

Marine dinoflagellate cysts in the ballast tank sediments of ships entering the Laurentian Great Lakes

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Introduction

One of the greatest threats to the ecological health of the Laurentian Great Lakes is the introduction of non-indigenous species (MILLS et al. 1993). The Laurentian Great Lakes have a long history of non-indigenous introductions with significant ecological and economic consequences. Since the opening of the St. Lawrence Seaway in 1959, approximately 65 % of all non-indigenous species introductions into the Great Lakes have been attributed to ballast water release (RICCIARDI 2006). Because of the concern of ballast water and its potential for introduction of non-indigenous species, the U.S. Coast Guard promulgated regulations in 1993, requiring ships with ballast water inbound to the Great Lakes to exchange ballast water with open ocean water. Despite these new regulations, the discovery rate of non-indigenous species increased after mandatory controls were implemented (HOLEK et al. 2004). Unfortunately, most of the ships entering the Great Lakes are exempt from the 1993 regulations. The majority of ships entering the Great Lakes since 1993 are loaded with cargo and are declared as 'no ballast water on board' (NOBOB) ships. These NOBOB ships carry residual water and sediments in their ballast tanks. Once in the Great Lakes these NOBOB ships discharge their residual water and sediment into the Great Lakes during the off-loading of inbound cargo and the loading of outbound cargo. These NOBOB ships represent a greater risk for introductions into the Great Lakes than ballasted ships (DUGGAN et al. 2005).

The residual sediment and water found in ballast tanks of NOBOB ships contain a variety of live organisms, and resting stages, cysts, spores, and eggs of algae and invertebrates (DUGGAN et al. 2005). Resting stages of many algae may remain viable in sediments for decades or even centuries (SICKO-GOAD et al. 1986) and can germinate under favorable environmental conditions. Dinoflagellate cysts have already been reported in the ballast tank sediments of ships entering ports in a variety of saltwater ports (HALLEGRAEFF & BOLCH 1992, HAMER et al. 2001). The non-indigenous, toxic dinoflagellate *Gymnodinium catenatum* Graham was introduced to Tasmania, Australia, and caused the closure of Tasmanian shellfish farms in 1986 and in 1987 (HALLEGRAEFF & BOLCH 1992).

The purpose of this study was to examine the residual water and sediments in tanks of NOBOB ships entering the Great Lakes for the presence of marine dinoflagellates. Marine dinoflagellates are a common component of the sediments of marine-going ships, but their abundance and composition has not been determined for ships entering freshwater ports, such as those in the Great Lakes.

Key words: harmful algae, NOBOB, non-indigenous species

Methods

Ballast tanks of NOBOB ships were sampled in 2002 and 2003 at various ports in the Great Lakes (e.g., Cleveland, Toledo). Deck hatches were used to gain access to ballast tanks, where water samples were collected by hand pump from the bottom of the ballast tank. Sediment samples were collected aseptically by using spatulas. In most cases, two tanks were sampled per ship. For each ballast tank, environmental measurements (temperature and salinity) and ship/tank identifiers, including ship's age, time since ballast tanks were cleaned, ballast tank type (double bottom, side, fore-peak, upper wing), and whether the tanks were flushed at sea on the most recent trip, were determined. The water and sediment samples were stored at 4 °C.

In the laboratory, phytoplankton slides were prepared (DOZIER & RICHESON 1975) from water samples and analyzed for cyst abundance and type, and total algae. This slide preparation technique consisted of filtering a water sample (both preserved and live) onto a membrane filter, clearing the mounted filter, and permanently mounting the filter on a slide for microscopic analysis. Lugol's solution was used to preserve phytoplankton samples. For sediment samples, a small (2–4 ml) sample was mixed with 30 ml of filtered water from the same tank, agitated using the super mixer for 2 minutes, washed with filtered water sample, and fractionated through 115 and 20 µm mesh sieves. The material retained on the 20-µm mesh was washed into a beaker and diluted to a known volume. Density gradient centrifugation was used to concentrate cysts types (BOLCH 1997). Both live

and preserved (Lugol's) samples were analyzed. All cyst identification and enumeration were conducted on a Leica DMR compound microscope. Photomicrographs of cysts were taken with a Optronix DEI-750 CE camera system using Image-Pro software. Cyst identification was based upon morphological criteria described in the literature (FUKUYO 1982, DALE 1983, BALDWIN 1987). The correspondences between ship/tank variables and dinoflagellate/cyst presence-absence and absolute/relative abundances within assemblages initially were determined using Pearson product moment correlation coefficients (SYSTAT 10 2000). Data were square root- or logarithmic-transformed (where appropriate) to stabilize variance and increase homogeneity of normalcy.

Results

Of the total 49 NOBOB ballast-tank samples analyzed, only 4 samples had no dinoflagellate cysts present. Dinoflagellate cysts represented 0–80 % of total algal abundance, with an average of 24 % (Fig. 1). The maximum number of dinoflagellate cysts taxa present in any one sample was 13 (Fig. 2).

A total of 35 cyst taxa were identified. Approximately 45 % of these taxa were found in only one sample, which included mostly *Protoperidinium* and *Gonyaulax* species. Four species were found in more than 20 % of the samples, including *Alexandrium minutum* (found in 15 of our 49 samples), *Protoperidinium oblongum*, *Alexandrium hiranoi*, and *Polykrikos schwartzii* (all found in 10

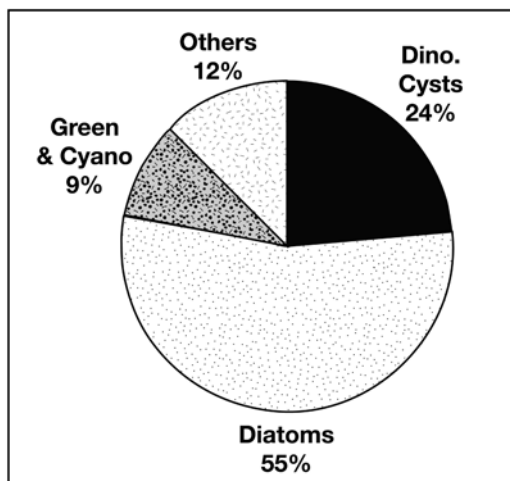


Fig. 1. Relative abundance of various algal groups in all ballast tank samples. Green & Cyano = Chlorophyceae and Cyanobacteria; Diatoms = Bacillariophyceae; Dino. Cysts = marine Dinophyceae (Dinoflagellate) cysts; Others = Other algae.

samples). Species more intermediate in their occurrence included: *Gonyaula polydra* and *Alexandrium lustanicum* (8 occurrences each); *Scrippsiella trochoidea*, *Protoperidinium excentricum*, and *Gymnodinium catenatum* (6 occurrences); *Alexandrium affinis*, *Gonyaulax verior*, *Protoperidinium sublinerme*, *P. leonis*, and *P. conicum* (5 occurrences); and *Alexandrium tarmarensense* (4 occurrences). Cysts of toxin-producing, marine dinoflagellates (5 taxa of *Alexandrium*, *Gonyaulax spinifera*, and *Gymnodinium catenatum*) were commonly observed in our samples. These taxa were found in 60 % of our samples and represented 31 % (range 0–100 %) of total cyst abundance.

An analysis of ship/tank variables and abundance of dinoflagellates showed that the number of dinoflagellates present in a particular sample was negatively correlated with ship's age ($r = -0.35$, $p < 0.05$; lower numbers associated with newer ships) and whether the tanks were flushed at sea ($r = -0.65$, $p < 0.01$; lower numbers associated with flushing).

Discussion

This study demonstrated that marine dinoflagellate cysts are common representatives in ballast tank sediments of transoceanic ships entering the freshwater Great Lakes. Despite the small and limited sampling size in this study, marine dinoflagellate cysts were found in more than 90 % of the ships and represented more than 20 % of total algal abundance. The number of ships where marine dinoflagellates were found is higher in the Great Lakes than in saltwater ports. In British ports, approximately 69 % of the ships had marine dinoflagellate cysts (HAMER et al. 2001), whereas in Australia, only 40 % of

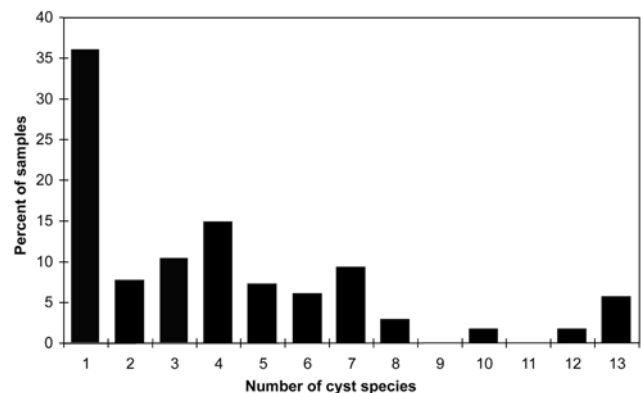


Fig. 2. The percentage of samples with number of dinoflagellate cysts found.

the ships contained dinoflagellate cysts (HALLEGRAEFF & BOLCH 1991). These differences are unexpected and are probably related to sampling and/or ship management practices.

The large number of marine dinoflagellates present in Great Lakes ships was also unexpected; approximately half of all known marine dinoflagellate cyst taxa were found in our ballast tank samples. Even though we only analyzed 53 samples, 38 taxa were identified, which is approximately half of the 80 known dinoflagellate cyst species (HALLEGRAEFF & BOLCH 1992). The number of dinoflagellate taxa found in ballast tanks in this study is similar to those published for ships entering British (31 taxa) and Australian (53 taxa) ports (HALLEGRAEFF & BOLCH 1992, HAMER et al. 2000).

The cysts of several harmful marine dinoflagellates were common in the sediments of ships entering the Great Lakes. Among them, cysts belonging to potentially toxin-producing species of the genus *Alexandrium* were the most frequently encountered. Five *Alexandrium* taxa were identified, and 2 taxa (*A. tarmarence/catenatum* and *A. minutum*) were found in 33% of our samples. Other harmful bloom-forming species, including *Gonyaulax polydra*, *Gymnodinium catenatum* and *Scrippsiella trochoidea*, were also found. The presence of harmful marine dinoflagellate cysts in ballast tanks has been documented worldwide and has been suggested as one of the dominant vectors responsible for the apparent global increase in harmful algal blooms (HALLEGRAEFF 1998).

The inoculation, germination, and growth of cysts and other resting stages are critical steps in the multi-phase process of success establishment of non-indigenous species. All the dinoflagellate cysts found in our study were marine species, and thus their invasion threat to the Great Lakes is extremely low. On a few occasions, cysts isolated from our experiments were placed in freshwater WC media (GUILLARD & LORENZEN 1972), and one species, *Scrippsiella trochoidea*, did germinate in freshwater media (Y. HONG, pers. comm.). Therefore, we cannot preclude the possibility that some taxa germinate in freshwater.

The ability to germinate in freshwater media does not equate with the ability to grow and establish large populations in the Great Lakes and other freshwater environments. While germination may be possible, we do not believe it is likely that any of these marine dinoflagellates will establish large and thriving populations in the Great Lakes. Several marine dinoflagellates can grow at low salinities (1–6 ppt; MAHONEY & McLAUGHLIN 1979, SULLIVAN & ANDERSEN 2001), but we found no evidence that any of the 35 marine dinoflagellates noted in this study can grow in freshwater (0 ppt).

Because marine dinoflagellate cysts have been suggested as model organisms to evaluate monitoring and treatment programs for controlling the spread of non-indigenous marine organisms (HALLEGRAEFF 1998), the results of our study may have broader implications for controlling the introduction of non-indigenous species in the Great Lakes.

Based on our results, ship management strategies may be useful to reduce the risk of transporting non-indigenous species in ballast water and sediment. In this study, flushing of ballast tanks significantly reduced the number of marine dinoflagellate species and should be considered an essential management strategy for NOBOB ships. Ships carrying pumpable ballast water have been required by U.S. regulations to conduct open-ocean exchange of their ballast water prior to entering the Great Lakes since 1993. The U.S. regulations, however, do not apply to NOBOB ships. In 2006, a new Canadian law resulted in regulations that require the salinity of all water in ballast tanks on all ships entering the Great Lakes to exceed 30 ppt. The NOBOB ships must flush their tanks with saltwater if they have not previously conducted an open-ocean exchange since last ballasting, and must also maintain on-board records demonstrating an active sediment reduction program. DUGGAN et al. (2005) also suggested that use of saltwater flushing by NOBOB ships would reduce the invasion risk from invertebrates. Until more effective permanent solutions are implemented, the use of saltwater flushing by NOBOB ships is a reasonable approach to reduce the potential for non-indigenous species introductions into the Great Lakes.

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