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The diatom *Chaetoceros* in ships' ballast waters – survivorship of stowaways

GEORGIA KLEIN[1*], IRENA KACZMARSKA[1], JAMES M. EHRMAN[2]

[1] Department of Biology, Mount Allison University, Sackville, NB, E4L 1G7, Canada

[2] Digital Microscopy Facility, Mount Allison University, Sackville, NB, E4L 1G7, Canada

Ship ballast water discharged by vessels into the receiving port is recognised today as an important vector for the spread of non-indigenous species and facilitates the introduction of potential invasive species. Here, we report on 18 species (of about 30 identified), both vegetative cells and spores, of the diatom genus *Chaetoceros* Ehrenberg found in ballast water collected from ships arriving at Canadian ports on the West Coast (WC), East Coast (EC) and the Great Lakes (GL). We found live, vegetative *Chaetoceros* cells (one of the most abundant taxa) in 49% of the 57 ballast water samples. The highest density of viable spores enumerated in our counts was 414 cells L^{-1}. In 62% of 52 samples examined using scanning electron microscopy (SEM), *Chaetoceros* spores were found, though fewer live, identifiable spores were found using light microscopy. Three reportedly harmful species, *C. convolutus*, *C. danicus*, *C. debilis* were encountered in WC samples, and additionally, *C.* cf. *hispidus*, a species not yet reported from Canada. *C. ceratosporus* and *C.* cf. *subsecundus*, to date reported only from the EC of the USA, now have been transported to the port of Vancouver, British Columbia. Our findings contribute to the assessment of the effectiveness of ballast water treatment via water exchange, and serve to evaluate the diversity of diatom vegetative cells and spores transported in ballast water tanks.

Keywords: Diatom, *Chaetoceros,* phytoplankton, non-indigenous species, ballast water, resting stage, spore, ultrastructure

Introduction

The rapid increase in large container shipping since the 1970s has facilitated rapid inter-connection between ports worldwide. This has provided marine biota with an unprecedented anthropogenic means of worldwide dispersal via ballast water (CARLTON 1996, RUIZ et al. 2000). Studies of invasive, non-indigenous aquatic macro-organisms in Canada (e.g. BREEN and METAXAS 2008, JOKELA and RICCIARDI 2008) also focused interest on ship ballast transport of microalgae, which, until recently, have received little systematic scientific attention. Extensive literature shows ballast waters and sediments to be vectors for the introduction of dinoflagellate resting stages into recipient ports (e.g. HALLEGRAEFF et al.

* Corresponding author: gklein@mta.ca

1990, ZHANG and DICKMAN 1999, HAMER et al. 2000, PERTOLA et al. 2006), particularly of potentially toxic dinoflagellates (e.g. HALLEGRAEFF and BOLCH 1991, BOLCH and DESALAS 2007). However, diatoms, one of the most species rich and abundant ballast water micro-biota, require more attention than they have thus far received. The literature on diatom resting stages in ballast water is scarce and only a few publications identify diatom spores (e.g. HALLEGRAEFF and BOLCH 1992), despite their potential for being harmful (BATES and STRAIN 2006) and/or invasive, and their long-term tolerance to growth-adverse conditions (MCQUOID and HOBSON 1995).

Depending on ship type and age, as well as weather conditions for ballast water exchange, 1–5% of the water plus sediment remains un-exchanged in the ballast tank due to limitation of pumping technology, but the sediments are likely to contain more species, some in resting stages, than the overlaying water. Diatom and dinoflagellate resting stages (spores, cysts) may remain viable in growth-limiting environments for years (SICKO-GOAD et al. 1989, HALLEGRAEFF and BOLCH 1992, MCQUOID et al. 2002).

There are both environmental and economical implications of the introduction of non-indigenous aquatic species. Damage due to invasive harmful algae can be considerable and it is not only toxin producing diatoms that are harmful (e.g. SKOV et al. 1999). *Chaetoceros* species can cause mechanical damage by piercing the gills of fish and mass die-off of high cell densities of blooming species can cause hypoxia in the water column (e.g. BATES and STRAIN 2006). Furthermore, biodiversity issues may result from the introduction of invasive species. There is potential for the successful establishment of non-indigenous aquatic species that may displace and replace native species (EDLUND et al. 2000). Local biodiversity may be compromised, leading to a cascade of changes with the potential for weakening or altering an ecosystem.

The aim of our study within the Canadian Aquatic Invasive Species Network (CAISN) was to evaluate the effectiveness of open-ocean exchange of ballast water prior to arriving in Canadian ports, as currently regulated by the IMO (International Maritime Organisation). We present an initial assessment of diversity and abundance of cells and resting spores from the diatom genus *Chaetoceros* arriving in ship ballasts in 2007.

Materials and methods

Ship sampling

Vegetative cells and spores were identified and enumerated in a total of 67 ballast water samples collected from the West Coast (WC, 30 samples; Vancouver Harbour) from 28 April to 6 September 2007; East Coast (EC, 24 samples; arrival ports Sept-Îles, Baie-Comeau and Port-Cartier) 29 April to 13 August 2007; Great Lakes (GL, 13 samples; arrival ports Toledo, Milwaukee, Detroit (USA), Sarnia and Windsor (Canada)) 30 July to 26 November 2007. The sampling protocol for water samples (developed by CAISN) targeted tanks that were at least 50% full. Bulk carriers were primarily sampled, but container ships and tankers were also included. With the use of a 5 L Niskin-bottle, water was collected at 3 depths to represent the tank water column (subsurface, about middle depth – depending on water level in the tank, and ca 1–2 m above bottom), combined to a total of 15 L and subsampled for various projects. For diatoms, 3 L of each sample were transferred into plastic bottles, preserved with acidified Lugol's solution and stored in a cool and dark loca-

tion until shipment to our laboratory. Specifics regarding ship type (e.g. double-bottom, wing-tank, top-side), location (starboard, portside, aft, peak), filling level of the tank, ballast water volume discharged, and physicochemical properties of the tank waters (temperature, salinity and pH) were recorded.

Laboratory procedures

Samples were carefully decanted and contents concentrated to a final volume of 20 mL and stored at 4 °C until processed. Diatom identification and enumeration were performed on a 10 mL sub-sample of the concentrate in a settling chamber (Hydrobios, Kiel, Germany) and expressed as cells L^{-1} or cells per ballast volume discharged as required. Autofluorescence of the chloroplast served as an indicator of cell viability at the time of fixation, and only fluorescing cells were counted. To reverse the quenching of chloroplast autofluorescence by Lugol's fixation, 4 drops of a saturated sodium thiosulfate solution in distilled water were added to the sub-sample prior to settling in the chamber. Counts were carried out using a ZEISS Axiovert 200 with epifluorescence illumination (HBO 50/AC, Mercury Shortarc) and 20 times and 40 times objectives. Using LM, fluorescing *Chaetoceros* cells (vegetative cells and spores ≥ 10 µm in shortest linear dimension) were identified to the lowest practical taxonomic level, most often to species. Cell morphometrics and scanning electron microscopy (SEM) were used to verify identification. Resting stages were counted when detected in LM however, many spores were too small (< 10 µm in longest dimension) for confident identification or enumeration. Therefore viable vegetative cells and larger spores were reliably identified and enumerated in LM, but much greater diversity and abundance of spores was found in SEM samples prepared directly from water mounts, due to the presence of the very small spores. For SEM examination, after LM counts were completed, the contents of the settling chamber were prepared by acid cleaning (modified from DREBES 1974) and dispersed onto filters as described in KACZMARSKA et al. (2005).

Statistical analysis

Hierarchical cluster analysis using average linking and Euclidean distance was performed on the entire data set using Systat version 10 (Systat Software, Inc., Chicago, IL).

Results

Ballast water

We found members of the genus *Chaetoceros* in 52 out of 67 ballast water (BW) samples. Each BW source differed either spatially or temporally (coast of Japan, Europe, America, mid-Pacific or mid-Atlantic) depending on if and where exchange took place. During the 2007 sampling season, a variety of BW treatments were applied to ships arriving at Canadian ports. Following International Maritime Organization (IMO) regulations, only ships that have undergone ballast water exchange may enter WC, EC or GL ports unless they commute from neighbouring waters (ports north of Cape Blanco, OR, USA, on the Pacific and north of Cape Cod, MA, USA, on the Atlantic coast). Ballast water exchange may occur anywhere on the route, adding to the variability of the biotic and

physicochemical properties of the tank waters. The application of cluster analysis to the entire sample set did not reveal any statistically significant patterns or similarities. This is not surprising considering the great variability of the ballast water sources, varying ballast water treatments and the varied abundance and species composition of very small spores (unidentifiable in light microscopy). Therefore, we did not pursue any more complex statistical analyses.

Among the 180 viable diatom species encountered in LM/SEM, we found the following resting stage or spore forming taxa: *Stephanopyxis* (with spore in formation)*, Leptocylindrus, Thalassiosira* and *Chaetoceros*. Here, we report on diversity and abundance of vegetative cells and spores belonging to the genus *Chaetoceros*. The volume of ballast water discharged (regardless of vessel-type or ballast water treatment) into the port ranged between 197 m³ and 22.897 m³. Depending on this discharged volume, we calculated between 2.2 x 10^5 (exchanged BW) and 3.6 x 10^8 (un-exchanged BW) *Chaetoceros* resting spores per tank for those found to contain *Chaetoceros* spores.

In 30 of 52 samples containing *Chaetoceros* (seen in LM) and prepared for SEM we found spores of approximately 30 species and other diatom and non-diatom taxa (e.g. Chrysophyceae). Due to the fact that many spores were smaller than 10–15 µm and could not be confidently identified and counted in the LM settling chambers, the data set comprising samples with enumerated spore densities is very conservative (Tab. 1). Using LM data, we recorded approximately 5 times higher (168–414 spores L^{-1} larger than 10 µm) spore densities in un-exchanged ships than in exchanged ships (1–75 spores L^{-1}; Tab. 1). The diversity of (large) spores encountered in LM counts is lower and not directly comparable to that found using SEM, which includes both large and small spores. Vegetative cells of species from the genus *Chaetoceros* were found in 49% of the samples. They were present in 18 WC, 13 EC and 2 GL samples. The most common and abundant *Chaetoceros* species in our samples identified to date by LM and SEM are listed below.

Most common species encountered

The following list details the most common *Chaetoceros* species found in the ballast water tanks (Figs. 1–16) and includes taxonomic references used to identify particular species, an indication of whether vegetative cells and/or spores were encountered, illustration of a specimen, size range (apical axis) of the cells, indication of abundance, and report of occurrence on Canadian or northern US-coasts, in that order. The abbreviation LA indicates length of apical axis.

1. *Chaetoceros* cf. *affinis* Lauder: PITCHER (1990), CUPP (1943); spores (Fig. 1); LA: 7.7–12.9 µm; abundant in one GL ballast sample; reported from EC/WC. *Note*: spores of *C. affinis* seem to bear more robust spines whereas ours feature rather delicate and thin spines. Apart from this character, the spore is closest to the description of *C. affinis* given by CUPP (1943).
2. *Chaetoceros ceratosporus* Ostenfeld: RINES and HARGRAVES (1988); vegetative cell and spore (Fig. 2); LA: 4.1–13.5 µm; found mainly in WC, rare in EC ballast; reported from WC (USA) and EC. *Note*: the allocation of the spore to C. *ceratosporus* might be disputable, however, it meets HUSTEDT'S (1962: p. 761) criteria describing it with the secondary valve smooth and the optional possession of spines: »Sekundärschale in der Mitte glatt, die langen Fortsätze sind zuweilen vorhanden«. In some of our species

spines were observed (as in RINES and HARGRAVES 1988), but these images are of low resolution and therefore not for publication.

3. *Chaetoceros cinctus* Gran: HASLE and SYVERTSEN (1997); spore with basal plate (Fig. 3); LA: 6.4–15 µm; found only in WC samples; reported from WC (USA) and EC.
4. *Chaetoceros compressus* Lauder: see PITCHER (1990) for spores; spores; LA: 5.5–9.8 µm; found in EC ballast only; reported from EC and WC.
5. *Chaetoceros* cf. *compressus* var. *hirtisetus* Rines et Hargraves: RINES and HARGRAVES (1990); spore size ca. 7 µm (Fig. 4); found in WC ballast; reported from EC.
6. *Chaetoceros constrictus* Gran: RINES and HARGRAVES (1988); vegetative cells and spores identified in LM; LA: 21–25 µm; found in WC samples; reported from EC and WC (USA).
7. *Chaetoceros convolutus* Castracane: RINES and HARGRAVES (1988); vegetative cells; LA: 9.1–17.4 µm; found only in WC ballast; reported from EC and WC.
8. *Chaetoceros debilis* Cleve: RINES and HARGRAVES (1988); vegetative cell and spore (Fig. 5); LA: 9.8–18.1 µm; found in EC and WC ballast; reported from EC and WC.
9. *Chaetoceros diadema* (Ehrenberg) Gran: RINES and HARGRAVES (1988); spore (Figs. 6,7) LA: 7.4 – 31 µm; found mainly in EC, with a few in WC ballast; reported from EC and WC.
10. *Chaetoceros* cf. *diadema* (Ehrenberg) Gran: HUSTEDT (1930); spore (Fig. 15); LA: 12.1 µm; found in WC ballast; reported from EC and WC (USA). *Note*: the closest match we could find in literature is the description by SUTO (2003) of *Monocladia humilis* and its assigned synonym *C. subsecundus* (syn. *C. diadema*). In general the literature describes the number of processes on the spore valve of *C. diadema* with more than 2, never with one singular process. However, the depicted spore might also represent C. *similis*.
11. *Chaetoceros didymus* Ehrenberg: RINES and HARGRAVES (1988); vegetative cell and spore (Fig. 8); LA: 8.8–29.8 µm; present only in WC ballast; reported from EC and WC.
12. *Chaetoceros furcillatus* Bailey: PETERSON et al (1999); spore (Fig. 9); size: 7.8 µm; found in one WC sample; reported from EC.
13. *Chaetoceros* cf. *hispidus* (Ehr.) Brightw.: CLEVE-EULER (1951); vegetative cell and spore (Fig. 10); LA: 11–27.4 µm; found only in WC ballast; no records on extant biogeography, poss. tertiary.
14. *Chaetoceros laciniosus* Schütt: RINES and HARGRAVES (1988); vegetative cell and spore; LA: 4.9– 0.5 µm; found in WC, EC and GL ballast; reported from EC and WC.
15. *Chaetoceros lorenzianus* Grunow: RINES and HARGRAVES (1988); vegetative cell and spore (Figs. 11, 12); LA: 5.3–24.6 µm; mainly in ballast from WC, rare in EC arrivals; reported from EC and WC. *Note*: Although Fig. 11 could also represent *C. decipiens*, RINES and HARGRAVES (1988) comment: »*C. lorenzianus* is extremely variable, and its varieties and forms are not well documented. Intermediate forms between *C. lorenzianus* and *C. decipiens* exist, at times making it difficult to separate these closely related taxa«, and a range of variability is also present in our material.
16. *Chaetoceros* cf. *radicans* Schütt: RINES and HARGRAVES (1988); vegetative cell and probable spore; LA: 5.7–16.2 µm; found mainly in WC, rare in EC ballast; reported from EC and WC.

17. *Chaetoceros* cf. *similis* Cleve: RINES and HARGRAVES (1988); vegetative cell and spore (Fig. 13); LA: 8.2–10.6 µm; found in WC ballast only; reported from EC and WC.
18. *Chaetoceros* cf. *socialis* var. *radians* (Schütt) PROSCHKINA-LAVRENKO: PROSCHKINA-LAVRENKO (1953); vegetative cell and spore (Fig. 14); LA: 5.1–10.4 µm; mainly in WC, rarely in EC ballast; reported from EC and WC.
19. Spore no.1 (Fig. 16); Identity could not be confirmed because we did not find valves of the vegetative stage attached. It is however similar to *Chaetoceros coronatus*.

Discussion

Quantities of vegetative and resting *Chaetoceros* propagules

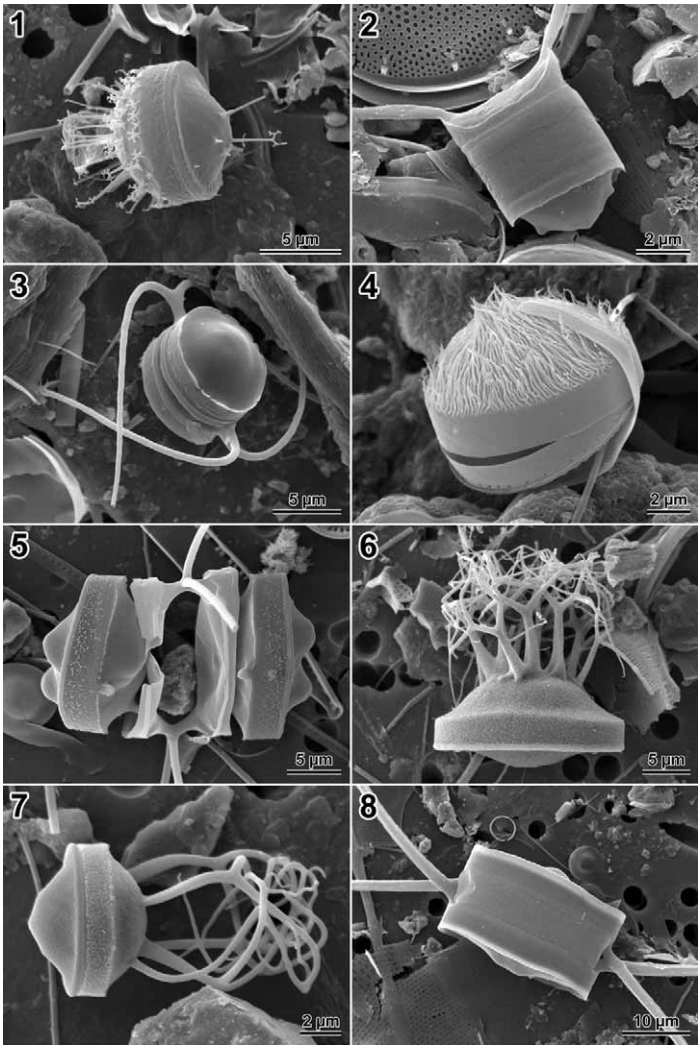
Our results serve to estimate *Chaetoceros* propagule pressure in Canadian coastal waters. Compared to *Pseudo-nitzschia* species found in the same ballast tanks (16 species; up to 1.2 x 10^{10} cells L^{-1}), *Chaetoceros* species arrive at Canadian ports in more moderate cell densities but higher species diversity (over 30) which we consider representative of ports receiving shipments from a wide variety of sources. Vegetative cell densities of *Chaetoceros* spp. ranged from 1–5.6 x 10^4 cells L^{-1}, while large spores identifiable using light microscopy (apical axis > 10 µm) were less frequent; with 1–414 live spores L^{-1}. We found ballast water cell densities comparable to that found in one sample from the receiving port of Vancouver, where a bloom of one *Chaetoceros* species produced 2.7 x 10^3 viable cells L^{-1} and 87 spores L^{-1}. However, late spring blooms of Canadian EC *Chaetoceros* spp. often carry cell densities that are an order of magnitude higher (MARTIN et al. 2001, 2006). The highest densities of spores carried to receiving ports were found in unexchanged ships traveling along the coasts of North America (414 spores L^{-1}), while the vessels that underwent mid-ocean exchange brought in, on average, fewer than 10 (large) live spores L^{-1}. Exchanged ships carried up to 75 spores L^{-1} in their ballast. If the low densities of large spores are also representative of the scarcity of all *Chaetoceros* spores in transoceanic vessels exchanging ballast in the open oceans, then the spore density and diversity originating from exotic coastal waters (across the ocean) were diminished due to ballast water exchange. This was particularly evident in ballast arriving in trans-Atlantic vessels, where spores were detected in SEM, but below countable quantities in LM.

Placed in the context of ballast tank volumes discharged, however, these moderate cell densities can become a sizable cell inoculate. We sampled tanks deballasting considerable inocula, up to 4.3 x 10^{10} live vegetative *Chaetoceros* spp. cells L^{-1} and 3.6 x 10^8 live *Chaetoceros* spores L^{-1} into the port waters. Apart from these particular examples of quantities discharged, the annual volumes of ballast waters discharged are considerable as well. If the compiled data set for 2007 is a true representation of a typical year of ballasted ship traffic in Canada, 22.7 x 10^6 t of ballast water were discharged in the Atlantic region, 16.3 x 10^6 t in the Pacific and 1.7 x 10^6 t in the Great Lakes/St. Lawrence regions (LO pers. com.). These volumes combined (just one year in one country) equal the total of 40.7 x 10^6 t. Divided by the population of Canada (33 million) this results in about 1.25 t of ballast water per capita in the country. Also, we found a higher than anticipated species diversity in our *Chaetoceros*-containing samples. Twenty nine species were encountered, with approximately half of these present as both vegetative cells and spores. Within the genus *Chaetoceros*, 73 spore forming species have been reported (STOCKWELL and HARGRAVES 1984).

Tab. 1. Summary of densities of viable vegetative cells and spores in examined samples (LM) and presence/absence information for spores from SEM.

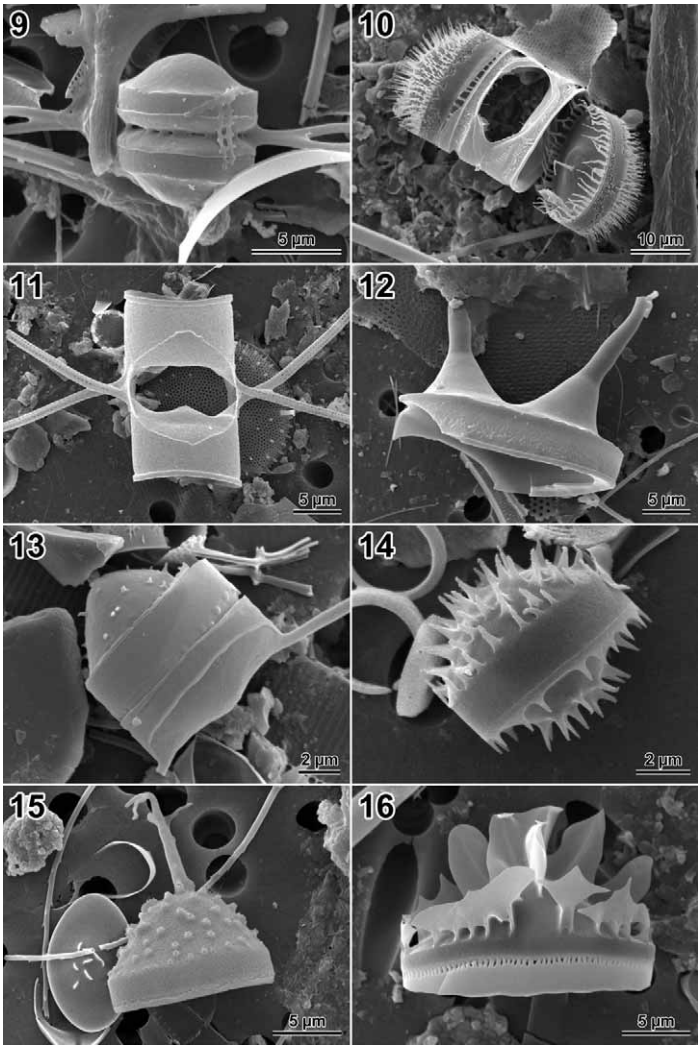
Sample	Exchange type	Total spores (cells/L)	Total vegetative cells (cells/L)	Sample	Exchange type	Total spores (cells/L)	Total vegetative cells (cells/L)
EC002	mo	–	–	WC001	nx	d	56,993
EC004	mo	d	7,441	WC002	nx	–	–
EC005	mo	–	11	WC003	mo	–	280
EC008	mo	d	–	WC004	mo	d	1,525
EC009	mo	d	1,690	WC005	nx	–	–
EC011	co	d	–	WC006	mo	d	18
EC013	mo	d	604	WC007	mo	–	11
EC016	co	–	–	WC008	mo	d	96
EC020	mo	d	–	WC009	mo	1	–
EC024	mo	d	128	WC010	mo	d	43
EC025	mo	–	26	WC011	mo	–	–
EC026	mo	–	–	WC013	co	75	163
EC027	mo	–	62	WC014	mo	5	–
EC030	co	–	1,176	WC015	co	d	–
EC032	co	d	–	WC016	nx	168	19
EC034	mo	–	–	WC017	nx	240	540
EC036	mo	–	–	WC018	co	–	–
EC038	mo	–	133	WC019	nx	d	991
EC039	mo	d	13	WC020	mo	–	1
EC041	mo	3	337	WC021	mo	d	–
EC044	mo	d	33	WC022	co	d	–
EC046	mo	d	–	WC023	co	d	–
EC049	mo	–	–	WC024	mo	–	–
EC050	mo	d	18	WC026	nx	414	523
GL001	mo	–	–	WC028	co	d	955
GL002	mo	–	–	WC029	mo	d	–
GL003	mo	–	–	WC030	mo	d	–
GL004	mo	27	–	WC032	mo	–	–
GL005	co	–	–	WC033	mo	67	5
GL006	mo	–	–	WC034	co	5	18
GL007	co	9	57				
GL008	nx	–	22				
GL009	mo	11	–				
GL010	mo	–	–				
GL011	co	–	–				
GL012	mo	–	8				
GL014	mo	–	–				

mo = mid-ocean exchange; co = coastal exchange; nx = no exchange; d = detected in SEM; – = not detected in LM or SEM



Figs. 1–8. *Chaetoceros* spores and/or vegetative cells (SEM). 1 – *C.* cf. *affinis*; 2 – *C. ceratosporus*; 3 – *C. cinctus*; 4 – *C.* cf. *compressus* var. *hirtisetus*; 5 – *C. debilis*; 6, 7 – *C. diadema*; 8 – *C. didymus*.

Our study distinguished 31 spore types, 18 of which could be identified to species level, albeit with uncertainty in some cases. In addition, 12 species (including those that are not known to form spores) were present as vegetative cells. This number of *Chaetoceros* species recovered from just 67 tanks arriving in Canada within a four month time-period rivals the diversity reported from multiannual monitoring in the entire Quoddy Region of the Bay of Fundy (MARTIN et al. 2001, 2006) and is comparable to the number of species reported from considerably larger water bodies such as the Gulf of St. Lawrence (BÉRARD-THERRIAULT et al. 1999) or Narragansett Bay (RINES and HARGRAVES 1988). Furthermore, residual ballast water and resuspended sediments increase the diversity we encountered, especially since the viability of spores found using only SEM could not be determined.



Figs. 9–16. *Chaetoceros* spores and/or vegetative cells (SEM). 9 – *C. furcillatus*; 10 – *C.* cf. *hispidus*; 11– *C. lorenzianus/C. decipiens?*, 12 – *C. lorenzianus*; 13 – *C.* cf. *similis*; 14 – *C.* cf. *socialis* var. *radians*; 15 – *C.* cf. *diadema* (close to *C. similis*); 16 – Spore no.1., similar to *C. coronatus*.

It is difficult to assess whether the observed diversity of spore bearing species is an unusual characteristic only of our samples (though this would be unexpected), because other ballast water diatom studies have rarely reported or identified *Chaetoceros* spores. Two major factors may have contributed to a high diversity of spores in our samples. First, most ballast examined was taken up during May–June, a time when a later spring diatom bloom becomes rich in *Chaetoceros* species in boreal temperate waters. Second, some spores we encountered may have been residual occupants of the tanks, that is they could have been re-suspended from the bottom of the tank sediment during ballast water exchange. De-

pending on ship type and atmospheric conditions during ballast exchange, up to 5% of the tank bottom waters and sediments are retained despite exchange. This would allow accumulation of sediments in ballast tanks and retention of associated biota, including resting spores (HALLEGRAEFF and BOLCH 1992).

Additional concern related to the discharge of non-indigenous organisms (or genotypes) is the presence of potentially harmful taxa. Among the species found in our study are those listed by BATES and STRAIN (2006) as harmful: *Chaetoceros concavicornis, C. convolutus* and *C. debilis*; implicated in fish mortality due to gill irritation on the Canadian West Coast and also reported from the East Coast, albeit in low cell numbers to date. *C. concavicornis, C. convolutus* and possibly *C. danicus* are listed by HORNER et al. (1997) as harmful species on the U.S. West Coast. Of these, we found *C. convolutus, C. danicus* and *C. debilis* (with spores) in both EC and WC samples.

In the natural environment, sporulation often occurs when growth conditions become suboptimal. Light and nutrient depletion, and to a lesser degree oscillations of water temperature (HOLLIBAUGH et al. 1981) may trigger spore production (MCQUOID and HOBSON 1995, 1996). The deprivation of nitrate in ambient waters, for example, has been repeatedly shown to induce spore formation (GARRISON 1981, KUWATA and TAKAHASHI 1999). It is possible that the physicochemistry within a ballast water tank facilitates sporulation, as prolonged darkness, decline of oxygen concentration, biomass decay and oscillation of mineral nutrient concentration are expected in ship ballast tanks.

Several studies have shown the survival potential of diatom dormant stages (spores and resting cells), some germinating after years (e.g. ANIL et al. 2007), even after exposure to high temperatures (ITAKURA et al. 1997). A period of dormancy of approximately two years seems to be common in many coastal species, with an upper limit of nine years for diatom spores (MCQUOID et al. 2002) and decades for resting cells (SICKO-GOAD et al. 1989). Conditions within residual ballast tank sediments are not unlike those found in coastal sediments, including anoxia, darkness and changes in temperature. Spore-producing coastal species, such as members of the genus *Chaetoceros*, may be well adapted to man-made environments such as ballast tanks, an advantage that may further contribute to their cosmopolitan distribution.

Our study also revealed that not only a considerable number of species, some potentially harmful, but also species not yet recorded from at least some Canadian coasts (such as *C.* cf. *hispidus*) are being discharged via ballast water. For example, abundant specimens of the cosmopolitan coastal marine species *C. affinis* were found in one ballast tank destined for the Great Lakes. In addition (including species found in BW samples but not represented in our list), *C.* cf. *compressus* var. *hirtisetus*, *C. coronatus*, *C. densus*, *C.* cf. *subsecundus*, and *C. simplex* var. *calcitrans* were found in ballast water samples arriving in Vancouver, while our extensive literature search shows them reported thus far only from the EC. Some species known from the Canadian Atlantic coast and more southern locations of the U.S. Pacific (*C. cinctus*, *C. ceratosporus*, *C. dichaeta*) were found in ballast destined for Vancouver BC, but not yet reported from western Canadian coastal waters, probably due to the scarcity of floristic studies. The latter example underscores challenges facing microbial invasive biology. The »waxing-and-waning« of diatom populations, scarcity of long term and historical base-line research for large regions of the world (SMAYDA 2007) and changing taxonomy (ROUND et al. 1990) all add to impairment of our capacity to detect and recognise non-indigenous micro-biota.

In summary, we found a considerable number of species from the genus *Chaetoceros* arriving live at Canadian ports in just 67 sampled ballast tanks. The highest densities of vegetative cells and spores were brought by vessels coming from the close southern neighbourhood, along the continental shelf from the same bioprovince. Because the species composition of this source of ballast waters is expected to be similar to that from Canada, such vessels do not undergo ballast water exchange. Vessels undergoing ballast water exchange carry smaller diatom inoculums per cargo vessel. Considering the shipping traffic magnitude and the volume of ballast waters globally translocated annually, the rate and consistency of the inocula and diversity of diatom species introduced to foreign ports should be of concern to Canada and other countries.

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