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Trans-Pacific shipboard trials on planktonic communities as indicators of open ocean ballast water exchange

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ABSTRACT: A trans-Pacific voyage from Japan to New Zealand via Singapore was used to assess planktonic (and sediment-dwelling) communities as indicators of open ocean ballast water exchange (BWE). The research was part of a larger project to develop methods for verifying whether international shipping has complied with mandatory controls and voluntary BWE guidelines. The dilution efficiency of exchanges was compared with changes in the composition and abundance of ballast tank phyto- and zooplankton taxa. Changes in the planktonic communities were measured by sampling the source port, the ballast water at the source and recipient ports and immediately before and after BWE, and the open ocean water during exchanges. Although exchanges appeared relatively effective at reducing the abundance (90 to 100%) of phyto- and zooplankton indicator taxa (i.e. taxa in the ballast water uploaded from the source port that were not present in open ocean samples or were present at <0.001 the original concentration), such reductions must be considered in the light of variation in survivorship in the control tank. Relatively high rates of mortality were associated with a rapid warming (i.e. 14.0 to 27.8°C) of the exchanged and control ballast tanks as the vessel entered the tropics. The rapid decline in the abundance of indicator taxa after BWE contrasted with a less effective reduction (i.e. 30.3 and 25.0% for the first and second exchanges, respectively) in the total number of phyto- and zooplankton taxa. Management controls and guidelines should place greater emphasis on whether the water uploaded during BWE is sufficiently oceanic to minimise the risk of uploading harmful coastal organisms.

KEY WORDS: Ballast water exchange · Tracer dye · Phytoplankton · Zooplankton · Sediment-dwelling species · Biological indicators · Open ocean · Trans-Pacific voyage

INTRODUCTION

Ballast water, which is carried onboard ships to provide stability and for trimming purposes, is recognised as a key mechanism for the translocation of non-indigenous marine species to new locations (Carlton 1985, Chu et al. 1997, Gollasch et al. 2000, Niimi 2000, Murphy & Ruiz 2001, Wonham et al. 2001). Examples of this include the zebra mussel *Dreissena polymorpha* in North America and the toxin-producing dinoflagellate *Gymnodinium catenatum* in the Derwent and Huon estuaries in Tasmania, which is now responsible for the regular closure of shellfish harvesting due to high levels of Paralytic Shellfish Poisoning (PSP) toxins (Hallegraeff & Bolch 1992).

The only current internationally accepted method of reducing the risk of new introductions via ballast water discharge is open ocean ballast water exchange (BWE). The aim of BWE is to replace all or most of the original ballast water from a port of origin with water from the open ocean. The open ocean is simply described by the International Maritime Organisation (IMO) in its Resolution A. 868(20) of 27 November 1997 (IMO 1997) as deep water as far as possible from shore. Ocean depths

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of up to 2000 m have been recommended by various authors and agencies (Williams et al. 1988, Dickman & Zhang 1999); however, many shipping routes, e.g. short voyages or those passing through island archipelagos, can render BWE at such depths impractical.

In February 2004, IMO member countries adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments. The convention called for BWE to be considered an interim measure, to be followed by the adoption of a BWE standard (Regulation D-1), which specifies an efficiency rating of 95% volumetric exchange of ballast water and a performance standard (Regulation D-2), limiting the number of organisms that ships could discharge in their ballast. The latter standard requires ships carrying out ballast water management to discharge less than 10 viable organisms ≥50 µm in size m^{-3} of water and less than 10 viable organisms <50 µm but >10 µm in size ml^{-1} of water. In addition, the ballast water performance standard sets limits on the discharge of a number of disease causing pathogens, including *Vibrio cholerae* and *Escherichia coli*. The legal status of Resolution A. 868(20) is not affected by the adoption of the Convention.

There are 2 main methods of carrying out BWE: empty-refill and flow-through dilution. The empty-refill method involves completely emptying then refilling the ballast tank (e.g. segregated tanks on container vessels) or hold (e.g. bulk carriers). The flow-through method involves pumping open ocean water into the tank or hold and then allowing the water to overflow. The IMO recommends that the tank volume should be pumped through the tank at least 3 times when using the flow-through method. Theoretically, in a perfect mixing environment, approximately 95% of the original coastal water in a ballast tank should be replaced by open ocean water if volumes of ocean water equal to 3 times the tank volume are pumped through (Rigby & Hallegraeff 1994). Ship design, cargo loading and safety considerations can limit the extent to which a BWE is carried out, however, particularly during rough sea conditions (Rigby & Hallegraeff 1994, Hay & Tanis 1998).

Exchange operations that realistically aim to exchange at least 95% of the original ballast water also aim to remove or kill at least 95% of the organisms in that water. The dilution of the original water is not the same as the removal of the organisms, however, as there is evidence for organisms having been retained in ballast tanks during exchanges (Rigby & Hallegraeff 1994, Wonham et al. 2001). Harvey et al. (1999) reported that although BWE has been shown to greatly reduce the diversity and abundance of planktonic organisms in the ballast water of ships entering the Great Lakes, coastal species remained in incoming vessels that had reported complete exchanges. These studies suggest that during BWE the rate of biological dilution may be less than that of the original water. The biota may be retained in the tanks either by entrainment or sedimentation, or by avoiding the outlets in the case of some of the larger species.

Most methods used for verifying compliance with ballast water controls and voluntary BWE guidelines are dependent on ships' documentation and log keeping (Hallegraeff 1998). Alternative ways of verifying compliance might include cross checking the volume of water claimed to have been pumped during the exchange (e.g. the 'Newcastle Method'), direct measurement of the volume of the original ballast water replaced (e.g. magnetic flow meters), and automated electronic systems (e.g. the 'black box') for interrogating variables such as ballast volumes pumped, geographic position and the time of BWE (Hay & Tanis 1998). Tracer dye studies have been proposed as a useful tool for measuring dilution after an exchange, but would probably be of relatively little value for routinely testing compliance (ibid). The optical characteristics of seawater can be used to distinguish between oceanic and coastal water types, and fluorescence spectra have considerable potential as a tool for verifying compliance (Hall et al. 2005, Hunt et al. 2006, Murphy et al. 2006). Identifying the different proportions of coastal and oceanic, and tropical and temperate, plankton communities present in the ballast water might also have potential as a compliance verification tool (Hallegraeff 1998). In the Port of Vancouver, for example, the relative proportions of coastal and oceanic copepod species have been used to corroborate claims that BWEs were made (see Hay & Tanis 1998).

The present study investigated the efficiency of BWE in terms of both the physical dilution of the original water and changes in the composition and abundance of phyto- and zooplankton communities uploaded from the source port. The study was part of a wider research project aimed at identifying methods for verifying whether international shipping has complied with ballast water controls and voluntary BWE guidelines, and establishing the effectiveness of ballast exchange at expelling the resident organisms inside ballast tanks. The research was undertaken for the New Zealand Ministry of Fisheries by the Cawthron Institute (New Zealand) in collaboration with Battelle (USA) between 1998 and 1999.

MATERIALS AND METHODS

Sampling design. BWE trials were carried out during a trans-Pacific voyage of the M/T 'Iver Stream' from Kawasaki Harbour, Japan, to Singapore Harbour between 23 February 1999 and 8 March 1999 (first leg), and from Singapore Harbour to New Plymouth (New Zealand) between 8 and 24 March 1999 (second leg). The ship's track-line, the locations of BWEs and coastal and open ocean sampling points during the voyage are shown in Hunt et al. (2007; their Fig. 1).

The 'Iver Stream' is a methanol carrier, which arrived in New Zealand fully ballasted. The vessel has a dead-weight tonnage (DWT) of 32 000 metric tonnes (t), and has 2 pairs of port and starboard ballast tanks (1435 m^3 capacity each: depth 15.0 m, width 6.5 m, length 14.8 m). Although the ballast tanks are not compartmentalised, they contain internal platforms and framing structures. All exchanges were carried out using the flow-through dilution method (pump rate 170 m^3 h^{-1}), and the vessel travelled 900 to 1200 km during the 24 to 40 h (depending on the number of pumps that were available) it took to exchange 3 times the volume of 1 ballast tank.

At the beginning of the first leg, all 4 ballast tanks were completely emptied and then cleaned of the remaining sediments to control for variation in sediment levels between tanks. The ship's ballast water intakes were located approximately 7.0 m below the waterline, and during ballasting the seawater depth in Kawasaki Harbour was approximately 10 m. The first exchange on the first leg was carried out from 26 to 28 February 1999 in the East China Sea in a gentle swell, over an approximate depth of 6500 m using a port ballast tank, while the second exchange was carried out from 1 to 2 March in the South China Sea in very similar sea conditions over an approximate depth of 3700 m using a starboard ballast tank. A second port ballast tank was used as a non-exchanged control for both exchanges; hence, Kawasaki Harbour water remained in this tank for the entire first leg and so traversed a wide latitudinal range from temperate regions to the tropics. For both exchanges on the first leg, 2 complete exchanges (i.e. 3 times the tank capacity) were carried out by pumping water through 4 to 6 large deck hoses into the man-hole at the top of the tank, and out via another pump at the bottom of the tank, near the aft end.

At the beginning of the second leg, a port and starboard tank were partly filled with Singapore Harbour water, and filling was completed as the 'Iver Stream' travelled out of the Harbour. The weather during this part of the voyage was very settled, resulting in very calm sea conditions. The first exchange, using the port tank, was carried out from 13 to 14 March 1999 in the west Pacific off the east coast of Irian Jaya over an approximate depth 1400 m. The second exchange, using the starboard tank, was carried out from 15 to 17 March off the north coast of Papua New Guinea over an approximate depth of 900 to 2000 m. The first exchange was complete (as described above); however, the second exchange was equal to only 2 times the tank volume owing to vessel time constraints. For both exchanges on the second leg, the water was pumped in at the bottom, near the aft end of the tanks, and allowed to overflow out of the hatches described above. No controls tanks were available on the second leg owing to vessel loading constraints; hence, analyses were limited to selected samples only. During the second exchange on the second leg of the voyage the 'Iver Stream' sailed in close proximity (10 to 15 km) to the mouth of the Sepik River.

Samples were collected from the ballast tanks several hours before and after exchanges, several hours after uplifting ballast, and at the end of each leg. All samples were collected via Butterworth hatches located on the seaward side of the top of the tanks.

Dilution efficiency. Laboratory experiments and preliminary shipboard trials on a New Zealand domestic container vessel, the M/V 'Spirit of Vision', were used to identify a suitable tracer dye for measuring the dilution efficiency of BWE. Rhodamine WT was chosen because, unlike histological stains, it is biologically inactive. It is a highly fluorescent material with the unique ability to absorb green light (optimum excitation wavelength 556 nm) and emit red light. Very few compounds have this property, so interference by other substances is uncommon. The dye is a highly specific tracer, and the amount of red light is directionally proportional to dye concentration down to at least 10^{-4} ppm for industrial type applications (Turner Designs 1999). We used Intracid® Rhodamine WT fluorescent water tracing dye. The active content of the commercial dye is 21.3% (or 2.13×10^5 ppm) and it has a specific gravity of 1.11 to 1.13 at 20°C. Dye fluorescence was measured using a Turner Designs™ 10-05 fluorometer, and Rhodamine WT concentration was calculated using a linear calibration of fluorescence and concentration as derived from standards of known concentrations of the dye.

The laboratory experiments and shipboard trials established that a Rhodamine WT concentration of 0.1 ppm was suitable for measuring the dilution efficiency of BWE (detection limit <0.001 ppm). The dye was also found to be stable over a range of environmental conditions (see Taylor & Bruce 1999). Relatively high concentrations of plankton and particulate matter had little or no effect on fluorescence, and the viability of phyto- and zooplankton was not affected by Rhodamine WT at a concentration of 0.1 ppm. It was also established that adding the dye to the bottom of ballast tanks prior to refilling ensured a high level of mixing prior to exchanges.

On the first leg of the voyage and during the early stages of filling the ballast tanks with water uploaded from Kawasaki Harbour, Rhodamine WT was added to the tanks such that the approximate concentration of the dye on filling was 0.1 ppm. On 24 February 1999, after the tanks had been filled and the dye was thoroughly mixed in the tanks, 1 l samples were collected from the surface (0.5 m), mid-depth (7.0 m) and bottom (14.0 m) using a 5 l van Dorn sampler to measure the exact dye concentration after filling the tanks. Replicate samples were taken from the mid-depth stratum to assess sampling variability and are described further in Taylor & Bruce (1999). This sampling procedure was also repeated several hours before and after BWE.

On the second leg, Rhodamine WT was added to the tanks in Singapore Harbour and van Dorn samples collected as described above. Additional depth-stratified time series samples were collected during the first exchange after 0.75, 1.5 and 2.25 times the tank volume had been pumped through the tank to assess the rate of dilution during exchanges. Fluorescence intensity was used to measure the concentration of Rhodamine WT in each sample and the dilution efficiency of each exchange was determined by calculating the reduction in Rhodamine WT concentration at each depth stratum.

Temperature and salinity. During the voyage, seawater temperature and salinity measurements were made of the source port (i.e. Kawasaki and Singapore Harbours), ballast (1 m depth-stratified sampling, several hours before and after exchanges on the first leg, and every 2 to 9 h during exchanges on the second leg) and open ocean surface water (daily en route, and every 1 to 9 h during all exchanges). All measurements were taken *in situ* using an Orion 140® temperature and salinity meter with the exception of the open ocean samples, which were collected using a stainless steel sampling bottle attached to a light rope.

Biological efficacy. At the beginning of the first leg, 50 l surface, mid-depth and bottom phyto- (20 µm filtered) and zooplankton (100 µm filtered), and 1 l surface, mid-depth and bottom phytoplankton (unfiltered) samples were collected from Kawasaki Harbour (at 0.5, 5.0 and 10.0 m) and the exchange and control tanks (at 0.5, 7.0 and 14.0 m) to establish the composition and abundance of plankton communities uploaded from Kawasaki Harbour. Five-litre samples were collected using a van Dorn sampler and the filtered samples consisted of 10 consecutive pooled samples (i.e. 50 l) at each depth stratum. Replicated 50 l samples were collected from the mid-depth stratum to assess sampling variability of selected taxa and are described further in Taylor & Bruce (1999). Additionally, vertical phyto- (20 µm filtered) and zooplankton (100 µm filtered) net hauls were collected for further qualitative analyses of the plankton communities. This procedure was repeated immediately before and after BWE and, for the tank used for the second exchange and the control tank, in Singapore Harbour at the end of the leg immediately prior to discharge of the ballast water. Samples collected from the beginning and end of the leg were pooled across depth strata to reduce processing time.

During BWE, daily 50 l phyto- and zooplankton samples were also collected via the deck hydrant to assess the composition and abundance of the plankton communities uploaded during the exchange. The deck hydrant was supplied by a pump and plumbing system similar to that of the ballast water system, with no additional intake or inline filters. Hence, the plankton samples obtained from the hydrant during each exchange were assumed to be representative of the plankton communities uploaded during the exchange. A similar sampling procedure to that described above was employed on the second leg of the voyage; however, because a control tank was not available for this leg, presentation of results on the biological efficiency of BWE is limited to descriptions of observations only. Data from the second leg are presented in Taylor & Bruce (1999).

Immediately after collection, all samples were examined using a light microscope to record key taxonomic features and assess the viability of planktonic taxa. The samples were then preserved using Lugol's iodine solution (phytoplankton) or 5% formalin (zooplankton) for identification and enumeration in the laboratory (Cawthron), where all phyto- and zooplankton were identified to the lowest taxonomic resolution possible. Identification of phytoplankton taxa followed the methods described in Thomas (1997). Samples were enumerated using an inverted light microscope and counts of live phytoplankton cells were based on slight modifications of the standard procedures described in Sournia (1978). Viability was assessed on the basis of the integrity of the cell contents. Zooplankton taxa were identified using various literature sources, and samples were enumerated using a Bogorov tray and a binocular light microscope. Viability was assessed on the basis of the integrity of each specimen. If the total zooplankton sample contained in excess of approximately 200 individuals of any one taxa, counts were based on 4 (1/8 evenly split) subsamples.

The biological efficiency of BWE was assessed by describing changes in the composition and abundance of the plankton communities, and measuring changes in the concentration and richness of the source port taxa on the first leg of the voyage. To evaluate changes in the composition and abundance of the plankton communities, a non-metric MDS (nMDS) ordination procedure based on the Bray-Curtis similarity measure (Primer v5.2.2) was used to describe changes in taxa richness and dominance among samples, following a $\log_{(x+1)}10$ transformation to down-weight the influence of the most dominant taxa (Clarke & Warwick 1994). Similarity thresholds were superimposed on the nMDS ordination pattern using group average clustering (Primer v5.2.2) (Clarke 1993). A SIMPER procedure (Clarke 1993) was used to identify the major taxa contributing to each group.

Indicator taxa, which were used for quantifying changes in phyto- and zooplankton concentrations after exchanges, were defined as those taxa present in the ballast water uploaded from the source port, but not present in open ocean samples or present at <0.001 the original concentration. Counts of dead plankton material were not carried out owing to considerable variation in the level of decomposition of dead specimens.

After emptying the ballast tanks at the end of the voyage, the remaining sludge in the control tank was inspected to assess the amount of sediment uploaded in Kawasaki Harbour that had remained in the tank after deballasting. Also, four 200 ml sediment samples were taken at different locations throughout the bottom of the tank to assess the survival of sediment-dwelling organisms and check for viable dinoflagellate cysts. Samples were refrigerated and transported to the laboratory for inspection and culturing of the cysts.

RESULTS

Dilution efficiency

After all 4 BWE, the concentration of Rhodamine WT showed a reduction equal to, or in excess of, 99% at all 3 depth strata. Time series sampling during the first exchange on the second leg demonstrated stratification of the dye after 0.75 the tank volume had been pumped through the tank (Rhodamine WT dilution = 47% at 0.5 m, 68% at 7.0 m, and 87% at 14.0 m). Stratification of the dye was less apparent after 1.5 times the tank volume had been pumped through, and at this stage the dye dilution was more than 95% at all 3 depth strata. These results provide strong evidence that, regardless of whether the ballast tanks on the 'Iver Stream' were filled from the top (first leg) or bottom (second leg) of the tanks, exchanges were very efficient at diluting the original ballast water. Interestingly, this was also the case during the second exchange on the second leg of the voyage, where only 2 times the tank volumes were exchanged.

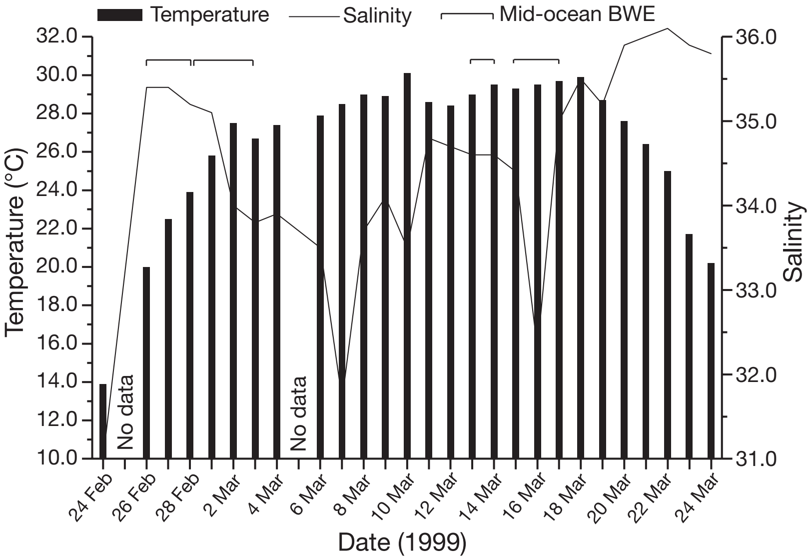

Fig. 1. Seawater surface temperature and salinity at approximately 24 h intervals during the entire 'Iver Stream' voyage. The times that ballast water exchange (BWE) was carried out for both the first and second legs are also shown

Temperature and salinity

Fig. 1 shows the daily surface seawater temperature (SST) and salinity measurements for the entire voyage. There was a steady increase in SST from 14.0°C in Kawasaki Harbour (24 February 1999) to 27.8°C in the South China Sea (2 March 1999). SST reached a maximum of 30.0°C in the tropics (10 March 1999), and began to decline again southwest of the Solomon Islands (19 March 1999). On the first leg, surface salinity increased rapidly from 31.0 on departure from Kawasaki Harbour to 35.5 after 2 d, but declined again during the time of BWE. This was attributed to the influence of land run-off to the South China Sea, possibly from the outflow of the Mekong River.

Both exchanges on the first leg resulted in an increase in temperature at all 3 depth strata from approximately 15.0°C (immediately prior) to 23.0°C after the first exchange, and from 20.0 to 27.5°C after the second exchange. Temperatures also increased, however, from approximately 15.0 to 22.0°, and from 20.0 to 27.5°C in the control tank at the corresponding times. Before and after both exchanges on the first leg, the salinity at all 3 depth strata reflected the salinity in the coastal water (32.7) and open ocean (35.2 and 34.1 for the first and second exchanges, respectively) at the corresponding times. There was no noticeable change in salinity in the control tank (32.7) during the corresponding time intervals.

Depth profiles of salinity during the first exchange on the second leg demonstrated initial stratification as the high salinity open ocean water entered the tank, followed by rapid displacement of the relatively low salinity water uploaded from Singapore Harbour. In this case, complete mixing occurred before 1.5 times the tank volume had been pumped through the tank. Similarly, depth profiles of salinity during the early stages of the second exchange indicated stratification, followed by rapid displacement of the original coastal water with open ocean water after 0.9 times the tank volume had been pumped through the tank (Fig. 2). Off New Guinea (16 March 1999), however, runoff from the Sepik River contributed lower salinity water, which formed a layer on top of the higher salinity water and effectively reduced the rate of dilution in the bottom layers of the tank.

Biological efficacy

The nMDS plot from the first leg shows that the phyto- and zooplankton communities uploaded in ballast water from Kawasaki Harbour closely resembled the corresponding harbour water (Fig. 3, Table 1). Although the open ocean community was clearly distinct from that found in the ballast tanks, a number of planktonic taxa such as *Skeletonema* spp., *Chaetoceros* spp., *Oithona* spp., bivalve larvae, Cirripedia larvae and *Microsetella novegica* were present in both the Kawasaki Harbour and open ocean samples. There was also a relatively high diversity and abundance of typical coastal taxa in the open ocean samples, such as the dinoflagellate *Dinophysis fortii* and several zooplankton genera (benthic harpacticoid copepods, and Cirripedia and bivalve larvae).

During both exchanges, there was a shift in species composition from a Kawasaki Harbour type community to a new community (Fig. 3, Table 1), which more closely resembled the communities sampled in the open ocean and was characterised by lower abundances overall, fewer dinoflagellate taxa and the ab-

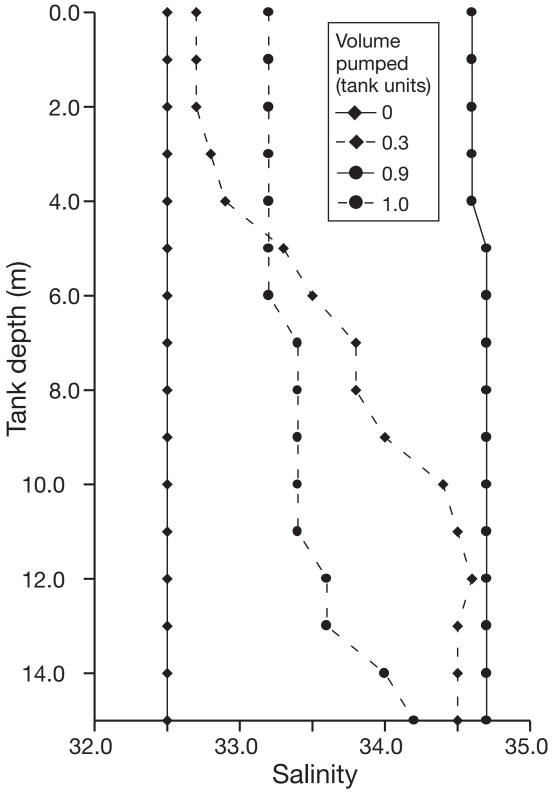

Fig. 2. Depth profiles of salinity after the second exchange (2 times the volume of the tank) on the second leg of the voyage

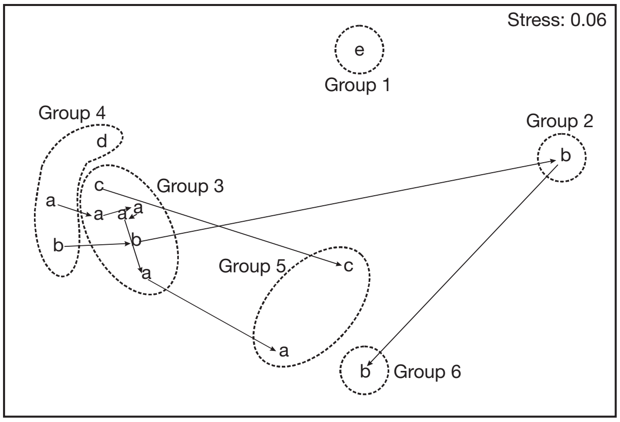

Fig. 3. Non-metric MDS ordination (2D stress = 0.06) showing trajectories of phyto- and zooplankton composition and abundance in depth-stratified ballast water samples collected from Kawasaki Harbour, immediately before and after ballast water exchange (BWE), and in Singapore Harbour. Groups (indicated by dotted lines) were formed based on approximately 65 % Bray-Curtis similarity. See Table 1 for further details

Table 1. Description of groups and sample types shown in Fig. 1; BWE: ballast water exchange

Group	Tank	Sample	BWE
1	e	Open ocean	
2	b	2nd exchange (starboard)	After
3	a	1st exchange (control)	Before
	a	1st exchange (control)	After
	a	2nd exchange (control)	Before
	a	2nd exchange (control)	After
	b	2nd exchange (starboard)	Before
	c	1st exchange (port)	Before
4	a	Kawasaki Harbour (control)	
	b	Kawasaki Harbour (starboard)	
	d	Kawasaki Harbour	
5	a	Singapore Harbour (control)	
	c	1st exchange (port)	After
6	b	Singapore Harbour (starboard)	

sence of a number of copepod taxa that were relatively abundant in the Kawasaki Harbour water (e.g. *Acartia omorii*, *Centropages abdominalis* and *Danielssenia* sp.) However, relatively diverse phyto- and zooplankton assemblages, including taxa of coastal origin, were uploaded during the exchanges. Taxa such as *Clausocalanus* spp. and the Oncaeidae were sampled in the open ocean, but not from Kawasaki Harbour, and were found to be relatively abundant in the tanks several hours after exchanges. The total number of phyto- and zooplankton taxa decreased from 33 in samples collected immediately before the first exchange to 23 taxa after the exchange (a 30.3% decrease), and from 24 to 18 during the time of the second exchange (25.0%).

Nevertheless, BWE did appear to be effective at expelling or killing the majority of organisms originally uploaded in the source port ballast water, with a marked reduction in the mean depth-stratified counts of Kawasaki Harbour phyto- and zooplankton source port indicator taxa in the exchanged tanks (Figs. 4 to 7). The reduction in the mean concentrations of the indicator taxa was 90 to 100%, except for the molluscan group, for which concentrations before exchanges were very low (i.e. 0 to 1 ind. per 50 l), and therefore the estimates of the change in concentration are unreliable.

More specifically, certain indicator planktonic taxa such as *Heterocapsa* spp. (Fig. 4) and polychaete larvae and juveniles (Fig. 5) were almost completely removed after the first exchange, and *Gyrodinium* spp., *Heterocapsa* spp., *Prorocentrum* spp. (Fig. 6), *Acartia omorii*, *Centropages abdominalis*, *Danielssenia* sp. and sediment-dwelling larval and juvenile polychaetes (Fig. 7) appeared to be completely removed after the second exchange.

Notable was the considerable variation in concentrations among the 3 depth strata for some taxa prior to both exchanges (e.g. all indicator copepod species combined, Figs. 5 & 7). A number of zooplankton taxa, including harpacticoid copepods, larval and juvenile mol-

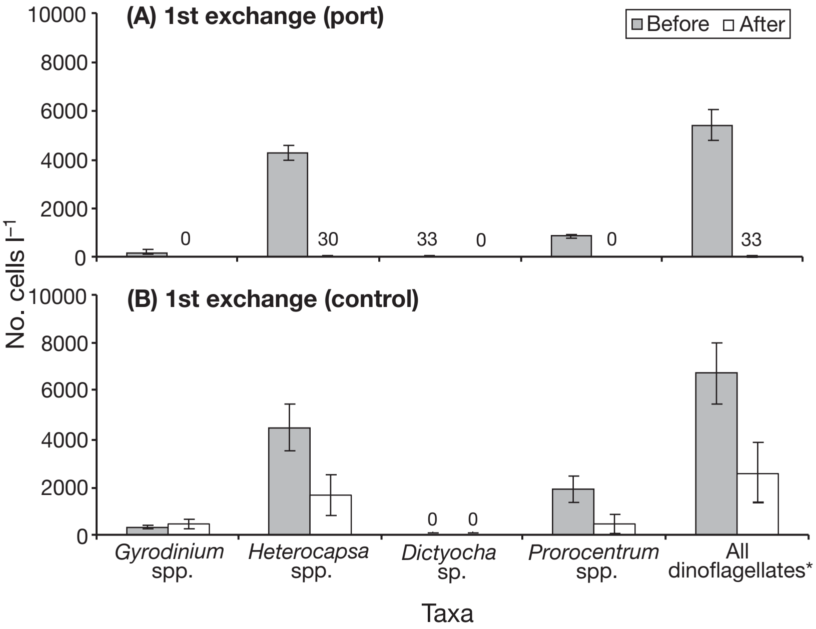

Fig. 4. Concentrations of indicator phytoplankton taxa in ballast water from (A) exchanged and (B) control tanks (means of depth-stratified samples ± SE) immediately before and after ballast water exchange (BWE) on the first exchange on the first leg of the voyage. Indicator taxa were defined as those present in the ballast water uploaded from the source port, but not present in open ocean samples or (*) present in open ocean samples at <0.001 the original concentration. Numbers above the bars are means <50

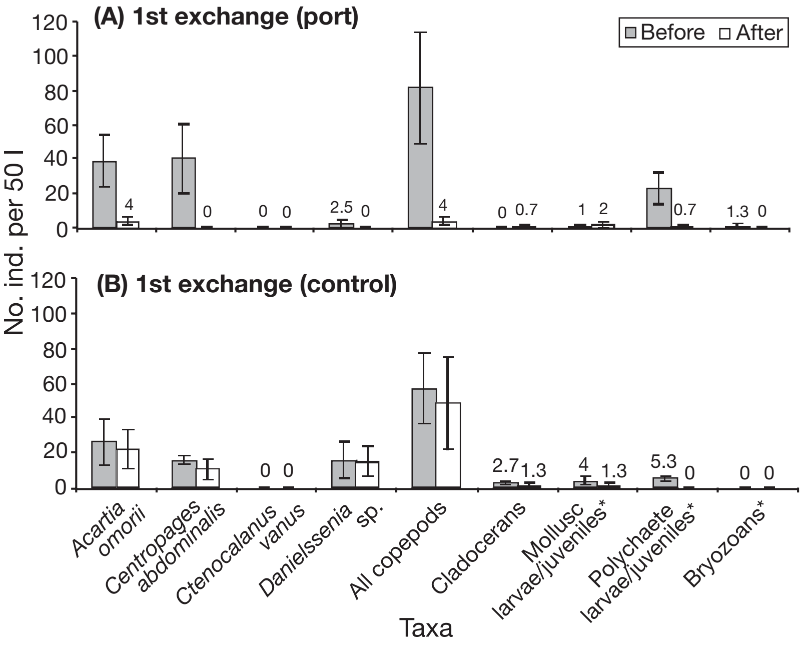

Fig. 5. Concentrations of indicator zooplankton taxa in ballast water from (A) exchanged and (B) control tanks (means of depth-stratified samples ± SE) immediately before and after ballast water exchange (BWE) on the first exchange on the first leg of the voyage. Indicator taxa were defined as those present in the ballast water uploaded from the source port, but not present in open ocean samples or (*) present in open ocean samples at <0.001 the original concentration. Numbers above the bars are means <10

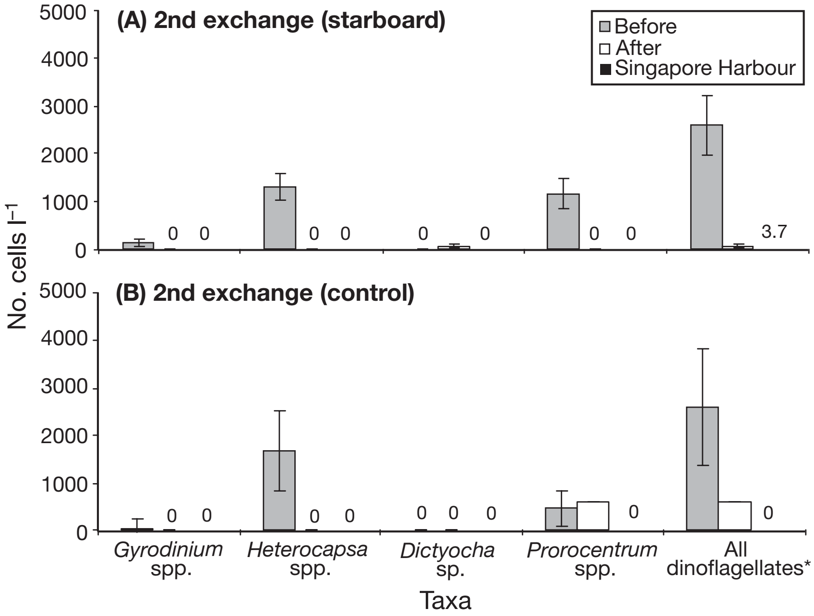

Fig. 6. Concentrations of indicator phytoplankton taxa in ballast water from (A) exchanged and (B) control tanks (means of depth-stratified samples ± SE), immediately before and after ballast water exchange (BWE) on the second exchange on the first leg of the voyage, and at Singapore Harbour (depth-strata pooled) immediately prior to discharge (not shown as all values are 0). Indicator taxa were defined as those present in the ballast water uploaded from the source port, but not present in open ocean samples or (*) present in open ocean samples at <0.001 the original concentration. Numbers above the bars are means <50

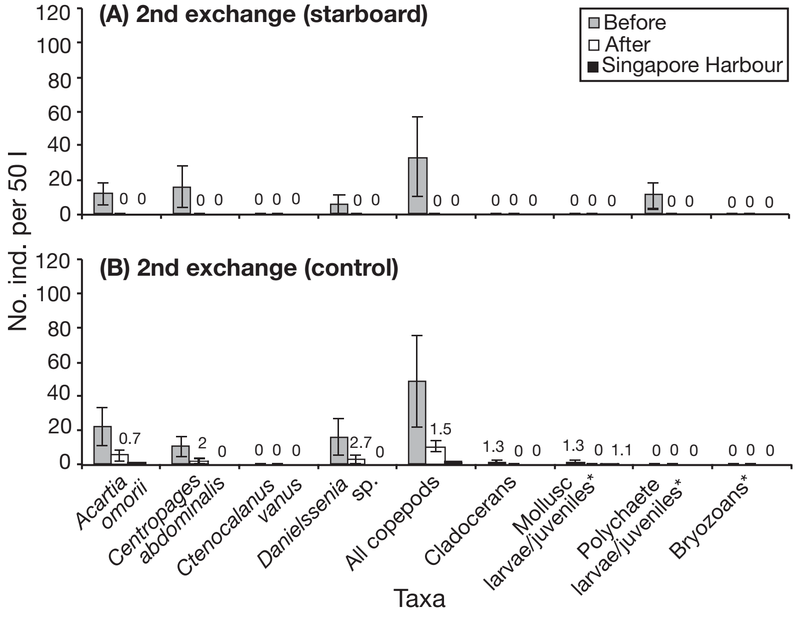

Fig. 7. Concentrations of indicator zooplankton taxa in ballast water from (A) exchanged and (B) control tanks (means of depth-stratified samples ± SE) immediately before and after ballast water exchange (BWE) on the second exchange on the first leg of the voyage, and at Singapore Harbour (depth-strata pooled) immediately prior to discharge. Indicator taxa were defined as those present in the ballast water uploaded from the source port, but not present in open ocean samples or (*) present in open ocean samples at <0.001 the original concentration. Numbers above the bars are means <10

luscs, and polychaetes uploaded from Kawasaki Harbour, were only found in the bottom stratum of the ballast tanks after BWE. This stratification may have been the result of certain sediment-dwelling species being brought into suspension during the exchange, then settling in the bottom layer of the tank after the exchange. One of the Kawasaki Harbour phytoplankton indicator taxa (*Dictyocha* sp.) was not collected before the second exchange on the first leg; however, viable *Dictyocha* sp. cells reappeared in samples collected after the exchange. *Dictyocha* spp. are silicioflagellates, and stratification in its distribution may be attributed to buoyancy differentials resulting from the siliceous skeleton of this organism. This signals the importance of sampling error associated when interpreting the results from ballast water sampling (Fig. 5).

Out of the total of 28 phyto- and zooplankton taxa identified in samples collected from the exchanged tank sampled in Kawasaki Harbour, 8 taxa were still present (71.5% decrease) at the end of the first leg in Singapore Harbour. Two of the indicator taxa, both from the dinoflagellate group (*Dinophysis fortii* and *Protoperidinium* spp.), remained in the tank. The other taxa present in the exchanged tank were the diatoms *Skeletonema* spp., *Chaetoceros* spp., and Oncaeidae, *Oithona* spp., Cirripedia nauplii and Ascidiacea larvae in the zooplankton.

During both exchanges on the first leg, the nMDS plot grouped samples collected from the control tank immediately before and after exchanges closely, indicating that the composition and abundance of the plankton community did not change markedly relative to the other samples (Fig. 3). A total of 30 planktonic taxa were found in the samples collected from the control tank immediately before and after the first exchange (0% decrease), whereas after the second exchange, the number decreased from 30 before to 19 (36.7% decrease).

There was, however, a relatively large decline in phyto- and zooplankton indicator taxa in the control tank during exchanges. These included *Prorocentrum* spp. during the first exchange (Fig. 4), and *Heterocapsa* spp. and all indicator dinoflagellates combined during both exchanges (Figs. 4 & 6), and larval and juvenile polychaetes, *Acartia omorii* and all copepods combined on the second exchange (Fig. 7).

During the second exchange on the first leg, there was a more significant decline in the overall diversity and abundance of both phyto- and zooplankton (including indicator taxa) in samples collected from the control tank compared to the control tank during the first exchange. Exceptions were indicator dinoflagellate *Prorocentrum* spp. (Fig. 6) and several (non-indicator) diatoms, e.g. *Skeletonema* spp., *Chaetoceros* spp. and *Pseudonitzschia* spp. Several of the indicator taxa in the control tank incurred particularly high levels of mortality during the time of the second exchange. For example, phyto- (*Heterocapsa* spp. and all dinoflagellates combined, Fig. 6); and zooplankton (*Acartia omorii, Centropages abdominalis*, *Danielssenia* sp., all copepods combined, Fig. 7). In addition, several other non-indicator taxa (e.g. the diatoms *Leptocylindricus* spp. and *Thalassiosira* spp.) also showed a considerable decline in concentration during exchanges. Other taxa, such as *Gyrodinium* spp., *Diplopsalis* spp. and *Skelotonema* spp., appeared to increase or remain relatively stable during the time of BWE.

A total of 33 plankton taxa were present in the control tank in Kawasaki Harbour, with 8 of these still present (75.8% decrease) at the end of the first leg in Singapore Harbour. Of the indicator taxa, no phytoplankton taxa were present (Fig. 6) and the zooplankton were represented by *Acartia omorii*, copepods, and larval and juvenile molluscs (Fig. 7). Other taxa present included diatoms *Skeletonema* spp., *Chaetoceros* spp., and *Pseudonitzchia* spp., and Anthozoa, *Oithona plumifera* and Cirripedia nauplii in the zooplankton.

The results from the second leg corroborated the main findings from the first leg. In particular, before and after the second exchange (2 times the volume of the tank only) there was a significant shift in species composition in the exchanged tank. After the exchange, the phytoplankton community changed from a community dominated by diatoms to one dominated by dinoflagellates, and there was a particularly marked increase in the richness of zooplankton taxa after the exchange. The relative abundance of both phyto- and zooplankton taxa was much lower prior to this exchange, however, than was observed before both exchanges on the first leg. This probably reflects the relatively high (8 d) containment time in the tropics prior to commencing the exchange.

At the end of the voyage low levels of sediment had accumulated in the control tank even though the ballast tanks were cleaned before the exchange trials. No invertebrates were found in the sediments; however, many empty phytoplankton frustules, typical of the species that were uploaded from Kawasaki Harbour (mainly *Skeletonema* spp., *Dinophysis* spp., *Prorocentrum* spp., and *Ceratium* spp.), were present. This supports the view that there were high levels of mortality in the exchanged and control tanks during the voyage. Significant, however, was the presence of a variety of viable, unidentified dinoflagellate cysts at the end of the voyage.

DISCUSSION

Ballast tanks are heterogeneous environments (for example, top of the water column versus the bottom sediments) with complex interactions between the mixing dynamics of the existing water and sediments, the incoming water during BWE, and between the water and the organisms themselves. This complexity is further increased by variation in the physical, physiological and behavioural tolerances of the different groups of ballast water organisms.

A trans-Pacific voyage of the 'Iver Stream' was used to test methodologies for assessing (1) the dilution efficiency of BWE, (2) changes in the composition and abundance of plankton communities in ballast tanks immediately before and after BWE, and from the time they had been uplifted in the source port to the time of discharge at the recipient port, and (3) the efficacy of exchanges at expelling source port indicator phyto- and zooplankton taxa that were resident in ballast tanks before BWE.

Dilution efficiency

It was established in a previous study by Taylor & Bruce (1999) that the tracer dye Rhodamine WT is useful for measuring dilution efficiency of BWE, including the possible retention of the original ballast water in parts of the tank during the exchange. Rhodamine WT was detected at low concentrations (<0.001 ppm) in samples collected after BWE on the 'Iver Stream', indicating a very high ($>99\%$) dilution efficiency. In addition, a high rate of dilution was achieved regardless of the pumping method used (i.e. the ballast tanks were either filled from the top and pumped out via the bottom, or tanks were filled from the bottom and allowed to overflow at the top). There was no sign of depth stratification of the tracer dye several hours before and after BWE. Depth-stratified sampling of physical parameters (such as temperature and salinity) and phyto- and zooplankton assemblages in the ballast tanks also confirmed that vertical mixing was relatively thorough after exchanges.

Time series sampling of salinity during BWE on the second leg demonstrated depth stratification in the early stages of both exchanges. This was probably due to the high salinity open ocean water entering the bottom of the tanks, which rapidly displaced the relatively low salinity coastal water. This suggests that salinity differentials between the existing ballast water and the open ocean water contributed to flow behaviours that caused an increased rate of dilution. This early stratification during BWE was confirmed by time series sampling of the tracer dye during the first exchange. Rigby & Hallegraeff (1994) reported preferential displacement of the original water in the early stages of an exchange, and attributed this result to flow behaviours between 'perfectly mixed' and 'plug flow' conditions.

A reversal in the salinity differential occurred during the second exchange on the second leg. Lower levels of salinity in the water entered the bottom of the tank as the 'Iver Stream' travelled in close proximity to the mouth of Sepik River, resulting in the retention of some of the higher salinity water in the bottom layer of the tank during the initial stages of the exchange. This result suggests that in certain situations, salinity differentials in ballast tanks may contribute to flow behaviours that cause a reduced rate of dilution.

Rigby & Hallegraeff (1994) carried out 2 ballast water exchange trials on the MV 'Iron Whyalla' using the flow-through dilution method and the tracer dye methylene blue. Both exchanges resulted in a dilution efficiency approximating the theoretical value of 95%. In contrast, the unexpectedly high rate of dilution achieved by BWE on the 'Iver Stream' voyage was closer to that expected when using the reballasting method in calm conditions (i.e. 95 to 99%; see Zhang & Dickman 1999).

Selective use of tracer dyes, such as adding the dye prior to refilling the ballast tanks, may have application in calibrating indirect measures of dilution (for example, the use of optical signatures, e.g. Hunt et al. 2006), or standardising the dilution efficiency of tanks with different configurations. This would enable implementation of BWE compliance requirements for different classes of vessels. In spite of this, given the logistical constraints of having to add the tracer dye to the tank before the exchange, it is unlikely that tracer dyes will be useful as practical compliance tools for routinely verifying that a BWE has taken place. The usefulness of salinity as a tool for verifying BWE is restricted to the detection of source port water that is relatively low in salinity (for example, brackish or freshwater).

Biological efficacy

Depth-stratified sampling before and after BWE on the 'Iver Stream' voyage showed a shift in the overall composition and abundance of phyto- and zooplankton from a community closely resembling the source port (Kawasaki Harbour) to a new community consisting of source port taxa, as well as taxa from the open ocean. In particular, coastal zooplankton taxa (e.g. bivalve and Cirripedia larvae, and copepods such as *Oithona* spp.) as well cosmopolitan phytoplankton taxa (e.g. dinoflagellates such as *Diplopsalis* spp. and diatoms such as *Skeletonema* spp.) appeared to replenish the tanks during exchanges.

Notwithstanding this result, both exchanges on the first leg suggest that BWE was effective at reducing the abundance of the phyto- and zooplankton taxa that were originally uplifted from Kawasaki Harbour, as evidenced by a 90 to 100% reduction in the mean concentrations of nearly all the indicator taxa. However, the rate of dilution of the biota did not always approach the rate of dilution of the water (i.e. >99%), and BWE was not as effective at reducing the overall number of plankton taxa. The total richness of phyto- and zooplankton taxa, as derived from depth-stratified sampling before and after BWE, decreased by 30.3 and 25.0% for the first and second exchanges, respectively. This compared to changes in phyto- and zooplankton richness of 0 and 37% for the first and second exchange, respectively, for the corresponding samples collected from the control tank.

Our findings are in general agreement with several other studies on the effectiveness of BWE which have compared the rate of dilution of the original coastal water with the rate of removal of the organisms. On a 16 d trans-Atlantic voyage from Israel to the USA, Wonham et al. (2001) found that although 93 to 100% of the original coastal water was removed from the ballast tanks during BWE, the removal of the coastal organisms ranged from 80 to 100%. These researchers also observed mixed communities of open ocean and coastal plankton in tanks that had been exchanged.

On a 17 d trans-Pacific voyage from Japan to Canada, Rigby & Hallegraeff (1994) found that under static conditions (i.e. the vessel was anchored in Singapore Harbour) a completed exchange resulted in the retention of 5% of the original water, but 25% of the original phytoplankton material remained. Similarly, Locke et al. (1983) reported that BWE were 67 to 89% effective in eliminating organisms commonly found in freshwater or brackish coastal environments. Dickman & Zhang (1999) claim that BWE by reballasting (i.e. empty-refill method) was 48% effective in reducing the mean number of diatoms and dinoflagellates. Zhang & Dickman (1999) described that, on average, BWE (also by reballasting) reduced the number of harmful diatoms and dinoflagellates from 4235 to 550 cells l^{-1}, i.e. 87% effective. The authors claim that the reason BWE failed to eliminate all harmful diatoms and dinoflagellates was probably due to the ballast tank not being completely emptied before being reballasted in the open ocean.

The ability of BWE to expel coastal organisms is determined by flow behaviours, the mixing characteristics of the ballast tank and the behaviour of the organisms themselves. Preferential flows and the motion of the ship prior to and during the exchange affect the degree of mixing, the behavioural responses of the various groups of organisms (for example, diatom versus dinoflagellate dominated phytoplankton communities) and the rate of sedimentation (Rigby & Hallegraeff 1994). Mixing characteristics may also be influenced by the configuration of the ballast tank, the extent of the internal structures (such as platforms, gussets and drainage girders) and the locations of the ballast intake and outlet points.

The first leg of the 'Iver Stream' voyage revealed considerable variation in the survivorship of phyto- and zooplankton taxa over the first 5 d (after the tanks were filled in Kawasaki Harbour) in both the exchanged and control tanks. However, there was then a marked decline in the survivorship of the majority of taxa in both the exchanged and control tanks over the final 7 d of the leg. Twenty-eight plankton taxa were originally sampled from the exchanged tank in Kawasaki Harbour, and 8 taxa were sampled from the tank (i.e. a 71.5% decrease) in Singapore Harbour. This compared with a reduction from 33 to 8 taxa (i.e. a 75.8% decrease) in the corresponding control tank samples. The concentrations of these remaining taxa were very low, however, for both the exchanged and control tanks. Furthermore, although taxa such as the heterotrophic dinoflagellate *Protoperidinium* spp., and larval zooplankton may have been viable at the end of the leg, diatoms such as *Skeletonema* spp. and *Chaetoceros* spp. found in both exchanged and control tanks are unlikely to have been viable, especially in the case of the control tank.

Wonham et al. (2001) found differential survival among ballast tank organisms on the 16 d voyage described above. Rigby & Hallegraeff (1994) also demonstrated differential survival among taxa, and that motile cells of photosynthetic dinoflagellates did not survive in ballast tanks for more than 3 d. The rapid mortality was attributed to the movement of the ballast water under seagoing conditions. In contrast, phytoplankton species that were non-photosynthetic, present in resting cyst stages or were able to produce resting cysts and some zooplankton persisted in the ballast tank environment for the duration of the 17 d voyage.

Sampling undertaken during the 'Iver Stream' voyage demonstrated that even when ballast water is not exchanged in open ocean, the contents of relatively large (i.e. 1435 m^3) ballast tanks can undergo marked fluctuations in temperature (14 to 26°C) due to travelling over a wide range of latitudes. Marked reductions in the diversity and abundance of ballast water organisms resulting from fluctuations in temperature and changes in other physical parameters (e.g. ballast water movement and light and oxygen limitation) lead to further effects at the biological level, such as collapse of the planktonic food chain.

The mortality rate of planktonic organisms originally uploaded in ballast water from source ports in temperate regions is likely to be particularly high on shipping routes spanning temperate to semi-tropical or tropical regions (e.g. Kawasaki Harbour to Singapore Harbour) and on trans-equatorial routes. Williams et al. (1988) reported a reduction in the survivorship of planktonic taxa on shipping routes (8 to 18 d) between Japan and Australia and attributed the mortality to temperature or food chain effects. This outcome is consistent with the results from the present study. The decline in abundance of the original organisms after BWE, however, may partly be attributed to mortality during the exchange, rather than expulsion from the tanks.

This situation may contrast with much lower mortality rates on voyages during which such changes do not occur; for example, over relatively short voyages (such as Australia to New Zealand) and shipping routes that lie within a relatively narrow latitudinal range (e.g. Chile to New Zealand). On a northern hemisphere trans-Pacific shipping route (i.e. no equatorial crossing), Zhang & Dickman (1999) found that the highest number of harmful ballast water organisms occurred when sea temperatures in the source (Oakland, California) and the recipient (Hong Kong, China) ports were both low, and was probably due to the minimal temperature variation between the 2 ports at this time. Also, low rates of mortality on coastal shipping routes may result in relatively high invasion risks compared to oceanic routes (Claudi & Ravishankar 2006).

While there was a relatively large reduction in the concentrations of the indicator plankton taxa during BWE on the first leg of the voyage, it is unclear exactly how much of the attrition was due to the exchanges expelling the biota, and how much was due to mortality in the tanks. Further evaluation of this question would require a more intensive sampling effort than was feasible in the present study, such as intensive time series analyses of plankton communities during exchanges, coupled with a more detailed analysis of the viability of ballast tank organisms (for an example see Hay et al. 1997).

The present study indicated that the uplift of coastal-type organisms, which may include harmful species, may be enhanced if BWE is carried out relatively close to the coast or near the influence of large rivers. Open ocean sampling on the first leg of the voyage revealed the presence of relatively high numbers of coastal-type taxa, e.g. including coastal phytoplankton genera, benthic harpacticoid copepods, and Cirripedia and bivalve larvae. Furthermore, although BWE on the first leg achieved a rapid decline in the number of dinoflagellates in the phytoplankton, an exchange on the second leg, which was carried out relatively close to the coast near the mouth of the Sepik River, resulted in a marked shift from a diatom dominated phytoplankton community to one dominated by dinoflagellates. Dinoflagellates are common in coastal and estuarine areas and often show associations with river plumes and rainfall events (Hallegraeff 1998). This has important implications with regard to the implementation of vessel ballast water management plans and compliance with BWE requirements, including specifying open ocean regions where BWE will be most effective. Importantly, such a determination of suitable open ocean regions for BWE also needs to include a consideration of the risk of invasion from where the ballast is discharged (Brickman 2006).

Our assessment of the efficacy of BWE at expelling the phyto- and zooplankton indicator taxa uploaded from the source ports (e.g. Kawasaki Harbour) was based on the assumption that none of these taxa were uploaded during the exchanges. Although sampling of the open ocean plankton communities was carried out at various times throughout the exchanges, and taxa that were common in both the source port and open ocean samples were eliminated as indicators of source port taxa, plankton communities are notoriously patchy in both space and time. This patchiness may confound quantitative assessments of the dilution of indicator taxa during BWE; hence, such assessments are unlikely to be useful as a direct means of verifying BWE.

Alternatively, although certain taxa (e.g. cosmopolitan diatom species, and phytoplankton such as *Dictyocha* spp. and invertebrate larvae that are common in both coastal waters and in the open ocean) are unlikely to be useful for directly quantifying changes in concentration during BWE, the open ocean plankton as a whole could be sampled during exchanges to characterise the composition and abundance of the community, with potential application as a measure for helping to verify whether exchanges have taken place.

However, regardless of depth recommendations for BWE (e.g. 2000 m), greater emphasis should be placed on whether the water uploaded in the open ocean is, in fact, sufficiently oceanic (that is, without coastal organisms) to minimise invasion risks. On some shipping routes, e.g. Japan to New Zealand (via Singapore), BWE are likely to be carried out in relatively shallow waters near the influence of large rivers. Such exchanges increase the potential to replenish ballast tanks with harmful organisms and salinity differentials that may influence the dilution efficiency and effectiveness of BWE at expelling the original organisms in the ballast tank.

In summary, we recommended that the use of phyto- and zooplankton communities as indicators of the efficacy of BWE should include assessment of:

- changes in species composition and abundance of the plankton assemblages and sediment-dwelling organisms brought about by the exchange, including sampling of the open ocean water;
- the incidence of coastal versus oceanic, and cold versus warm water species found in ballast tanks and, with regard to the survivorship potential of planktonic organisms during a ship's voyage, the incidence of taxa known to be relatively tolerant of such environments (e.g. dinoflagellate cysts; see Hallegraeff & Bolch 1992, Hallegraeff et al. 1997, and Mountfort et al. 1999);
- the relative abundance of viable phytoplankton communities, which may be indicative of recent BWE (Hallegraeff & Bolch 1992, Hay et al. 1997);
- the presence of selected harmful organisms (e.g. *Asterias amurensis*).

FUTURE RESEARCH

The biological aspects of this study focused on changes in the composition and abundance of planktonic communities and suspended sediment-dwelling organisms, e.g. juvenile spionid worms, which are readily collected in plankton samples. Although such organisms are potentially harmful if translocated to suitable new environments, a greater range of potentially harmful organisms (e.g. coastal fish species and pathogenic bacteria) can be translocated in ships' ballast water than were sampled here. Future studies on the efficacy of BWE for expelling unwanted organisms should consider variation in the effects of exchanges on these additional groups of organisms.

Trading patterns have been shown to have a significant effect on invasion rates in various parts of the world, including New Zealand (Taylor et al. 2000). Ruiz et al. (2000) collated information on coastal marine communities in North America and established that the rate of invasions has increased exponentially over the last 200 yr, and that shipping has been the main vector. The authors concluded that the source regions for invasions corresponded to patterns of trade. The results of the 'Iver Stream' voyage indicated that, in certain situations, shipping routes spanning temperate to tropical regions may be as equally or more effective than conducting BWE. Controlled shipboard trials (ideally on the same ship using the same ballasting procedures but on different voyages) comparing survivorship rates of ballast water organisms on shipping routes that span a range of latitudes would help to clarify the importance of temperature changes within ballast tanks. This would help identify high risk shipping routes, thus facilitating the development of appropriate ballast water regulations and management options. Studies on the survivorship of ballast water organsims should also consider the importance of ship type.

Future research on the usefulness of plankton communities as indicators of BWE should include the collation of baseline information. These include a broad scale biogeographical database on coastal and open ocean plankton communities occurring at different times of the year, while encompassing the main shipping routes. This would improve our understanding of the significance of plankton communities uploaded during BWE. Data on the phyto- and zooplankton communities uploaded on coastal shipping routes also requires consideration (Claudi & Ravishankar 2006). Further, the selection of easily applied indicators of the biological efficacy of BWE requires adequate knowledge on the spatio-temporal distributions of a representative range of ballast water taxa, as well as identification and quantification of the key factors that determine species survivorship en route. These factors include the physiological tolerances of the organisms, food supply, reproductive capacity and containment time, and the interactions between these factors. This baseline information could be used for designing multiple ship surveys on ballast water risks, including further studies on the effectiveness of BWE, and for targeting compliance.

The limited efficacy of BWE as a treatment method for minimising potential future species introductions via ballast water has led to the investigation of technologies to provide more reliable alternatives, some of which show promise, e.g. cyclonic separation, filtration, ultraviolet radiation, oxygen deprivation, heat treatment, ozonation, or a combination of these. Many technological challenges remain, however, including the design of effective as well as economically robust treatment systems that are compact enough them to be retrofitted to merchant ships.

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