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Research Article

Ballast sediment-mediated transport of non-indigenous species of dinoflagellates on the East Coast of Canada

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Abstract

The presence and abundance of non-indigenous, and/or harmful or toxic dinoflagellate species in ballast sediments is examined for 65 cargo ships visiting ports on the East coast of Canada, as part of the Canadian Aquatic Invasive Species Network (CAISN). Ships visiting several ports in the provinces of Quebec, New Brunswick and Nova Scotia were sampled during three summers (2007, 2008, 2009). These ships included general cargo, bulk carriers and oil tankers, and they represented two major categories: ships undergoing continental and trans-oceanic voyages. Our results show that potentially viable dinoflagellate cysts are present in ballast sediments of all the categories of ships arriving to the East coast of Canada. The concentrations of all types of dinoflagellate cysts are higher in continental ships without ballast water exchange (BWE) than in ships with BWE, including trans-oceanic ships, which presented lower risk of introduction of non-indigenous species (NIS) of dinoflagellates. We identified 14 non-indigenous dinoflagellate cyst species not yet reported from Canadian coasts, including 4 potentially harmful/toxic species, representing a possibility of new introductions. These introductions of toxic NIS could represent a problem for marine Canadian ecosystems, with potentially disastrous effects on fish communities, aquaculture and human health. This potential risk may be facilitated with climate change.

Key words: invasive species, dinoflagellates, cyst introductions, ballast tank, sediment, ballast water exchange, harmful algae

Introduction

Ship transport is a major vector for biological invasions, namely through the seawater carried in ballast tanks (Medcof 1975; Carlton 1985; Ruiz et al. 2000; Fofonoff et al. 2003). Ballast water is carried onboard ships to provide balance stability, and maintain safe transit conditions. Ballast tanks may also carry unpumpable residual water and sediments that can contain viable organisms (Williams et al. 1988; Bailey et al. 2005a). The invasion risk posed by live organisms associated with sediments or residual water from ships visiting the Great Lakes has been examined by Bailey et al. (2005a, b), but these studies did not look at the presence of phytoplankton species. Fahnenstiel et al. (2009) examined dinoflagellate cysts in ballast tank sediments of ships entering the Great Lakes but only for NOBOB (no ballast on board) ships. Ballast sediments from other regions have been shown to contain viable long-lived resting stages of phytoplankton (named cysts or dinocysts in the case of dinoflagellates), notably of non-indigenous and toxic species of dinoflagellates (Hallegraeff and Bolch 1992; Kelly 1993; Hamer et al. 2001; Persson 2002; Pertola et al. 2006). For the present study, we considered "non-indigenous" all species of dinoflagellates that had not been reported before in the scientific literature for the Gulf of St. Lawrence and Eastern Canada.

Approximately 2000 species of living dinoflagellates have been identified and less than 150 are known to produce cysts (Head 1996). At least 90 of these cyst producing species are known to be harmful (Sournia 1995) and a minimum of 45 species are considered as toxic (Sournia 1995; Smayda 1997; Hallegraeff 2003). Cysts are generally produced as part of the life cycle of dinoflagellates, and in some cases in response to adverse environmental conditions (low oxygen concentration, low temperature or light intensity) that may occur in ballast tanks. When conditions improve, dinoflagellate cysts can germinate (e.g. in a new environment), and resume the pelagic phase of their life cycle. Thus, encystment may help the cells survive the voyage, or cysts may be picked up with suspended sediment when ballast tanks are filled. Cases of non-indigenous dinoflagellate species introduction mediated by ballast sediments have been recorded in Australia (Hallegraeff and Bolch 1992; Hay et al. 1997), North America (Kelly 1993; Harvey et al. 1999) and Northern Europe (Hamer et al. 2001; Pertola et al. 2006). The transfer of dinoflagellate species via ballast sediments contributes to the spread of harmful and toxic species and increases the frequency, intensity and geographic distribution of toxic poisoning such as paralytic shellfish poisoning (PSP) (Hallegraeff 1998), which can have a direct impact on human health, fisheries and aquaculture (Hallegraeff and Bolch 1991, 1992; Hallegraeff 2003).

The most commonly used method to reduce the risk of non-indigenous species introductions with current technologies is either open-ocean or mid-ocean ballast water exchange (BWE) (McCollin et al. 2007). The purpose of these exchanges is to replace almost all of the original ballast water from the coastal region of origin, and hopefully remove the sediments as well (IMO 2004). Under perfect conditions, approximately 95% of the ballast water from the original port should be replaced (Rigby and Hallegraeff 1994). In Canada, mandatory ballast water control and management regulations have been applied since June 2006 through the Canada Shipping Act (Office of the Auditor General of Canada 2008). This measure aims to prevent or reduce the release of foreign aquatic organisms and it is enforced for all ships arriving in Canada's coastal waters and particularly in the Great Lakes. There are three categories of ships traveling to Canadian coasts: 1) trans-oceanic ships (TOE) for which BWE is mandatory 2) continental ships traveling within North American waters that are required to do a ballast water exchange (CE) or continental ships for which BWE is not required (CNE) (includes most often oil tankers engaged in coastwise trade or vessels operating exclusively between ports on the East coast of the United States north of Cape Cod) (Canada Shipping Act 2001; United States Coast Guard 1993; Simard and Hardy 2004). There were no NOBOBs examined in this study.

In this study, we examine whether cargo ships visiting the East coast of Canada may be carrying non-indigenous, and/or harmful or toxic dinoflagellate species in their ballast sediments, which could inoculate local waters if discharged. The composition, abundance and percentage of occurrence of dinoflagellates present in ballast sediments from 65 ships is presented, along with pertinent ship information, details of ballast water exchange, including strategies used (empty-refill, flow-trough and no exchange) and age of ballast water. We identified both empty and live dinoflagellate cysts, since an empty cyst may represent a cell that germinated while inside the ballast tank. We also compared results among all categories of ships mentioned above (CE, CNE, TOE) and examined the efficiency of ballast water exchange to remove dinoflagellates from ballast tank sediments.

Methods

Sediment ballast sampling and laboratory procedures

Sampling was carried out during three summers as part of the Canadian Aquatic Invasive Species Network (CAISN). Ships visiting the ports of Sept-Îles, Port Cartier and Baie-Comeau (Quebec) were sampled from June to August 2007. In 2008, ships visiting the ports of Hantsport and St. John (New Brunswick) and Canso, Halifax and Liverpool (Nova Scotia) were sampled from May to July. Finally, in 2009 several CNE ships were sampled in St. John (New Brunswick) (Figure 1). A total of 65 ballast sediment samples were collected from general cargo, bulk carriers and oil tankers (see Appendix 1) - these represent roughly 10% of the yearly number of foreign ships visiting the marine sector of the St. Lawrence (Bourgeois et al. 2001). The CAISN program aimed to sample at least 20 ships from each of the three ship categories (TOE, CE, CNE). Hence ships were selected on an opportunity basis until we had reached a maximum of about 20 ships for each category. Once the ships arrived in port, the sampling team met the captains and asked for the ballast reporting form (required by Transport Canada for all ships entering Canadian waters), they sampled the water column (data not presented here) and waited, when possible, until a tank was completely empty in order to go into the tank for sediment sampling. Sediment samples were taken from one tank per ship, mostly from wing tanks or top side and forepeak tanks according to the quantities of sediments found at the bottom of the tanks (Appendix 1), and the accessibility of the tank. In either case, sediment samples were collected through direct

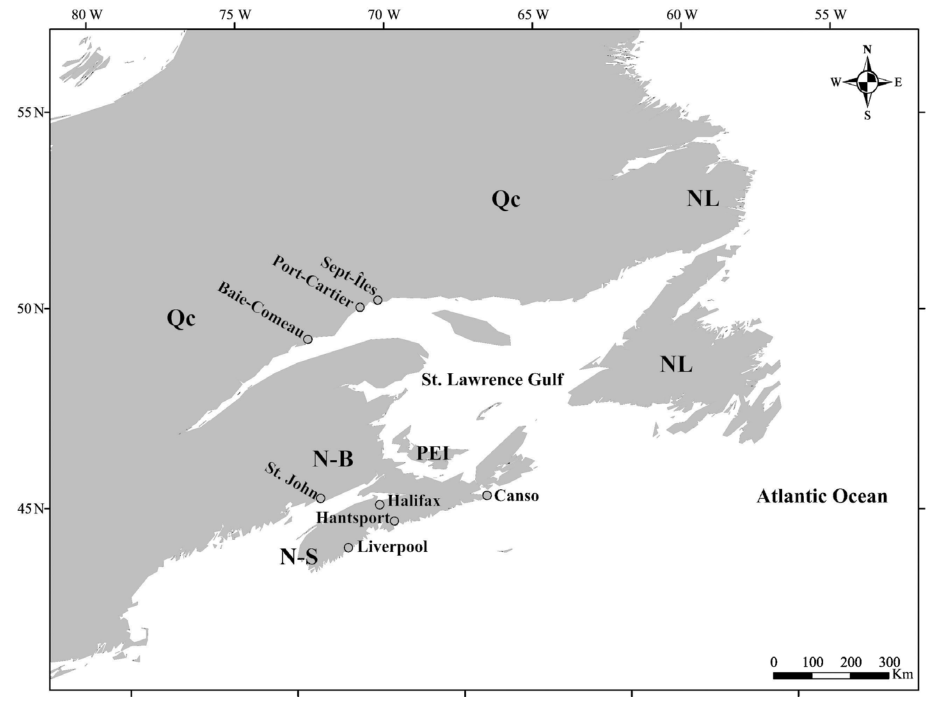

Figure 1. Location of ports where the ships were sampled on the East coast of Canada. QC = Quebec, NL = Newfoundland, N-B New Brunswick, N-S Nova Scotia, PEI = Prince Edward Island. The map was made using the World Robinson projection.

access to the tanks, after complying with security conditions (e.g. prior tank oxygenation). Approximately 500 g of the top 10 cm of the bottom sediments were collected from different areas within each tank, placed in a single plastic bag and stored in the dark at 4°C for a maximum of 12 months, prior to laboratory analysis. The quantities of ballast sediment were estimated by multiplying the surface area covered with sediment, with the average thickness of the sediment layer and correcting for the percent coverage of the sediment in the tank bottom.

All sediment samples were processed at the Institut des sciences de la mer de Rimouski (ISMER), of the University of Québec at Rimouski (UQAR). In the laboratory, each sample was mixed in the bag prior to the analysis and two sub-samples of 5 cm^3 of sediment were taken. The first one was for sediment dry weight determination and the second one for dinoflagellate cysts identification and counts. The first wet sub-sample was weighted and dried in an Imperial II radiant heat oven (Lab Line Instruments Inc.), at 55°C. Dry weight was taken after 48 h. The second wet sub-sample was transferred into a 250 mL beaker with 30 mL of filtered seawater originating from the Sept-Îles region. This seawater had a salinity of 26 and was pre-filtered on hydrophilic polysulfone 0.2 µm pore size membranes (Mini Capsule, Pall Corporation). Samples were manually homogenized and sonicated with a Fisherbrand FS-20® sonicator for 2 – 3 min to help detach the cysts from sand and detritus. These samples were then sieved on a 73 µm Nytex mesh to remove coarse sand followed by a 20 µm Nytex sieve. The fraction larger than 20 µm (which contains the cysts), was sieved twice on the 20 µm Nytex and washed with filtered sea water. Finally, the coarse particles were concentrated in the centre of a Petri dish by circular movements. Dinocysts were identified and counted (more than 200 cysts per sample) using an inverted microscope (Olympus) at 200× to 400× magnification. All specimens were mounted in a "micro slide chamber" following Horiguchi et al. (2000) and observed in a transmitted light microscope (Zeiss Axiovert) at 250× to 1000× in order to improve the identification. Dinocyst concentrations are given per gram of dry sediment.

Germination tests

Dinoflagellate cysts were separated into empty cysts (not viable) and full cysts, which were deemed viable (more precisely potentially viable, using a criterion of cellular content). As there were too many samples to do germination tests for all cysts, we randomly selected over 800 cysts that were tested for germination. To do this, viable dinocysts were isolated, cleaned and cultured in sterilized f/2 medium without silica prepared with filtered seawater from the Sept-Îles region (salinity = 26), sterilized in an autoclave (Guillard 1975). Dinocysts were washed through successive transfers through three Petri dishes with f/2 medium. They were then put in a 24-well Falcon® non-tissue culture treated plate, with each well filled with 2 ml of f/2 medium. These plates were placed in an environmental chamber at a temperature of 15°C, and a 14L: 10D photoperiod, provided by Phillips cool white fluorescent lamps with a light intensity between 100 and 120 µE m^{-2} s^{-1}. Each Falcon® plate was observed daily during 10 days and then once a week during 4 weeks. Successful germination of cysts was used to verify their viability and to observe the archeopyle, which facilitates identification.

Nomenclature of dinoflagellate cysts

The taxonomical nomenclature used in this work follows Blanco (1989a, 1989b), Fensome et al. (1993) and Rochon et al. (1999), with help also from de Vernal et al. (2001), Head et al. (2001, 2005), Mudie and Rochon (2001), Louwye et al. (2004) and Head (2007). Some species in our samples are similar to those from Russian East coasts described by Orlova et al. (2004) and from Baja California, Mexico as illustrated by Peña-Manjarrez et al. (2005). Cysts of *Polykrikos kofoidii* (Chatton, 1914) were compared with cysts described by Matsuoka and Fukuyo (2000) and with illustrations in Radi et al. (2001). Some taxa of e.g. *Brigantedinium cariacoense* (Wall, 1967), *B. irregulare* (Matsuoka, 1987) and *B. simplex* (Wall, 1965) were identified from the shape of the archeopyle and compared with description and illustrations in Rochon et al. (1999) and McMinn et al. (2010). Finally, dinoflagellate cyst names (paleontological names) and their corresponding biological affinities (thecate names) were provided following Head (1996), Lewis et al. (1999), Pospelova et al. (2002, 2006) and Ellegaard et al. (2003) (see Table 1).

Statistical analysis

Ships were separated into three categories depending on the type of voyage and ballast water exchange (see above). We verified whether the location of ballast exchange was indeed done in coastal or oceanic waters by comparing the location of the exchange with the Exclusive Economic Zone (EEZ) which defines coastal waters as those within ~370 km or 200 nautical miles from coastal regions (United Nations 1982), using ArcView version 9.3. Since this definition of coastal waters is more political than biological, we also plotted the location of ballast exchange sites with respect to Longhurst's biogeographical provinces or biomes (Longhurst 1998).

Cysts were separated into five categories: viable cysts (VC), empty cysts (EC) and total cysts (TC = VC + EC), as well as total cysts of NIS (TC NIS), empty cysts of NIS (EC NIS) and viable cysts of NIS (VC NIS). One-way ANOVA was performed using PERMANOVA for PRIMER v.6 (Primer-E Ltd). *P*-values were obtained by permutation, not requiring data normality. In addition, independence and homogeneity of dispersions are directly implied by the permutation procedure (Anderson et al. 2008). Analyses were applied in order to 1) examine differences for each dependent cyst variable among ship categories, 2) characterize relationships among trans-oceanic (TOE), continental exchanged (CE) and continental non-exchanged (CNE) transport, as well as the influence of ballast water exchange strategies, including Flow-Through (FT), Empty-Refill (ER) or No-Exchange (NE). The ballast water age (BWA), defined as the residence time of water within ballast tanks, was calculated as the number of days after ballast water exchange (for TOE and CE ships) or after ballast water uptake at the port of origin (for CNE ships). Finally, dinoflagellate concentrations were compared with BWA and the volume of the sediment contained in the bottom of the tanks.

Results

Vessels sampled

A total of 65 sediment samples were collected from 66 foreign ships visiting ports of Atlantic Canada (for one ship visited, there was not enough sediments). Ballast sediments were collected mostly from bulk carriers (58% of all ships), oil tankers (33%), general cargo (8%) and

Table 1. Dinoflagellate cyst names found in this study (paleontological names) and their corresponding biological affinities (thecate names). In some cases, the same name is used for both nomenclatures.

Cyst species Paleontological name	Dinoflagellate motile cell Biological name
Brigantedinium cariacoense (Wall, 1967)	*Protoperidinium avellana* (Meunier, 1919) Balech, 1974
Brigantedinium simplex (Wall, 1965)	*Protoperidinium conicoides* (Paulsen, 1905) Balech, 1973
Brigantedinium irregulare (Matsuoka, 1987)	*Protoperidinium denticulatum* (Gran & Braarud, 1935) Balech, 1974
Bitectatodinium tepikiense (Wilson, 1973)	*Gonyaulax digitalis* (Pouchet, 1883) Kofoid, 1911
Cyst of *Alexandrium tamarense* (Lebour, 1925) Balech, 1985)	*Alexandrium tamarense* *
Cyst of *Cochlodinium polykrikoides* (Margalef, 1961)	*Cochlodinium polykrikoides* *
Cyst of *Gymnodinium catenatum* (Graham, 1943)	*Gymnodinium catenatum* *
Cyst of *Gymnodinium nolleri* (Ellegaard & Moestrup, 1998)	*Gymnodinium nolleri*
Cysts of *Protoperidinium americanum* (Gran & Braarud, 1935) Balech, 1974	*Protoperidinium americanum*
Cyst of *Pentapharsodinium dalei* (Indelicato & Loeblich, 1986)	*Pentapharsodinium dalei*
Cyst of *Polykrikos kofoidii* (Chatton, 1914)	*Polykrikos kofoidii*
Cyst of *Polykrikos schwartzii* (Bütschli, 1873)	*Polykrikos schwartzii*
Cyst of *Scrippsiella trochoidea* (Braarud, 1957)	*Scrippsiella trochoidea* *
Dubridinium caperatum (Reid, 1977)	*Preperidinium meunieri* (Pavillard, 1907) Elbrächter, 1993
Ensiculifera spp. (Balech, 1967)	Cf. *Pirumella quiltyi*
Impagidinium aculeatum (Wall, 1967) Lentin & Williams (1981)	*Gonyaulax* sp. indet.
Impagidinium pallidum (Bujak, 1984)	*Gonyaulax* sp. indet.
Impagidinium paradoxum (Wall, 1967) Stover & Evitt, 1978	*Gonyaulax* sp. indet.
Impagidinium sphaericum (Wall, 1967) Lentin & Williams (1981)	*Gonyaulax* sp. indet.
Islandinium minutum (Harland & Reid in Harland et al., 1981) Head et al., 2001	*Protoperidinium* sp. indet.
Lingulodinium machaerophorum (Deflandre & Cookson, 1955; Wall, 1967)	*Lingulodinium polyedrum* * (Stein, 1883) Dodge, 1989
Nematosphaeropsis labyrinthus (Ostenfeld, 1903) Reid (1974)	*Gonyaulax spinifera* complex
Operculodinium centrocarpum sensu (Wall & Dale, 1966)	*Protoceratium reticulatum** (Claparède & Lachmann, 1859) Bütschli, 1885
Operculodinium janduchenei (Wall & Dale, 1967)	
Polysphaeridium zoharyi (Rossignol, 1962) Bujak et al., 1980	*Pyrodinium bahamense* * Plate, 1906 var. *compressum* (Böhm, 1931)
Quinquecuspis concreta (Matsuoka, 1985)	*Protoperidinium leonis* (Pavillard, 1916) Balech, 1974
Selenopemphix quanta (Wall & Dale, 1968)	*Protoperidinium conicum* (Gran, 1900) Balech, 1974
Spiniferites belerius (Reid, 1974)	*Gonyaulax scrippsae* (Kofoid, 1911)
Spiniferites bentorii (Rossignol, 1964) Wall & Dale (1970)	*Gonyaulax digitalis*
Spiniferites delicatus (Reid, 1974)	*Gonyaulax* sp. indet.
Spiniferites elongatus (Reid, 1974) Ellegaard et al., 2003	*Gonyaulax elongata* (Reid, 1974) Ellegaard et al., 2003 *Gonyaulax spinifera-Spiniferites group*
Spiniferites membranaceus (Rossignol, 1964) Sarjeant (1970)	*Gonyaulax membranacea* (Rossignol, 1964) Ellegaard et al., 2003
Spiniferites mirabilis (Rossignol, 1967) Sarjeant (1970)	*Gonyaulax spinifera* complex
Spiniferites ramosus (Ehrenberg, 1838) Mantell (1854)	*Gonyaulax scrippsae* *Gonyaulax spinifera* complex
Spiniferites spp.	
Stelladinium reidii (Bradford, 1975)	*Protoperidinium* sp. cf. *P. compressum* (Abé, 1927) Balech, 1974
Trinovantedinium applanatum (Bradford, 1977) Bujak & Davies, 1983	*Protoperidinium pentagonum* (Gran, 1902) Balech, 1974)
Tuberculodinium vancampoae (Rossignol, 1962) Wall, 1967	*Pyrophacus steinii* (Schiller, 1935) Wall & Dale, 1971
Votadinium calvum (Wall & Dale, 1968)	*Protoperidinium oblongum* (Aurivillius, 1898) Parke & Dodge, 1976
Votadinium spinosum (Reid, 1977)	*Protoperidinium claudicans* (Paulsen, 1907) Balech, 1974

* Harmful or toxic species

Ro/Ro type ships (1%). Sediment samples were collected mostly from wing tanks or topside tanks (65%), 28% from forepeak tanks (where we found the highest quantities of sediments), 6% from double bottom tanks and only 1% from cargo holds (Appendix 1). These last tanks are the only ones used to transport merchandise, but once the merchandise is downloaded, they are filled up with water in a similar way to the other tanks, to provide stability during voyages.

Information from the Ballast Reporting Forms showed that, of the 65 ships sampled, 65% undertook ballast water exchange (BWE). About 54% of these ships exchanged their ballast waters in a coastal region and 46% in mid-ocean, according to the International Maritime Organization (IMO) forms provided by ship crews. To verify that BWE was done, we used salinity data from ballast water prior to emptying the tank before sediment sampling (whenever this was possible) or from another tank with the same ballast water source. These data showed that the TOE ships had a mean salinity of 34 ± 0.36 (n=12) while CE ships had a mean salinity of 32 ± 0.43 (n=9), which can be used to corroborate that BWE was done in waters of a salinity typical of truly marine environments.

Concerning the mode of BWE, about 33% of all ships exchanged ballast water using the empty/refill method, 32% used the flow-through method (ca 300% tank volume overflow, to achieve about 95% water replacement), 30% did not do any BWE, 2% used alternative methods such as a mix between flow-through and empty/refill methods, and for the remaining 3% of ships, ballast water management was undetermined. The method of ballast water exchange mostly used for TOE ships was FT while CE ships used mostly the ER method (with the exception of EC-28 that used an alternative method with only 20% of ballast volume replacement). We also examined where exactly BWE was done (whether it was in coastal or oceanic waters, according to the EEZ and to Longhurst's (1998) biogeographic provinces – see Appendix 1). About 78% of TOE ships exchanged their ballast water in oceanic waters (at a distance between 417 and 998 km from any coast) and 13% of these ships did the exchange inside the EEZ (9% were undetermined). Based on Longhurst's provinces, most of the TOE ships exchanged ballast in the NADR (North Atlantic Drift) province. For CE ships, 95% of these did the exchange in coastal waters according to EEZ limits, while 5% exchanged ballast outside of this area. Fourteen of 21 CE ships exchanged ballast in Longhurst's NWCS (Northwest Atlantic Shelves) province. The minimum and maximum distance from the coast where CE ships undertook ballast exchange was 30 and 378 km respectively, while the average distance was 203 km. These distances were calculated using geographic information systems analysis (ArcView, version 9.3, Figure 2).

Dinocyst diversity and identification

Dinoflagellate cyst assemblages were characterized by a relatively high diversity, with species belonging to the orders Gonyaulacales, Gymnodiniales and Peridiniales, including Calciodinelloides and Diplopsaloides. In terms of number of species, diversity varied from 2 to 24 species for TOE ships, with an average of 15; from 3 to 17 species for CE ships, with an average of 16; and from 8 to 17 species for CNE ships with an average of 10. Appendix 2 gives a detailed list of all species identified.

A total of 51 taxa belonging to 40 genera were identified in the ballast sediments among which, 9 that could not be identified to the species level. Eighteen different species of cysts were common to all categories of ships and *Brigantedinium simplex*, *B. cariacoense* and the round brown protoperidinioid cysts dominated dinoflagellate cyst assemblages. Those assemblages were accompanied by *Dubridinium caperatum*, *Quinquecuspis concreta*, and *Votadinium calvum*. Cysts of the Gonyaulacales were dominated by the potentially harmful species (indicated with an asterisk after the name) *Operculodinium centrocarpum** and *Scrippsiella trochoidea**. Cysts belonging to the order Gymnodiniales were dominated by *Polykrikos schwartzii*. Seven of these 51 taxa are considered as potentially harmful taxa (*Alexandrium tamarense**, *Cochlodinium polykrikoides**, *Gymnodinium catenatum**, *Lingulodinium machaerophorum**, *Operculodinium centrocarpum**, *Polysphaeridium zoharyi** and *Scrippsiella trochoidea**).

In addition, 14 of these 51 taxa are considered as non-indigenous species for the East coast of Canada. Dominant non-indigenous species included cf. *Polysphaeridinium zoharyi**, *Polykrikos kofoidii*, *Lingulodinium machaerophorum** and *Gymnodinium catenatum**. These species were recorded with empty and potentially viable cysts, posing a potential risk to marine ecosystems in case they germinate. In addition, 4 of these 14 non-indigenous species

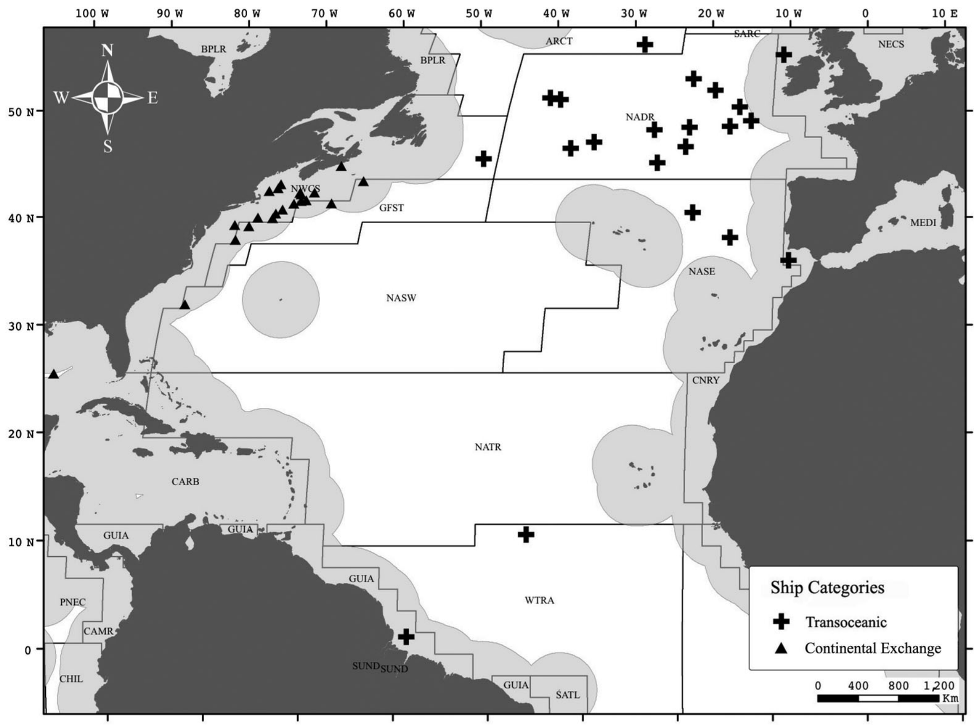

Figure 2. Location of ballast water exchange sites for ships arriving to the East coast of Canada. The light grey zone indicates the exclusive economic zone EEZ (370 km or 200 n.m.). The map was made using the World Robinson projection. Partitions correspond to the provinces defined by Longhurst (1998), ARCT Atlantic Arctic Province, SARC Atlantic Subarctic Province (Atlantic Polar Biome); NADR North Atlantic Drift Province, GFST Gulf Stream Province, NASW, NASE North Atlantic Subtropical Gyral Province West and East (Atlantic Westerly Winds Biome); WTRA Western Tropical Atlantic Province (Atlantic Trade Wind Biome); NECS Northeast Atlantic Shelves Province, NWCS Northwest Atlantic Shelves Province, CNRY Eastern Canary Coastal Province, GUIA Guinea Coastal Province (Atlantic Costal Biome); CARB Caribbean Province (Atlantic Trade Wind Biome).

(29%) are potentially harmful or toxic. Six unidentified cyst species were also recorded (Appendix 2).

Abundance of dinoflagellate cysts in ballast sediments

The East coast ballast sediment samples revealed cyst concentrations that ranged from 1 to 220 total cysts g^{-1} dry sediment. Total cyst abundances were significantly different among ship categories (Permanova, $p = 0.01$) (Figure 3). Ships that did not exchange ballast water (CNE), reached the East coast of Canada with the highest abundances of all types of cysts. Empty cyst concentrations were also significantly higher in CNE ships (mean = 46 ± 5 cysts g^{-1} dry sediment; median = 42 cysts g^{-1} dry sediment) than in TOE and CE ships (Permanova, $p < 0.001$). For these two later ship categories, higher values of empty cysts were recorded in TOE ships (mean = 30 ± 6 cysts g^{-1} dry sediment; median = 18 cysts g^{-1} dry sediment) than in CE ships (mean = 22 ± 5 cysts g^{-1} dry sediment; median = 15 cysts g^{-1} dry sediment).

Cysts that were full were considered viable; random tests on over 480 full cysts from various species showed an average percentage of excystment of 38%, indicating that a significant proportion of all full cysts could germinate. Although not significant (Permanova test, $p > 0.05$), the mean concentrations of viable dinocysts were almost the same between TOE ships (mean = 4 ± 1 cysts g^{-1} dry sediment; median = 1 cyst g^{-1} dry sediment) and CE ships (3 ± 1 cysts g^{-1} dry sediment; median = 1 cyst g^{-1} dry sediment), whereas it was higher in CNE ships (mean = 28 ± 10 cysts g^{-1} dry sediment; median = 9 cysts g^{-1} dry sediment) (Figure 4).

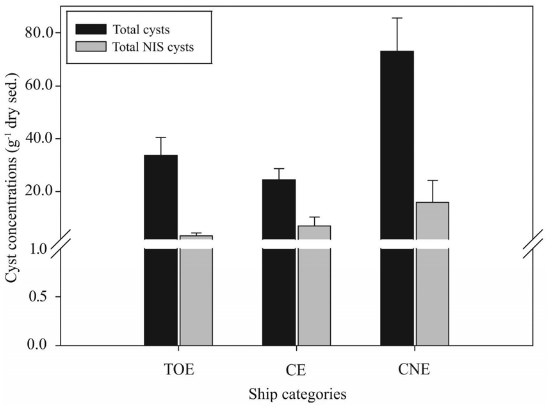

Figure 3. Mean concentration (± standard error) of total cysts (TC) and total NIS cysts (TC NIS) present in ballast sediment for each category of ships. There are significant differences between continental ships without ballast water exchange (CNE) and both transoceanic (TOE) and continental ships with exchange (CE), in terms of TC (PERMANOVA test; $p < 0.05$).

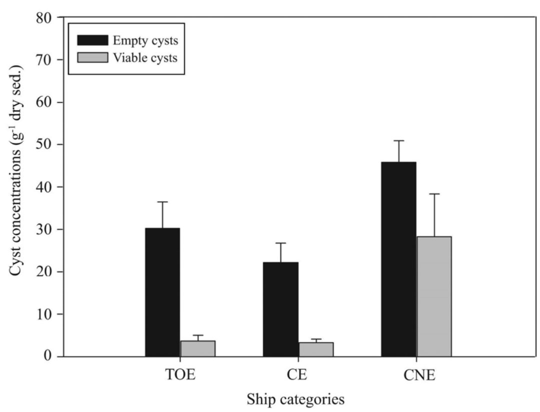

Figure 4. Mean concentration (± standard error) of empty and viable cysts of dinoflagellates present in ballast sediment for each category of ships. Although not significant, values for viable NIS cysts were seemingly higher in CNE vessels compared with CE and TOE vessels (significantly higher for empty cysts: PERMANOVA test, $p < 0.05$).

For empty and viable NIS cysts, there were no significant differences among the three ship categories (Permanova test, $p > 0.05$). Concentrations for empty NIS cysts were similar in CNE ships and CE (mean for both categories = 7 ± 3 cysts g^{-1} dry sediment; median = 3 cysts g^{-1} dry sediment) in contrast with TOE ships (mean = 2 ± 0 cysts g^{-1} dry sediment; median = 0 cysts g^{-1} dry sediment). Although not significant, values for viable NIS cysts were seemingly higher in CNE vessels (mean = 9 ± 5 cysts g^{-1} dry sediment; median = 1 cysts g^{-1} dry

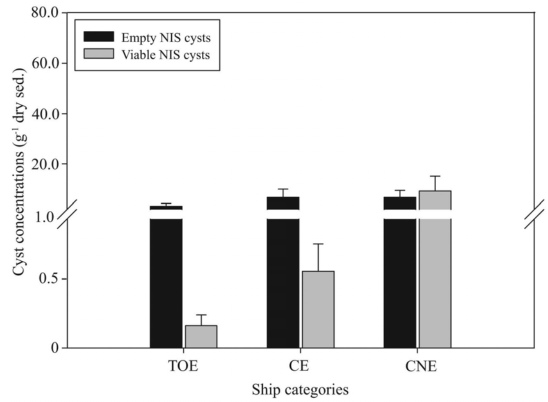

Figure 5. Mean concentration (± standard error) of empty and viable NIS cysts of dinoflagellates present in each category of ships. There are no significant differences among ship categories (PERMANOVA test; $p > 0.05$).

sediment), compared with CE and TOE vessels (mean for both categories = 1 ± 0.1 cysts g^{-1} dry sediment; median = 0 cysts g^{-1} dry sediment), respectively. Finally, the concentrations of viable NIS cysts in CNE ships were also seemingly higher than that of empty NIS cysts, although the differences were not significant (Figure 5).

Percentage of occurrence

Table 2 presents the percentage of occurrence for the dominant dinocyst taxa. *Polykrikos schwartzii* was the most commonly observed species present in the ballast sediment samples (in 78.5% of all ships sampled), across all ship categories, followed by *Brigantedinium simplex* (70.8%), *B. cariacoense* (60.0%), *Quinquecuspis concreta* (55.4%) and *Votadinium calvum* (55.4%) (Table 2).

However there were some dinoflagellate cyst species more commonly observed in categories that performed ballast water exchange. For TOE ships, these were *Bitectatodinium tepikiense* (in 6 ships out of 24), *Operculodinium centrocarpum* (in 12 ships out of 24) and *Spiniferites elongatus* (in 7 ships out of 24), while for CE ships, there was only *Spiniferites ramosus* (in 11 ships out of 21). Finally, *Dubridinium caperatum* (in 10 ships out of 20), the cyst of *Protoperidinium americanum* (in 14 out of 20) and *Selenopemphix quanta* (in 11 ships out of 20) were among the most commonly-present dinoflagellate cyst species for CNE ships, apart from those listed above.

Table 2. Percentage of occurrence for the seven numerically-dominant taxa of dinoflagellate cyst species from sediment samples collected on ships arriving to Canada's East coast. Values in columns 2 to 5 are the number of samples containing the indicated species found in ships from the three categories and from all ship categories (column 5). The last column presents values (as totals for all ship categories) expressed as percentage of the total number of ships (i.e. 24 TOE + 21 CE + 20 CNE = 65 ships). Occ. = occurrence; Nd= no data.

Species	TOE n=24	CE n=21	CNE n=20	All categories	All categories
	Number of ship samples			Total	% Occurrence
Bitectatodinium tepikiense	6	Nd	Nd	6	9.2
Brigantedinium cariacoense	15	12	12	39	60.0
B. simplex	15	13	18	46	70.8
Polykrikos schwartzii	16	17	18	51	78.5
Scrippsiella trochoidea	13	12	9	34	52.3
Quinquecuspis concreta	14	11	11	36	55.4
Votadinium calvum	11	13	12	36	55.4

Percentage of occurrence of NIS cysts

Table 3 presents the percentage of occurrence for all non-indigenous dinocyst taxa. *Polysphaeridium zoharyi** (30.8%), *Lingulodinium machaerophorum** (26.2%) and *Polykrikos kofoidii* (20.0%) were the most frequently found non-indigenous species for all categories of ships (Table 3; Figure 6).

In addition, *Trinovantedinium applanatum* (in 5 ships out of 24) and *Votadinium spinosum* (in 4 ships out of 24) were the most frequently found species in TOE ships. The most commonly observed non-indigenous species for continental ships (with and without ballast water exchange) were *Brigantedinium irregulare* (in 8 ships out of 41) and *Cochlodinium polykrikoides** (in 10 ships out of 41).

Ballast water exchange strategies

Figure 7 and the statistical analyses using Permanova show that total and empty cyst abundances were significantly higher in ships with no BWE compared to ships with BWE. Viable cyst abundances were also significantly higher in non-exchanged ships ($p < 0.05$). In contrast, ballast water management practices (FT, ER) have no significant effect on total or viable cysts of NIS ($p = 0.766$ and $p = 0.069$, respectively).

Ballast water age

Ballast water age was significantly different among TOE and continental (CE and CNE) ships (Permanova test, $p < 0.05$) (Figure 8), while there was no significant difference between the two continental ship categories. The average water age (number of days) was higher for TOE ships (mean = 6 ± 0.5 days; median = 6) than for CE (mean = 3 ± 0.5 days; median = 2) and CNE ships (mean = 4 ± 1 days; median = 2). Ballast water age shows no clear statistically-significant effect on dinoflagellate cyst abundances, even though the two continental categories, with generally shorter trip duration, have higher abundances of total and viable dinoflagellate cysts of NIS. Ships with "younger" ballast water, less than 4 days old, have the highest abundances of viable cysts of NIS.

Ballast sediments

The volume of sediments was significantly different between CNE ships and BWE categories (P< 0.001) (Figure 9A). The highest sediment volumes were recorded in CE ships (mean= 2.02 ± 0.8 m^3; median = 0.31 m^3) followed by TOE ships (mean = 1.3 ± 0.5 ± m^3; median = 0.68 m^3). The lowest quantities were recorded in CNE ships (mean = 0.1 ± 0.05 m^3; median = 0.067 m^3). The two categories performing BWE were not significantly different. Regardless of the ship category, in some ballast tanks, sediments were uniformly distributed on the bottom. In other tanks, sediments were mostly found in tank corners. The shapes and dimensions of tank compartments varied depending on the tank sampled, type of ship (e.g. bulk carrier, oil tanker or RO/RO) (Appendix 1) and/or gross tonnage of ships visited (data not shown), making it difficult to get an accurate estimate of the volume of sediment per tank or per ship. Since the shape and dimensions of the various types of tanks may affect the accumulation of bottom sediment, we plotted the sediment volume for the major types of tanks encountered (Figure 9B). The highest

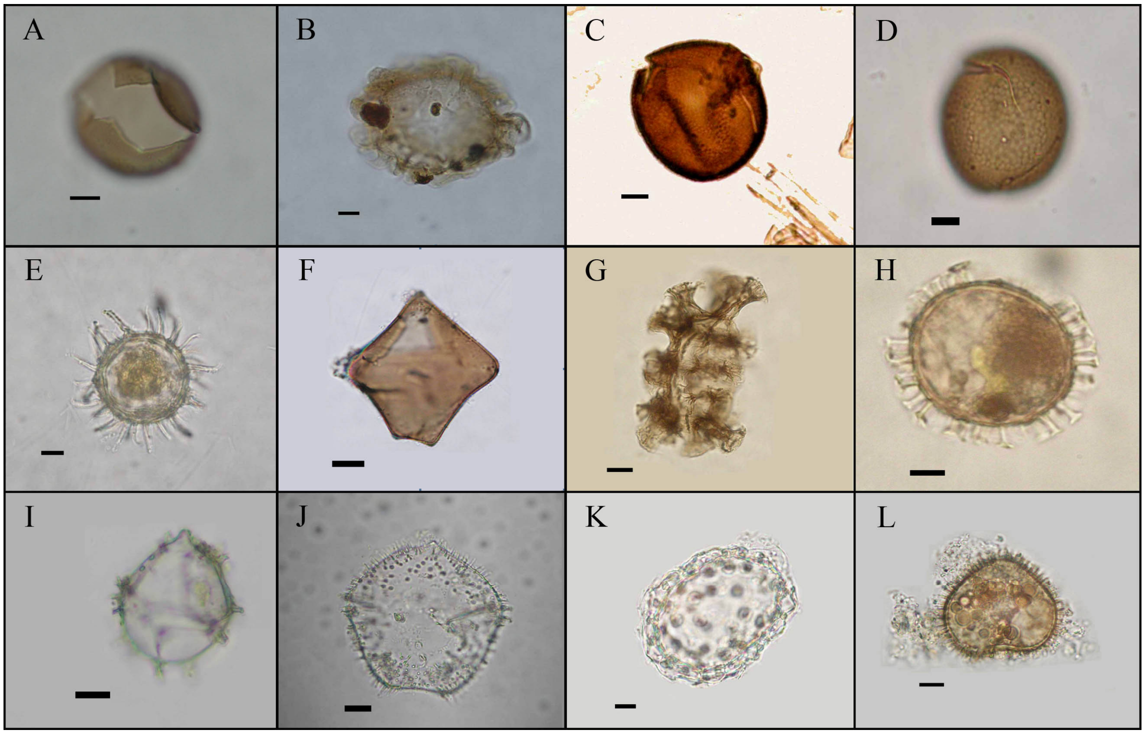

Figure 6. Non-indigenous species found in the ballast sediment of ships sampled on the East coast of Canada: *Brigantedinium irregulare* (A), *Cochlodinium polykrikoides** (B), *Gymnodinium catenatum** (C), *Gymnodinium nolleri* (D), *Lingulodinium machaerophorum** (E), *Protoperidinium latissimum* (F), *Polykrikos kofoidii* (G), *Polysphaeridium zoharyi** (H), *Spiniferites bentorii* (I), *Trinovantedinium applanatum* (J), *Tuberculodinium vancampoae* (K), *Votadinium spinosum* (L). *Fragilidinium mexicanum* and *Stelladinium reidii* are not shown in the plate. Harmful or toxic species are indicated with an asterisk. Horizontal bars indicate 10 µm. Photographs by Oscar Casas-Monroy.

Table 3. Percentage of occurrence for all non-indigenous dinoflagellate cyst taxa from sediment samples collected on ships arriving to Canada's East coast. Values in columns 2 to 5 are the number of samples containing the indicated species found in ships from each ship category and from all of them (column 5). The last column presents values (as totals for all ship categories) expressed as percentage of the total number of ships (65 ships). Occ. = occurrence.

Species	TOE	CE	CNE		
	n=24	n=21	n=20	All categories	All categories
	Number of ship samples			Total	% Occurrence
Brigantedinium irregulare	4	2	6	12	18.5
*Cochlodinium polykrikoides**	1	5	5	11	16.9
Cyst of *Polykrikos kofoidii*	7	3	3	13	20.0
Cyst of *Protoperidinium latissimum*	0	1	2	3	4.6
Fragilidium mexicanum	5	2	1	8	12.3
*Gymnodinium catenatum***	4	5	2	11	16.9
G. nolleri	1	1	0	2	3.1
*Lingulodinium machaerophorum***	8	7	2	17	26.2
*Polysphaeridium zoharyi***	2	13	5	20	30.8
Spiniferites bentorii	0	1	1	2	3.1
Stelladinium reidii	0	0	4	4	6.2
Trinovantedinium applanatum	5	4	1	10	15.4
Turbeculodinium vancampoae	0	1	0	1	1.5
Votadinium spinosum	4	2	0	6	9.2

* Harmful species

** Saxitoxin producers. *L. machaerophorum* is mostly associated with yessotoxin production

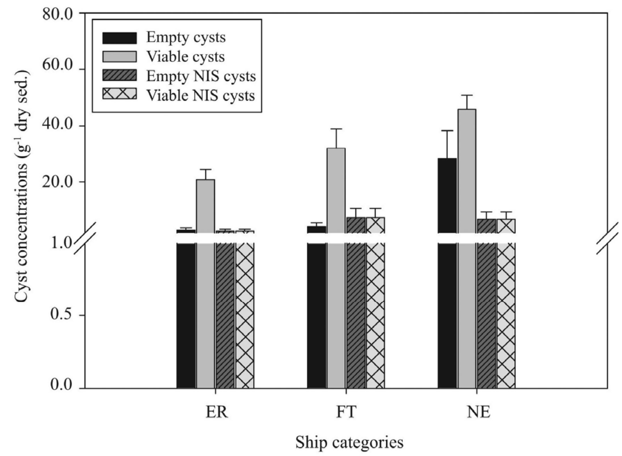

Figure 7. Mean concentration (± standard error) of empty cysts (EC), viable cysts (VC), empty NIS cysts (EC), and viable NIS cysts (VC NIS) of dinoflagellates present in ballast sediments for each ballast water exchange strategy. There are significant differences among ships without ballast water exchange (NE) and ships with exchange (ER = empty-refill, FT = flow-through), in terms of VC and EC (PERMANOVA test; $p < 0.05$).

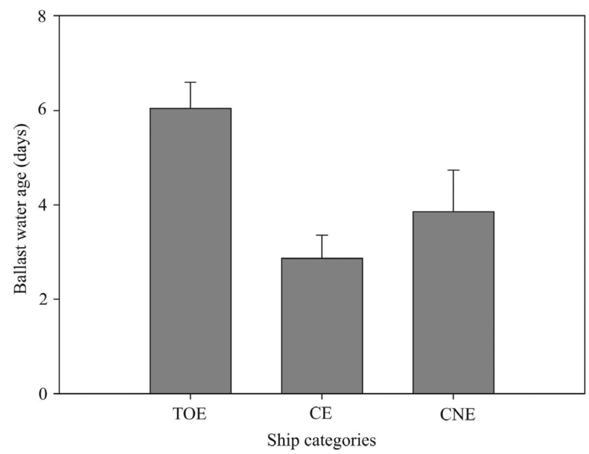

Figure 8. Mean ballast water age estimated for each category of ships. There are significant differences among trans-oceanic and continental ships (CE and CNE) (PERMANOVA test; $p < 0.05$), but no significant difference between the CE and CNE ships.

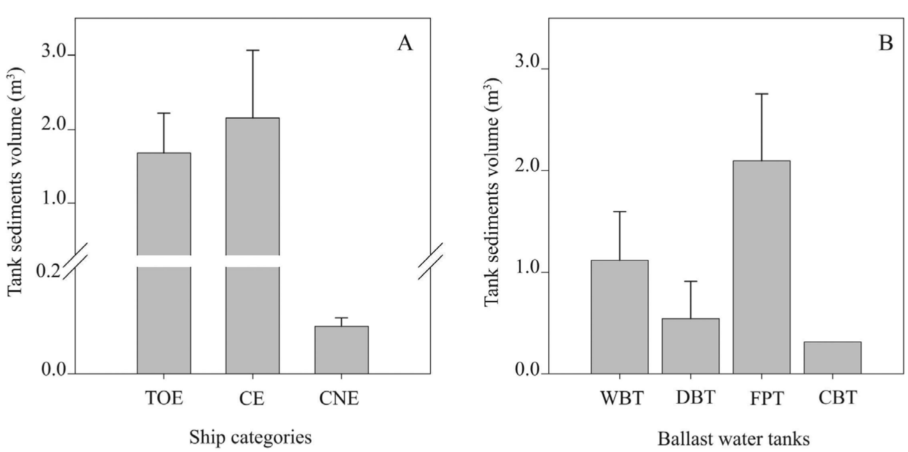

Figure 9. A) Volume of sediments estimated for all ship categories. Differences are significant between continental non-exchanged ships (CNE) and the two ship categories with ballast water exchange (PERMANOVA test; $p < 0.001$). B) Volume of sediments estimated in each type of tanks for all ship categories. WBT = wing tanks; DBT = Double bottom tanks; FPT = forepeak tanks; CBT = cargo ballast tank.

mean sediment volumes were recorded in forepeak tanks (2.1 m^3 ± 0.7 m^3) followed by wing tanks (1.2 m^3 ± 0.5 m^3). The lowest mean quantities were recorded in double bottom and cargo tanks (less than 1 m^3). Sampling was done mostly in wing tanks in the two continental ship categories (15/21 ships for CE and 19/20 ships for CNE), while forepeak and wing tanks were used for trans-oceanic ships (22/24 ships). Although the same type of tanks was sampled for CE and CNE ships, the amount of sediments was clearly different between the two (Figure 9A). Part of the explanation may lie in the different types of ships sampled, with oil or chemical tankers dominating the CNE category while there are more bulk carriers in the CE category (Appendix 1).

Discussion

There are a few studies examining the composition and abundance of planktonic species in ballast water of ships visiting ports of the East coast of Canada, but this is the first extensive study dealing with phytoplankton in ballast sediments for this region. Our results show that cysts of dinoflagellates are present in ballast sediments and many of those are potentially viable (cyst not broken and with cell content, some that successfully excysted in the laboratory). Non-indigenous viable species are present (total abundance = 206 cysts g^{-1} dry sediment for all viable NIS cyst species over 65 ships), as well as some harmful/toxic species, some of which are also non-indigenous. Along with studies from other regions of the world, this confirms the importance of ballast sediment as a vector for the potential introduction of non-indigenous viable organisms between and within different biogeographic zones. Our results also highlight a significant transfer of dinoflagellate cysts, including non-indigenous, harmful/toxic species through continental transport, especially by non-exchanged ships traveling in coastal waters of the East coast of North America.

Previous studies in North America also reported viable phytoplankton contained in ballast sediments of bulk cargo ships using various ballasting practices (Kelly 1993; Harvey et al. 1999). Most studies, including ours, sampled mostly bulk carriers for two principal reasons: 1) these ships carry a large volume of ballast water because, in addition to the tanks generally used for ballast water (such as fore peak, after peak, double bottom and wing tanks), they also often fill empty cargo holds with ballast water to improve stability (Kelly 1993), and 2) they represent 64% of the ships arriving to the East coast of Canada (Bourgeois et al. 2001; Simard and Hardy 2004). In the present study 31% of ships sampled were oil or chemical tankers, and most of these were non-exchanged. Our results show that these ships reached the East coast of Canada with the highest abundances of total NIS cysts (144 cysts g^{-1} dry sediment) and of potentially viable NIS cysts (101 cysts g^{-1} dry sediment) (maximum values found per ship). A previous study carried out for ships arriving into Atlantic Canadian waters, also showed higher phytoplankton abundances in ballast water from oil tankers compared with bulk carriers, general cargo and container ships (Carver and Mallet 2002), however ballast sediments were not examined in that study. Simkanin et al. (2009) found that bulk carriers and oil tankers discharged a greater volume of water per ship than other ship types, (perhaps removing some of the ballast sediment at the same time, which would explain the smaller sediment volume in the CNE ship category in the present work), and oil tankers undertaking coastal voyages discharged significantly greater volumes compared to tankers arriving from overseas to ports of the United States. Moreover, the United States are considered one of the main export regions of ballast water by crude oil carriers (Endresen et al. 2004), contributing with a high number of ship arrivals (higher frequency in the discharge events) on the East coast of Canada. Internationally, oil tankers account for some 37% of the ballast water transported annually, while dry bulk cargo carriers account for 39% (coal, iron ore, grains and other bulk commodities) (Endresen et al. 2004). For this study, we only have data from 13 oil and chemical tankers that discharged about 2.5×10^5 MT (Metric Tonnes) of ballast water. The Canada Port State Control National Ballast Water Database from Transport Canada indicates that bulk carriers discharged more than 10.4×10^6 MT of ballast water during 2008 at all ports of the marine coastal region of the East coast of Canada, while oil and chemical tankers discharged the same volume during the same period. Hence, both categories of ship (carriers and tankers) can strongly contribute to the introduction of species into a new region.

Continental exchanged ships may have implications for the spread of non-indigenous or harmful/toxic species. Our results show that non-living and potentially viable cysts of non-indigenous species are present in 30% of ships arriving to the East coast of Canada. Just in 2008, more than 25×10^6 MT of ballast water were discharged in all ports on this coast (Transport Canada, unpublished data). This transport of water and sediments in ballast tanks of continental exchanged ships poses a risk of introduction and spread of NIS, in spite of the ballast water exchange practice. The role of ballast water exchange is to reduce to a large extent the abundances of non-indigenous species and to increase salinity to protect in-land fresh-water ecosystems (Rigby and Hallegraeff 1994). If we compare the two continental categories CNE and CE, we see that the CNE ships presented significantly higher concentrations for all categories of dinoflagellate cysts compared to CE, for which water exchange relative to the ballast tank volume varied between 90% (E-R) and more than 300% (F-T, see Appendix 1). Ballast exchange in mid-ocean should reduce the amount of sediment (and associated organisms) picked up during ballast intake, and consistent with this, we find lower volumes of sediment in the trans-oceanic ships (TOE) compared to the continental ships with a BWE (CE) (Figure 9). Indeed, a majority of the CE ships exchanged their ballast water within the EEZ or in areas under continental influence, according to the concept of coastal biomes proposed by Longhurst (1998) (cf. Appendix 1). However, the very low sediment volumes observed in the continental non-exchanged ships (CNE) is counter-intuitive. We believe this may be due to the fact that ships of the CNE category were mostly tankers while ships of the CE category were mostly bulk carriers (Appendix 1) hence differences between them cannot be attributed only to the presence or absence of BWE or the specific location to perform BWE. These results also indicate that 1) cyst abundances are highly variable and 2) continental ship traffic (with and without ballast water exchange) represents a risk of introduction of dinoflagellate species as cyst forms, including NIS. These observations, particularly the highest dinocyst abundance in CNE ships, are in accordance with those of Burkholder et al. (2007), who found phyto-plankton abundances 4-fold higher in tanks with coastal water than in tanks with ballast water from the open-ocean.

In addition, the voyages are of shorter duration for continental ships (3–4 days) compared with trans-oceanic ships (6–12 days). The shorter trip duration within the coastal waters of North America can favor the survival of dinoflagellate cysts. Consequently, the shorter voyage duration for continental compared to trans-oceanic ships may also have contributed to the higher concentrations of potentially viable cysts in continental ships. In addition, continental ships (CE) seemingly carry more sediment, increasing the concentration of cysts per tank. When comparing the cysts concentrations and the ballast tank sediment volumes between continental ships, our results show that CNE presented the highest cyst concentrations but the lowest sediment volumes, while CE was the opposite. These results suggest that both categories of continental ships present a risk of NIS introduction. More potentially viable cysts may be found in the upper layers of the ballast sediments if they have been deposited recently, explaining the highest concentration in ships with shorter voyage duration. In that case, more viable cysts could be discharged with sediment slurries when ballast water is discharged in ports. Scientists from Fisheries and Oceans Canada are currently examining whether or not sediments can be discharged along with ballast water during loading operations in port. Preliminary results show that little suspended particulate matter is discharged with ballast water at the beginning of the de-ballasting procedures but it increases towards the end (Nathalie Simard, Maurice Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli QC, personal communication). These results indicate that ballast sediments can be flushed out of ballast tanks and thus could play an important role in the introduction of non-indigenous species. Methods used to estimate the ballast sediment quantities per ship were set-up by the CAISN program and have been used by others in this program (Briski et al. 2010). These results show estimations close to 5 tonnes of sediments per ship (averaged over 17 ships), while our data show an average close to 15 tonnes (over 65 ships). Nevertheless, only a single tank per ship was sampled and often spots and areas with higher sediment accumulation were observed in double bottom, topside or forepeak tanks. Forepeak tanks, due their size, usually have the most sediment (personal observations). Here, the maximum sediment volumes recorded per tank were 16.8 m^3 or 12 tonnes in a topside tank and 12.1 m^3 or 10 tonnes in a forepeak tank.

This study is the first one describing in detail the dinoflagellate cysts present in ballast sediments from ships arriving to the East coast of Canada. The dinoflagellate cyst diversity was relatively higher in CNE ships than in TOE and CE. There were also differences in the dinoflagellate cyst assemblages, for example CE ships were characterized by a higher proportion of Gonyaulacales cysts (37%) while in TOE and CNE ships, this proportion was lower (24% and 19%, respectively). In addition, Gymnodiniales such as *Polykrikos schwartzii* and Peridiniales such as *Brigantedinium* spp., *Dubridinium* spp., *Quinquecuspis concreta* and *Votadinium calvum* were dominant species with highest percentage of occurrence in all categories of ships. Except for *Quinquecuspis concreta* that may be present in open ocean environments around the British Isles (Dodge and Harland 1991), these species occur mostly in neritic areas, in line with the origin of the water used for ballast water exchange or to fill the ballast tanks of CE and CNE ships. Moreover, these dominant species are often considered as biological indicators of eutrophic water conditions in coastal systems (Pospeslova et al. 2006). Considering their usual distribution, it is not surprising to find more Gonyaulacales in CE ships instead of the TOE, even though certain genera such as *Impagidinium*, *Nematosphaeropsis* and *Spiniferites* are often present in open ocean environments (Mudie and Rochon 2001). Species such as *Spiniferites ramosus*, associated with both neritic and oceanic domains (Dodge and Harland 1991), were here more abundant in continental exchanged ships.

Of the 51 dinoflagellate species found in all ballast sediment samples (TOE, CE and CNE), more than 50% are cosmopolitan and distributed from coastal to oceanic environments, and from temperate to tropical regions. Of these, 14 have not yet been reported in Canada's East Coast waters (Appendix 2). Our data show that these non-indigenous species presented higher empty and potentially viable NIS cyst concentrations in continental exchanged ships than in trans-oceanic ships, suggesting that continental traffic could contribute to future invasions. In addition, out of these 14 NIS, 4 are considered as potentially harmful/toxic and their concentrations ranged from 2 to 70 cysts g^{-1} dry sediment. Some of these NIS harmful/toxic species are restricted to tropical and subtropical regions and their invasion threat is low for temperate regions, although climate change may facilitate this. Once a non-native population is successfully established within a port, the port system itself may become a source for subsequent introductions (particularly if cyst resuspension occurs) through passive range expansion, and continental exchange ships may influence this secondary spread of NIS (Wasson et al. 2001; Ruiz et al. 2006).

The proliferation of non-indigenous species of dinoflagellates poses enormous risks, particularly when these are harmful or toxic. These proliferations have had major economic consequences for the Australian aquaculture industry (Hallegraeff 2002). Cysts of potentially toxic algal species found in this study such as PSP producers *Alexandrium* spp., *Gymnodinium catenatum* and *Polysphaeridium zoharyi* or yessotoxin producers such as *Lingulodinium machaerophorum* can remain dormant in sediments for a period up to 10–20 years (Hallegraeff and Bolch 1992). Cysts of species such as *Gymnodinium nolleri* and *Operculodinium centrocarpum** have even been found in viable form after about 37 years in the sediments of Koljö Fjord on the west coast of Sweden (McQuoid et al. 2002). Hence according to our results, coastal shipping represents a higher risk than trans-oceanic shipping in terms of these organisms despite lower abundances (9–541 cysts cm^{-3}, this work) than in previous studies for ships visiting Scottish ports (5–1450 cysts cm^{-3} in ballast water discharge, Macdonald 1995) and Australian ports (40–22 500 cysts cm^{-3}, Hallegraeff and Bolch 1992). For some species, such as *G. catenatum**, the chances of survival under typical Canadian winter conditions are low because this species is restricted to tropical/subtropical regions. It is the same case for species such as the cyst of *Fragilidium mexicanum* mostly found in coastal waters of Mexico, Japan, Russia and Europe (Blanco 1989a; Selina and Orlova 2009), or *Polysphaeridium zoharyi* which was abundant in our samples and is mostly distributed in the Caribbean Sea and Gulf of Mexico. In the case of *L. machaerophorum**, it has been recorded occasionally in sites with mean winter temperature as low as -1.5°C, but this species is generally found mostly in coastal sediments from the subtropical/tropical Southern Hemisphere and from tropical/temperate regions of the Northern Hemisphere. This species is generally reported to be restricted to regions with summer temperatures between 10 to 12°C (Rochon et al. 1999), however, temperatures as low as 4°C do not prevent survival of the cysts (Lewis and Hallet 1997). The presence of cysts of *Cochlodinium polykrikoides** is also worth noting here, since this harmful species seems to be expanding its range and it is generally found in temperate environments (Kudela et al. 2008). Climate change may facilitate the introduction of these toxic species (Dukes and Mooney 1999; Vitousek et al. 1997), increasing the risk to Canada's coastal waters.

In summary, our data show that potentially viable dinoflagellate cysts are present in ballast sediments of three categories of ships arriving to the East coast of Canada. The concentrations of all types of dinoflagellate cysts are higher in continental ships without ballast water exchange than in ships with BWE, including trans-oceanic ships that presented the lowest risk of introduction of NIS for these ballast sediment organisms. Even though CE ships had a lower concentration of dinocysts than CNE ships, sediment volume was higher in CE ships, suggesting that they also present a significant risk to coastal environments. The presence of non-indigenous species in continental ships (particularly non-exchanged ones) may be related to the upsurge in aquatic invasions that have affected some ports on the US Eastern seaboard, such as Chesapeake Bay (Ruiz and Reid 2007).

We identified 14 non-indigenous dinoflagellate cyst species not yet reported from Canadian coasts, including 4 harmful/toxic species, representing a possibility of new introductions. In addition, non-indigenous species were present in close to 30% of the ships arriving to the East coast ports. The risk of new invasions increases with the volume of ballast water discharged in receiving ports and the frequency of ships' visits, but also with the number of NIS released (Carlton 1996; Verling et al. 2005). Some of these non-indigenous dinoflagellate species have a wide distribution (Dodge 1982). However some species found in TOE ships (e.g. *L. machaeorophorum**) live in marine environments with relatively similar temperature and salinity conditions as local coastal ecosystems, which could facilitate their successful establishment. Other species, particularly harmful/toxic species, have a more restricted distribution limited to the tropical and subtropical regions. A warming climate may facilitate the introduction, establishment and spread of these species. Further research on NIS and on ballast water and sediment discharge can help us to understand, prevent and predict new possible introductions of non-indigenous phytoplankton species. Ballast water treatment methods presently available or proposed seem to have limited efficacy for phytoplankton, depending mostly on cost, biological efficacy and environmental impact (Gregg et al 2009; Tsolaki and Diamadopoulos 2010). Dinoflagellate cysts pose significant technological challenges because of their resistance to most treatments (except heat when applicable), and difficulties associated with high flow rates, and large volumes of ballast water and sediments. A combination of two or more methods may increase the efficacy when feasible but further research on IMO-approved treatment methods are needed (Gregg et al. 2009; Tsolaki and Diamadopoulos 2010).

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Supplementary material

The following supplementary material is available for this article.

Appendix 1. Summary of ships sampled, ballast water exchange practice and location.

Appendix 2. Summary of dinoflagellate cyst species found in the sediment samples.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2011/AI_2011_6_3_Casas-Monroy_etal_Supplement.pdf