tanks and control tanks throughout a single voyage, although concentrations were higher after exchange than in control tanks (Johengen et al. 2005).

On a related note, total phytoplankton concentrations were approximately 4-fold higher in exchanged ballast water taken up in coastal areas—ranging in distance from 3 to 222 km from the nearest shoreline—than in the open ocean (Burkholder et al. 2007). When median phytoplankton concentrations were compared by region, tanks with water from the Atlantic and Pacific Oceans were not significantly different from each other, yet they had significantly higher concentrations than water from the Indian Ocean (Burkholder et al. 2007). Lower concentrations in Indian Ocean water may have been due to longer distances between ports and the attendant sampling of protist communities (Burkholder et al. 2007). A 48% (Dickman and Zhang 1999) and 53% (Zhang and Dickman 1999) reduction in diatoms and dinoflagellates was identified after exchange in the northern Pacific Ocean. On a voyage from Japan to Australia, with increasing time after ballast exchange,

heterotrophic nanoflagellate concentrations increased while autotrophic nanoflagellates decreased (Tomaru et al. 2014). In this circumstance, it was hypothesized that death of autotrophic nanoflagellates, due to a lack of sunlight, provides a food source for hetero-trophs, resulting in an increase in concentrations of heterotrophic nanoflagellates (Tomaru et al. 2014). In addition, increases in protist concentrations may be a consequence of bacterivory, which has been reported to have decreased bacterial concentrations in estuaries by a mean of 84% (Iriarte et al. 2003). Differing outcomes in protist removal show a discrepancy in ballast water exchange as control of the concentration of protists.

Comparing types of exchange from voyages over two consecutive years, in 1999, flow-through exchange had greater removal of microplankton (phytoplankton and microzooplankton) than empty-refill exchange (80 and 59%, respectively) (Simard et al. 2011). On the other hand, in 2000, flow-through and empty-refill had nearly the same removal (49 and 51%, respectively) (Simard et al. 2011). When compared to control tanks, phytoplankton concentrations decreased 75% after empty-refill exchange in a containership compared to a 93% removal in commercial oil tankers (Ruiz and Reid 2007).

The effect on the protist community structure due to exchange has varied. A total of 21 culturable species of protists, including several potentially harmful species, were detected in un-exchanged ballast water from 13 ships surveyed after crossing the Atlantic Ocean into Galveston Bay (Steichen et al. 2014). The presence of these toxin-producing and bloom-forming protists provides additional evidence that unexchanged ballast water has the potential to transfer harmful organisms. In one study, a total of 100 phytoplankton species were identified with species richness ranging from 5 to 47 species per tank among exchanged ballast tanks, and the composition was dominated by chain-forming diatoms and dinoflagellates (Burkholder et al. 2007). There was a larger concentration of diatom and dinoflagellate species in ships undergoing exchange between Manzanillo, Mexico and Hong Kong, China, compared to those that did not undergo exchange (34 and 53 species, respectively) (Dickman and Zhang 1999). In another study, plankton density and diversity also increased after exchange; here, the ballast water was taken up in a harbor with salinity ranging from 36 to 39 psu, which is much closer to open-ocean salinity than most coastal waters (Wonham et al. 2001). In contrast, there was a decrease in species richness on a voyage between the Baltic Sea and the Atlantic Coast of Europe when tanks underwent exchange (Olenin et al. 2000).

Another study showed the number of microplankton (and zooplankton) taxa in both types of exchange decreased when compared to a control tank, although flow-through exchange had slightly greater removal efficiency when compared to empty-refill exchange for both voyages in 1999 (37 and 29%, respectively) and 2000 (40 and 34%, respectively) (Simard et al. 2011). Natural cell death and voyage duration may play an important role in organism densities. This further demonstrates the need for control and test tank comparisons for the evaluation of exchange efficiency.

Residual sediment in ballast tanks can provide a habitat for not only zooplankton and benthic micro-invertebrates, but it can also provide a seed bed for pelagic protist communities. Diatom concentrations and the number of taxa increased with an increasing amount of sediment in ballast tanks, and further investigation showed concentrations in the sediments may be orders of magnitude higher than overlying water (Villac et al. 2013). Viable *Aureococcus anophagefferens* cells, also known as brown tide, were detected in residual water in two ships that had undergone open-ocean exchange followed by freshwater exchange (Doblin et al. 2004). This is notable because the salinity in the tanks following freshwater exchange was ≤5 psu, and the *Aureococcus* species' preferred salinity range is 18 to 30 psu (Doblin et al. 2004; Anderson et al. 1989). Such broad tolerance of salinity may be a consequence of sediment buffering (Doblin et al. 2004). The presence of *A. anopha-gefferens* at salinities much lower than its preferred salinity range, and its detection in some—but not all—tanks surveyed within the study, suggests that exchange may not remove all problematic organisms. This proposal, in addition to the organism's mixo-trophic nature and capability to survive in darkness for long periods of time (Doblin et al. 2004), illustrates that exchange alone may not completely remove the possibility of translocation and introduction of nuisance protist species.

Bacteria and virus-like particles

Examining the efficacy of exchange as a means to remove bacteria and VLPs, variable outcomes have been reported. Studies have described both a reduction of bacteria and VLPs after exchange, as well as no difference in concentrations before and after exchange (Figure 2). For example, in ships sampled on the West Coast of Canada, bacteria concentrations decreased after exchange and were lower in exchanged than in un-exchanged ballast water (Sun et al. 2010). In another study, concentrations of total bacteria and VLPs both decreased after

exchange on a voyage from Japan to Australia (Tomaru et al. 2014). Further, microbial biomass concentrations decreased 1.8-fold over 15 days in exchanged tanks on a voyage from Israel to the US, but there were no significant differences in concentrations when comparing samples directly before and after exchange (Drake et al. 2002). In the same study, it is also noteworthy that there were no significant differences in concentrations between control and exchange tanks at the end of the voyage (on Day 15) (Drake et al. 2002). Similarly, concentrations of viable bacterial cells were not reduced as a result of exchange on a voyage from Japan to Qatar (Mimura et al. 2005), nor en route from Japan to the West Coast of Canada, in which bacterial densities initially decreased, yet were not significantly different when compared to control tanks at the end of the voyage (Seiden et al. 2010). Concentrations of VLPs were also not significantly different between exchanged and un-exchanged tanks during two trans-Pacific voyages, although a lack of detectable differences may have been a consequence of highly variable total VLP concentrations throughout both voyages (Leichsenring and Lawrence 2011). It has also been reported that bacterial concentrations in ballast tanks were similar (within one order of magnitude) among ballast tanks, and between coastal and open-ocean waters, indicating bacterial concentrations were unrelated to ballast exchange (Burkholder et al. 2007).

These conflicting reports indicate that bacteria and VLP concentrations may not be affected by ballast water exchange alone. A decrease in concentrations may not indicate exchange has removed bacteria and VLPs but instead may reflect lower concentrations of microorganisms in oceanic ballast water (following exchange) as opposed to coastal waters. For example, oceanic heterotrophic bacteria concentrations can range from 1×10^4 to 1×10^6 cells mL^{-1} in comparison to estuarine or coastal organism concentrations, which typically range from 1×10^6 to 5×10^6 cells mL^{-1} (e.g., as summarized in Tomaru et al. 2014). Oceanic and coastal bacterial concentrations that fall within the same range indicate that bacteria concentrations may not provide sufficient information regarding organism exchange efficacy. On the other hand, the microbial community may be refreshed with oceanic water compared to the community in un-exchanged tanks, which can undergo decay. Regardless, in addition to making direct counts of bacteria and VLPs, it is helpful to measure exchange efficacy by also evaluating microorganism composition and diversity. For example, denaturing gradient gel electrophoresis (DGGE) profiles of uncultured bacteria before and after exchange showed a change in community structure, indicating successful ballast water exchange, while DGGE profiles for culturable bacteria indicated no difference in community profiles (Tomaru et al. 2014).

Identifying pathogenic bacteria is important not only to meet the discharge standard, but also to prevent the introduction of invasive species to coastal locations. A study measuring the concentrations of pathogenic *Vibrio cholerae* O1 and O139 serotypes indicated both species were present on 93% of the ships tested (Ruiz et al. 2000). When investigating microbial species composition among exchanged ballast tanks, the highest concentrations of pathogenic bacteria found were in 37% (*Escherichia coli*), 26% (*Vibrio* spp.), and 6.5% (*Pseudomonas aeruginosa* [Schroeter, 1872]) of tanks (Burkholder et al. 2007). Detecting these species shows potentially harmful bacteria can be delivered to coastal waters even after exchange.

Another consideration when evaluating the organism removal efficacy of exchange is grazing on protists and bacteria by zooplankton and microzooplankton. Reports of low concentrations of zooplankton in ballast tanks may suggest little to no grazing on phytoplankton and microbial assemblages (Burkholder et al. 2007), yet it has also been reported that grazing in ballast tanks controls microorganism concentrations (Seiden and Rivkin 2014). Although exchange may remove zooplankton in some instances, microzooplankton concentrations may affect concentrations of microorganisms. As described in Drake et al. (2002), microzooplankton grazing could be a factor influencing bacteria and VLP densities, reducing bacteria concentrations (and concentrations of VLPs). In concert (or separately), the depletion of available organic matter by the bacterial community could result in a steady state of low microbial concentrations (Drake et al. 2002).

Water quality

Temperature

Regarding the relationship between the concentration of living organisms and temperature, different outcomes have been reported. While a positive relationship was found between temperature and bacterial concentrations (Seiden et al. 2010), in other studies, temperature had no correlation with bacterial concentrations (Sun et al. 2010) or zooplankton and phytoplankton densities (Smith et al. 1996). An increase in bacterial concentrations (or any poikilotherms, for that matter) in response to increasing temperature is not surprising, as it is a fundamental tenet of biology (e.g., Hoch and Kirchman 1993). Conversely, phytoplankton

concentrations do not necessarily correlate with temperature, as light and nutrients play large roles in primary productivity (e.g., Hoch and Kirchman 1993). By entering dormant stages, diapausing eggs and cyst-forming organisms are capable of resisting extreme temperature changes (Bailey et al. 2004).

Salinity

In general, the salinity of open-ocean water is higher and has a narrower range than that of water originating from coastal areas. Due to this salinity difference, it would be expected that many organisms inhabiting either open-ocean or coastal locations would not survive the salinity changes resulting from ballast exchange. Specifically, it has been reported that ballast water exchange can be more effective when water is discharged in freshwater ports, owing to osmotic shock (Bailey et al. 2011). In a modeling study, the efficacy of exchange when exchange occurred between freshwater ports was >99% due to a combination of physical ballast exchange and osmotic stress (Bailey et al. 2011).

Surprisingly, exchange has been shown to have various responses on living organisms in conjunction with salinity. While salinity has been reported to have a non-significant relationship to ballast water bacteria (Seiden et al. 2010; Sun et al. 2010), and a negative relationship to zooplankton and phytoplankton abundance (Smith et al. 1996), it has also been shown to have contradicting effects. Laboratory testing with two copepods (*Temora* sp. and a copepod in the family Corycaeidae) and an ostracod, all collected from a cargo hold with a salinity of 35 psu, were placed into water of varying salinity. The results showed 100% mortality of all organisms at 8 psu, and no significant difference in survival at 22 and 35 psu for the *Temora* sp. and the ostracod, which both had high survival rates (Beeton et al. 1998). In addition to having high survival rates, *Temora* sp. successfully reproduced at both 22 and 35 psu (Beeton et al. 1998). Conversely, copepods of the family Corycaeidae survived only at 22 psu (Beeton et al. 1998). In a separate study in which the living number of organisms was visually counted every 24 h after introduction to salinities ranging from 0 to 25 psu, concentrations of the euryhaline amphipod *Dikerogammarus villosus* tolerated salinities up to 20 psu (Bruijs et al. 2001). Additionally, in a study in which three cladocerans (*Bosmina coregoni*, *Bythotrephes longimanus*, and *Cercopagis pengoi*) were placed in incubation tanks with either a progressive exposure to a salinity of 4 psu to a salinity of 8 psu, or a direct exposure to water with a salinity of 30 psu, all resulted in 100%

mortality (Ellis and MacIsaac 2009). The viability of eggs placed in incubation chambers within a ballast tank that had undergone exchange was not significantly different when compared to eggs placed in control ballast tanks, once again indicating possible salinity tolerances (Gray and MacIsaac 2010). Finally, laboratory experiments simulating empty-refill and flow-through exchange using salinity exposures showed organisms originating from freshwater and oligohaline (0 to 2 psu) environments experience high mortality rates when exposed to 34 psu salinity (Santagata et al. 2008).

Laboratory exposures play an important role in organism behavior and tolerance, yet they may not tell the full story of organism physiology as it pertains to ballast water exchange. Indeed, shipboard testing may elucidate responses of ambient organisms that differ from those encountered in laboratory testing with cultured species. As described by Doblin et al. (2004), *Aureococcus anophagefferens* collected from ballast water had unexpected viability at salinities ≤5 psu, which is surprising because the microalgae show a salinity preference of >22 psu in laboratory studies (Anderson et al. 1989) and 18 to 32 psu in field studies (Cosper et al. 1989). A range of salinity tolerances may also contribute to the varying results encountered among different studies. Further, because most studies evaluate the direct effects of salinity on organisms, a lack of information on the responses of diapausing eggs and cysts may leave a void in the understanding of exchange efficiency (Bailey et al. 2004).

Other water quality parameters

The effect of dissolved oxygen on organisms is inconsistent across studies. Dissolved oxygen concentration increased following exchange, and in one of two voyages, was negatively correlated with VLP abundance (Leichsenring and Lawrence 2011). Likewise, an inverse relationship between bacterial concentration and dissolved oxygen concentrations was reported on a voyage from Japan to the West Coast of Canada (Seiden et al. 2010). In a separate study, there was no relationship between dissolved oxygen and phytoplankton or bacteria concentrations (Burkholder et al. 2007).

Chromophoric dissolved organic matter (CDOM), which has concentrations generally lower in open-ocean water than in coastal water (Chang and Wood 2004), is another parameter that can be used to measure the volumetric efficacy of exchange. Measurements of CDOM (determined by fluorescence) decreased in ballast tanks that had undergone exchange (Murphy et al. 2004). This result was different in

control tanks, which showed consistent concentrations or a slight increase in CDOM fluorescence over the duration of the voyage (Murphy et al. 2004). In an additional study of nine voyages, fluorescent signatures of two humic materials from exchanged tanks showed lower fluorescence than un-exchanged tanks (Murphy et al. 2006). Chromophoric dissolved organic matter was deemed a promising measurement to indicate exchange, showing signatures within ballast tanks that mirrored those of the surrounding coastal or oceanic waters (Murphy et al. 2009; Doblin et al. 2010). In addition, CDOM was reported to demonstrate the largest change in concentrations when compared to salinity and trace elements in port water versus ocean water (Doblin et al. 2010). In a study to examine CDOM as a potential tracer, it was reported that a single humic-like component in conjunction with salinity and absorption could be suitable as an indicator, and it would be suitable to determine open-ocean exchange (Li et al. 2010). These findings suggest that CDOM may be a useful proxy for determining exchange by comparing fluorescence profiles in tanks to those in open-ocean waters.

Trace elements have also been investigated as an indicator of exchange. In one study, the initial concentrations before exchange were high, and following exchange, they were lower or similar to concentrations found in the open ocean (Murphy et al. 2004; Murphy et al. 2008). Specifically, barium and manganese decreased in all four pairs of ballast tanks where exchange occurred (Murphy et al. 2004; Murphy et al. 2008; Murphy et al. 2009). Additional studies measuring trace elements may lead to more precise measurements of exchange and may even allow for ocean signatures that could determine the location in which exchange occurred.

Conclusions

The two types of exchanges implemented, empty-refill and flow-through, both generally showed an efficient volumetric exchange of ballast water. The survey of reports evaluating biological exchange efficacy (by means of organism detection and quantification), however, indicated that organisms were not consistently removed from tanks following exchange. Furthermore, the diversity and physiological state of organisms can cause differences in responses, providing a less-than-definitive solution for determining exchange efficiency.

Most environmental parameters investigated in this review were shown to be inconsistent when used as an indicator for exchange. That is, biological and water quality measurements indicated that effectiveness of exchange varied greatly. Water depth and

salinity differences between exchange sites may additionally influence organism concentrations within exchanged ballast tanks. Moreover, sampling methods surely influence the measurement of exchange efficacy. Because of varying results, it would be necessary to establish a standardized technique to ensure representative sampling and measurements.

Across studies, zooplankton were the most common biological parameter examined, and while most (11 of 14) studies showed a decrease in concentrations, varying responses in community structure were evident. Other biological measurements (of protists, bacteria, and VLPs) exhibited different outcomes—both in terms of concentrations and community structure—in response to exchange. Protists varied widely (both decreases and increases in concentrations of organisms were documented), while bacteria and VLP concentrations mostly (in 5 of 8 studies) showed no change in concentrations. On the other hand, CDOM and trace elements, such as barium and manganese, may prove to be useful indicators after further investigation. In conclusion, no biological, chemical, or water quality parameter alone provided a definitive indicator of exchange.

Acknowledgements

References

Anderson DM, Kulis DM, Cosper EM (1989) Immunofluorescent detection of the brown tide organism, *Aureococcus anophagefferens*. In: Cosper EM, Bricelj VM, Carpenter EJ (eds), Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms formerly lecture notes on coastal and estuarine studies. Springer-Verlag, New York, NY, pp 213–228

Baier RE, Forsberg RL, Meyer AE, Lundquist DC (2014) Ballast tank biofilms resist water exchange but distribute dominant species. *Management of Biological Invasions* 5: 241–244, http://dx.doi.org/10.3391/mbi.2014.5.3.07

Bailey SA, Deneau MG, Jean L, Wiley CJ, Leung B, MacIsaac HJ (2011) Evaluating efficacy of an environmental policy to prevent biological invasions. *Environmental Science and Technology* 45: 2554–2561, http://dx.doi.org/10.1021/es102655j

Bailey SA, Duggan IC, Van Overdijk CDA, Johengen TH, Reid DF, Macisaac HJ (2004) Salinity tolerance of diapausing eggs of freshwater zooplankton. *Freshwater Biology* 49: 286–295, http://dx.doi.org/10.1111/j.1365-2427.2004.01185.x

Bailey SA, Nandakumar K, MacIsaac HJ (2006) Does saltwater flushing reduce viability of diapausing eggs in ship ballast sediment? *Diversity and Distributions* 12: 328–335, http://dx.doi.org/10.1111/j.1366-9516.2006.00222.x

Beeton AM, Carlton JT, Holohan BA, Wheless GH, Valle-levinson A, Drake LA, Ruiz G, Mccann L, Walton W, Frese A, Fofonoff P, Godwin S, Toft J, Hartman L, Von Holle E (1998) Ballast exchange study: Consideration of back-up exchange zones and environmental effects of ballast exchange and ballast release. Report from the Cooperative Institute for Limnology and Ecosystem Research (CILER), 239 pp

Briski E, Bailey SA, Cristescu ME, MacIssac H (2010) Efficacy of "saltwater flushing" in protecting the Great Lakes from biological invasions by invertebrate eggs in ships' ballast sediment. *Freshwater Biology* 11: 2414–2424, http://dx.doi.org/10.1111/j.1365-2427.2010.02449.x

Briski E, Ghabooli S, Bailey SA, MacIsaac HJ (2012) Invasion risk posed by macroinvertebrates transported in ships' ballast tanks. *Biological Invasions* 14: 1843–1850, http://dx.doi.org/10.1007/s10530-012-0194-0

Briski E, Gollasch S, David M, Linley RD, Casas-Monroy O, Rajakaruna H, Bailey SA (2015) Combining ballast water exchange and treatment to maximize prevention of species introductions to freshwater ecosystems. *Environmental Science & Technology* 49: 9566–9573, http://dx.doi.org/10.1021/acs.est.5b01795

Briski E, Linley RD, Adams JK, Bailey SA (2014) Evaluating efficacy of a ballast water filtration system for reducing spread of aquatic species in freshwater ecosystems. *Management of Biological Invasions* 5: 245–253, http://dx.doi.org/10.3391/mbi.2014.5.3.08

Bruijs MCM, Kelleher B, van der Velde G, de Vaate AB (2001) Oxygen consumption, temperature and salinity tolerance of the invasive amphipod *Dikerogammarus villosus*: indicators of further dispersal via ballast water transport. *Archiv für Hydrobiologie* 152: 633–646, http://dx.doi.org/10.1127/archiv-hydrobiol/152/2001/633

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 U.S. military ships as a function of port of origin, voyage time, and ocean exchange practices. *Harmful Algae* 6: 486–518, http://dx.doi.org/10.1016/j.hal.2006.11.006

Cangelosi A, Mays N, Reid D, Knight I (2013) Shipboard sampling approaches and recommendations by the Great Lakes Ballast Technology Demonstration Project. 1st International Workshop on Guidelines and Standards for Ballast Water Sampling: Rio de Janeiro, Brazil, 7–11 April, 15 pp

Chang G, Wood AM (2004) Colored dissolved organic in the coastal ocean. *Oceanography* 17: 50–59, http://dx.doi.org/10.5670/oceanog.2004.47

Choi K, Kimmerer W, Smith G, Ruiz GM, Lion K (2005) Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. *Journal of Plankton Research* 27: 707–714, http://dx.doi.org/10.1093/plankt/fbi044

Cordell J, Kalata O, Pleus A, Newsom A, Strieck K, Gertsen G (2015) Effectiveness of ballast water exchange in protecting Puget Sound from invasive species. Phase 3 Final Report, University of Washington and Washington Department of Fish and Wildlife, 55 pp

Cosper EM, Dennison WC, Milligan A, Carpenter EJ, Lee C, Holzapfel J, Milanese LL (1989) An examination of the environmental factors important to initiating and sustaining "brown tide" blooms. In: Cosper EM, Bricelj VM, Carpenter EJ (eds), Novel phytoplankton blooms: causes and impacts of recurrent brown tides and other unusual blooms lecture notes on coastal and estuarine studies. Springer-Verlag, New York, NY, pp 317–336

Dibacco C, Humphrey DB, Nasmith LE, Levings CD (2012) Ballast water transport of non-indigenous zooplankton to Canadian ports. *ICES Journal of Marine Science* 69: 483–491, http://dx.doi.org/10.1093/icesjms/fsr133

Dickman M, Zhang F (1999) Mid-ocean exchange of container vessel ballast water. 2: Effects of vessel type in the transport of diatoms and dinoflagellates from Manzanillo, Mexico, to Hong Kong, China. *Marine Ecology Progress Series* 176: 253–262, http://dx.doi.org/10.3354/meps176253

Doblin MA, Murphy KR, Ruiz GM (2010) Thresholds for tracing ships' ballast water: an Australian case study. *Marine Ecology Progress Series* 408: 19–32, http://dx.doi.org/10.3354/meps08599

Doblin MA, Popels LC, Coyne KJ, Hutchins DA, Cary SC, Dobbs FC (2004) Transport of the harmful bloom alga *Aureococcus anophagefferens* by oceangoing ships and coastal boats. *Applied Environmental Microbiology* 70: 6495–6500, http://dx.doi.org/10.1128/AEM.70.11.6495-6500.2004

Drake LA, Ruiz GM, Galil BS, Mullady TI, 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, http://dx.doi.org/10.3354/meps233013

Drake LA, Moser CS, Robbins-Wamsley SH, Riley SC, Wier TP, Grant JF, Herring PR, First MR (2014) Validation trials of a Shipboard Filter Skid (p3SFS) demonstrate its utility for collecting zooplankton. *Marine Pollution Bulletin* 79: 77–86, http://dx.doi.org/10.1016/j.marpolbul.2013.12.044

Ellis S, MacIsaac HJ (2009) Salinity tolerance of Great Lakes invaders. *Freshwater Biology* 54: 77–89, http://dx.doi.org/10.1111/j.1365-2427.2008.02098.x

Endresen Ø, Lee Behrens H, Brynestad S, Bjørn Andersen A, Skjong R (2004) Challenges in global ballast water management. *Marine Pollution Bulletin* 48: 615–623, http://dx.doi.org/10.1016/j.marpolbul.2004.01.016

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

Gollasch S, Rosenthal H, Botnen H, Hamer J, Laing I, Leppäkoski E, Macdonald E, Minchin D, Nauke M, Olenin S, Utting S, Voigt M, Wallentinus I (2000b) Fluctuations of zooplankton taxa in ballast water during short-term and long-term ocean-going voyages. *International Review of Hydrobiology* 85: 597–606, http://dx.doi.org/10.1002/1522-2632(200011)85:5/6<597::AID-IROH597>3.0.CO;2-4

Gollasch S, Rosenthal H, Botnen H, Crncevic M, Gilbert M, Hamer J, Hülsmann N, Mauro C, McCann L, Minchin D, Öztürk B, Robertson M, Sutton C, Villac MC (2003) Species richness and invasion vectors: sampling techniques and biases. *Biological Invasions* 5: 365–377, http://doi:10.1023/B:BINV.0000005569.81791.25

Gollasch S, Matej D (2011) Sampling methodologies and approaches for ballast water management compliance monitoring. *Traffic and Environment* 33: 397–405

Gray DK, Bailey S, Duggan IC, MacIsaac HJ (2005) Viability of invertebrate diapausing eggs exposed to saltwater: implications for Great Lakes' ship ballast management. *Biological Invasions* 7: 531–539, http://dx.doi.org/10.1007/s10530-004-6347-z

Gray DK, Johengen TH, Reid DF, MacIsaac HJ (2007) Efficacy of open-ocean ballast water exchange as a means of preventing invertebrate invasions between freshwater ports. *Limnology and Oceanography* 52: 2386–2397, http://dx.doi.org/10.4319/lo.2007.52.6.2386

Gray DK, MacIsaac HJ (2010) Diapausing zooplankton eggs remain viable despite exposure to open-ocean ballast water exchange: evidence from in situ exposure experiments. *Canadian Journal of Fisheries and Aquatic Sciences* 67: 417–426, http://dx.doi.org/10.1139/F09-192

Hay CH, Tanis D (1998) Mid-ocean ballast water exchange: procedures, effectiveness, and verification for BAL9701 Examination of efficiency of ballast water exchange practices and degree of ship compliance with NZ ballast-water mandatory controls and voluntary guidelines. Cawthron Report No 468, 51 pp

Hewson I, O'Neil JM, Fuhrman JA, Dennison WC (2001) Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. *Limnology and Oceanography* 46: 1734–1746, http://dx.doi.org/10.4319/lo.2001.46.7.1734

Hoch M, Kirchman D (1993) Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. *Marine Ecology Progress Series* 98: 283–295, http://dx.doi.org/10.3354/meps098283

International Maritime Organization (IMO) (2004) International Convention for the Control and Management of Ships' Ballast Water and Sediments. Convention BWM/CONF/36

Iriarte A, Madariaga I, Revilla M, Sarobe A (2003) Short-term variability in microbial food web dynamics in a shallow tidal estuary. *Aquatic Microbial Ecology* 31: 145–161, http://dx.doi.org/10.3354/ame031145

Johengen T, Reid D, Fahnenstiel G, Dobbs F, Doblin M, Ruiz G, Jenkins P (2005) Assessment of transoceanic NOBOB vessels and low-salinity ballast water as vectors for non-indigenous species introductions to the great lakes. Final Report, 287 pp

Karaminas L (2000) An investigation of ballast water management methods with particular emphasis on the risks of the sequential method. Lloyd's Register of Shipping. UJNR/MFP 24th Joint Meeting, 24 pp

Leichsenring J, Lawrence J (2011) Effect of mid-oceanic ballast water exchange on virus-like particle abundance during two trans-Pacific voyages. *Marine Pollution Bulletin* 62: 1103–1108, http://dx.doi.org/10.1016/j.marpolbul.2011.01.034

Li C, Zhang Y, Guo W, Zhu Y, Xu J, Deng X (2010) Verification of Ballast Water Exchange for International Ships Anchored in Xiamen Port by CDOM Tracer. *Spectroscopy and Spectral Analysis* 30: 2541–2545

Locke A, Reid DM, van Leeuwen HC, Sprules WG, Carlton JT (1993) Ballast water exchange as a means of controlling dispersal of freshwater organisms by ships. *Canadian Journal of Fisheries and Aquatic Science* 50: 2086–2093, http://dx.doi.org/10.1139/f93-232

McCollin T, Shanks AM, Dunn J (2007) The efficiency of regional ballast water exchange: Changes in phytoplankton abundance and diversity. *Harmful Algae* 6: 531–546, http://dx.doi.org/10.1016/j.hal.2006.04.015

McCollin T, Shanks AM, Dunn J (2008) Changes in zooplankton abundance and diversity after ballast water exchange in regional seas. *Marine Pollution Bulletin* 56: 834–844, http://dx.doi.org/10.1016/j.marpolbul.2008.02.004

Mimura H, Katakura R, Ishida H (2005) Changes of microbial populations in a ship's ballast water and sediments on a voyage from Japan to Qatar. *Marine Pollution Bulletin* 50: 751–757, http://dx.doi.org/10.1016/j.marpolbul.2005.02.006

Minton MS, Verling E, Miller AW, Ruiz GM (2005) Reducing propagule supply and coastal invasions via ships: effects of emerging strategies. *Frontiers in Ecology and the Environment* 3: 304–308, http://dx.doi.org/10.1890/1540-9295(2005)003[0304:RPSACI]2.0.CO;2

Murphy K, Boehme J, Coble P, Cullen J, Field P, Moore W, Perry E, Sherrell R, Ruiz G (2004) Verification of mid-ocean ballast water exchange using naturally occurring coastal tracers. *Marine Pollution Bulletin* 48: 711–730, http://dx.doi.org/10.1016/j.marpolbul.2003.10.015

Murphy K, Boehme JR, Noble M, Smith G, Ruiz G (2009) Deducing ballast water sources in ships arriving in New Zealand from southeastern Australia. *Marine Ecology Progress Series* 390: 39–53, http://dx.doi.org/10.3354/meps08178

Murphy KR, Field Paul M, Waite D, Ruiz Gregory M (2008) Trace elements in ships' ballast water as tracers of mid-ocean exchange. *Science of the Total Environment* 393: 11–26, http://dx.doi.org/10.1016/j.scitotenv.2007.12.011

Murphy KR, Ruiz GM, Dunsmuir WTM, Waite TD (2006) Optimized parameters for fluorescence-based verification of ballast water exchange by ships. *Environmental Science & Technology* 40: 2357–2362, http://dx.doi.org/10.1021/es0519381

Olenin S, Gollasch S, Jonušas S, Rimkutè I (2000) En-route investigations of plankton in ballast water on a ship's voyage from the Baltic Sea to the open Atlantic coast of Europe. *International Review of Hydrobiology* 85: 577–596, http://dx.doi.org/10.1002/1522-2632(200011)85:5/6<577::AID-IROH577>3.0.CO;2-C

Ruiz GM, Murphy KR, Verling E, Smith G, Chaves S, Hines AH (2005) Ballast water exchange: efficacy of treating ships' ballast water to reduce marine species transfers and invasion success?

Smithsonian Environmental Research Center. Final Report 925.410.051110.BWE&NIS.doc, 17 pp

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, http://dx.doi.org/10.1038/35040695

Ruiz GM, Reid DF (2007) Current state of understanding about the effectiveness of ballast water exchange (BWE) in reducing aquatic nonindigenous species (ANS) introductions to the Great Lakes Basin and Chesapeake Bay, USA: synthesis and analysis of existing information. NOAA Technical Memorandum GLERL-142, 143 pp

Santagata S, Gasiunaite ZR, Verling E, Cordell JR, Eason K, Cohen JS, Bacela K, Quilez-Badia G, Johengen, TH, Reid DF, Ruiz, GM (2008) Effect of osmotic shock as a management strategy to reduce transfers of nonindigenous species among low-salinity ports by ships. *Aquatic Invasions* 3: 61–76, http://dx.doi.org/10.3391/ai.2008.3.1.10

Seiden JM, Rivkin RB (2014) Biological controls on bacterial populations in ballast water during ocean transit. *Marine Pollution Bulletin* 78: 7–14, http://dx.doi.org/10.1016/j.marpolbul.2013.09.003

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

Simard N, Plourde S, Gilbert M, Gollasch S (2011) Net efficacy of open ocean ballast water exchange on plankton communities. *Journal of Plankton Research* 33: 1378–1395, http://dx.doi.org/10.1093/plankt/fbr038

Smith DL, Wonham MJW, McCann LD, Reid DM, Carlton JT, Ruiz GM (1996) Shipping study II. Biological invasions by nonindigenous species in United States waters: quantifying the role of ballast water and sediments Parts I and II. Report No CG-D-02-97, 164 pp

Steichen JL, Schulze A, Brinkmeyer R, Quigg A (2014) All aboard! A biological survey of ballast water onboard vessels spanning the North Atlantic Ocean. *Marine Pollution Bulletin* 87: 201–210, http://dx.doi.org/10.1016/j.marpolbul.2014.07.058

Sun B, Mouland R, Way C, Rivkin R (2010) Redistribution of heterotrophic prokaryotes through ballast water: A case study from the west coast of Canada. *Aquatic Invasions* 5: 5–11, http://dx.doi.org/10.3391/ai.2010.5.1.2

Taylor MD, Bruce EJ (2000) Mid ocean ballast water exchange?: shipboard trials of methods for verifying efficiency. Cawthorn Report No 524, 64 pp

Taylor M, MacKenzie L, Dodgshun T, Hopkins G, de Zwart E, Hunt C (2007) Trans-Pacific shipboard trials on planktonic communities as indicators of open ocean ballast water exchange. *Marine Ecology Progress Series* 350: 41–54, http://dx.doi.org/10.3354/meps07016

Tomaru A, Kawachi M, Fukuyo Y (2010) Denaturing gradient gel electrophoresis shows that bacterial communities change with mid-ocean ballast water exchange. *Marine Pollution Bulletin* 60: 299–302, http://dx.doi.org/10.1016/j.marpolbul.2009.11.019

Tomaru A, Kawachi M, Demura M, Fukuyo Y (2014) Changes in microbial communities, including both uncultured and culturable bacteria, with mid-ocean ballast-water exchange during a voyage from Japan to Australia. *PLOS ONE* 9: e96274, http://dx.doi.org/10.1371/journal.pone.0096274

Tsolaki E, Diamadopoulos E (2010) Technologies for ballast water treatment: a review. *Journal of Chemical Technology and Biotechnology* 85: 19–32, http://dx.doi.org/10.1002/jctb.2276

US Coast Guard (2009) Standards for living organisms in ships' ballast water discharged in U.S. waters (Draft programmatic environmental impact statement, proposed rule and notice). *Federal Register* 74: 44631–44672

US Coast Guard (2012) Standards for living organisms in ships' ballast water discharged in U.S. waters (Final rule). *Federal Register* 77: 17254–17304

US Environmental Protection Agency (EPA) (2013) Vessel general permit for discharges incidental to the normal operation of vessels (VGP) authorization to discharge under the national pollutant discharge elimination system (NPDES). Washington, DC, 194 pp, http://www.epa.gov/npdes/pubs/vgp_permit2013.pdf

US National Archives and Records Administration (2012) Code of Federal Regulations. Title 46. Ballast Water Management Systems. Washington, DC

Villac MC, Kaczmarska I, Ehrman JM (2013) The diversity of diatom assemblages in ships' ballast sediments: colonization and propagule pressure on Canadian ports. *Journal of Plankton Research* 35: 1267–1282, http://dx.doi.org/10.1093/plankt/fbt090

Wonham MJ, Walton WC, Ruiz GM, Frese AM, Galil BS (2001) Going to the source?: role of the invasion pathway in determining potential invaders. *Marine Ecology Program Series* 215: 1–12, http://dx.doi.org/10.3354/meps215001

Zhang F, Dickman M (1999) Mid-ocean exchange of container vessel ballast water. 1: Seasonal factors affecting the transport of harmful diatoms and dinoflagellates. *Marine Ecology Program Series* 176: 243–251, http://dx.doi.org/10.3354/meps176243