

Diatom survivorship in ballast water during trans-Pacific crossings

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Abstract Ship ballast water is believed to be responsible for global dispersal of alien biota; mid-ocean ballast water exchange is most commonly used to mitigate this process. Diatoms are among the most abundant biotic-component in ballast water, yet their invasive biology is poorly understood. To test effectiveness of MOE we examined diatom species composition and cell density in two sets of samples. First, we examined samples collected daily during one 24 days long trans-Pacific crossing in tanks with and without ballast exchanged. Second, we used samples from 23 trans oceanic vessels arriving at Vancouver harbour where diatoms were collected on arrival. Up to 86,429 live diatom cells/l were found in the tanks, ~50% of the samples share up to eight species consistently present. Cell densities and species richness declined over time and with replacement of coastal ballast water by mid-oceanic water. In both data sets diatoms survive in the tanks for as long as 33 days despite ballast exchange.

Keywords Ballast water · Diatoms · Ballast exchange · Ballast water chemistry · Propagule pressure

Introduction

Natural physical and chemical barriers historically provided the separation required for ecosystems to evolve independently and diversify individually. Currently, these barriers are being bridged with globalization and the increase in world trade, facilitating homogenization of the earth's biota (Eno 1996). More conspicuous non-indigenous biota receive greater research attention (e.g., the zebra mussel in the Great Lakes); however, microbes such as exotic diatoms may also carry environmental, economical and health-related detriments (Kawecka and Sanecki 2003; Edlund et al. 2000; Edwards et al. 2001), but often remain unstudied.

Ballast water is the most important vector for transporting aquatic protists, plants and animals (Edwards et al. 2001; McCarthy and Crowder 2000; Lavoie et al. 1999; Cohen and Carlton 1998; Ruiz et al. 1997). Diatoms, microscopic photoautotrophic protists, are one of the most abundant, consistent and diverse microbiota found in ballast water. Their invasive potential, active and effective propagule pressure, is poorly understood and documented only

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in a very few studies (Carlton and Geller 1993; Coste and Ector 2000; Burkholder et al. 2007). This lack of studies likely stems from the assumption that microbiota, in general, are cosmopolitan (e.g., Finlay 2002), an assumption challenged by several recent studies (Casteleyn et al. 2008; Kaczmarska et al. 2009; Kooistra et al. 2008; Medlin 2007). Moreover, our current understanding of diatom α -level morphotaxonomy may not be sufficient to distinguish between native and non-indigenous morphologically similar (semi-cryptic) species.

Available studies demonstrate a decline in abundance of most of the ballast biota during the transit due to stressors such as lack of light (for photoautotrophs), predation, physical damage, and the presence of harmful substances within the tank waters (Dickman and Zhang 1999; Lavoie et al. 1999; Zhang and Dickman 1999). Even with many causes for mortality (Carlton 1985) in tanks, ballast waters may still carry large numbers of viable organisms to the receiving port, whether in vegetative or resting stages (Ohtsuka et al. 2004). Following their release to receiving port waters, many microorganisms, including diatoms, propagate asexually whereby rapidly enlarging their population size (Lavoie et al. 1999). For such microbes, even small initial propagules (in cells per liter) injected repeatedly into a receiving port present an effective means of facilitating alien species establishment. Presently, mid-ocean ballast water exchange is the primary method of propagule pressure reduction (Hallegraeff 1998; Carver and Mallet 2002; Gollasch et al. 2007). Replacement of coastal ballast water by oceanic waters is believed to lower the probability of alien biota survival in the tanks. However, it has been shown that although port water may be close to 100% exchange during ballast treatment, the removal of foreign organisms is less reliable (McCollin et al. 2007).

The first necessary condition for alien diatom invasion is their arrival to an exotic location, or actual propagule pressure. Therefore, to evaluate diatom propagule pressure on the receiving port, we examined the survival capacity of diatoms in ballast tanks in two sets of samples: (1) from ballast water collected daily during a 3 weeks long trans-Pacific voyage in ballast tanks that underwent mid-ocean ballast water exchange (MOE) and those that did not (MON), thereafter referred to as TPV sample set; and (2) analysis of 23 ballast water samples from

transoceanic ships that underwent ballast water exchange (age after exchange ranged between 7 and 33 days) prior to arrival in Vancouver harbour (called Tank Set). Samples from the Tank Set serve as “time in the tank” replicates of the TPV samples where the number of days in the tanks for exchanged samples was 8 days while 27 days in un-exchanged tanks. To the best of our knowledge this is the first study documenting and quantifying daily changes in viable diatom species richness, composition and abundance in relation to chemical and physical properties of the tank waters (e.g., salinity, temperature and mineral nutrients), over the duration of transit in mid-ocean exchanged and un-exchanged ballast tanks and comparing them to typical MOE ballast.

Materials and methods

Sampling procedure

In the following we are describing two data sets (TPV and Tank Set with $n = 23$ ballast samples) that share (1) the biogeographical origin (Pacific), (2) the treatment in form of trans-oceanic exchange (except the two untreated control tanks from the TPV) and (3) the sampling protocol.

All samples were collected in 2007 and 2008 by the same Canadian Aquatic Invasive Species Network (CAISN) sampling team from vessels arriving in Vancouver with the origin of the following ports: Japan, Korea, China, Hawaii, Ecuador, Guatemala and Mexico. The majority of ship types comprised bulk carriers. The age of ballast water in the tanks was calculated in days following mid-ocean exchange and ranged between 7 and 33 days. Ballast and port water samples examined here were integrated from three casts of a 5 l Niskin bottle collected from three depths (0, 2 and 4 m), pooled in a 20 l carboy container and thoroughly mixed. From this, 3 l of water were subsampled for Tank Set samples, preserved with acidified Lugol's solution and shipped to our laboratory.

For TPV samples, ballast water was sampled daily onboard a bulk carrier during crossing of the Northern Pacific Ocean from Hakata, Japan (24 July, 2007) to Vancouver, Canada (20 August, 2007). Four ballast tanks were sampled; tanks 4 port and starboard

(4P, 4S) were control, un-exchanged waters (in total 27 days in tanks) while tanks 5 port and starboard (5P, 5S) underwent mid-Pacific ballast water exchange, in the form of empty-refill, *after sampling* on the day 15 of the voyage (12 days in tanks). Ambient harbour water samples from the source-ports of Hakata and discharge-port of Vancouver were also collected and processed in the same manner as the ballast water samples. From integrated ballast water from three casts (as described above), a known volume between 3 and 6 l of ballast water was filtered through a 20 µm plankton net, and the residue was carefully and thoroughly rinsed into a beaker and withdrawn using a filtering syringe with a 25 µm pore size filter. The filter was placed in a 15 ml Falcon tube filled with 95% ethanol to cover and preserve the concentrated phytoplankton and stored for the duration of the voyage in dark cool storage until processing.

Concurrently with diatoms, tank water salinity, temperature, pH and dissolved oxygen were measured daily in all four tanks at three depths using a YSI 85 probe (Rikly Hydrological Company, Columbus, Ohio). Measurements were taken at three depths (0, 2, 4 m) and mean values were used for analyses. Integrated (as described above) samples for nutrient analysis (PO₄, Si) were filtered (GF/F, pore size 0.47 µm Derlin filter holders) and stored at -20°C until analysis. Measurements were carried out with a Bran & Luebbe Autoanalyzer 3[®] (Norderstedt, Germany) at the University of British Columbia using air-segmented continuous-flow analysis, following procedures detailed by Murphy and Riley (1962) and Armstrong et al. (1967) for soluble orthophosphate and silica, respectively. Chlorophyll *a* (Chl *a*) samples were collected by filtering of a known volume of water (500–1,000 ml) from each carboy onto a 47 mm GF/F filter using vacuum pump and filtration manifold. Filters were kept frozen at -20°C until lab processing. Chl *a* concentrations were measured using the fluorometric method following the protocol described by Parsons et al. (1984). Samples were read after a 24 h extraction in 90% acetone and after acidification.

Diatom sample processing and counts

Two port samples and 54 ballast water samples were processed from the crossing. Into the first week of

the crossing, every sample from ballast tank 5P was selected for detailed examination of species composition and abundances; until day 23 of sampling, every second sample was examined from this tank. Samples from the remaining three tanks were examined every other day for the duration of the crossing.

The treatment for the TPV samples followed the method described below after several protocols were tested for consistency and quality of subsample used for diatom analyses. The method proved reliable in removing cells from the filter and preparing them for investigation with minimal loss of protoplast-containing diatoms. All samples were split and half was archived. For diatom identification and enumeration, the other half-filter (normally 1–3 l or an equivalent of 30–50% of collected sample) was vortexed and removed from 95% ethanol for further cleaning. This filter was gently scraped in seawater to dislodge cells remaining on the filter after vortexing. This solution was passed through a 253 µm sieve to separate large filter fibers. The filtrate was then combined with the 95% ethanol solution containing cells which were initially dislodged via vortexing. At this stage, cells were cleaned two times via centrifugation and re-suspended in filtered seawater. With the final pellet intact, the supernatant was decanted to a final volume of 20 ml. 10 ml were settled in an Utermöhl chamber for at least 15 h, the remaining 10 ml were archived or used for taxonomic examination. Following the removal of all cells, filters were randomly examined with LM and scanning electron microscopy (SEM) to ensure all cells had been dislodged.

Subsamples from the Tank Set were concentrated and 10 ml settled in an Utermöhl chamber. In order to quench the masking effect of Lugol's solution, subsamples of the Tank Set were treated with sodium thiosulfate prior to application of epifluorescence. Cells were counted using a Zeiss Axiovert 200 LM (Oberkochen, Germany) with epifluorescence (HBO 50/AC, Mercury Shortarc). The natural fluorescence of the chloroplast in cells was used as an indication that cells were alive at the time of fixation. Calculated cell densities of the most abundant diatom, *Nanofrustulum shiloi* were rounded to the nearest 100 cells/l. Diatoms were identified to species level whenever possible. Some taxa were also investigated using SEM (JEOL JSM-5600 SEM, Peabody, Mass.) for which diatoms were prepared following Kaczmarzka

et al. (2005) and operated at 10 kV and 8 mm working distance at the Digital Microscopy Facility, Mount Allison University.

Among the diatoms identified we refer to a species commonly reported as *Nitzschia longissima*. It is likely that there exists an undescribed species resembling the latter (Kaczmarek et al. 2007) and for this reason we use the name “*Nitzschia pseudo-longissima*” in quotation marks.

Statistical analysis

Statistical tests were performed on the TPV data set in order to evaluate the effect of environmental variables on the densities of the two most consistently present diatoms in the ballast tanks using Pearson correlation. The correlation coefficients were tested for significance with the Bonferroni test (SYSTAT 10, 2000). Ballast water properties tested included days in the ballast tank or since exchange, temperature, salinity, dissolved oxygen, mineral nutrients (PO₄, Si) and chlorophyll *a*, a proxy for total phytoplankton abundance. Because of the heterogeneity of the 23 ballast samples and absence of environmental tank water data, no statistical analysis was performed on this sample set.

Results

Trans-Pacific voyage—ballast water properties

All chemical and physical properties of tank waters varied over time and with the exchange (Figs. 2, 3), except for pH, which was invariable at 8.0, and so was not included in statistical analyses or discussed further.

Following the uptake of port water, temperature showed a slight gradual increase in both exchanged and un-exchanged tanks, followed by a decrease of roughly 10°C after mid-ocean exchange (Fig. 1). In the exchanged tanks, this decrease was more rapid. Ballast water salinity showed an inverse pattern, and was elevated in exchanged tanks (Fig. 1). Dissolved oxygen concentrations rapidly declined to trace amounts within the first 8 days in all tanks but showed a large increase following exchange, with a gradual decline thereafter in tanks 5P and 5S (Fig. 2). Chlorophyll *a* concentrations declined rapidly in the

first 6 days in all tanks (Fig. 3). Following exchange, chlorophyll *a* concentrations increased slightly then fell once again. Phosphate concentrations closely mirrored the oxygen concentration pattern including a sharp increase, rising from approximately 0.10 to 0.60 µg/l following ballast exchange (Fig. 2). In un-exchanged tanks, phosphate concentrations oscillated between 0.01 and 0.10 µg/l for the duration of the crossing. Silica concentrations increased in all four tanks (Fig. 3). A more marked increase occurred after mid-ocean exchange.

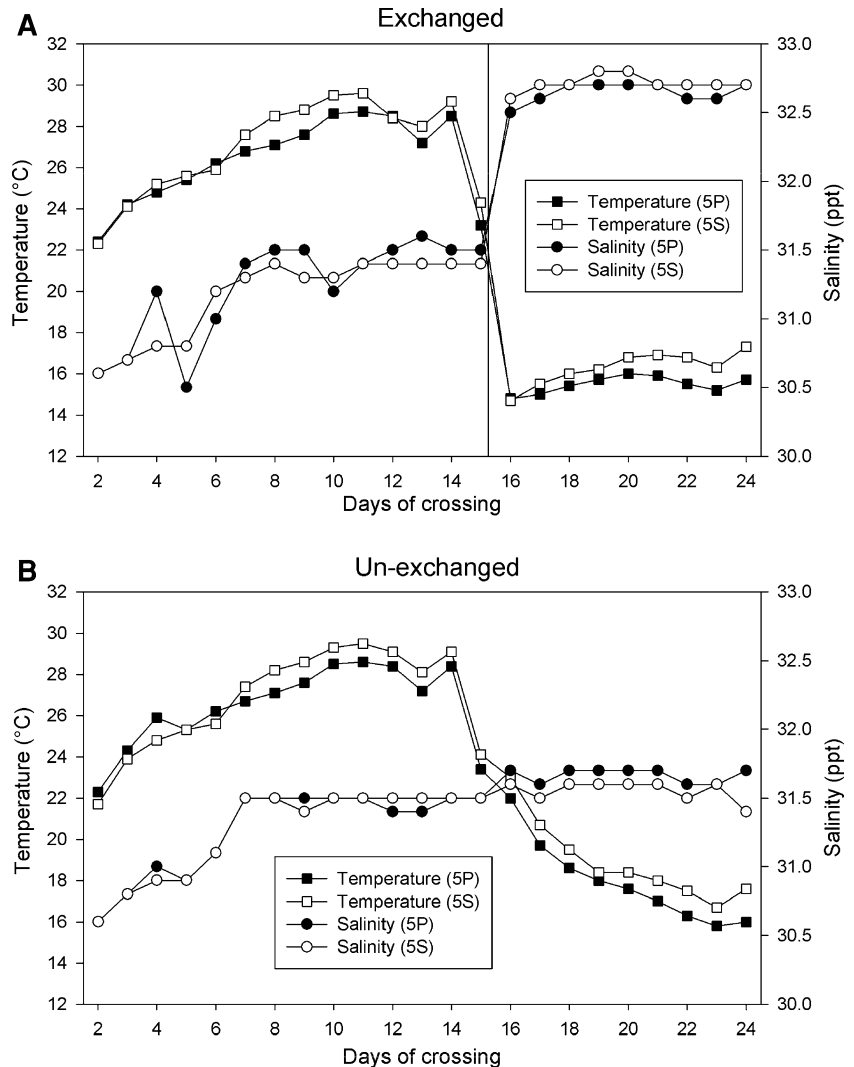
Pearson correlation between the physicochemical properties of the ballast water showed several significant relationships: ballast water salinity and silica concentration were positively correlated to days since ballast water uptake, including uptake at the port and mid-ocean exchange. In contrast, days since ballast water uptake correlated negatively with temperature, dissolved oxygen and phosphate concentrations. Chlorophyll *a* concentrations correlated negatively with salinity and positively with dissolved oxygen. Correlations between physicochemical factors and two most persistent occupants of the ballast tanks (*Nanofrustulum shiloi* and *Cylindrotheca closterium*) are shown in Table 2. In case of *C. closterium* significant correlations were found with salinity ($P = 0.001$), dissolved oxygen ($P = 0.010$) and PO₄ ($P = 0.002$), which suggest a capacity to adapt to adverse environmental conditions as present in ballast tanks.

Diatom species composition

Trans-Pacific voyage

Forty-one diatom taxa (29 species, Table 1) containing epifluorescent chloroplasts were identified in the four ballast tanks. The occurrence of most species was sporadic, present in less than three samples, with very low cell densities. However, consistently present taxa in the exchanged tanks (5P, 5S) could be grouped into three categories: (1) species present over the entire voyage, (2) species present only prior to exchange and (3) species present only after exchange. For example, *Cylindrotheca closterium* and *Thalassiosira eccentrica* were present throughout the crossing in tank 5P while *Actinoptychus senarius* was present only prior to mid-ocean ballast water exchange in tank

Fig. 1 Temperature and salinity in the water of A exchanged (5P, 5S) and B un-exchanged (4P, 4S) ballast tanks. Vertical line indicates mid-ocean ballast water exchange

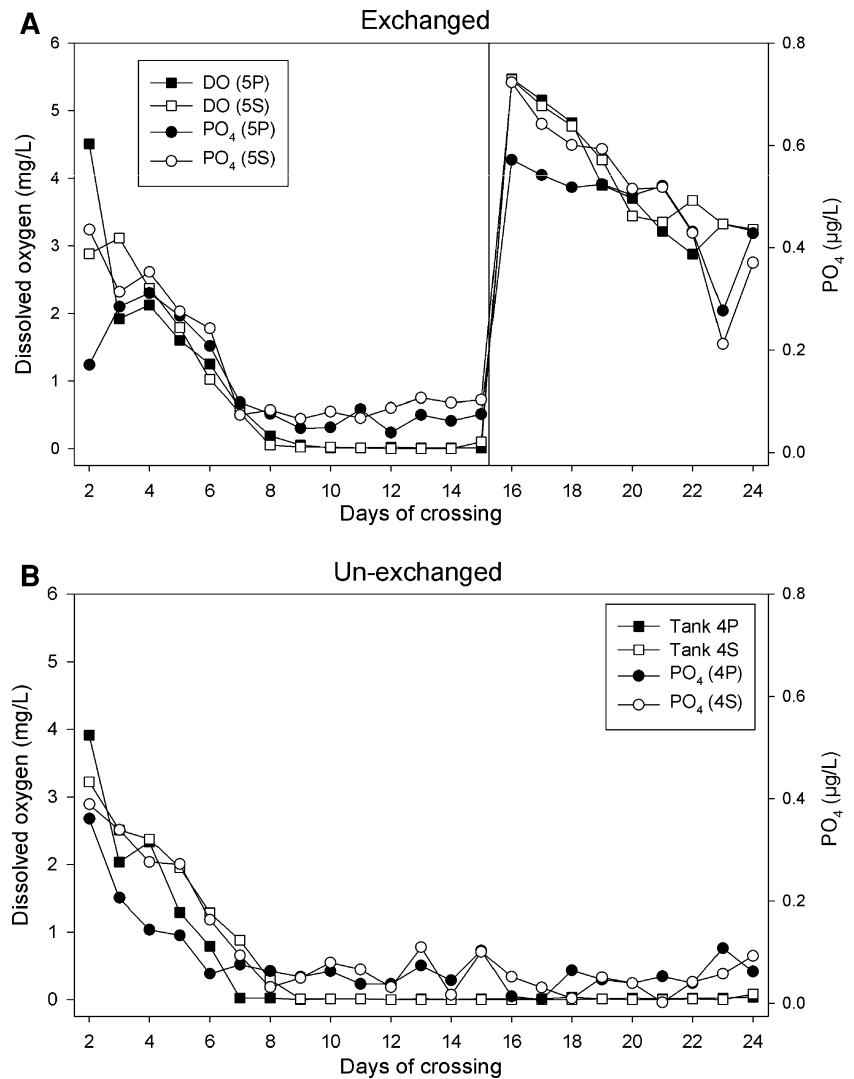


5P. *Corethron hystrix* appeared in samples after mid-ocean ballast water exchange, and was absent in the un-exchanged tanks (Table 1). No pattern in species composition or abundance was evident in the un-exchanged tanks. On arrival in Vancouver after 27 days of voyage, cell densities in the exchanged tanks were four times higher compared to the untreated tanks.

Excluded from the observation is a colony forming, araphid pennate, *Nanofrustulum shiloi* due to high cell densities and obvious exemplary position among the diatoms present in the tanks. This species was present in all but one sample and its populations in exchanged tanks 5P and 5S showed densities ranging from 20 to 29 cells/l to upwards of 10,000 and

3,500 cells/l, respectively. It reached concentrations of 113,300 cells/l in un-exchanged tank 4P on day 7 of the voyage. In both MOE and MON, cell densities rose greatly in some samples (5 of 54 samples), likely as an artifact of the colonial habit of *N. shiloi* when a larger than usual colony happened to be encountered during counts. These five samples did not affect overall trends observed through the voyage. Upon arrival at the receiving port, cell densities were still over 5,000 and 21,000 cells/l in the two exchanged tanks (5P, 5S, respectively), and over 20,000 and 3,000 cells/l in the two un-exchanged tanks (4P, 4S, respectively). *N. shiloi* was also present in Hakata surface water port samples (108,000 cells/l) and Vancouver port samples (90 cells/l).

Fig. 2 Dissolved oxygen and phosphorus concentrations in the water of A exchanged (5P, 5S) and B un-exchanged (4P, 4S) ballast tanks. Vertical line indicates mid-ocean ballast water exchange

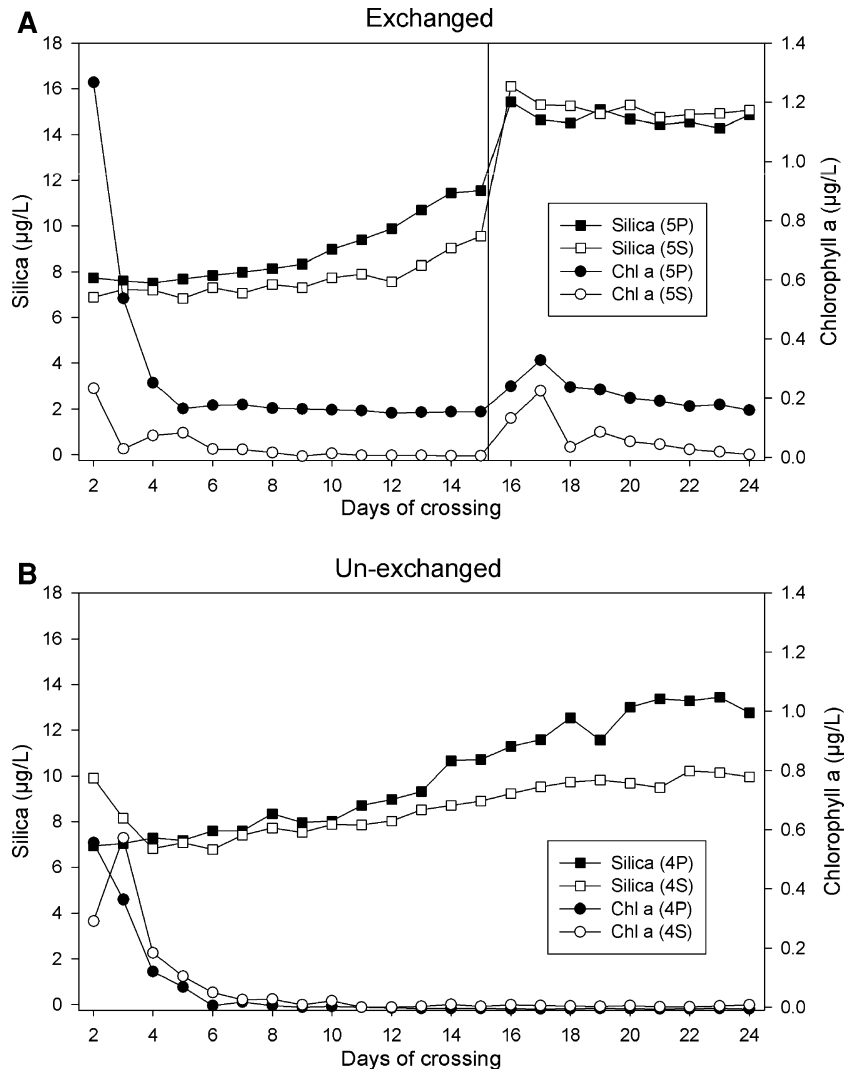


Relating changes in environmental variables and their effects on populations of the majority of species would be meaningless due to the sporadic frequency of most species. Exceptions were *Nanofrustulum shiloi* which was consistently and abundantly present in all tanks, and *Cylindrotheca closterium*, which was present in low cell densities but was persistent in both exchanged and un-exchanged tanks. *N. shiloi* populations were not statistically correlated with any of the environmental variables measured (Table 2). The presence of *C. closterium* in the tanks was negatively correlated with ballast water salinity, and positively correlated with dissolved oxygen and phosphate concentrations.

Tank set

From the previous section it is apparent that some species of diatoms survive a long time in the stressful environment of ballast tanks (Figs. 1, 2, 3) in MOE water for up to 12 days, and even in MON for up to 27 days (Table 1). To establish whether diatom tolerance to the ballast tank environment found in our TPV should be anticipated in other ballast tanks or should be considered an artifact of small sample size, we investigated species abundance and species richness in a total of 23 MOE ballast samples from ships arriving in Vancouver harbour after Pacific passages with ballast waters of at least 7–33 days in

Fig. 3 Silica and chlorophyll *a* concentrations in the water of A exchanged (5P, 5S) and B un-exchanged (4P, 4S) ballast tanks. Vertical line indicates mid-ocean ballast water exchange



the tanks; the minimum age of the ballast water diatoms.

We found viable diatoms in all samples; cell densities ranged from 9 to 86,429 cells/l. We identified 46 diatom genera and 63 species (Table 1). Unexpectedly, freshwater diatoms such as *Asterionella formosa*, *Fragilaria crotonensis* and *Aulacoseira* spp., taken up in the port of origin, were also found epifluorescing in 5 samples representing 9–33 days old ballast waters. Eight of the 46 taxa were found more or less regularly (16 out of 23 samples) and these were: *Skeletonema* cf. *costatum*, *Nitzschia* “*pseudo-longissima*”, *Chaetoceros* spp. (see Table 1), *Cylindrotheca closterium*, *Thalassionema nitzschioides*, *Paralia sulcata*, *Thalassiosira eccentrica* and various *Pseudo-nitzschia* species.

Discussion

Ballast diatom species composition and environment

Literature documenting the survival of diatom cells in ship’s ballast water is scarce compared to the attention paid to dinoflagellates. The reason for this is at least in part the wide spread belief that most diatom species show a cosmopolitan distribution and that photosynthesizing organisms would not survive the adverse conditions during a passage in a ballast tank (Finlay 2002; Drake et al. 2002). Less than a dozen publications published since the 1990s deal explicitly with the examination of diatom survival in

Table 1 Species present (cells l⁻¹) in TPV exchanged (MOE) and un-exchanged (MON) ballast tanks and in Tank Set

Species	Cells l ⁻¹ (n)		
	Exchanged tanks (5P, 5S)	Un-exchanged tanks (4P, 4S)	Tank set (total no. of ships: n = 23)
<i>Actinocyclus</i> Ehrenberg sp.	1–22 (3) B	–	1–3 (3)
<i>A. ochotensis</i> A.P. Jousé ^b	1–3 (2) B	1 (1)	–
<i>Actinoptychus senarius</i> (Ehrenberg) Ehrenberg	1–8 (8) B	4–20 (7)	27–610 (2)
<i>A. splendens</i> (Shad.) Ralfs	–	–	11–131 (2)
<i>Asterionella formosa</i> Hassall	–	–	15–144 (3)
<i>Asterionellopsis glacialis</i> (Castracane) Round	1–24 (5) B	1–36 (3)	3–21 (3)
<i>Asteromphalus</i> Ehrenberg sp.	–	–	13–23 (2)
<i>A. hyalinus</i> Karsten	1–13 (8) A	1 (2)	–
<i>Aulacoseira</i> Thwaites sp.	–	–	1–19 (4)
<i>Bellerochea malleus</i> (Brightwell) van Heurck ^c	–	–	21 (1)
<i>Biddulphia alternans</i> (Bailey) van Heurck. ^c	–	–	3 (1)
<i>Brachysira vitrea</i> (Grunow) Ross	1 (1) B	–	–
<i>Cerataulina</i> Peragallo ex Schütt sp.	1 (1) B	–	–
<i>C. pelagica</i> (Cleve) Hendey ^a	–	–	3 (1)
<i>Chaetoceros</i> Ehrenberg spp.	1–8 (3)	4–12 (4)	5–1,525 (7)
<i>C. atlanticus</i> Cleve	1 (1) A	–	–
<i>C. constrictus</i> Gran	–	–	48 (1)
<i>C. convolutus</i> Castracane ^a	–	–	1 (1)
<i>C. danicus</i> Cleve ^a	–	–	1 (1)
<i>C. debilis</i> Cleve ^a	–	–	3–88 (3)
<i>C. didymus</i> Ehrenberg	15 (1) B	–	–
<i>C. lacinosus</i> Schütt	–	–	56–112 (2)
<i>C. simplex</i> Ostenfeld ^c	–	–	1 (1)
<i>Chaetoceros</i> spores	–	–	1–67 (8)
<i>Coscinodiscus</i> Ehrenberg sp.	1–9 (3)	2–7 (2)	1–8 (2)
<i>C. asteromphalus</i> Ehrenberg ^c	–	–	44 (1)
<i>C. radiatus</i> Ehrenberg & H.L. Smith	–	–	1–3 (3)
<i>Corethron</i> Castracane sp.	–	–	3 (1)
<i>C. hystrix</i> Hensen	2–15 (9) A	1 (2)	–
<i>Cyclotella</i> (Kützing) Brebisson sp.	–	–	3–13 (3)
<i>C. striata</i> (Kützing) Grunow	1–11 (3) B	1–4 (2)	–
<i>C. radiosa</i> (Grunow) Lemmermann	1 (1)	–	–
<i>Cylindrotheca closterium</i> (Ehrenberg) Lewin & Reimann ^a	1–9 (11)	1–13 (18)	3–25 (8)
<i>Dactyliosolen fragilissimus</i> (Bergon) Hasle	–	–	1–12 (4)
<i>Delphineis suriella</i> (Ehrenberg) G.W. Andrews	1 (2)	–	–
<i>Denticula</i> Kützing sp.	–	–	296 (1)
<i>Diploneis</i> Ehrenberg ex Cleve sp.	1–2 (3) B	–	1–3 (2)

Table 1 continued

Species	Cells l ⁻¹ (n)		
	Exchanged tanks (5P, 5S)	Un-exchanged tanks (4P, 4S)	Tank set (total no. of ships: n = 23)
<i>Detonula pumila</i> (Castracane) Gran	–	–	11 (1)
<i>Ditylum brightwellii</i> (West) Grunow	–	–	1–44 (3)
<i>Entomoneis paludosa</i> (W. Smith) C.W. Reimer ^b	–	–	11 (1)
<i>Eucampia</i> Ehrenberg sp.	1 (1) B	–	–
<i>E. zodiacus</i> Ehrenberg ^a	–	–	1–218 (2)
<i>Fragilaria</i> Lyngbye sp.	–	–	83 (1)
<i>F. crotonensis</i> Kitton	–	–	8 (1)
<i>F. investiens</i> (W. Smith) Cleve-Euler ^b	–	–	12 (1)
<i>Grammatophora</i> Ehrenberg sp.	–	–	1–3 (2)
<i>Guinardia delicatula</i> (Cleve) Hasle ^a	13–19 (2) B	–	1–1,002 (5)
<i>Hemiaulus</i> Heiberg sp.	–	–	1 (1)
<i>Leptocylindrus danicus</i> Cleve ^a	–	–	3–8 (4)
<i>L. minimus</i> Gran ^a	–	–	3–261 (2)
<i>Minidiscus trioculatus</i> (Taylor) Hasle	1 (1) B	–	–
<i>Nanofrustulum shiloi</i> (Lee, Reiman & McEnery) Round, Hallsteinsen & Paasche ^b	15–96,765 (27)	73–113,333 (27)	137 (1)
<i>Navicula</i> Bory spp.	2 (2) B	–	1–37 (5)
<i>N. cf. directa</i> (W. Smith) Ralfs ex Pritchard ^c	–	–	3 (1)
<i>Nitzschia</i> Hassall sp.	–	–	13 (1)
<i>N. longissima</i> (Brebisson) Ralfs	–	–	1 (1)
<i>N. “pseudo-longissima”</i>	–	–	1–411 (14)
<i>N. sicula</i> (Castracane) Hustedt ^c	–	–	16 (1)
<i>Odontella aurita</i> (Lyngbye) C.A. Agardh	3 (1) B	–	11–64 (2)
<i>O. mobiliensis</i> (Bailey) Grunow	–	–	27 (1)
<i>O. sinensis</i> (Greville) Grunow ^c	–	–	4–5 (2)
<i>Paralia sulcata</i> (Ehrenberg) Cleve	12 (1) B	–	5–28 (6)
<i>Pinnularia</i> Ehrenberg sp.	–	–	1–3 (2)
<i>Plagiotropis</i> Pfitzer sp.	–	–	3 (1)
<i>P. vanheurckii</i> Grunow ^c	12 (1) B	1–11 (3)	–
<i>Pleurosigma elongatum</i> Wm. Smith	1–3 (6)	1–8 (3)	–
<i>Porosira glacialis</i> (Grunow) E. Jørgensen	–	1 (1)	–
<i>Pseudo-nitzschia</i> Peragallo spp.	2–5 (3)	1–5 (7)	5–1,345 (20)
<i>P. delicatissima</i> (Cleve) Heiden ^a	1–17 (10) A	–	–
<i>P. multiseriis</i> (Hasle) Hasle ^a	6 (1) B	–	–
<i>P. turgidula</i> (Hustedt) Hasle ^{a,b}	1–90 (6) A	–	–
<i>Raphoneis</i> Ehrenberg sp.	1 (2) B	–	–
<i>Rhizosolenia setigera</i> Brightwell	–	–	1–87 (4)
<i>Skeletonema cf. costatum</i> Greville sp.	–	–	3–1,846 (14)
<i>S. costatum</i> (Greville) Cleve ^a	1–3 (2) A	–	–

Table 1 continued

Species	Cells l ⁻¹ (n)		
	Exchanged tanks (5P, 5S)	Un-exchanged tanks (4P, 4S)	Tank set (total no. of ships: n = 23)
<i>Staurosira</i> Ehrenberg sp.	1 (1) B	–	–
<i>Stephanopyxis turris</i> (Greville) Ralfs ex Pritchard	–	–	11 (1)
<i>Synedra</i> Ehrenberg sp.	–	–	3–5 (2)
<i>Tabularia</i> (Kützing) Williams & Round sp.	–	–	1–3 (2)
<i>T. fasciculata</i> (Agardh) Williams & Round	–	–	5–14 (2)
<i>Thalassionema</i> Grunow ex Hustedt sp.	3–9 (3) B	3 (2)	–
<i>T. frauenfeldii</i> (Grunow) Hallegraeff	–	–	1–4 (2)
<i>T. nitzschoides</i> (Grunow) Mereschowsky	–	–	4–436 (8)
<i>Thalassiosira</i> (Cleve) Hasle sp.	1–7 (3) B	1–4 (4)	1–9 (3)
<i>T. angusta-lineata</i> (A. Schmidt) Fryxell & Hasle	–	–	13 (1)
<i>T. antarctica</i> Comber ^b	–	–	96 (1)
<i>T. curviseriata</i> Takano ^b	–	1 (1)	–
<i>T. excentrica</i> (Ehrenberg) Cleve	1–16 (12)	1–12 (11)	1–16 (6)
<i>T. hendeyi</i> Hasle & Fryxell ^b	–	–	1 (1)
<i>T. hyalina</i> (Grunow) Gran ^c	–	–	35–41 (2)
<i>T. nordenskjöldii</i> Cleve	–	–	27–69 (2)
<i>T. proschkinae</i> Makarova ^c	1–5 (4)	1–3 (2)	1–3 (3)
<i>T. punctigera</i> (Castracane) Hasle	–	–	5 (1)
<i>T. tenera</i> Proschkina-Lavrenko	1–3 (2)	1 (1)	–
<i>Thalassiothrix</i> cf. <i>longissima</i> Cleve & Grunow ^c	–	–	5 (1)
<i>Paralia sulcata</i> (Ehrenberg) Cleve	12 (1) B	–	5–28 (6)
<i>Pinnularia</i> Ehrenberg sp.	–	–	1–3 (2)
<i>Plagiotropis</i> Pfitzer sp.	–	–	3 (1)

Number in parentheses following cell count indicates number of samples in which the species was detected. Species' presence in exchanged tank before or after exchange denoted by B and A, respectively

^a Harmful species

^b Not reported from all of Canada

^c Not reported from West Coast of Canada

Table 2 Pearson correlation coefficients for *Nanofrustulum shiloi* and *Cylindrotheca closterium* with environmental variables

	<i>N. shiloi</i>	<i>C. closterium</i>
Days since exchange	–0.111	–0.451
Temperature	0.132	–0.033
Salinity	0.163	–0.606 (0.001)
Dissolved oxygen	–0.128	0.542 (0.010)
PO ₄	–0.154	0.591 (0.002)
Si	–0.150	–0.172
Chlorophyll <i>a</i>	–0.093	0.289

Significant coefficients in bold with Bonferroni probabilities in parentheses

ship's ballast water. Among the pioneers, Dickman and Zhang (1999), Zhang and Dickman (1999) and Hallegraeff and co-workers (references below) were the first to raise attention to the capacity of diatoms to

survive adverse conditions such as found in ballast tanks (e.g., darkness, changes in temperature and salinity) and also their potential to cause harm once established in a new environment (e.g., Hallegraeff and Bolch 1992; Forbes and Hallegraeff 2002). Recent publications by Burkholder et al. (2007) and Zvyagintsev et al. (2009) add to a better understanding by presenting a holistic approach covering processes within ballast tanks and survival of numerous phyla among plankton organisms, thus complementing our findings of species overlap and proximate cell densities. However, the focus was in most cases on tank conditions and in a wider sense zoo/phytoplankton/bacterial composition on arrival. The life conditions of cells (the intact state of the chloroplast serves as proof of viability) were rarely verified which is crucial in order to substantiate the capacity of certain species to survive long periods of time under adverse conditions.

The present results are unique inasmuch as they (1) document daily changes in tank environment beyond routine measurements of salinity, temperature, etc.; (2) we relate these to viable diatom species richness, composition and abundance to a 24 days long TPV; (3) compare viable diatom richness, composition and abundance of the voyage at the receiving port to the viable diatom species richness, composition and abundance in the MOE ballast tanks arriving at the same destination with ballast tank water of similar age; (4) extensive literature search enables us to direct attention to possibly non-indigenous species.

Our data representing the TPV show that many changes observed in the ballast water chemistry, diatom species composition and abundance were time dependent and generally similar in all four tanks up to the date of ballast water exchange. This is in accordance with Dickman and Zhang (1999) who suggest that changes in temperature during the voyage (plus lack of light) impacts the survival of especially tropical species. The authors also report a 87% effectiveness of open ocean exchange in reducing the total abundance of diatoms and dinoflagellates. We calculated the same number comprising the diatom community in our tanks. This high percentage, however, should not obscure the fact that ultimately the high numbers of viable cells discharged (over 86,000 cells/l) present a high propagule pressure and establishment potential. In addition we could show that changes in water chemistry that occurred with mid-ocean ballast exchange were also reflected in the declining phytoplankton abundance (measured as chlorophyll *a*). Chl *a* in all ballast tanks showed that plant mortality occurred quickly after initial uptake of water into the ballast tanks, in conditions of no light and drop in concentrations of nitrate, phosphate and oxygen.

Increasing silica concentrations in the ballast water column was likely due to decomposition of siliceous plankton. The disappearance of organic phosphate from the water column of the tanks, which took place quickly after taking up ballast but increased following ballast water exchange, is often accelerated by precipitation with oxidized metal salts such as aluminum, calcium and iron (Miao et al. 2006). These components are very likely present in many ballast tank waters, and upon a mixing event of mid-ocean exchange, phosphate that has precipitated in

anoxic sediment could be re-mobilized (Miao et al. 2006) and may become biologically active (e.g., heavy metals) and affect diatoms surviving in the ballast tanks.

Of the 84 diatom species found in all ballast water samples (MOE & MON) examined here, most are cosmopolitan and eurytopic. Of these 10 have not yet been reported in Canadian coastal waters (see Table 1), which can be attributed to new introduction but also to the scarcity of publications documenting coastal diatom species composition in our region. Our data show that mid-ocean exchange affected the composition of diatom assemblages within the ballast tanks by introducing new species, and perhaps stirring up the ballast tank residual sediments with accumulated microbiota. The majority of our species (65%) are of coastal origin, only 15% are primarily found in the open ocean such as *Corethron hystrix* and *Asteromphalus hyalinus* (Horner 2002; Hasle and Syvertsen 1997). The latter group of species is thought to be less likely to tolerate the low salinities of coastal ports (Locke et al. 1993). Although the uptake of such species into the ballast tanks was anticipated, they have thus far generated little concern in regard to their potential introduction to new, coastal environments. However, more eurytopic diatom species were also taken up during exchange, such as *Pseudo-nitzschia turgidula*, a species reported as toxigenic in New Zealand (Rhodes 1998) and has not yet established in Canada. The same accounts for other species known to be harmful when occurring in mass blooms, such as *Eucampia zodiacus*, or *Chaetoceros* (e.g., *C. constrictus*, *C. convolutus*) and the presumably cosmopolitan *Skeletonema*. Future studies will show how members of this taxa differentiate/diversify, building up on the study by Kooistra et al. (2008) who found not only semi-cryptic species but also distinct biogeographical ranges. The above named species are considered also eurytopic and cosmopolitan (Hasle and Syvertsen 1997); but in some cases (e.g., *Skeletonema*, *Cyclotella*) the cosmopolitan distribution has been refuted (Medlin 2007). Surprisingly, several freshwater species (contributing ca. 10% to the species identified) were also found in our samples surviving in seawater and mid-ocean exchange, up to 33 days in one case of *Aulacoseira* spp. What should be a natural biogeographical and ecological barrier does not exist for these species which are transported in ballast water

(or sediment) straight into the receiving habitats, of inland waterways or Great Lakes.

Possible survival strategies in ballast water tanks

Two species consistently recovered in our TPV samples illustrate different survival strategies of diatoms. The decline of *Cylindrotheca closterium* correlates to lowered phosphates, dissolved oxygen and length of time in darkness. *Nanofrustulum shiloi* on the other hand showed no interactions with any of the variables measured, indicating that its population was controlled by factors other than those measured in our study (e.g., oxygen, nutrient concentration) or the absence of light, possibly representing one of the more tolerant diatom species. These two species examples underscore the difficulty in formulating general predictions as to which species will respond to stressors acting on biota in the ship ballast environment and over time.

Our unpublished experiments with cultured *N. shiloi* kept in light and dark conditions confirm the long term survival potential of the species kept in darkness. We noted a decline in cell numbers of about 80%, from 24,000 to 5,000 cells/ml within 6 weeks, thus 3 weeks longer than our trans-oceanic voyage. Concurrent LM examination of the chloroplast showed continuous fluorescence and no condensation or shrinking of the cell contents after 6 weeks in darkness, which suggests that cells maintained normal vegetative protoplast constitution, unlike the changes taking place in species such as *Ditylum brightwellii* or *Skeletonema* during resting stage formation (e.g., condensation of protoplast).

Survival in darkness is known among diatoms. Forbes and Hallegraeff (2002) could successfully culture 31 diatom species in light for up to 20 days after transport in ballast water tanks and some were kept alive for up to 37 weeks in darkness. In contrast, Zvyagintsev et al. (2009) reports that the phytoplankton found in oil tanker ballast waters expired within 10 days and attributed this to leaking of oil derived toxins into the ballast water.

Frequent occurrence in tanks of certain diatoms, e.g., *Paralia*, *Skeletonema* and *Nanofrustulum*, suggests that these diatoms may supplement their metabolic requirements within ballast tanks by the use of alternative trophic strategies independent of light

energy. Under conditions of reduced or complete loss of light or low concentrations of mineral nutrients, heterotrophy has been reported in some species of microalgae including diatoms, particularly in benthic habitats (Bavestrello et al. 2000; García et al. 2005; Radchenko et al. 2004). Biosynthetic steps known from heterotrophs and the often found close association of heterotrophic flagellates and bacteria with diatoms suggest evolutionary retainment of genes from the secondary host or horizontal gene transfers from bacteria (Bowler et al. 2008). The photoautotroph-unconventional production of enzymes responsible for energy storage, uptake of reduced carbon and use of multiple forms of nitrogen explain survival in light deprived conditions and fast regeneration after change to light conditions (Armbrust et al. 2004; Weber et al. 2009). *N. shiloi* is likely well adapted to use small biogenic molecules; it was first described as a weakly silicified endosymbiont of the foraminiferan species *Amphistegina lobifera* Larsen, *A. lessonii* d'Orbigny and *A. papillosa* Said. It was only later discovered free living in benthic environments worldwide. It is possible that the adaptations *N. shiloi* evolved for facultative heterotrophy (exchange of organic compounds between host and the symbiont), make this, and perhaps similar diatoms, particularly suited for survival in ship ballast waters, frequently rich in organic compounds. For example, *P. tricornutum* developed strategies to assimilate organic carbon and reduce the light requirement for microalgal biomass production (García et al. 2005). Given the fact that there is a rich supply of organic matter due to decomposition processes in the tank, heterotrophy as a means of supporting survival among diatom species seems reasonable.

With its propensity to form large colonies, *N. shiloi* represents species which could be difficult to integrate into ecological risk assessment models (Hayes 2002). Once entrained within the ballast tank, globular, gelatinous colonies of *N. shiloi* may readily be caught in tank crevices and structures. Our results show that colonies could be retained in the tanks during mid-ocean exchange or de-ballasting at a port. This, coupled with the ability to withstand long periods of darkness, increases the probability of these colony types seeding other ports. Our extensive literature search shows that this species has not yet been reported as an inhabitant of the western coast of North America.

In summary, our data indicate that long term crossing and mid-ocean ballast water exchange is an effective means to reduce transfer of live diatom inocula from coast to coast for the majority of species encountered in this study. However, we also observed that up to 50% of the ballast samples share at least 4 of the 7 regularly occurring species on their arrival. We found that several oceanic species taken up during MOE survive the transfer and were released into the receiving port, some known to be toxicogenic. Furthermore, 10 samples (>20%) carry viable freshwater/brackish water species (e.g., *Asterionella formosa*), with tolerance for low salinities, from the port of origin. They remain within the ballast after MOE and are poised to be released to the receiving, possibly low-salinity port water environment. The number of species combined with the high abundance of cells repetitively discharged into a Canadian port represents a high actual propagule pressure and accompanying potential of successful establishment of a species.

We identified 11 diatom species not yet reported from Canadian coasts, possibly representing new introductions. Considerable cell densities and numerous diatom species arrive alive at the receiving ports after several weeks in the ballast tanks and MOE. Some of these diatoms are true cosmopolites, but others have more restricted distribution and ecology which may involve the capability to supplement their normal photoautotrophy with a heterotrophic mode of nutrition. The latter adaptations render such biota particularly suited to global dispersal through ballast waters despite mid-ocean exchange because ballast tanks often contain residual sediments and settled, decaying biota as a source of organic matter. Considering the fact that also harmful taxa (e.g., *Pseudo-nitzschia*, *Skeletonema*) are transported into new habitats, building expertise in research and monitoring discharge from ballast tanks into receiving ports is important in understanding and preventing the introduction of non-indigenous and potentially invasive microbes.

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