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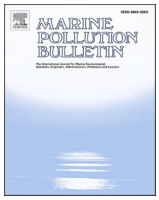

Discharge of ballast residual sediments during de-ballasting procedures: A more realistic estimate of propagule pressure

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ABSTRACT

Ship ballast residual sediments are an important vector of introduction for non-indigenous species. We evaluated the proportion of residual sediments and associated organisms released during de-ballasting operations of a commercial bulk carrier and estimated a total residual sediment accumulation of ~13 t, with accumulations of up to 20 cm in some tank areas that had accumulated over 11 years. We observed interior hull-fouling (anemones, hydrozoans, and bryozoans) and high abundances of viable invertebrate resting stages and dinoflagellate cysts in sediments. Although we determined that <1 % of residual sediments and associated resting stages were resuspended and released into the environment during individual de-ballasting events, this represents a substantial inoculum of 21×10^7 viable dinoflagellate cysts and 7.5×10^5 invertebrate resting stages with many taxa being nonindigenous, cryptogenic, or toxic/harmful species. The methods used and results will help estimate propagule pressure associated with this pathway and will be relevant for residual sediments and nonindigenous species management.

1. Introduction

The introduction and spread of non-indigenous species (NIS) are considered one of the greatest ecological threats to aquatic ecosystems around the world and cost billions (Grosholz, 2002; Molnar et al., 2008; Simberloff et al., 2013; Pyšek et al., 2020; Cuthbert et al., 2021). Ship ballast water and associated sediments are considered an important pathway for species introductions beyond their natural ranges (Carlton, 1985; Ruiz et al., 1997; IPBES, 2023). Although ballast water has been better studied, various studies have raised awareness about the invasion risk associated with residual sediments, which may harbor a large variety of taxa, including phytoplankton, protozoans, invertebrates, and bacterial species (e.g., Bailey et al., 2005; Mimura et al., 2005; Briski et al., 2011a; Casas-Monroy et al., 2013; Villac et al., 2013; Lv et al., 2017; Shang et al., 2019; Lin et al., 2021).

Sediments and associated biota are pumped into ballast tanks during ballasting operations, especially if conducted in ports located in rivers or estuaries and in shallow waters. Thus, sediments may accumulate in tank bottoms as ships cannot be completely emptied during de-ballasting due to structural and pumping limitations (Prange and Pereira, 2013). Sediment accumulation depends on many factors, including the structural complexity within tanks, ballast management practices, sediment loads in entrained ballast water, trade patterns, and ship dry dock frequency (Hamer, 2002; Johengen et al., 2005; Wilson et al., 2006; Bilgin Güney et al., 2018). Consequently, residual sediments ranging from <1 t up to several hundred tonnes may accumulate in ballast tanks (Lucas et al., 1999; Hamer et al., 2000; Duggan et al., 2005; Johengen et al., 2005; Drake et al., 2007; Briski et al., 2010; Casas-Monroy et al., 2011). Estimates of sediment depth in ships varies greatly between studies, ranging from a few centimeters to >50 cm (Lucas et al., 1999; Hamer et al., 2000; Bailey et al., 2005; Gollasch and David, 2016). Even ships using a ballast water management system (BWMS) may have sediment accumulations on tank bottoms (Bailey et al., 2022).

Numerous taxa (e.g., dinoflagellates, copepods, cladocerans, polychaetes) produce resting stages to ensure reproductive success and avoid adverse conditions. During dormancy resting stages may accumulate on sediment surfaces where they may be resuspended during ballasting operations and loaded with ballast water. Residual sediments in ballast

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tanks can provide suitable habitat for dinoflagellate cysts and invertebrate resting stages that may display high richness and abundance, with resting stages remaining viable for decades and able to hatch or germinate when conditions are favourable (e.g., Hallegraeff and Bolch, 1992; Hamer et al., 2000; Bailey et al., 2003; Briski et al., 2011a; Casas-Monroy et al., 2011; Branstrator et al., 2015). Ballast sediments and the biological assemblages they contain may pose risks for receiving regions even if ships have BWMS on board as evidence suggests that some resting stages may be tolerant to ballast water treatment (Bolch and Hallegraeff, 1993; Hallegraeff, 1998; Gregg and Hallegraeff, 2007; Wang et al., 2018; Nwigwe and Kiyokazu, 2023).

Ballast discharge processes may create high-velocity and turbulent conditions on the tank-bottom environment, creating a mixture of ballast water and resuspended sediment, particularly at the end of de-ballasting procedure (Reid et al., 2007). However, several studies have acknowledged that the proportion of sediments expelled from tanks during de-ballasting operations is unknown (Drake et al., 2007; Villac et al., 2013; Gollasch et al., 2019). This information is crucial to obtain more accurate estimates of propagule pressure associated with ballast sediments discharged with ballast water and to improve risk assessments for this pathway.

This study evaluates the proportion of ballast sediments and associated organisms released during de-ballasting operations to improve estimates of propagule pressure. Specific objectives were to: 1) measure the concentration of suspended particulate matter (SPM) in discharged ballast water at regular intervals during de-ballasting operations to estimate the quantity of sediments released; 2) examine in situ sediment dynamics by mapping the distribution of sediments and organisms; 3) estimate the quantity of residual sediments accumulated in ballast tanks by undertaking a detailed quantification of the spatial distribution of residual sediments to estimate the proportion of sediments released; and 4) measure concentrations of dinoflagellate cysts and invertebrate resting stages in residual sediments and their depth-dependent viability.

2. Material and methods

2.1. Ship description and sampling design

Ballast water and residual sediments were collected from a commercial bulk carrier that regularly trades between northern Europe and Canada with occasional trips to Brazil. Built in 1997, the ship's regular trading pattern is such that it sails fully loaded with cargo to Europe and is in full ballast on its return trip to Canada/Brazil. The vessel has a gross register of ca. 100,000 t and a transport capacity of ca. 220,000 t; it has 14 ballast tanks that are 24-m deep, giving a maximum ballast water capacity of ca. 120,000 m^3 (Fig. 1). Ten of the 14 ballast tanks are routinely filled at the port of origin and exchanged before entering Canadian waters (based on the analysis of 20 ballast water reporting forms from 2007 to 2009). Prior to our sampling campaigns, the ship was in dry dock in 2000 (Korea), 2002 (Rotterdam) and 2007 (Portugal) (pers. communication, ship's captain), when accumulated sediments were supposed to be manually removed.

Our original sampling design consisted of sampling water and sediments from the same ballast tanks before and after the ship's return-trip to Rotterdam, Netherlands. However, due to market demands, the ship was diverted from its usual route between sampling campaigns and sailed to Asia between our two samplings. Two sampling campaigns were conducted in May and September 2009 when the ship arrived in eastern Canada. Owing to repeated changes in the de-ballasting schedule and time constraints, we were unable to sample the same ballast tank during both campaigns as originally planned. As such, the #4 starboard (4S; capacity of 5832 m^3) and #1 starboard (1S; 9671 m^3) ballast tanks were sampled in May and September 2009, respectively. Since this ship usually fills all tanks at the same time and location, we assumed that quantities of accumulated sediments and associated biota would be similar between tanks. The 4S tank is divided into three sections (referred to as A, B, and C in Fig. 1) by large transverse frames and each section is perpendicularly subdivided into 14 further sub-sections (5.6 m × 0.9 m) (Fig. 2). Sections were divided by 0.56 m high longitudinal frames spaced 0.86 m apart. Samples were collected in each section (A, B and C) in the 4S tank. The 1S tank is larger and divided into six sections and 13 sub-sections with three of the six sections (referred to as sections D, E, and F in Fig. 1) being sampled (Fig. 3). Because this ballast tank was near the front of the ship, sub-sections D5 through D12 were curved, as were F1 through F5.

Ballast water is introduced and evacuated in ballast tanks through a bellmouth located in the after end (back) of each tank towards the centerline of the ship. The ballast bellmouth had a clearance off the bottom shell of nine centimeters. In addition to the main ballast pumps, the ship was equipped with low volume stripping pumps (eductors) to evacuate residual ballast waters at the end of de-ballasting operations. Clearance of the eductors was four centimeters above the bottom and these pumps work on a venturi system using high-pressure water from the port to create suction.

2.2. De-ballasting operations and ballast water sample collection

During de-ballasting operations, ballast water is first released by gravity. For the purpose of this study, ballast water was diverted to the ballast pump such that water samples could be collected from a spigot located on the structure. Water samples (3 L) were collected from the spigot to assess the concentration of SPM and organic matter (OM) released during de-ballasting operations. During the May sampling campaign, six tanks were de-ballasted simultaneously (1S + 1P, 4S + 4P, and 7S + 7P), first by gravity then followed by pumping. Water samples were collected every 15 min during the first 1 h15 when water was released by gravity (hereafter GO for gravity out). Thereafter, ballast water was released through pumping for a further 2 h20 (hereafter BO for bellmouth pump out). Difficulties were encountered when pumps were turned on, as suction occurred at high pump speeds and it became

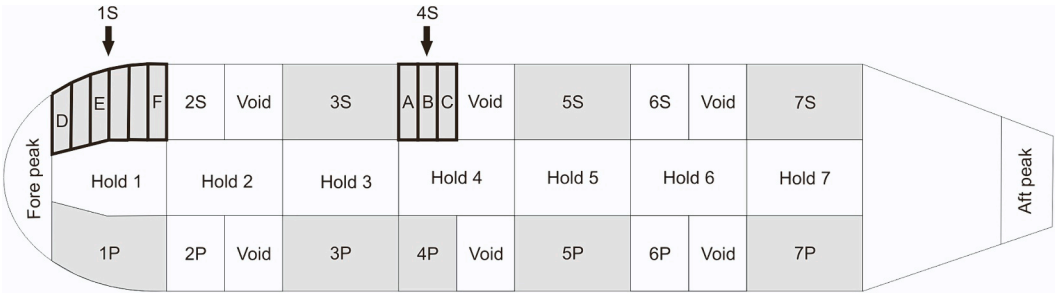

Fig. 1. Schematic diagram (not to scale) showing the vessel's tank arrangement. The grey shaded tanks were in ballast prior to the two sampling campaigns. The three sections identified as "A, B, and C" in the 4S tank (starboard ballast water tank #4) were sampled during the May sampling campaign, and the three sections identified as "D, E, and F" in the 1S tank (starboard ballast water tank #1) were sampled during the September sampling campaign.

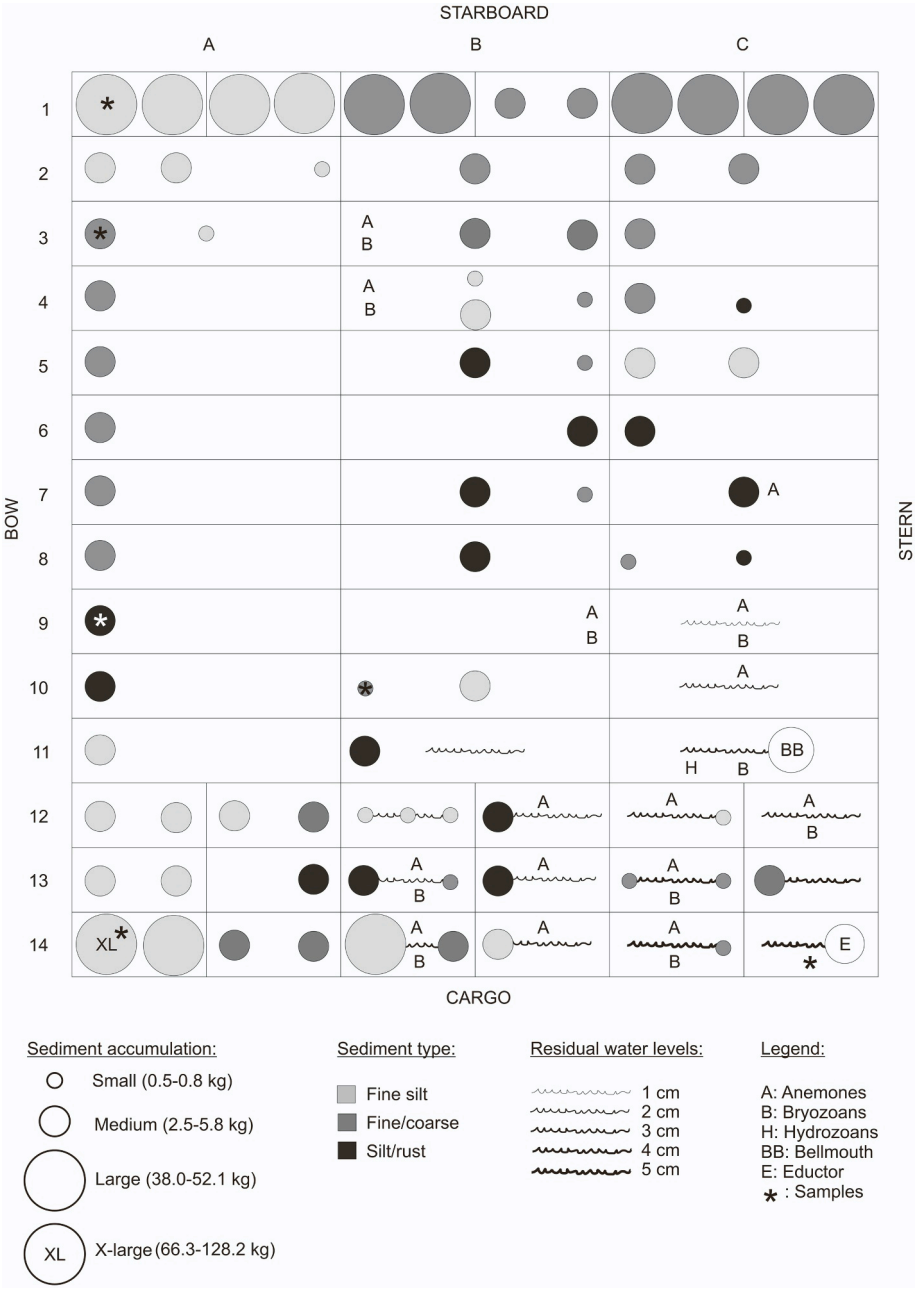

Fig. 2. Schematic diagram of the spatial distribution of residual sediments, sediment types, live organisms, and residual water levels in tank 4S (May). Circles indicate sediment accumulations (estimated minimum to maximum kg wet sediment). Tank sub-sections were flat with the exception of sub-section 1 which had inclined tank compartments (starboard side of ship). The asterisk indicates where sediment samples were collected (A1, A3, A9, A14, B10, and C14). Sediment cores were collected in sections A1 (10 cm) and A14 (18 cm).

difficult to collect water from the spigot. Water samples were thus collected when ballast pumps were turned off momentarily (five times); it was not possible to collect water samples at the end of the de-ballasting operations owing to suction from ballast pumps. During the September sampling campaign, the ship's crew installed a valve near the pump's manifold to overcome the suction problem to allow water samples to be collected every 15 min during the first 5 h of de-ballasting operations. Thereafter, de-ballasting operations were temporarily interrupted for ca. 5 h on the sampled tank as other tanks were being de-ballasted. We resumed collection of water samples once the pumps had been turned on again and collected samples every 5 min during the last 30 min of de-ballasting when the eductor pump was activated (hereafter EO for eductor pump out).

2.3. Suspended particulate and organic matter

To determine SPM, all water samples were immediately brought to the laboratory and three subsamples (500–1000 ml) filtered on pre-burned and pre-weighed Whatman GF/F filters and rinsed with ammonium formate to remove salts. Filters were kept frozen in air-tight plastic Petri dishes until weighed and dried to constant weight at 60 °C for 24 h. The percent organic matter was calculated as the weight loss of dried material combusted at 450 °C for 5 h (adapted from Strickland and Parsons, 1972).

2.4. Residual sediment sample collection

Sediment accumulation and distribution in tanks were quantitatively

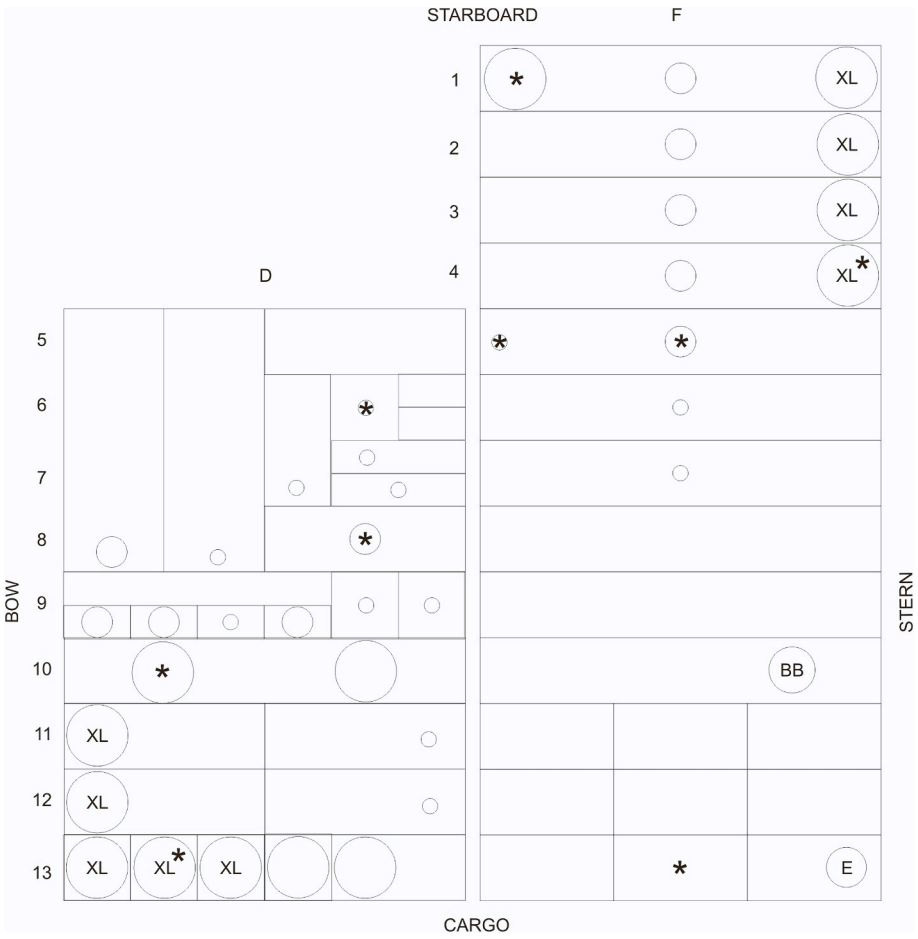

Fig. 3. Schematic diagram of the spatial distribution of residual sediments in tank 1S (September) (see Fig. 2 for a detailed legend). For conciseness, only sections D and F are shown. Circles indicate sediment accumulations (see Fig. 2 for the legend). Twelve small sediment accumulations and two X-large sediment accumulations were observed in section E (not shown). Residual water and bryozoans were present in the compartments near the ballast bellmouth and eductor (data not shown due to logistical and time constraints on the ship). Sub-sections F1 to F5 and all tank sub-sections of section D (with the exception of D13) were inclined. The asterisk indicates where sediment samples were collected (D6, D8, D10, D13, E13, F1, F4, F5, and F13). Sediment cores were collected in sections F4 (13 cm), E13 (10 cm) and D13 (20 cm). No data was collected on sediment type due to time constraints.

assessed during the two in-tank sampling campaigns. Ship and enclosed space entry procedures were followed prior to entering the ship ballast tanks. The spatial distribution of sediments was carefully mapped and a size category (small, medium, large or very large) was assigned to each sediment accumulation present in 4S and 1S tanks. Type (fine silt, mix of fine and coarse sediments, and mix of silt and rust), volume, and weight of residual sediments were assessed, and the presence of live organisms and residual water levels noted. Sediment samples were randomly collected from each of three sections of tanks 4S (A, B and C) and 1S (D, E and F) and from four different sediment accumulation sizes (small, medium, large, and very large) (Figs. 1–3). The volume of each sampled sediment accumulation was assessed by shovelling the accumulated sediments into a pre-marked bucket. Sediment volumes were later converted to wet sediment weight from weighing 250 ml sub-samples in the laboratory. Using our sediment samples, we calculated that 1 m^3 of residual sediment corresponded with an average of 1.3 t of residual sediment.

As sediment accumulations were much greater than expected in the tanks, we modified the sampling protocol to include sediment cores from tank sub-sections with the greatest accumulations. During the May sampling campaign, we collected sediment cores with the equipment on hand (i.e. 60 cc plastic cut-off syringes). A core was collected from a sediment accumulation in sub-section A1 (Fig. 2). Sediments were extruded from the syringes at two cm intervals resulting in five depth strata (0–2, 2–4, 4–6, 6–8, and 8–10 cm). We collected sediments from the top (0–6 cm), middle (6–12 cm) and bottom (12–18 cm) from another sub-section (A14) with high sediment accumulation. During the September sampling, 20 cores (25 cm long and 5 cm diameter) were collected from sub-section D13 where great sediment accumulation had occurred (20 cm) (Fig. 3). Sediments were extruded in the laboratory at two cm intervals from 0 to 20 cm, with the exception of surficial sediments, which were divided into 0–1 and 1–2 cm depth strata, resulting in 11 depth strata. As time was limited and since we could not collect an additional series of cores, we collected sediments from the top, middle, and bottom of two other high sediment accumulations of the tank (E13: 0–3 cm, 3–6 cm and 6–10 cm; F4: 0–4 cm, 4–8 cm and 8–13 cm). A total of 14 and 26 sediment samples was collected during the May and September sampling campaigns, respectively. Sediment samples were subdivided and analyzed for % water content (WC), % OM, granulometry, dinoflagellate cysts, and invertebrate resting stages (methods described in the following sections).

2.5. *Residual sediment characteristics*

Sediment samples were kept cool and in the dark during transport to the laboratory. Approximately 5 cm^3 of sediments were extruded into pre-ashed and pre-weighed aluminum cups, weighed, and dried to constant weight at 60 °C for 24 h. Sub-samples were re-weighed and the difference in weights recorded as water content (%). The percent organic matter was calculated as the weight loss of dried material combusted at

450 °C for 5 h. Sediment grain-size analyses were performed using a Beckman-Coulter LS13320 laser diffraction grain-size analyzer, which has a detection range of 0.04–2000 μm. Samples were deflocculated with sodium hexametaphosphate and mixed for 3 h prior to analyses. The grain size distributions and statistical parameters were calculated using GRADISTAT software (Blott and Pye, 2001).

2.6. Radiometric dating of sediment slices

Sediment age and accumulation rate were estimated from non-destructive determinations of ^{210}Pb and ^{226}Ra (Joshi, 1987, 1989) in 11 sediment slices from the D13 cores. Sediment sub-samples (45 g) were dried and homogenized for each of the 0–2 cm thick slices (0 to 20 cm depth), with the top slice further divided into 0–1 and 1–2 cm sub-slices. Activity concentrations of ^{210}Pb and ^{226}Ra isotopes were measured by gamma spectrometry method using a high purity germamium (HPGe) detector with a relative efficiency of 50 %.

2.7. Isolation, identification and viability of dinoflagellate cysts

One gram of wet sediment was sieved onto a 20 μm nylon mesh with sterile seawater (32 ppt) to remove fine sand, silt and clay. The >20 μm fraction was then transferred into a 15 ml centrifuge tube with sterile seawater. The tube was agitated for at least 2 min to homogenize contents and 1 ml of the sediment/water solution collected with a glass Pasteur pipette and transferred into a Sedgwick Rafter counting chamber where dinoflagellate cysts (with and without cell content) were identified and counted to the genus or species level at 200× using a Nikon Eclipse TE2000-U inverted microscope, based on Rochon et al. (1999) and Gómez (2012, 2013). Counts varied between 188 and 718 specimens and cyst concentrations are expressed in cysts/g of wet sediment. The remaining fraction was transferred into a 90 mm plastic Petri dish and individual cysts with cell content (n = 100 to 300) were isolated using a glass micropipette and washed twice in sterile seawater. Following the second wash, individual cysts were transferred into a well plate (Corning, 24 wells/plate) filled with 2 ml of sterile f/2 – silica culture medium (salinity 32). Plates and Petri dishes were incubated in a Sanyo MLR-351H Environmental chamber at 10 °C with a 12:12 light: dark cycle, and examined daily to monitor excystments, for a maximum of two weeks.

2.8. Enumeration, identification and viability of invertebrate resting stages

Before processing, sediments were homogenized by thorough mixing. To determine resting stage abundance, four 40 g subsamples (not considered as replicates for statistical analysis) were taken from each sediment sample and processed by the sugar flotation method (Briski et al., 2013). Extracted resting stages were counted and a maximum of 20 of them per morphological group was taken for molecular identification (Briski et al., 2011b). Following enumeration and identification, hatching experiments were conducted to determine their viability. To this end, sediment samples were stored in the dark at 4 °C for four weeks to try to break the diapause of resting stages (Schwartz and Hebert, 1987; Dahms, 1995). Four samples (too little quantity of sediment in other samples) from the first sampling campaign (i.e., A3, A14 (0–6 cm), A14 (6–12 cm), and A14 (12–18 cm)) and all samples from the second sampling campaign (D6, D8, D10, D13, E13, F1, F4, F5 and F13) were used for hatching experiments (Figs. 2 and 3). After being extracted from sediments, resting stages destined for hatching experiments were placed into vials containing sterile synthetic pond water (0 ppt; Hebert and Crease, 1980) or a sterile seawater medium with salinity of 15 or 30 ppt. The seawater medium was prepared using natural seawater collected from a vessel loaded with ocean-water ballast, filtered through 2.5 μm Whatman paper filter, and diluted to 15 or 30 ppt with synthetic pond water. Four replicates were placed into each of the 0, 15 and 30 ppt treatments at 20 °C. All experiments were conducted using a light:dark cycle of 16:8 h. Dishes were checked for emergence of animals every 24 h for the first ten days, and every 48 h for the next ten days. Hatched individuals were removed to separate vials for enumeration and identification. Hatching success was calculated by dividing the total number of animals hatched by the total number of resting stages isolated for hatching, and multiplying by 100.

2.9. Proportion of residual sediments released during de-ballasting

To estimate the proportion of residual sediments released during de-ballasting operations, we only considered sediments resuspended during these operations (not suspended particulates present in ballast waters). Thus, the percent (%) of residual sediments released during de-ballasting operations was calculated using the following equations:

$$\textit{Discharged resuspended sediments}\ (DRS) = BW - RS \times SPM - EO \quad (1)$$

where:

- Discharged resuspended sediments (DRS): quantity of residual sediments (kg) that is resuspended at the end of the de-ballasting operations and is discharged through ballast waters in the environment;
- Ballast water with resuspended sediments (BW-RS): ballast water volume (m^3) below the longitudinal frames containing resuspended sediments during EO. This represents the estimated remaining and pumpable ballast water volume present in the tanks when SPM concentration started to increase during EO operations. We assumed this volume to be when the ballast water level reached the height of the longitudinal frames (0.56 m). See Eq. (2).
- Suspended particulate matter during EO (SPM-EO): mean concentration of SPM measured during the last 30 min EO de-ballasting operations.

$$\textit{Ballast water with resuspended sediment}\ (BW - RS) = \textit{Total tank surface area} \times \textit{Height of the pumpable water below the longitudinal frame} \quad (2)$$

where:

- Total tank surface area. See Eq. (3).
- Height of pumpable water below the longitudinal frame = 0.52 m. A height of 0.52 m is used in the calculation to reflect the 0.04 m of unpumpable water (height of the eductor pump from bottom)

$$\textit{Total tank surface area}\ (m^2) = BW - TT\ (m^3)/\textit{Ballast tank height}\ (m) \quad (3)$$

where:

- BW − TT: Total ship ballast water volume = 95,160 m^3; Table 1
- Ballast tank height = 24 m

Thus, total tank surface area = 95,160 m^3/24 m = 3965 m^2.

Finally, we calculated the percent (%) of residual sediments released during de-ballasting as:

$$\textit{Percent}\ (\%)\ \textit{of residual sediments released} = \textit{Discharged resuspended sediments}\ (DRS)/\textit{Total dried residual sediments} \times 100 \quad (4)$$

where:

- Total dried residual sediments: total estimated amount of sediments (wet weight) accumulated in the ship and corrected for WC (Table 1)

2.10. Propagule pressure associated with sediments for dinoflagellate cysts and invertebrate resting stages

The propagule pressure associated with released sediments for dinoflagellate cysts and invertebrate resting stages was calculated as:

$$\textit{Quantity of dinoflagellate cysts or invertebrate resting stages released} = \textit{Total residual sediments} \times \textit{proportion of residual sediments released} \times \textit{mean concentration of organisms} \quad (5)$$

where:

- Quantity of dinoflagellate cysts or invertebrate resting stages: number of dinoflagellate cysts or invertebrate resting stages that are discharged in the environment by this ship at each voyage;
- Total residual sediments: average total estimated amount of sediments (wet weight) accumulated in the ship;
- Proportion of residual sediments released;
- Mean concentration of organisms: mean dinoflagellate cysts concentration or mean invertebrate resting stages density measured in sediment samples.

2.11. Statistical analyses

Variation in suspended particulate matter concentrations measured in ballast waters during GO, BO and EO de-ballasting periods was evaluated using repeated measures ANOVA. Variation in the density of dinoflagellate cysts in sediments was compared using a *t*-test. In both cases, data were square-root transformed to satisfy assumptions of normality and homoscedasticity for the statistical models. Significant differences were evaluated using a posteriori pairwise multiple comparison tests (Holm-Sidak method). Variation in resting stage densities in sediments between tanks was evaluated using Mann-Whitney rank sum test, as data transformations were unable to constrain the data to meet assumptions for parametric tests. A significance level of 95 % was used for all statistical analyses.

Multivariate dinoflagellate community data were compared using non-parametric multivariate analysis of variance using PRIMER 7 (v. 7.0.13) and PERMANOVA+ (v. 1.0.2) (PERMANOVA, Anderson, 2001; McArdle and Anderson, 2001). Data were fourth-root transformed to give greater weight to more rare taxa and the Bray-Curtis similarity coefficient used to calculate the matrix of similarities between samples. Relative similarity among samples is represented graphically using non-metric multi-dimensional scaling ordination and a dummy of 1 was added to address issues related to plots that include samples with zero abundances (Clarke et al., 2006). SIMPER analysis (Clarke, 1993) was used to identify the taxa that contributed the most to the dissimilarity among groups.

3. Results

3.1. Suspended particulate matter release during de-ballasting operations

During the May sampling campaign, SPM concentrations (mean ± SE) measured in water samples were 2.8 ± 1.7 mg/L during the first 1.5 h when water was released through gravity (GO) and 1.0 ± 0.4 mg/L during the following 2.3 h when the pumps were turned on (BO). Overall, SPM measured in water remained low during GO and the first part of de-ballasting operations (BO) and did not differ significantly between periods ($F = 1.634$; $p = 0.223$), averaging 1.9 ± 0.9 mg/L (with 30 ± 2 % for the organic matter). Since no data are available at the end of de-ballasting operations in May due to technical problems (suction from the ballast pump in tank 4S), these results were not used to estimate the quantity of SPM released by this ship during de-ballasting operations. Data obtained from the September sampling campaign were used (see following sections).

During the September sampling campaign, SPM concentrations in water samples differed significantly between GO, BO and EO de-ballasting operations ($F = 71.572$; $p < 0.001$) (Fig. 4). Similar to the May sampling campaign, SPM measured in water samples collected during GO (2.0 ± 0.6 mg/L) and BO (3.1 ± 0.5 mg/L) remained low and did not differ significantly ($t = 1.283$; $p = 0.222$), averaging 2.7 ± 0.4 mg/L (with 18 ± 2 % OM). During the last 30 min of de-ballasting (when the eductor pump (EO) was activated), water samples were visibly cloudier in appearance and SPM concentrations (21.9 ± 1.8 mg/L with 21 ± 2 % OM) increased significantly relative to GO ($t = 9.043$; $p < 0.001$) and BO ($t = 9.857$; $p < 0.001$).

3.2. Characteristics and spatial distribution of residual sediments

Sediments in both tanks were mainly composed of silt (90 ± 2 %), clay (9 ± 1 %), and sand (1 ± 2 %), with a mean grain size of 14.4 ± 2.5 μm. Organic matter averaged 12 ± 1 % and 20 ± 9 % in sediment accumulations in tanks 4S and 1S, respectively; water content averaged 61 ± 3 % (4S) and 51 ± 1 % (1S) (or 54.7 ± 1 % average for both tanks).

The spatial distribution of residual sediments in tanks 4S and 1S are presented in Figs. 2 and 3, respectively. In tank 4S, sediment accumulations varied in size (small, medium, large, and X-large accumulations) between areas of the tanks and generally increased in size with distance from the ballast bellmouth and the eductor (Table 1, Fig. 2). Small (0.7 ± 0.1 kg wet sediment) and medium-sized (4.1 ± 0.7 kg wet sediment) accumulations were mainly observed in the middle and front subsections of the tank (Fig. 2). Mainly "large" sediment accumulations (42.9 ± 4.6 kg wet sediment) were observed in the starboard subsections (A1, B1, and C1) and front cargo side sub-sections (A14 and B14), and the greatest sediment accumulations ("X-large", 87.5 ± 20.3 kg wet sediment) were found in the tank's front corners in A1 (Fig. 2). A similar pattern of residual sediment accumulation was observed in tank 1S during the second sampling (Fig. 3). As for tank 4S, the greatest sediment accumulations were recorded in the front corner of tank 1S with five "X-large" sediment accumulations in sub-sections D11, D12, and D13. Four "X-large" sediment accumulations were also recorded in the curved F1, F2, F3, and F4 starboard sub-sections (Fig. 3).

In tank 4S, we noted up to ca 5 cm of residual water in the compartments adjacent to the ballast bellmouth and eductor, corresponding to the height between the floor and the eductor (Fig. 2). Fouling organisms such as small anemones (*Sagartiogeton undatus*), bryozoans (*Membranipora membranacea* and *Electra pilosa*), polychaetes, and hydrozoans were observed in the compartments with residual waters (Figs. 2 and 5). For example, a total of 53 anemones were counted in B12, and a dozen each in B9, B13, and B14, while a live polychaete (*Alitta succinea*) was recovered in a sediment core collected in subsection A1 (in the 6 to 8 cm depth strata). In tank 1S, no substantial sediment accumulations were recorded in the compartments adjacent to the ballast bellmouth and eductor, but residual water and bryozoans were present in these compartments (Fig. 3).

3.3. Total quantity of accumulated sediment in the entire ship

To estimate sediment accumulations for the entire ship, we assumed that starboard and port tanks had accumulated similar amounts of sediment. Based on the recorded number of sediment accumulations of different sizes in tanks 4S and 1S, we estimated the total average (± SE), minimum and maximum sediment accumulations per tank sections

Table 1
Estimated total sediment (average ± SE, minimum, and maximum, kg wet weight) per accumulation sizes (S, M, L, and XL), sections (A through F) and tanks (1, 3, 4, 5, and 7). Refer to Fig. 1 for schematic diagram and Figs. 2 and 3 for details on sediment accumulations. Total sediment accumulation per tank sections (A through F) were based on the number of different sized sediment accumulations (see the number of S, M, L, and XL accumulations in the notes). The total wet sediment per tank was then estimated based on the total calculated for each section and the number of sections per tank (see notes). Total dried sediment weights are given in parentheses.

Sediment (accumulation sizes)		Total sediment (wet, kg)		Number of sediment accumulations
		Average	Min - Max	
S	Small	0.7 ± 0.1	0.5–0.8	3
M	Medium	4.1 ± 0.7	2.5–5.8	4
L	Large	42.9 ± 4.6	38.0–52.1	3
XL	X-large	87.5 ± 20.3	66.3–128.2	4
Tank (sections)	**Section location in tank**	**Average**	**Min - Max**	**Notes**
A	Front	386 ± 57	307–507	2S + 20 M + 5 L + 1XL
B	Middle	205 ± 26	161–263	9S + 17 M + 3 L
C	Back	213 ± 25	178–267	7S + 9 M + 4 L
D	Front	632 ± 124	499–881	10S + 4 M + 4 L + 5XL
E	Middle	183 ± 42	139–266	12S + 2XL
F	Back	415 ± 90	317–596	3S + 5 M + 1 L + 4XL
Ship (tank nos.)	**BW capacity (m^3)**	**Average**	**Min - Max**	**Notes**
1S, 1P[1]	9671	1779 ± 380 (872)[6]	1373–2540	Sum of sections D, E (four times), and F
2S, 2P[2, 3]	5775	–	–	–
3S, 3P[3]	11,665	1418 ± 188 (553)[6]	1131–1825	Based on tank 4S, with the sum of sections A, B (four times), and C
4S, 4P[2]	5833	804 ± 109 (314)[6]	647–1037	Sum of sections A, B, and C
5S, 5P[3]	11,648	1418 ± 188 (553)[6]	1131–1825	Based on tank 4S, with the sum of sections A, B (four times), and C
6S, 6P[2, 3]	5725	–	–	–
7S, 7P[4]	8763	1073 ± 145 (418)[6]	863–1384	Based on tank 4S, with an additional 33.5 %
Total (10 tanks)	**95160[5]**	**12,984 ± 2020 (5420)[6]**	**10,288–17,221**	**Sum of 10 tanks**

[1] Tanks 1 differ from the others (more curved).
[2] Tanks 2, 4, and 6 have similar dimensions but are half those of tanks 3 and 5.
[3] Tanks 2 and 6 were not in ballast (only on one occasion of 20 trips between 2007 and 2009 according to the ballast water reporting forms provided to Transport Canada).
[4] Tanks 7 were 33.5 % larger than tank 4, thus estimated sediment accumulations are based on accumulations in tank 4S, to which we added 33.5 %.
[5] Does not include tank capacity for fore peak and aft peak tanks and tanks 2 and 6 (not used on a regular basis to carry ballast waters).
[6] Total dried sediment. WC% measured in 4S tank (61 %) was used for 3, 4, 5, 7 tanks and WC% in 1S (51 %) for 1 tanks.

(Table 1). Mean estimated total wet sediment per section varied between 183 and 632 kg. We considered the total sediment accumulation in tanks 4S and 4P to be the sum of sections A, B, and C, thus averaging 804 ± 109 kg (wet sediment) with a minimum of 647 to a maximum of 1037 kg. We assumed that starboard and port tanks 3 and 5 had similar sediment accumulations as those recorded in tank 4S. However, since their size was twice that of tanks 4 (starboard and portside), total sediment accumulation in tanks 3 and 5 was estimated as the sum of sections A, B (4 times), and C, giving an estimated average of 1418 ± 188 kg (wet sediment) with a minimum of 1131 to a maximum of 1825 kg. Tanks 7 were 33.5 % larger than tanks 4 and as such, estimated sediment accumulations are based on accumulations in tank 4S to which we added 33.5 % to give an estimated average of 1073 ± 145 kg of wet sediment (863–1284 kg). For tank 1S, we estimated the total sediment accumulation to be the sum of sections D, E (4 times), and F, resulting in an average of 1779 ± 380 kg (wet sediment) with a minimum to maximum range of 1373 to 2540 kg. Thus, estimated sediment accumulation in the ship was 12,984 ± 2020 kg (10288–17,221 kg) for the ten tanks that were in ballast prior to sampling (Table 1; Fig. 1). A review of the ship's ballast history during the two years prior to our sampling campaigns (data not presented here) showed that these ten tanks were in ballast 85 % of the time, all 14 tanks 10 % of the time, and 8 tanks 5 % of the time.

3.4. Sediment accumulation rate and age

Substantial sediment accumulations were most notable in the front corners and starboard and cargo sides of tanks, near the ship's centerline (Figs. 2 and 3). For example, total sediment accumulations were 12 cm (A1), 18 cm (A14), 20 cm (D13 middle), 30 cm (D13 corner), 26 cm (F3), and 13 cm (F4) deep. Sediment accumulation rate and age were estimated from cores collected from section D13 in tank 1S, where sediment accumulations had reached 20 cm (Fig. 3). Cores collected in this section showed sediments to be brown in color at the surface (0–2 cm) and black through the rest of the core (Fig. 6). ^{210}Pb and ^{226}Ra analyses of these

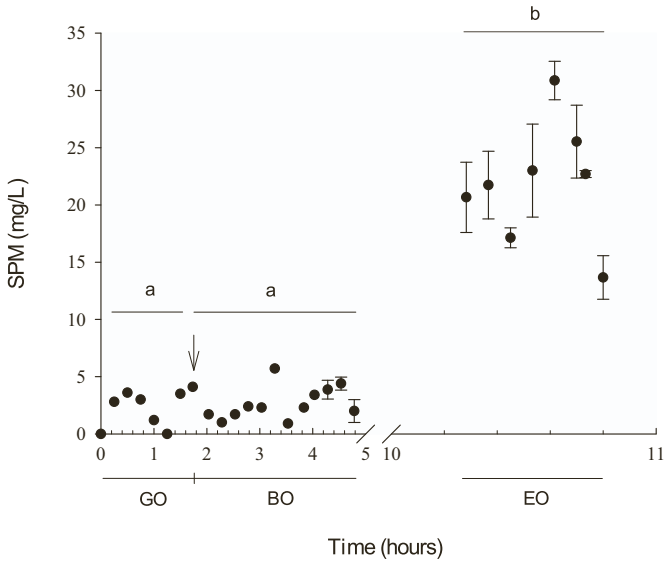

Fig. 4. Suspended particulate matter (mg/L) in water samples collected from ballast pumps during de-ballasting operations in September (mean SPM ± SE from 4.5 h to the end). Water samples were collected every 15 min during the first 5 h and every 5 min during the last 30 min. De-ballasting operations were temporarily stopped on the sampled tank between ca. hours 5 and 10 while other tanks were being deballasted. The arrow indicates the time when the ballast pump was turned on. GO: Gravity Out, BO: Bellmouth pump Out, EO: Eductor pump Out. Different letters above bars indicate significant ($p < 0.05$) differences between de-ballasting operations (GO, BO, EO).

Fig. 5. Examples of residual sediment accumulations and live organisms observed in tank 4S: (A) "small" sediment accumulation with bryozoans and anemones in sub-section B4, (B) "medium" sediment accumulation with bryozoans and anemones in B3, (C) "large" sediment accumulation in C1, (D) "X-large" sediment accumulation in A14, (E) ballast bellmouth (BB), bryozoans, and residual water in C11, (F) eductor (E), fine silt, and residual water in C14, (G) *Sagartiogeton undatus* (ca. 2 cm) in C12, (H) *Sagartiogeton undatus* (ca. 2 cm) in C13, (I) *Membranipora membranacea* (ca. 5 cm) in C11, (J) *Electra pilosa* (ca. 7 cm) in C11, (K) *Hydroids* spp. (<5 mm) in C11, and (L) *Alitta succinea* (ca. 3 cm) from a sediment sample. Sections A, B, and C refer to Fig. 2. Longitudinal frames can be seen in C through F.

sediment cores revealed that the sediment accumulation rate was ca. 1.67 $cm \cdot yr^{-1}$ and that sediments from the bottom 2 cm strata were ca. 11 years old, which corresponds to the year the ship was commissioned (Fig. 7).

3.5. Abundance of organisms and communities in sediments

3.5.1. Dinoflagellates

The concentration of dinoflagellate cysts with viable cell content was significantly higher in tank 4S (812 to 3732 cysts/g of wet sediment) than that in tank 1S (178 to 899 cysts/g wet sediment) ($t = 5.149$; $p < 0.001$) (Table 2, Fig. 8). Viable cysts represent 58 ± 5 % (mean ± SE) and 33 ± 6 % of the total abundance of dinocyst assemblages (with and without cell content) in tanks 4S and 1S, respectively. Calculated excystment rates varied between 38 and 47 % (average 40 %). A total of 38 viable dinoflagellate taxa (15 autotrophic and 23 heterotrophic taxa) was identified to the genus or species level in sediment samples collected during the two campaigns. The number of taxa per sediment sample varied from 7 to 26 (14 ± 4) (4S) and 8 to 31 (16 ± 3) (1S) (Fig. 8). The dinoflagellate community was dominated by 15 species that constituted >91 % of total abundance in each sample. *Brigantedinium cariacoense* and *Brigantedinium simplex* were the most abundant species in all samples from both tanks, with the exception of E13, F1 and F4 (*B. cariacoense* and *Votadinium calvum*). The third dominant species varied slightly between samples and was *Protoperidinium* sp. A (A3, A9, B10), *V. calvum* (A14, A1, C14, D6, D8, D10, D13, E13, F13), *B. simplex* (F1 and F4) and *Dubridinium* sp. (F5). The three dominant species from each sample represent 65–95 % (4S) and 52–91 % (1S) of total abundance. Ten toxic/harmful species (*Operculodinium centrocarpum*, *Operculodinium centrocarpum* short spines, cyst of *Scrippsiella acuminata*,

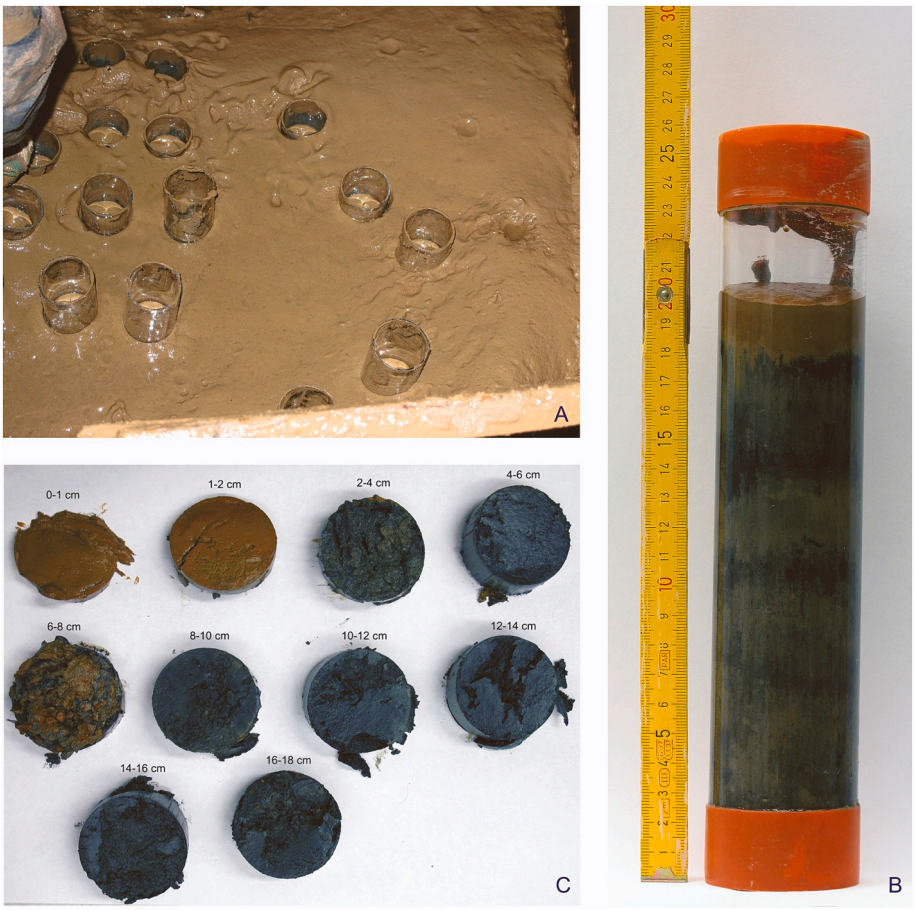

Fig. 6. Photographs showing (A) sediment cores inserted into the sediments of sub-section D13 in tank 1S during the second sampling, (B) a sediment core, and (C) sediment slices (0–18 cm depth) from a core.

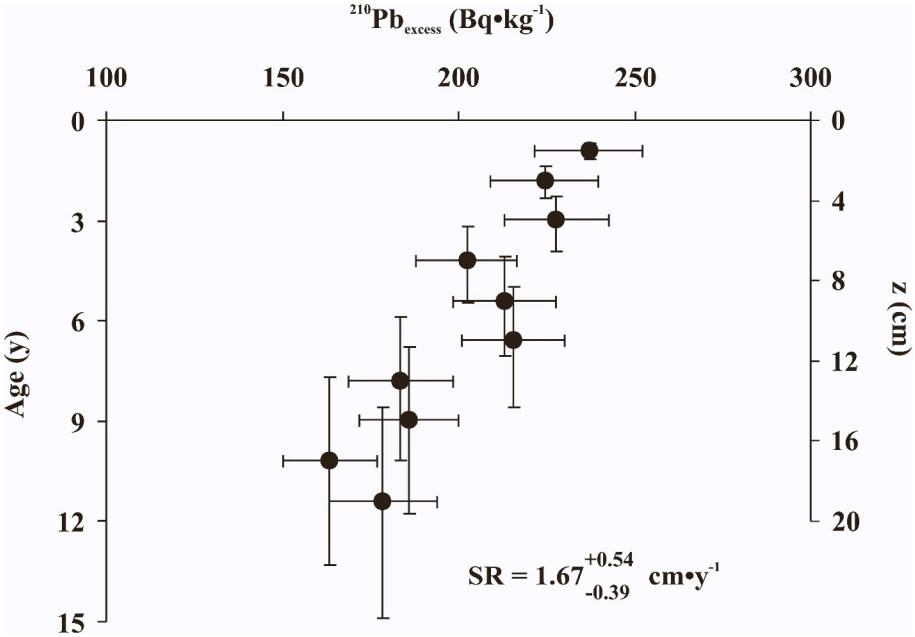

Fig. 7. Estimated sediment age for the 2 cm sediment slices, from the surface to 18 cm depth, from the sediment cores collected in section D13 in tank 1S. Sediment age was estimated on the basis of ^{210}Pb and ^{226}Ra vertical profiles. The sedimentation rate was estimated at 1.67 cm yr^{-1}.

Table 2
Dinoflagellate cyst concentrations (cysts with cell content/g wet sediment) and invertebrate resting stage densities (eggs/40 g wet sediment ± S.E.) in residual sediments from ballast tanks 4S (collected in May) and 1S (collected in September). IDs refer to Figs. 2 and 3. Size refers to the sediment accumulation size (fluff, small, medium, large, or X-large). *Vertical profiles (cores) were done for A1, A14, D13, E13, and F4 sediment samples; and mean (± S.E) dinoflagellate cyst concentrations and invertebrate resting stage densities measured in different strata are presented and detailed results are in Figs. 9 and 10. Surface strata data are presented in parentheses. Unid.: unidentified.

Tank	ID	Size	Dinocysts (# cysts/g wet sediment)	Invertebrate resting stages (# eggs/40 g wet sediment)									
				Total	Copepods		Polychaetes				Cladocerans			
					Acartia bifilosa	Unid. Calanoida	*Hediste diversicolor*	*Alitta succinea*	*Phyllodoce mucosa*	Unid.	*Evadne nordmanni*	*Bosmina* sp.	*Moina* sp.	*Pleopis polyphemoides*
4S	A1*	L	812 ± 266 (1603)	448 ± 60 (443 ± 39)	157 ± 41	0	289 ± 61	0	0	0	2.2 ± 0.4	0.9 ± 0.3	0.1 ± 0.1	0
4S	A3	L	2394	648 ± 37	0	0	251 ± 69	397 ± 45	0	0	0.3 ± 0.3	0	0	0
4S	A9	M	1881	387 ± 5	0	0	299 ± 18	86 ± 13	0	0	0.3 ± 0.3	1.5 ± 0.6	0	0
4S	A14*	XL	1213 ± 655 (2489)	262 ± 66 (356 ± 51)	0	157 ± 44	99 ± 25	0	0	0	4.6 ± 0.9	1.3 ± 0.6	0.2 ± 0.1	0
4S	B10	S	1623	142 ± 45	0	0	0	0	142 ± 45	0	0	0	0	0
4S	C14	fluff	3732	127 ± 6	0	0	0	0	0	125 ± 7	0.8 ± 0.5	0.8 ± 0.5	0	0
1S	D6	S	178	0	0	0	0	0	0	0	0	0	0	0
1S	D8	M	370	0.8 ± 0.3	0	0	0	0	0	0	0	0	0	0.8 ± 0.3
1S	D10	L	732	0	0	0	0	0	0	0	0	0	0	0
1S	D13*	XL	899 ± 206 (2249)	0.9 ± 0.1 (0.5 ± 0.3)	0	0	0	0	0	0	0	0	0	0.9 ± 0.1
1S	E13*	XL	384 ± 134 (141)	0.7 ± 0.2 (1 ± 0.4)	0	0	0	0	0	0	0	0	0	0.7 ± 0.2
1S	F1	L	205	1.3 ± 0.5	0	0	0	0	0	0	0	0	0	1.3 ± 0.5
1S	F4*	XL	584 ± 210 (999)	0.8 ± 0.3 (0.8 ± 0.3)	0	0	0	0	0	0	0	0	0	0.8 ± 0.3
1S	F5	M	249	0.1 ± 0.1	0	0	0	0	0	0	0	0	0	0.1 ± 0.1
1S	F13	fluff	259	1.3 ± 0.3	0	0	0	0	0	0	0	0	0	1.3 ± 0.3
Ship[1]		–	1949 ± 555	281 ± 80	–	–	–	–	–	–	–	–	–	–

[1] Mean concentration of organisms (dinoflagellate cysts and invertebrate resting stages) in residual sediments. Concentration measured at the surface strata was used for A1, A14, D13, E13 and F4 (cores). Mean concentration measured in tank 4S was used for tanks 3, 4, 5 and 7, and mean concentration in 1S was used for tanks 1.

Spiniferites belerius, *Spiniferites delicatus*, *Spiniferites* cf. *delicatus*, *Spiniferites elongatus*, *Spiniferites membranaceus*, *Spiniferites mirabilis* and *Spiniferites ramosus*) were found in sediments and represented 0–8 % (2 ± 1.5 %) and 2–6 % (4 ± 0.5 %) of total abundance in tanks 4S and 1S, respectively. Two non-indigenous taxa (*Votadinium calvum*; https://www.algaebase.org/search/species/detail/?species_id=81733 and *V. spinosum*; https://www.algaebase.org/search/species/detail/?species_id=120640) not previously reported on Canada's east coast were identified and represented 1–13 % (6 ± 1.8 %) and 7–65 % (21 ± 6.2 %) of total abundance in 4S and 1S samples, respectively (see details in A1 Supplementary material). Overall, dinoflagellate communities varied significantly between tanks (Pseudo-F = 4.3151; p = 0.002) but no significant difference was found between the size of sediment accumulations (Pseudo-F = 1.2387; p = 0.256) and for the interaction between sediment accumulation size and ballast tank (Pseudo-F = 0.7539; p = 0.786) (Fig. 9). Sixteen taxa explained 71 % of the dissimilarity between ballast tanks (4S versus 1S) and most of these taxa (13) were more abundant in the former (SIMPER analysis; see details in A2 Supplementary material).

3.5.2. Invertebrate resting stages

As observed for dinoflagellate cysts, invertebrate resting stage abundance also varied significantly between ballast tanks, with abundances in tank 4S being greater than that in tank 1S (T = 75; p = 0.002; Table 2). Concentrations ranged from 127 to 648 resting stages/40 g (mean of 335 ± 82 resting stages per 40 g of wet sediment) and from 0 to 3 resting stages/40 g (0.63 ± 0.16 resting stages per 40 g of wet sediment) in tanks 4S and 1S, respectively (Table 2). Ten taxa were identified in the study: four Cladocera (*Evadne nordmanni* and *Pleopis polyphemoides* – both native to the Baltic Sea, *Bosmina* sp., *Moina* sp.),

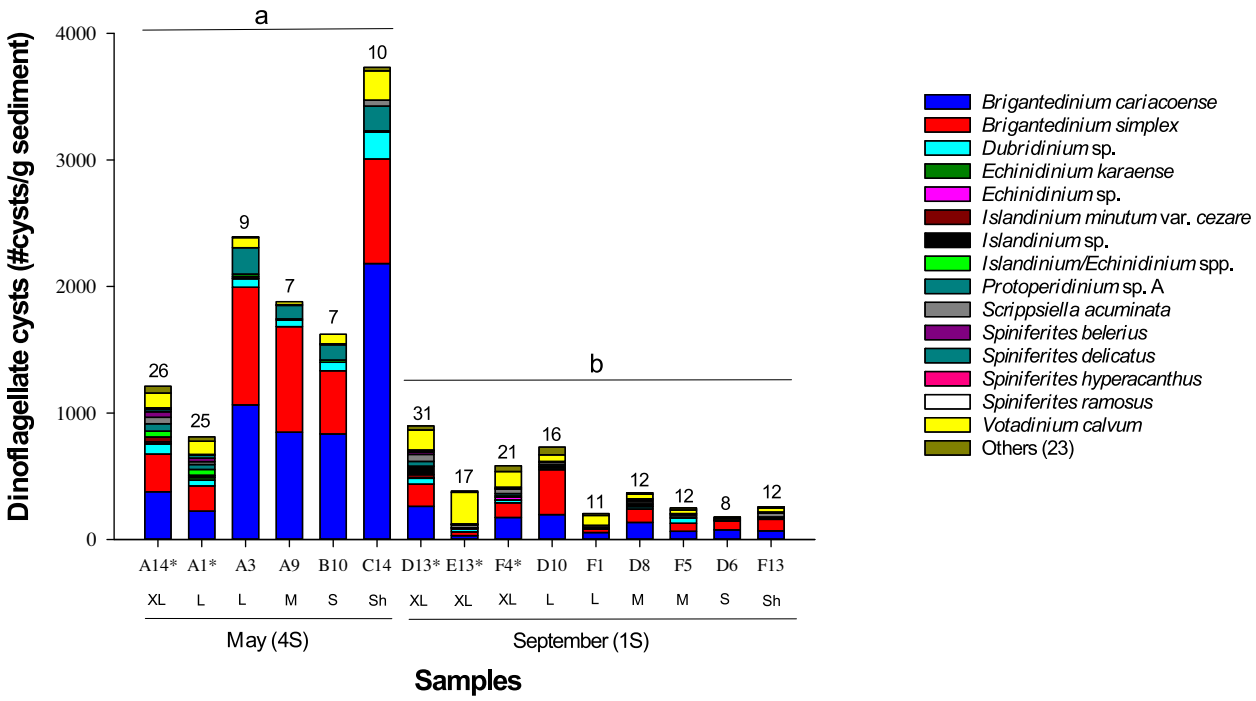

Fig. 8. Dinoflagellate cyst concentrations (cysts with cell content) per gram of wet sediment from ballast tanks 4S (samples A through C) and 1S (samples D through F), collected during the May and September sampling campaigns, respectively. The number of taxa identified in each sample are indicated over the bars. Letters beneath the x-axis indicate the size of the sediment accumulations (XL: X-large, L: large, M: medium, S: small, Sh: shelf). Sediment accumulations with * (e.g. A14*) present mean dinoflagellate cyst concentrations measured in the different strata and detailed results are presented in Fig. 10. Different letters above bars indicate significant ($p < 0.05$) differences between dinoflagellate cyst concentrations measured in tanks (4S and 1S).

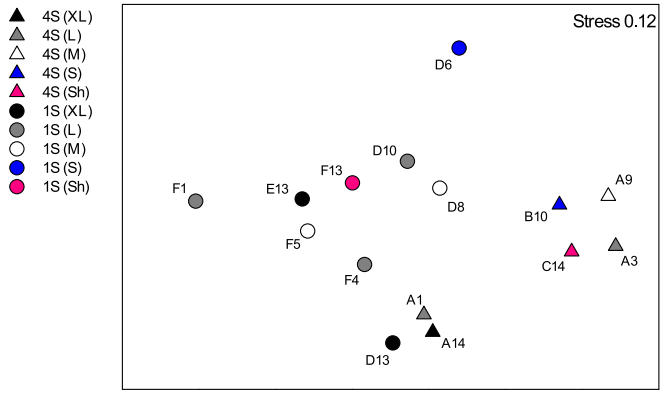

Fig. 9. Non-metric multi-dimensional scaling ordination of dinoflagellate cyst communities found in residual sediment samples from ballast tanks 4S (triangle) and 1S (circle). Labels indicate sample identification (see Table 2 for details). Letters in parentheses indicate the size of sediment accumulations (XL: X-large, L: large, M: medium, S: small, Sh: shelf).

four Polychaeta (*Hediste diversicolor* – native to the north-east Atlantic, but introduced to the north-west Atlantic), *Alitta succinea* – considered cryptogenic to both sides of North Atlantic, *Phyllodoce mucosa* – cryptic status on the west coast of the North America, an unidentified polychaete, and two copepods (*Acartia bifilosa* – native to the eastern Atlantic, and an unidentified Calanoida). The number of taxa per sediment sampled varied from 1 to 5 (4 ± 1) for tank 4S and 0 to 1 (1 ± 0.1) taxa for tank 1S. The community was dominated by NIS or cryptogenic species, which represented between 1 and 100 % (mean of 67 %) and 0–100 % (80 %) of the total abundance in 4S and 1S samples, respectively.

During the May sampling campaign (4S), copepods, polychaetes, and cladocerans represented on average 16 %, 83 %, and 1 % of the total number of resting stages found in all samples, respectively. Copepods (*A. bifilosa* and unidentified Calanoida) were only found in two sediment samples (A1 and A14) collected from sides of the tank (Table 2) and unidentified Calanoida was the only taxa that hatched during the hatching trials (0.3 %). However, polychaete resting stages were spread throughout the sediment samples (middle and sides) and were the most abundant taxa. The highest densities of *A. succinea* (397 ± 45 resting stages per 40 g of sediment) and of *H. diversicolor* (299 ± 18 resting stages per 40 g of sediment) resting stages were found in A3 and A9, respectively. Cladocerans were observed in most sediment accumulations, but at low abundances (highest density 4.6 ± 0.9 resting stages per 40 g of sediment), and were mainly represented by *Evadne nordmanni*. In contrast to 4S, only one species (*P. polyphemoides*) was found in 1S and was equally distributed throughout the samples (0 to 1.3 resting stages/40 g), with a mean density of 0.63 ± 0.16 resting stages/40 g. Hatching experiments resulted in no hatching of any resting stages from the September sampling campaign.

3.6. Dinoflagellate cyst and invertebrate resting stage abundances vs. depth

The vertical distribution of dinoflagellate cyst concentrations and invertebrate resting stage densities was examined in six sediment accumulations (A1, A14, F4, E13 and D13; Fig. 10). Although no statistical inferences can be made due to different sampling methods, general patterns in the vertical distribution of organisms can be highlighted. Overall dinoflagellate cyst concentrations (surface concentration in parentheses) ranged as follows: A1: 493–1603 (1603); A14: 318–2489 (2489); F4: 323–999 (999); E13: 141–604 (141); and D13: 115–2249 (2249) cysts/g sediment. The greatest concentration of dinoflagellate cysts was found in surface sediments, with the exception of E13 (Fig. 10), with *B. cariacoense* and *B. simplex* representing 69–80 % of these abundances. The opposite pattern in E13 was explained by the presence of the non-indigenous *V. calvum*, which represented 79 % of the

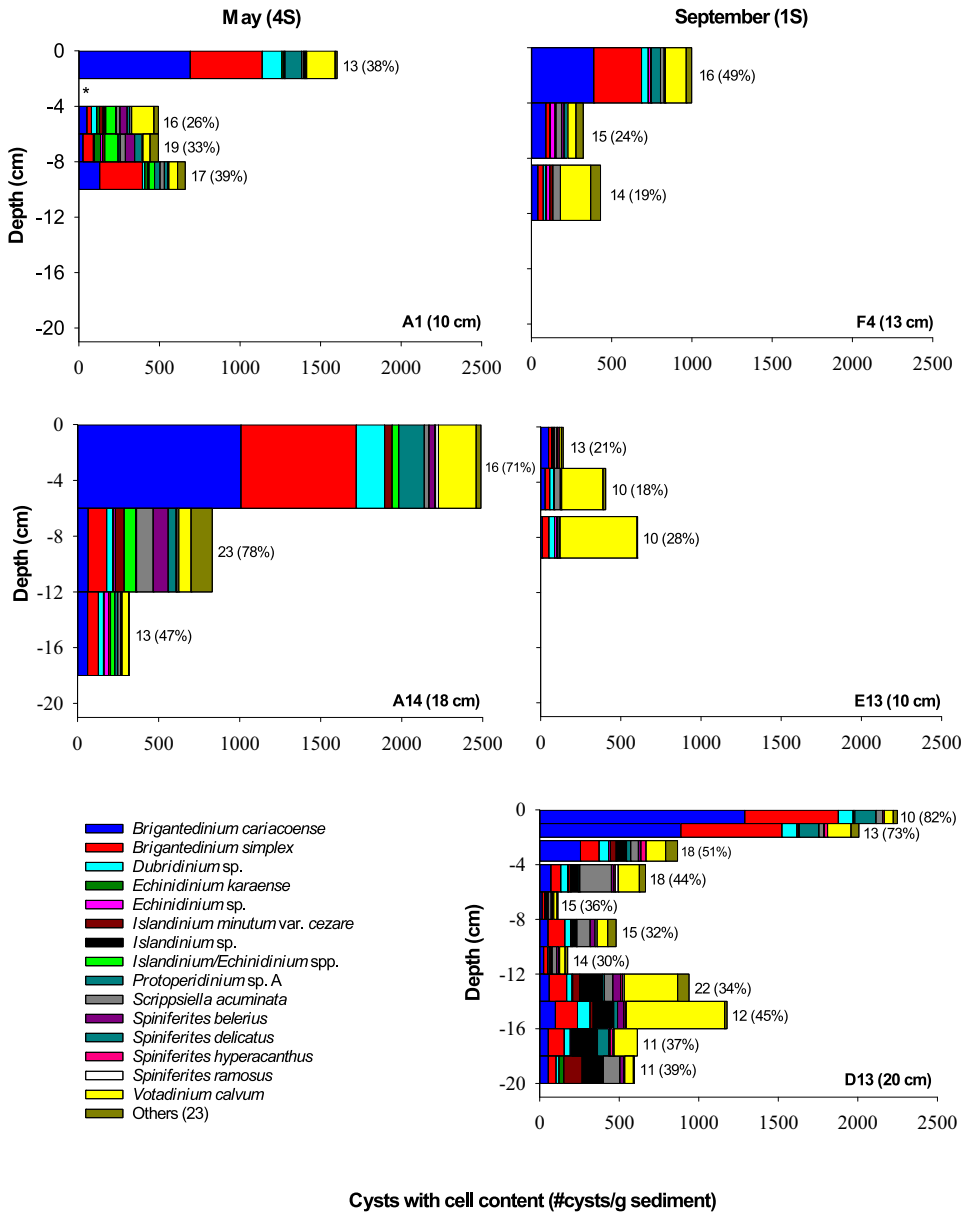

Fig. 10. Vertical profiles of viable dinoflagellate communities (cysts with cell content/g wet sediment) in residual sediment accumulations from ballast tanks 4S (A1 and A14) and 1S (F4, E13, and D13), collected in cores (up to 20 cm depth) during the May and September sampling campaigns, respectively. The number of taxa (with cell content) per stratum are indicated beside each bar. The number in parentheses represents the percent (%) of cysts with cell content present in the overall community (with and without cell content cysts). *: no data.

abundance in the deepest stratum (6–10 cm). The highest percentage of cysts with cell contents (82 %) occurred in surface sediments (D13) but a high proportion (up to 45 %) also occurred in deep strata. We observed no general pattern with respect to taxonomic richness of dinoflagellate cysts (i.e., number of taxa) by depth. The total number of taxa in each depth stratum varied from 13 to 23 and 10 to 22 taxa in the 4S and 1S tanks, respectively (Fig. 10). We observed the highest richness in surface sediments for F4 and E13 and mid-depth for A1, A14, and D13 (Fig. 10).

Invertebrate resting stages exhibited no abundance pattern in either of the two tanks (Fig. 11). Invertebrate abundance (surface abundance in parentheses) ranged as follows A1: 282–593 (443); A14: 136–356 (356); F4: 0.5–1 (0.75); E13: 0.5–1 (1); and D13: 0–1.5 (0.5) resting stages per 40 g of sediment. The highest resting stage abundance was observed in the 6–8 cm depth stratum of A1 (593 resting stages/40 g sediment; same depth stratum as the live polychaete *A. succinea*) and in the 0–6 cm stratum of A14 (356 resting stages/40 g). However, no *A. succinea* resting stages were found in the different strata of sample A1. The number of taxa in the different strata varied from 3 to 5 taxa in tank 4S, while in tank 1S all strata contained only one species (*P. polyphemoides*). In 4S, copepods (*A. bifilosa* and unidentified Calanoida) were only present in the top layers of sediment cores. In contrast, *H. diversicolor* was absent in the top layers.

3.7. Proportion of residual sediments released during de-ballasting

To estimate the proportion of residual sediments released during de-ballasting operations, we only considered sediments resuspended during these operations (not suspended particulates present in ballast waters).

Based on Eq. (2), we estimated that the volume of ballast water with resuspended sediment (BW-RS) amounted to 3965 m^2 (total tank surface area) × 0.52 m (height of the pumpable water below the longitudinal frame) = 2062 m^3. Then, based on Eq. (1), we estimated that discharged resuspended sediments (DRS) amounted to 2062 m^3 (BW-RS) × 0.0219 kg/m^3 (SPM during EO de-ballasting operations; Fig. 4) = 45.2 kg (dry

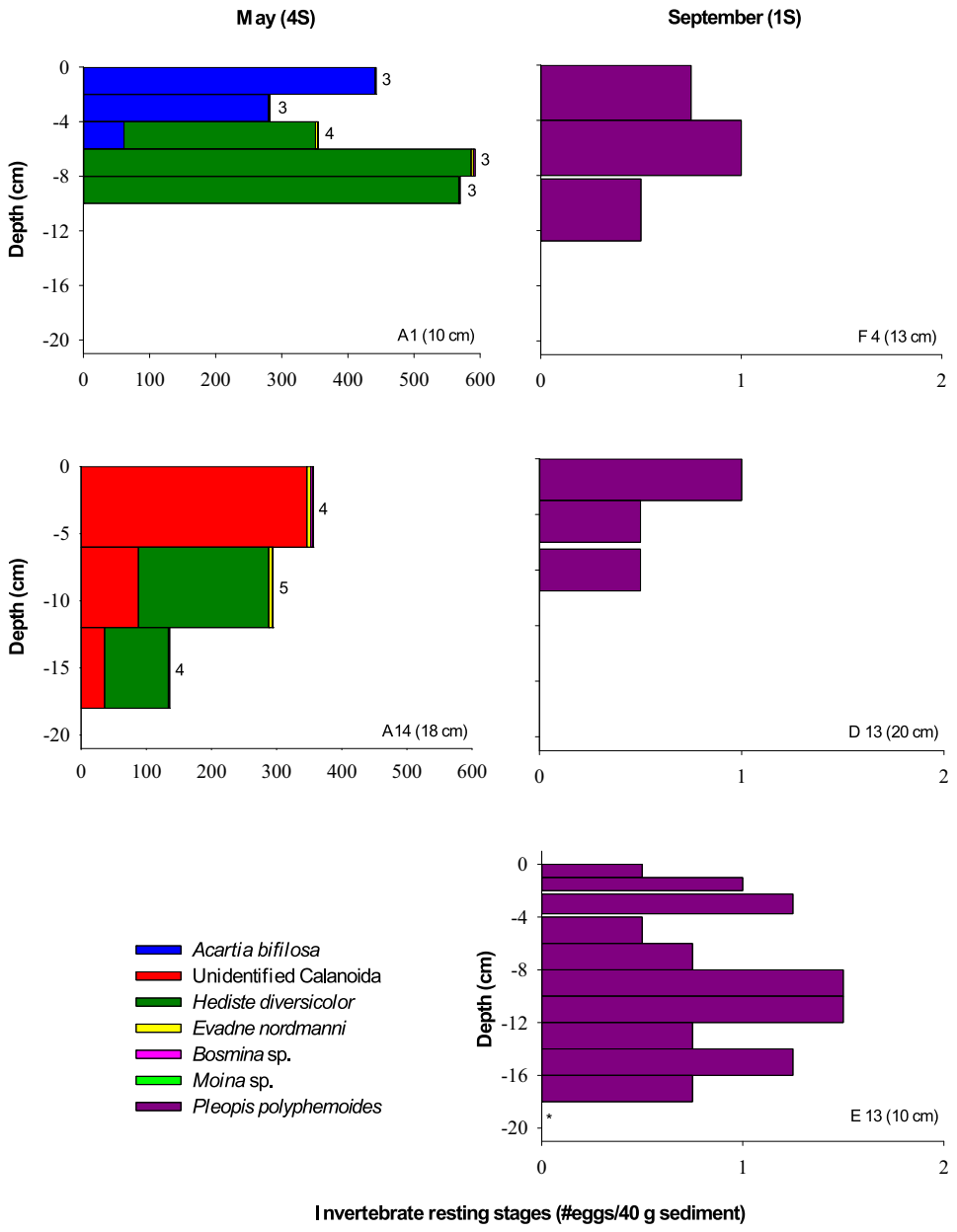

Fig. 11. Vertical profiles of invertebrate resting stages (#eggs/40 g sediment) in residual sediment accumulations from ballast tank 4S (A1, A14) and tank 1S (F4, E13, D13), collected in cores during the May and September sampling campaigns. Note the different scale for counts between May and September. Numbers at the end of the bars indicate the number of taxa, *: 0 eggs observed in this strata.

sediment). Thereafter, based on Eq. (4), we estimated that the percent (%) of residual sediments released was 45.2 kg / (5420 kg + 45.2 kg) × 100 = 0.83 %. As such, we estimate that 45.2 kg of sediment (dry sediments) was released into the environment during de-ballasting operations, representing 0.83 % of total residual sediments accumulated in the ship.

3.8. Propagule pressure associated with sediments for dinoflagellate cysts and invertebrate resting stages

Based on Eq. (5), we estimated that the quantity of viable dinoflagellate cysts released during de-ballasting operations amounted to 210 × 10^6 dinoflagellate cysts for the ship (or 84 × 10^6 dinoflagellate cysts if the 40 % excystment rate is applied), which corresponds to the product of 12,984 000 g wet sediment (total residual sediments; Table 1) × 0.83 % (as calculated above) × 1949 cysts/g wet sediment (mean concentration; Table 2). In addition, we estimated that the quantity of viable invertebrate resting stages released during de-ballasting operations amounted to 7.5 × 10^5 invertebrate resting stages for the ship (or 2.3 × 10^3 invertebrate resting stages if the 0.3 % hatching rate is applied), which corresponds to 12,984 000 g wet sediment (total residual sediments; Table 1) × 0.83 % (as calculated above) × 7 resting stages/g wet sediment (mean concentration of 281 resting stages/40 g wet sediment; Table 2).

4. Discussion

To our knowledge, this is the first study to examine the fine details of spatial variation of sediment accumulations in ballast tanks and to estimate the abundance of organisms (dinoflagellate cysts and invertebrate resting stages) in sediments that may be resuspended and released during de-ballasting operations. This more precise quantitative approach allowed us to estimate the volume of residual sediments released during de-ballasting operations (<1 % of total sediments and

organisms contained therein) and to calculate more realistic propagule pressure estimates associated with this pathway.

4.1. Residual sediment accumulation

We used precise quantitative measurements to provide better estimates of residual sediment accumulations than those estimated in previous studies, most of which have been based on visual approximations of sediment cover inside tanks and observations of sediment depth (see Drake et al., 2007; Briski et al., 2010; Casas-Monroy et al., 2011). We observed significant accumulations of residual sediments in ballast tanks, consistent with the aforementioned studies. We estimate that sediment accumulation in the sampled ship totalled ~13 t (wet sediment) for the ten tanks that were in ballast prior to sampling. This estimate falls within the range of sediment accumulation reported in previous studies (Lucas et al., 1999; Hamer et al., 2000; Duggan et al., 2005; Johengen et al., 2005; Drake et al., 2007; Briski et al., 2010; Casas-Monroy et al., 2011). For example, Casas-Monroy et al. (2011) estimated an average of 15 t of residual sediments per ship based on 65 cargo ships surveyed in Eastern Canada. Similarly, Briski et al. (2010) noted that residual sediment loads ranged from <1 to 45 t (mean of 5 t) in ships arriving in the Great Lakes based on 17 ships, and Bailey et al. (2005) reported <1 to 65 t per ship, with an average of 14 t from 39 ships entering the Great Lakes. Johengen et al. (2005) surveyed 103 non-ballasted vessels (called NOBOB) entering the Great Lakes and found that sediment accumulated within the tanks may reach up to 100 t, with 60 % of ships estimated to be carrying <10 t. Casas-Monroy et al. (2011) also reported that the volume of residual sediments per tank was significantly higher in coastal ships traveling within North American waters (average volume of 2.02 m^3 corresponding to 2.6 t) for which ballast water exchange (BWE) was not required versus transoceanic ships (1.3 m^3 or 1.7 t) for which BWE was mandatory. In comparison, we estimated that ca. 1.3 t of residual sediments accumulate per tank for a transoceanic ship that conducted BWE. Bailey et al. (2022) observed fine sediments in one third of the samples collected from 29 ships with operational ballast water management systems (BWMS), suggesting that even ships designed with such systems may have sediment accumulation in ballast tanks. This may result from small particles passing through pre-filtration systems, as most BWMS typically use mesh sizes of 35–50 μm (David et al., 2015; Bilgin Güney et al., 2020). As such, sediment may continue to accumulate in tanks as the dominant sediment grain size found in ballast tanks may be smaller than the typical filter mesh size mentioned above, which is supported by our results (mean grain size of 14.4 ± 2.5 μm) and previous studies (Hamer, 2002; Maglić et al., 2016, 2019). As many BWMS combine pre-filtration with a second procedure – often a strong oxidizing agent or UV light – to further reduce risk, an open question is whether fine cysts or resting stages entrained in ballast water that pass through the initial treatment stage will be rendered nonviable by the second step.

Our results showed that sediment accumulation generally increased with distance from the ballast bellmouth and eductor, with greatest accumulations (up to 20 cm) in the front corners and starboard and cargo sides of tanks. Casas-Monroy et al. (2011) similarly reported that sediments accumulated in tank corners but also observed tanks with sediments uniformly distributed on the bottom and hypothesized that these contrasting results were due to the high variability between ballast tanks in terms of size and shape. Sediment accumulation is highly dependant on the structural complexity within a ballast tank that causes a complex flow regime during ballasting and de-ballasting operations (Wilson et al., 2006; Bilgin Güney et al., 2018). Flushing efficiency is affected by the positions of inlets and outlets (Qi et al., 2014; Qi and Eames, 2015). Indeed, ballast tanks may contain many compartments separated by longitudinal and transversal structural frames with various openings and dead spots characterized by low local flow velocities that make them susceptible to sediment accumulation during de-ballasting operations (Wilson et al., 2006). Prange and Pereira (2013) suggested that sediment accumulation is due to obstruction by scallops (small apertures) in tank structures that prevent sediments from being directed towards the ballast water bellmouth and eductor. The same authors also suggested that 10 to 15 cm deep sediment layers are commonly observed in ballast tanks and that some of these areas are not easily accessible and therefore sediments must be manually removed when in dry dock. The largest sediment accumulations in our study were also located in such areas. It is interesting to note that these sediments appear to have accumulated since the ship began sailing as the radiometric dating of sediment cores indicates that the bottom 2 cm sediment stratum was ca. 11 years old, corresponding to the year the ship was commissioned rather than after the most recent dry dock event. This indicates that sediments were not removed during previous dry dock operations or during regular maintenance and cleaning schedules. This is supported by Johengen et al. (2005) who found that residual sediments in ships was related to maintenance quality and management as well as the origin of ballast water and not the capacity or age of the ship.

4.2. Presence of live organisms in ballast tanks

Ballast tanks can be favourable environments for the survival and growth of organisms as shown by the presence of live anemones, polychaetes, bryozoans, and hydrozoans on the framing and bottom of tanks and a live polychaete found in residual sediments of the ship. Such “interior hull fouling” was also noted by Drake et al. (2005), who observed biofilms containing bacteria, microalgae, and associated protozoans in ballast tanks. The anemones were identified as *Sagartiogeton undatus* which is native to northern Europe and is a NIS to Canadian waters (https://www.gbif.org/species/155460820). Other observed species (*M. membranacea*, *E. pilosa* and *A. succinea*) are, respectively, introduced (Scheibling et al., 1999), native (https://www.marinespecies.org/aphia.php?p=taxdetails&id=111355), and cryptogenic (https://invasions.si.edu/nemesis/species_summary/-48) species to Canada’s East coast. However, their presence in ballast tanks suggests that several species can survive ocean transits and constitute a potential risk of NIS introduction.

4.3. Taxonomic richness and abundance of organisms present in sediments

We observed high richness and abundance of viable dinoflagellate cysts in residual sediments. Consistent with Casas-Monroy et al. (2011), we considered that cysts with cell content were viable. These authors conducted tests on over 480 cysts with cell content from various species and obtained an average excystment rate of 38 %, which is similar to our results (average 40 %), indicating that a significant proportion of all sediment-borne cysts could germinate if released into suitable waterbodies. In terms of the number of dinoflagellate cyst taxa, we observed a mean taxonomic richness (15 taxa), which is also similar to that observed by Casas-Monroy et al. (2011), who also found an average of 15 dinoflagellate taxa (2 to 24 species) in sediments from 24 transoceanic ships. As observed in these ships, the dinoflagellate community in sediments from our study was dominated by *B. cariacoense* and *B. simplex* and we found several potentially harmful/toxic species and NIS that represent a high risk to marine ecosystems. Viable dinoflagellate cyst concentrations measured in residual sediments in our study (178–3732 cysts/g wet sediment = 269–6008 cysts/g dry sediment) are similar to the highest concentrations (887–1779 cysts/g dry sediment) reported by Casas-Monroy et al. (2013) for all types of ships arriving on the East coast of Canada. Macdonald (1995) reported similar concentrations (5–1450 cysts/cm^3), while Lin et al. (2021) and Hallegraeff and Bolch (1992) reported lower (36–448 cysts/g dry sediment) and higher (40–22,500 cysts/cm^3) concentrations, respectively.

To our knowledge, this study represents the first time that cores of residual sediments were collected from ballast tanks and the sediment layers analyzed. The concentration and proportion of viable

dinoflagellate cysts (% of viable cysts with cell content present in the overall community) measured in cores were generally higher at the surface of sediment accumulations, which is likely the only portion of the sediments that is resuspended and discharged during de-ballasting operations. However, high concentrations of viable cysts were also measured in the deeper strata of cores. Of the total 38 dinoflagellate taxa identified, two were NIS (*V. calvum* and *V. spinosum*), both first described from the surface sediments around the British Isles (Reid, 1977). *Votadinium calvum* can be considered a (sub-polar) temperate to equatorial coastal species and occurs in hypersaline and hyposaline environments, whereas *V. spinosum* is a coastal species whose distribution is restricted to saline environments (Zonneveld et al., 2013; Zonneveld and Pospelova, 2015). Highest abundances for both species occur in the Sea of Japan and East China Sea (*V. calvum*) and East China Sea (*V. spinosum*). *Votadinium calvum* was abundant in some of our sediment samples, reaching a maximum of 79 % of the total abundance of viable cysts in the deepest strata of one core. This species was also relatively abundant (53 %) in the third deepest sediment layer (14–16 cm) of a second core, which corresponded to a 9-year-old sediment layer (2000) based on radiometric dating. The ship's travel history indicates dry docking in Korea in 2000, where this species is relatively abundant (Zonneveld et al., 2013). Our data not only show that sediments can accumulate in certain areas of tanks over several years, but also that variation in dinoflagellate cyst communities may be linked to geographic areas visited by ships.

We observed high richness and abundance of invertebrate resting stages in residual sediments. Taxonomic richness was lower (0 to 5 taxa per sediment sampled for a single ship) than that reported by Bailey et al. (2005) and Briski et al. (2011a), who found 0 to 20 taxa (39 NOBOB ships) and a mean of 13 taxa (22 transoceanic vessels), respectively. Of the ten taxa identified in our samples, three were NIS (30 % of the total number of taxa) and two were cryptogenic to North Atlantic coasts, similar to previous studies that observed 29 % (Bailey et al., 2005) and 31 % (Briski et al., 2011a) of NIS in samples. Invertebrate resting stage densities in our study (281 ± 80 resting stages/40 g wet sediment) were similar to those (129 ± 48 resting stages/40 g wet sediment) reported by Briski et al. (2011a) for the same type of ship. However, the invertebrate community composition observed in sampled ships arriving in the Atlantic region differed greatly from those observed in our study (Briski et al., 2011a). Copepods (88 %), cladocerans (7 %) and rotifers (3 %) were the dominant taxonomic groups observed in residual sediments of the ships studied by Briski et al. (2011a), whereas the invertebrate community in our study was represented by polychaetes (83 %), copepods (16 %) and cladocerans (1 %). We observed very low hatching success (0.3 %) during our trials with an unidentified Calanoida being the only taxon that hatched. This differs from previous observations by Bailey et al. (2003), Briski et al. (2011a) and Branstrator et al. (2015) who obtained mean proportions of resting stages hatched ranging from 33 to 40 % (NOBOB vessels), 3.5 % (transoceanic vessels) and 31–75 % (domestic Great Lakes cargo ships), respectively. A few hypotheses may explain our poor hatching success. While the sugar flotation method is well known to have no adverse effects on the viability of cladocerans (Lukić et al., 2016) and copepods (Viitasalo, 2007), we could not find scientific literature on the effects of this method on polychaete resting stages. In addition, as no polychaete resting stages successfully hatched in previous studies/trials (E. Briski, unpublished data), we are uncertain if appropriate conditions were used during our trials to induce polychaete resting stages to hatch and cannot therefore consider unhatched resting stages as non-viable. Cáceres (1997) found that unhatched resting stages may not have received the appropriate hatching cues and therefore considered all invertebrate resting stages present in residual sediments to be potentially viable. We thus follow this approach in our interpretation of resting stage viability.

Copepod resting stages (*A. bifilosa* and unidentified Calanoida) were only found in two sediment samples (A1 and A14) in the furthest corners from the pump, and were mostly present in the top sediment core layers. Copepod resting stages in ballast sediments degrade very quickly and disappear over time (Briski et al., 2011c; Dong et al., 2021). In previous studies, ca. 50 % of resting stages degraded in less than six months (Briski et al., 2011c). This high degradation rate could explain why they did not accumulate in the sampled ballast tanks. Copepod resting stages were mainly distributed in the top layers of sediment cores, with decreasing density with depth, suggesting that ballasting and de-ballasting operations disturbed only the top layers (i.e., 2–3 cm) of sediments. Additionally, when copepod resting stages are found in ballast sediments, they typically occur at high densities, suggesting that resting stages are pumped into the tanks at high densities (Briski et al., 2010, 2011c).

In contrast to copepods, resting stages of the polychaete *H. diversicolor* were only present in the deepest layer of the same sediment cores (A1 and A14). This species is a dominant organism in soft bottoms and widely distributed throughout European coasts (García-Arberas and Rallo, 2002), is non-indigenous to the Canadian east coast, but is introduced elsewhere in the Northwest Atlantic. *Hediste diversicolor* is a euryhaline species that can withstand large variations in salinity (Smith, 1956). As the species has a holobenthic life cycle without pelagic larvae (Dales, 1950), we assume that once introduced into a ballast tank, it will stay and reproduce there. The lack of free pelagic larvae in *H. diversicolor*, together with the assumption that large accumulations of sediments do not move significantly, may explain why this species was only found in one section of the tank. This section is the most remote compartment from the pump, suggesting that the worm was at the end of the tank and remained there.

Cladocerans (*Evadne nordmanni*, *Bosmina* sp., *Moina* sp., and *Pleopis polyphemoides*) are taxa with resting stages that are very resistant and do not degrade readily in sediment (Briski et al., 2011c). For example, *Evadne nordmanni* and *Pleopis polyphemoides* did not degrade after one year (Briski et al., 2011c). Their presence in all strata of all cores collected in both tanks supports this hypothesis. Unlike copepod resting stages, Cladocera resting stages were never found at high density in ballast sediments (Bailey et al., 2005; Briski et al., 2010; Briski et al., 2011c). The equal distribution of Cladocera resting stages throughout the tanks suggests a slow but constant accumulation of resting stages in tanks.

4.4. Resuspension of residual sediments and associated organisms

Suspended particulate matter concentrations were low during the first part of de-ballasting operations but increased significantly (ten-fold) during the last 30 min. Consistent with Reid et al. (2007), we believe that increased turbidity, corresponding to increased SPM concentrations, occurred when water in tanks dropped below the longitudinal framing. In a study on NOBOB vessels, these authors observed large increases in turbidity of de-ballasted waters during most de-ballasting events. Spikes in turbidity were recorded when the water depth dropped below ca. 0.6 m, i.e. below the shell framing ("stiffeners") where drainage to the bellmouth becomes restricted and water is forced through the limber holes resulting in narrow, high-speed jets that have sufficient energy to resuspend accumulated sediment along the flow paths (Reid et al., 2007). Observed sediment deposition patterns and observations of sediment scouring in the two tanks support this hypothesis. Indeed, the spatial distribution of sediments in tanks 4S and 1S showed that the greatest sediment accumulations occurred on the sides and front of tanks. Sediments appeared to be resuspended and partially removed from central areas of tanks and completely removed near the ballast bellmouth and eductor pumps. Reid et al. (2007) estimated that resuspension and removal of sediments occur during discharge in variable amounts, affecting 30 to 80 % of bottom areas, depending on tank design, de-ballasting flow rate, and the nature of residual sediments, which can vary widely among ships. However, they did not assess the quantity of sediments discharged during de-ballasting operations. The proportion of sediments expelled from tanks during ballast discharge

was unclear until recently (Gollasch et al., 2019). Quantitative measurements of sediment accumulation, and mapping of the spatial sediment distribution combined with SPM measurements during de-ballasting operations, allowed us to better estimate the proportion of residual sediments released during de-ballasting operations. This, in turn, allows more realistic estimates of propagule pressure associated with this pathway.

4.5. Propagule pressure estimates

This is the first study to determine the proportion of accumulated sediments and associated organisms released into the environment during de-ballasting operations. Although we determined that only ~1 % of residual sediments and associated resting stages are resuspended and released during de-ballasting operations, this still represents a very substantial inoculum of $\sim 21 \times 10^7$ viable dinoflagellate cysts (84×10^6 if we apply the 40 % germinated cysts) and 7.5×10^5 invertebrate resting stages (2.3×10^3 if we apply the 0.3 % hatched resting stages) discharged into the environment per de-ballasting event, with a considerable proportion of these organisms being NIS, cryptogenic, or potentially toxic/harmful species. As concentrations are generally higher in the top sediment layers for both types of resting stages, we considered our propagule estimates to be conservative as top layer concentrations were measured for only one third of the samples, whereas other samples may contain sediments from deeper layers. In addition, transoceanic ships may not represent the worst-case scenario, as Casas-Monroy et al. (2011) observed that NIS cyst concentrations are higher in continental exchanged ships than in transoceanic ships. Previous estimates of propagule pressure associated with residual sediments may have overestimated values as they were based on the total quantity of sediments and total quantity of organisms carried on ships, and not the actual quantity resuspended and discharged during de-ballasting operations. For example, Bailey et al. (2005) estimated that an average NOBOB ship entering the Great Lakes carries about 3.6×10^5 resting stages t^{-1}, which is three orders of magnitude greater than our estimate of propagule pressure. Similarly, Casas-Monroy et al. (2011) estimated that transoceanic ships transport on average 4×10^9 dinoflagellate cysts per tonne of dry sediment (calculated from mean concentration of 4 cysts/g dry sediments), which is two orders of magnitude greater than our estimate. The estimates in this study for resuspended sediments (and associated organisms) during de-ballasting operations can be applied to most ships, even those equipped with treatment systems, as sediments continue to accumulate in ballast tanks (Bailey et al., 2022) and as evidence suggests that some resting stages may tolerate ballast water treatments (Gregg and Hallegraeff, 2007; Wang et al., 2018; Nwigwe and Kiyokazu, 2023).

4.6. Management of ballast residual sediments

The International Convention for The Control and Management of Ships Ballast Water and Sediments (BWM Convention) was adopted in February 2004, ratified in 2017, and ships will have to meet the IMO discharge standard in 2024. It will require all affected vessels to manage their ballast water to meet D-2 performance standards (IMO, 2004). According to the Convention, the management of residual sediments should be part of a ships' ballast water management plan (BWMP) and ballast tank sediments must be removed when ships dry dock (but not during normal ballasting and de-ballasting operations). Our results highlight that sediments may accumulate in some tank areas over several years, and suggest that cleaning procedures may be ineffective and that management of ballast sediments needs to be improved. Given that sediments may contain high abundances of viable dinoflagellate cysts and invertebrate resting stages, their collection and deposition in appropriate reception facilities is essential (IMO, 2006). Despite management strategies (i.e., exchanges, treatments) and regular maintenance schedules, ballast sediments and the biological assemblages they contain may continue to accumulate in ballast tanks even if ships have ballast water treatment systems on board (Bilgin Güney et al., 2020; Bilgin Güney, 2022). Even if only 1 % of residual sediments and associated resting stages are expelled during de-ballasting, this represents a high number of propagules being discharged each voyage that may pose risks for receiving regions as dinoflagellate cysts and invertebrate resting stages may germinate when conditions are favourable.

According to the D-2 standard of the IMO Convention, vessels may only discharge ballast water that contains viable organisms within specified limits (i.e., allowable numbers within certain size class of organisms in the ballast water) (IMO, 2004). In order to better evaluate ship compliance with this standard, further research should evaluate ballast sediment propagules – especially resting stages and cysts - under operational conditions by collecting water samples during the last portion of de-ballasting operations (when resting stages are most likely to be resuspended and discharged) to ensure compliance with the D-2 performance standards.

5. Conclusion

To our knowledge, our study is the first attempt to estimate the quantity of residual sediments and taxa accumulated in and discharged from ballast tanks. This more precise quantitative approach allowed us to estimate the volume of residual sediments released during de-ballasting operations (<1 % of total sediments and organisms contained therein) and to calculate more realistic propagule pressure estimates associated with this pathway. Our results may provide guidance in the on-going evolution of best management practices for ballast sediments to better understand and control this important invasion pathway.

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CRediT authorship contribution statement

Nathalie Simard: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Andrea M. Weise:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **André Rochon:** Writing – review & editing, Resources, Investigation, Funding acquisition. **Elizabeta Briski:** Writing – review & editing, Investigation. **Hugh J. MacIsaac:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Christopher W. McKindsey:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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