Diversity and Distributions, (Diversity Distrib.) (2010) **16**, 132–143

BIODIVERSITY RESEARCH

Is vessel hull fouling an invasion threat to the Great Lakes?

Francisco Sylvester* and Hugh J. MacIsaac

Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON, Canada N9B 3P4

*Correspondence: Francisco Sylvester, Great Lakes Institute for Environmental Research, University of Windsor, Windsor, ON, Canada N9B 3P4.
E-mail: fsf@uwindsor.ca

ABSTRACT

Aim Hull fouling is a leading vector for the introduction of marine, non-indigenous species (NIS) worldwide, yet its importance to freshwater habitats is poorly understood. We aimed to establish the complement of NIS transported via this vector to the Great Lakes and to determine if they pose an invasion risk.

Location Laurentian Great Lakes.

Methods During 2007 and 2008, we collected scrapings from exterior surfaces as well as underwater video-transects from 20 vessels shortly after their arrival in Great Lakes' ports. Invertebrates present were sorted and identified in the laboratory.

Results Total estimated abundance averaged > 170,000 invertebrates per ship belonging to 109 taxa. Most (72%) of these taxa were freshwater species already present in the Great Lakes, whereas 11 and 31% were native to estuarine and marine habitats respectively, and would not be expected to survive in this habitat. Abundance was dominated by barnacles (51%), cladocerans (19%), bivalves (12%) and amphipods (11%). Sea-chest grating and the rudder were hot-spots for biofouling. Invertebrate diversity and total abundance were positively associated with total time spent in port during the last year and time in Pacific South American ports and negatively related to time in high latitudes and sailing speed. Although we found some live, established invaders such as *Gammarus tigrinus* and *Dreissena rostriformis bugensis*, only one individual of a freshwater NIS (*Alexandrovia onegensis*, Oligochaeta) not yet reported in the Great Lakes was detected. The animal's poor condition and seemingly low population abundance indicated the risk of live introduction by this vector was likely quite low.

Main conclusion Our results indicate that hull fouling appears to pose a low risk of introductions of new species capable of surviving in the Great Lakes, unlike foreign-sourced freshwater ballast water that historically was discharged by these transoceanic vessels.

Keywords
Biological invasions, Great Lakes, hull fouling, invasion vectors, non-indigenous species, ships.

INTRODUCTION

The introduction of species to habitats to which they are non-indigenous is a principal form of global change (Lawler *et al.*, 2006). Introduction of non-indigenous species (NIS) has increased in both terrestrial and aquatic environments worldwide commensurate with increased global trade and travel (see Ruiz & Carlton, 2003). Human populations are utterly dependent on ships for global commerce, with over 40% of the world's imports transported by sea by a fleet of more than 50,000 ships (Lloyd's Register Fairplay, 2008; World Trade Organization, 2008). Not surprisingly, shipping has played a key role in the spread of NIS globally and is a dominant mechanism by which marine species have been introduced to a number of coastal habitats (Molnar *et al.*, 2008). Shipping activities present a number of diverse vectors, by which NIS may be transported including ballast water, ballast sediment and fouling of the sea-chest and of numerous

DOI:10.1111/j.1472-4642.2009.00622.x
www.blackwellpublishing.com/ddi

external surfaces (hereafter generalized as 'hull fouling') (see Carlton, 1985; Carlton & Hodder, 1995; Gollasch, 2002, 2007; Fofonoff *et al.*, 2003; Minchin & Gollasch, 2003; Coutts & Taylor, 2004).

Hull fouling is recognized as a very important vector of introduction, having played a central role in introduction of aquatic NIS to many coastal, marine habitats (e.g. Carlton, 1985; Gollasch, 2002; Coutts *et al.*, 2003; Godwin, 2003; Farrapeira *et al.*, 2007). The importance of this vector reflects the many centuries of marine shipping by wooden and later steel-hulled vessels, the large number of vessels engaged in global trade and the large surface areas available to be colonized by NIS (Carlton & Hodder, 1995; and references cited therein). Despite this, hull fouling typically has not been included in government invasive species management programmes, Australia being an exception.

The Laurentian Great Lakes have sustained a steady increase in the number of NIS that has been reported established over the past 150 years (Holeck *et al.*, 2004; Ricciardi, 2006). The most important vector of NIS introduction since the modern St. Lawrence Seaway began operation in 1959 is ballast water released from transoceanic vessels, which accounts for between 55 and 70% of new established NIS during this period (Holeck *et al.*, 2004; Kelly *et al.*, 2009). Hull fouling seems comparatively unimportant, accounting for likely introduction of only two (< 4%) species, the red alga *Bangia atropurpurea* (Agardh, 1824) and the green alga *Enteromorpha flexuosa* (Bliding, 1963) (Kelly *et al.*, 2009). To date, only a single study has examined hull fouling on the Great Lakes. Patch samples collected from a single vessel that was sampled during emergency dry-dock on Lake Ontario included 74 distinct marine and freshwater fouling taxa, although the total community may have included between 100 and 200 species (Drake & Lodge, 2007). Eight of 29 taxa identified to species level had never been reported in the Great Lakes (Drake & Lodge, 2007). Considering that between 439 and 622 transoceanic vessels entered the Great Lakes each year between 1994 and 2000 (Colautti *et al.*, 2003), hull fouling would at first glance appear to be a potentially important mode of species introduction. This vector could be enhanced by the implementation in 2008 of a ban on use of highly effective tributyl tin-based antifouling paints, as required by International Convention of the Control of Harmful Antifouling Systems on Ships (Evans *et al.*, 2000).

It is possible that freshwater species attached to hull surfaces could be regularly introduced to the Great Lakes, yet fail to colonize successfully because they arrive dead or in very poor condition following open-ocean exposure (Colautti & MacIsaac, 2004). The present lack of regulation of hull fouling relative to ballast water vectors reflects an assumption that the Great Lakes are invulnerable to invasions via hull fouling species. In this study, we present the first large-scale assessment of fouling communities on transoceanic vessels operating on the Great Lakes to explore the invasion risk posed by this vector and whether management is required.

METHODS

During the summers of 2007 and 2008, we surveyed exterior surfaces of 20 commercial transoceanic vessels visiting ports on the Great Lakes. Vessels were sampled in both Canadian (Clarkson, Hamilton) and USA (Cleveland and Toledo) ports. All surveys were conducted by contract divers while ships were stationary in port. We sampled bulk carriers (16 vessels) and chemical tankers (four vessels), two of the most prevalent types of transoceanic vessels entering the Great Lakes. Sampling was opportunistic and based on availability of vessels and dive teams, although an effort was made to cover a range of ships in terms of time since last dry-dock and time since application of antifouling paint. The first ship surveyed had been dry-docked only 3 months earlier, and its hull surfaces were free of fouling. Using the results of this survey and divers' hull inspection experience, we then set a minimum time of 10 months out of dry-dock for the rest of ships surveyed. This sampling limitation would tend to ensure that we sampled more moderately and heavily fouled ships than would be represented by all vessels inbound to the Great Lakes. We also sought to include vessels from different shipping companies to better reflect the diversity of vessel types, operational patterns and maintenance practices of the shipping community servicing the Great Lakes.

Divers surveyed both sides of the hull of each ship from bow to stern and bottom to waterline (Fig. 1). However, access to the mid-ship section was normally limited by the size of the gap between the hull and the berth bed and wall. The following locations were inspected in all ships surveyed: the rudder sides, bottom, leading and trailing edges, propeller nose and blades, rope guard, stern tube, sea-chests, bow-thruster tunnel and grating, bulbous bow, stem and main hull (Fig. 1). Other locations sampled included the bilge keel, kort nozzle, skeg, anodes, cathodic protections and water discharge holes. However, these locations were found only on a low percentage of the ships, normally associated with low abundances of fouling organisms, and were excluded from the analysis. Our in-water sampling design did not allow us to open and sample inside sea-chests and other protected locations. We did, however, sample from the grating covering the sea-chests. Therefore, hull fouling was probably underestimated in such locations. Two visually identifiable categories of hull fouling were determined: algae and shells. A preliminary analysis showed that shells were mainly barnacles, and will be referred hereafter as 'barnacles'. Physical samples were collected at each location where growth was observed, whereas a value of zero organisms was recorded in locations where no growth was found. Thus, physical sampling was not random, but aimed to include the highest possible number of organisms and species. To estimate average abundances m^{-2} and for the whole ship, we used percent cover information from random video-transects of the entire hull (see below). Sampling was conducted at each location with 1–3 replicate 20 × 20 cm magnetic quadrats attached to the hull of each vessel, or equivalent surface area at uneven locations where the use of the

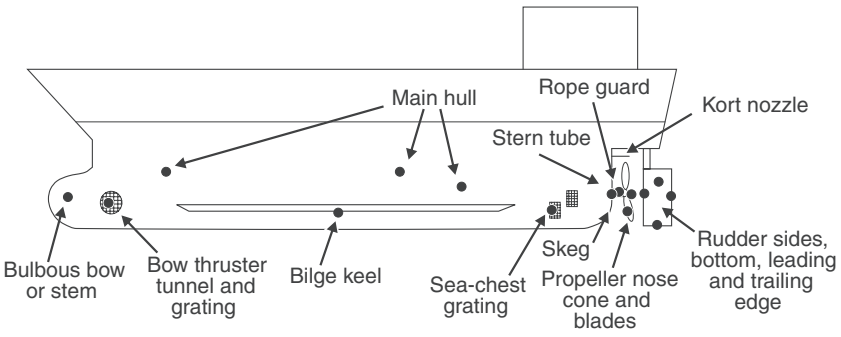

Figure 1 Locations of exterior surfaces, from which physical samples were collected on transoceanic vessels operating on the Great Lakes. Modified from Coutts & Taylor (2004).

quadrat was not practical. Barnacle samples were scraped into re-sealable plastic bags and a suction device mounted on a scraping blade was used to collect soft growth. As each sample invariably included a volume of water surrounding the ship, we also collected 2 L water samples from the dock at mid-hull depth to be used as controls. The water volume of each hull fouling sample was measured at the dock using a graduated cylinder, and organisms in the equivalent volume of control water sample were subtracted from abundance counts (see below). Species present in control water samples were subtracted from species richness estimations (see below). For five ships, hull samples were examined with naked eye at the dock, prior to fixation, to determine if organisms were dead or alive when collected. However, this analysis was limited to barnacles, amphipods, chironomids, mites, large bivalves and gastropods. The remaining groups were not large enough to be checked reliably. Specimens that appeared alive (e.g. movement, valve/plate closure) were preserved separately. All samples were sieved through a 40 μm mesh and preserved in 95% ethanol at the dock. Information including the list of ports-of-call since last dry-dock or 1 year previous to sampling, antifouling protection and the sheet of particulars were collected from the crews of each vessel.

All samples were processed in the lab to estimate abundances and conduct taxonomic identifications. We did not find any macroalgae in our samples. Instead, algal cover was restricted to relatively thin films lacking reproductive structures, thus only organisms from selected samples were identified at a coarse taxonomic level. For this reason, we focused our efforts on invertebrate animals, with consideration of algae restricted to their potential as habitat for invertebrates.

All specimens of non-barnacle invertebrates larger than 1 mm were sorted in the lab, identified under a dissecting microscope and used to estimate abundance. As barnacles often broke during sampling, abundances per sample were estimated based on specimen (all entire shells) and plate (volume-fractionated shell fragments) counts. All entire barnacles and representative samples of fragments were collected for identification. The < 1 mm fraction of the samples was split in the lab using a Folsom splitter (McEwen *et al.*, 1954). At least half of that fraction was sorted under a dissecting microscope and all organisms were identified to main taxonomic groups to estimate abundances. All organisms sorted, or a minimum of 30 individuals of each cladoceran and copepod morphotype found, were collected from each sample to conduct taxonomic identification. Taxa were identified to the lowest possible taxonomic level and classified as native, established NIS or non-established NIS to the Great Lakes by consultation with global taxonomists (see Acknowledgements). Many specimens were not identified to species level and therefore our analysis provides lower-bound estimation of species richness. As a result of the colonial nature of polychaetes and hydrozoans and poor condition of the specimens in our samples, we were not able to quantify abundances and taxonomic identifications were limited for these groups. Rotifers and nauplii were excluded from our analysis.

Between 1 and 3 h, random video-transects were shot of each ship at all sampled locations to estimate total percent cover, abundances m^{-2} and total abundances. We subsequently analysed *c.* 60 still images per ship using a random stratified design per location. Percent cover of algae and barnacles in the images was estimated by superimposing a 50-point grid to the images. Surface area of each underwater location on the hull was calculated by approximation to simple geometric forms, using technical information and plans of underwater parts provided by the ships' crews. Taxa abundances per location were calculated by multiplying average abundances in 20 × 20 cm physical samples (by estimated % cover at that location) by the location's surface area (m^2) and dividing by the surface area of the sampling quadrat (0.04 m^2). Abundances for the whole ship were calculated as the sum of abundances in all locations. Abundances per wetted surface area were obtained by dividing total abundances per ship by total surface area of underwater locations. These calculations were not possible for one ship, for which we were unable to get video footage and therefore that ship was only used for analyses related to species richness.

Statistical analysis

To examine whether the sampling design used in the study adequately estimated the likely species pool on a vessel, we estimated species richness for each ship sampled using the Chao-2 species richness estimate. Chao-2 estimate is recommended for quadrat sampling over a heterogeneous habitat, such as the sampling conducted here (Chao, 1987; Chao & Shen, 2003). We estimated Chao-1 species richness for all ships pooled to make a

comparison between that estimation and the Chao-1 value reported for the ship sampled by Drake & Lodge (2007). We generated an individual-based species rarefaction curve pooling samples from all ships to make comparisons with a curve generated using the data in Drake & Lodge (2007). Additionally, we estimated Chao-2 richness for different external surfaces on the hull to compare species richness across locations in the ships surveyed. Sample-based species rarefaction curves were graphed for hull surfaces with non-overlapping confidence intervals. As a result of insufficient sample size, these calculations could not be made for the bulbous bow and stem.

We estimated Chao-2 species richness of non-barnacle invertebrates on algae and barnacles. Chao-1 and Chao-2 estimates were calculated using SPADE software (Chao & Shen, 2006). Species rarefaction curves were generated with 5000 random iterations using ECOSIM software (Gotelli & Entsminger, 2006). Confidence intervals (95%) were generated with the same software to test for significant differences (Chao & Shen, 2006; Gotelli & Entsminger, 2006). To analyse species richness at comparable levels of sampling effort, the *x*-axis of sample-based rarefaction curves was rescaled to number of individuals based on the average number of individuals per sample (Gotelli & Colwell, 2001).

We tested for differences in the abundance of non-barnacle invertebrates on algal versus barnacle cover, as well as for differences in % cover between locations on the hull, to detect potential hot-spots for hull fouling. This analysis was carried out using Mann–Whitney *U*-test. Parametric tests were not used because the assumptions of those tests could not be met.

A principal component analysis (PCA) was conducted to explore the relationship between the assemblages found on a vessel and a range of variables relating to the ship's travel and maintenance history (Statistica 7.0, Statsoft, Tulsa, OK, USA). We looked for relationships between hull fouling invertebrate community variables and those that integrated the sailing speed, hull husbandry and voyage history, each of which was collected from the ships' crews. Variables in this analysis included total abundance of invertebrates, total abundance excluding barnacles, percent cover of barnacles, total observed richness, Chao-2 species richness estimate, total number of samples per ship, sailing speed, time since last dry-dock, time since last painted with antifouling coating, total and maximum residence time at port per region over the previous year. We classified ports-of-call according to the following regions: (1) North America; (2) Europe and North Africa (for simplicity, referred to as 'Europe'); (3) Central America (Gulf of Mexico, Caribbean Sea and Pacific coast of Central America); (4) Atlantic South America; (5) Pacific South America; (6) India, South and Central Africa; (7) Norwegian Sea and Arctic region (referred to as 'Arctic'). Additionally, we included in the analysis the time since last Panama Canal crossing. This coarse classification was aimed to separate ports according to factors that can potentially affect fouling communities, such as long transits across oceans, hemisphere switching, freshwater exposure in the Panama Canal, and adverse environmental conditions in high latitudes (Coutts, 1999; Coutts & Taylor, 2004). The output of the PCA allowed us to detect visually patterns of relatedness between hull fouling and other variables studied. In this type of analysis, correlated variables are plotted close together on the graph, and the length of the vector along two principal axes indicates the relative importance of that variable in explaining variability of the data.

We constructed regression models to analyse total abundance of invertebrates and species richness per ship as a function of total time in port during the year prior to sampling, time since last dry-dock, time since last painted and typical sailing speed. We tested several linear and nonlinear, least square regression models (R, R Foundation for Statistical Computing, Vienna). Nested models were compared using a likelihood ratio test (chi-square), and the simplest, significant model was selected. Variables that did not improve the full model were eliminated. For non-nested models, we used AICc (bias-corrected Akaike Information Criterion for small samples; Burnham & Anderson, 2004) to evaluate relative model performance. A significance level of 95% was used for all statistical analyses.

RESULTS

The hulls of 20 ships sampled ranged between 3600 and 8400 m^2 of underwater surface area, the vessels had gone between four and 55 months since last being dry-docked and painted with antifouling coatings. Overall, the vessels had relatively low cover of biofouling organisms (mean 1.5%; Table 1). We collected 98 hull fouling samples from 18 ships surveyed, with two vessels lacking any fouling. Average invertebrate density was 172,000 individuals $ship^{-1}$ (max. 1.5×10^6 individuals $ship^{-1}$) or 23 individuals m^{-2} (max. 215 individuals m^{-2}) (Table 1). We examined 8250 hull fouling organisms, which belonged to 109 distinct taxa and 57 identified species (see Appendices S1 and S2 in Supporting Information).

Barnacles (51%), cladocerans (19%), bivalves (12%) and amphipods (11%) had the largest contributions to total abundance of organisms per ship (Fig. 2a,b). While bivalves were very abundant, the number of species was the modest. Copepods and oligochaetes were not abundant, but accounted for a large fraction of the species found (Fig. 2c). With the exception of a single specimen of the oligochaete *Alexandrovia onegensis* (Hrabe, 1962), which was in poor condition, we did not find any fresh or brackish-water species that have not already been reported in the Great Lakes (Fig. 2c; Appendix S1). Nevertheless, we did find several established NIS, including *Cercopagis pengoi* (Ostroumov, 1891), *Bythotrephes longimanus* (Leydig, 1860), *Daphnia lumholtzi* (Sars, 1885), *Dreissena rostriformis bugensis* (Andrusov, 1897), and *Gammarus tigrinus* (Sexton, 1939) (Appendix S1).

We found live specimens belonging to seven species and five genera of amphipods, mites and chironomids, which accounted for 66, 27, and 22%, respectively, of the total abundances of those groups in the ships for which live samples were examined (Appendices S1 and S2). Live copepods, cladocerans and oligochaetes were also collected (Appen-

Table 1 Ship characteristics and abundance of invertebrates on hull surfaces. Ships are arranged in increasing order of invertebrate abundance. Abundance could not be estimated for one vessel (NA).

Ship	Underwater surface area (m^2)	Time since last dry-dock (d)	Time since last painted (d)	Percent cover (%)	Invertebrate abundance (individuals m^{-2})	Invertebrate abundance (individuals $ship^{-1}$)
	6589	110	110	0.0	0.00	0
	6615	478	493	0.0	0.00	0
	7696	429	429	4.1	0.00	20
	5082	348	340	0.0	0.01	48
5	4899	286	275	0.0	0.01	57
	8214	1528	1528	0.1	0.02	202
	7702	1021	1021	0.0	0.09	714
	3602	478	478	0.0	0.22	803
	8387	936	936	8.9	0.17	1455
10	7705	909	1398	0.1	0.49	3770
	7809	538	1218	1.2	1.39	10,875
	8389	537	537	0.0	1.35	11,305
	7265	931	931	1.0	2.11	15,296
	3577	1097	1138	0.9	5.18	18,521
15	4207	623	623	6.0	6.04	25,415
	7652	924	934	0.3	4.14	31,676
	7673	861	861	0.5	61.17	469,374
	7086	763	751	1.9	157.30	1,114,698
	7251	512	512	3.6	214.95	1,558,661
20	7719	502	1671	NA	NA	NA
Mean	6756	691	809	1.5	23.93	171,731

dix S1). This combination of freshwater and marine species posed no risk of invasion because each of the former species was already present in the Great Lakes.

We also found 21 marine taxa not found in the Great Lakes in our samples. With the exception of the amphipod *Marinogammarus obtusatus* (Dahl, 1938), these taxa were always in poor condition and probably dead when collected (Appendices S1 and S2). Algae were dominated by small Phaeophyceae (brown algae). The abundance of invertebrates excluding barnacles was an order of magnitude larger on barnacles than on algae ($P < 0.01$, Mann–Whitney *U*-test).

Estimated richness averaged < 25 species for 12 vessels, with a maximum of 78, and was 2.4 times higher than observed richness for all ships (Fig. 3). These richness estimates are substantially lower than that observed by Drake & Lodge (2007). However, estimated species richness for the Drake and Lodge ship was not statistically different from that based on pooled samples from our 20 ships (Fig. 4). Nonetheless, the accumulation rate was much steeper for the Drake and Lodge ship than for our pooled values (Fig. 4).

Overall, 51% of the underwater locations examined lacked fouling. Leading edge of the rudder and sea-chest grating accumulated the most species, whereas propeller, hull, and rudder sides and bottom had the fewest species (Fig. 5b). Sea-chest grating also accumulated species at a much greater rate than did other surfaces, particularly the hull (Fig. 5a). Although we could not estimate species richness for the bulbous bow and stem, we only found five different species on two ships at these locations. For this reason, we anticipate species richness was not high at these sites. Sea-chest grating, hull, bulbous bow, stem and bow thruster had comparatively low levels of fouling, whereas the rudder leading edge, rudder trailing edge and rope guard were among the most heavily fouled locations (Fig. 5c). Invertebrate fouling was almost always associated with barnacles on locations including the sea-chest grating, bow thruster and rope guard, whereas that on rudder sides and bottom and bulbous bow and stem was much less reliant on barnacle presence (Fig. 5).

PCA revealed a strong negative correlation between longest and total stationary time spent in the Arctic and hull fouling variables (Fig. 6). Species richness and invertebrate abundance were also negatively correlated with typical sailing speed. Conversely, fouling was strongly associated with total and maximum time spent in ports in the Pacific coast of South America and, to a lesser extent, with time since last crossing through the Panama Canal (Fig. 6). Time since dry-dock and time since painted with an antifouling coating were not related to hull fouling variables.

The most significant variables in both single variable and full regression models were time spent in port during the year prior to sampling ($P < 0.001$) and sailing speed ($P < 0.05$). The best model (AICc = 62.02) predicted total abundance of invertebrates (A, in individuals $ship^{-1}$) as

$$\log A = 0.0539TP - 0.2439S - 1 \quad (1)$$

where TP (in days) is total time in port during the year prior to sampling, and S (in knots) is sailing speed. Species richness per

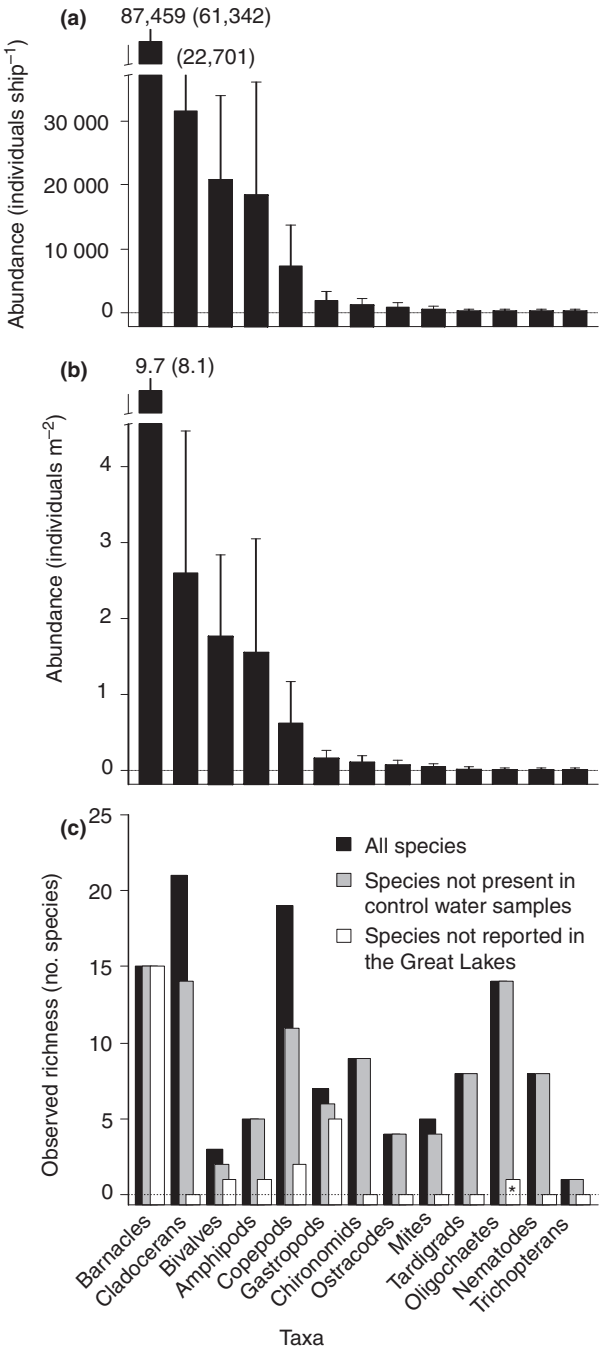

Figure 2 Mean (+SE) abundance of invertebrate taxa per vessel (a), per wetted surface area (b), and total invertebrate species richness (c) for all ships. Organisms present in port (control) water samples were subtracted from a, b, and c as indicated. Only one freshwater species that has not been observed in the Great Lakes was found in hull fouling samples (indicated with an asterisk). Values off the scale are indicated.

ship (R; Chao-2 estimation of the number of species) was best predicted as a linear function of time in port

$$R = 0.2228TP - 1 \quad (2)$$

with AICc = 168.36. There was no relationship between either total abundance of invertebrates or species richness and time since the vessel was last dry-docked and painted ($P > 0.05$).

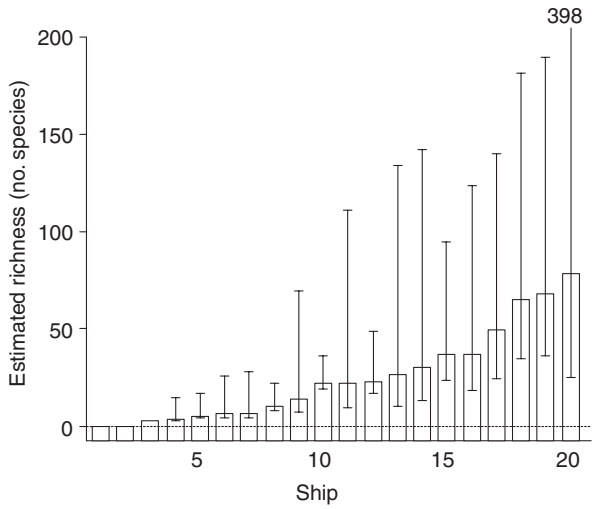

Figure 3 Chao-2 species richness estimate for ships in this study. Error bars indicate 95% confidence intervals. Values off the scale are indicated.

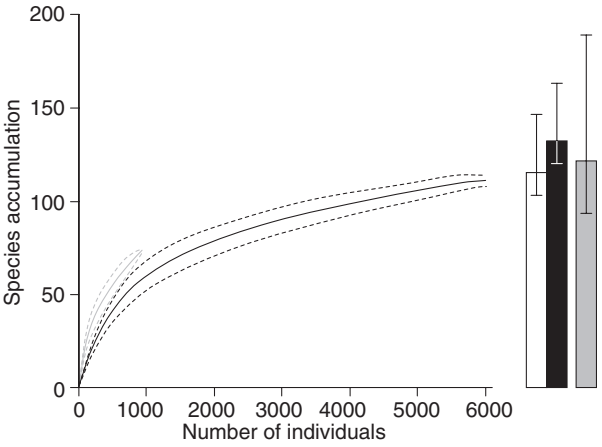

Figure 4 Individual-based rarefaction curves for all ships pooled from this study (dark line ± 95% CI) and the ship sampled by Drake & Lodge (2007) (grey line (± 95% CI)). Also shown are Chao-1 species richness estimates (± 95% CI) for our study and that of Drake and Lodge. Species richness estimates for our study either excluded species found in Great Lakes' port water (open bar) or included them (solid bar), whereas Drake and Lodge's estimate includes these species (grey bar). Chao-2 richness estimates for our study are 133 and 150 species without and with port water samples considered, respectively.

DISCUSSION

Great Lakes fouling patterns

This is the first comprehensive study of ship hull fouling from a freshwater perspective. Our results suggest that this vector does not pose the same risk for NIS transport and introduction

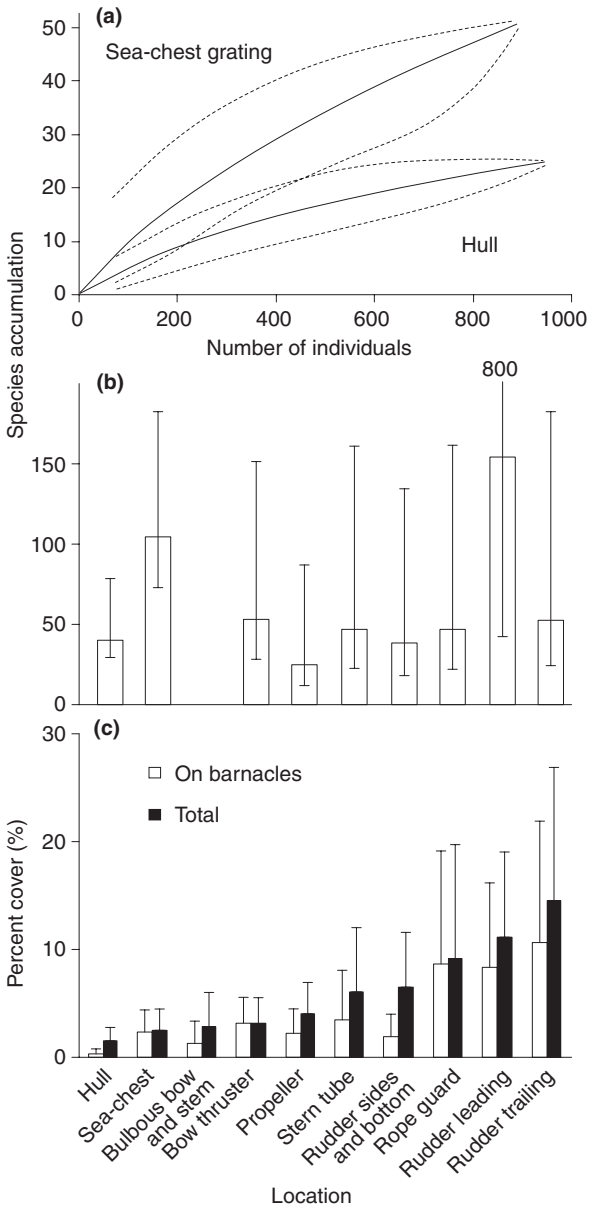

Figure 5 Sample-based rarefaction curves for underwater locations with significantly different asymptotic richness (a), Chao-2 species richness estimate (b) and average percent cover (c) for all locations across all ships in this study. The *x*-axis of the upper graph has been rescaled to number of individuals sampled. Dotted lines and error bars indicate 95% confidence intervals. Values off the scale are indicated. Sea-chest refers to the protective grating covering its exterior surface.

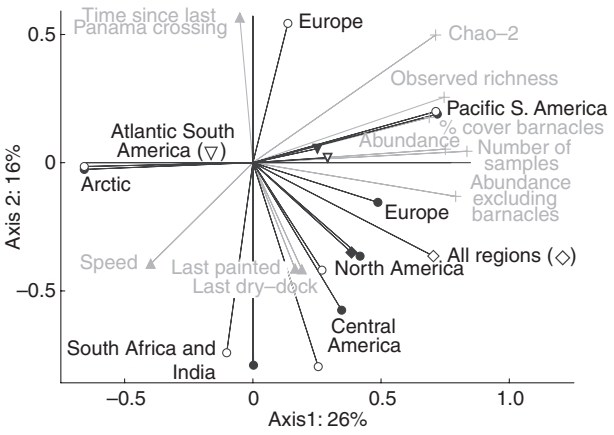

Figure 6 Principal component analysis of invertebrate communities fouled on hulls of vessels sampled in the Great Lakes based on ports visited over the year prior to sampling. Geographical identities represent total (black open symbols) and maximum (black solid symbols) residence time of vessels in port in that region over the preceding year. Variables related to fouling extent are indicated with grey crosses. Other variables are indicated with grey triangles. Last painted and last dry-dock refer to times since application of an antifouling agent and since the vessel was removed from the water for maintenance; speed refers to the vessels' reported cruising speed; % cover barnacles refers to total percent cover of barnacles on the ship. Percentage of the total variance explained along the first and second PCA axes is indicated.

to the Great Lakes as it does in marine environments (e.g. Gollasch, 2002; Coutts & Taylor, 2004). After intensively sampling 20 ships, we did not find a single species new to the Great Lakes that had a reasonable chance of establishing a population. Although our study confirms the potential for live species to be transported *via* hull fouling (Gollasch, 2002; Coutts & Taylor, 2004; Ruiz & Smith, 2005), the majority of live species found were freshwater organisms already present in the Great Lakes. We suspect that these species were picked up by ships as they travelled through the St. Lawrence Seaway and Lakes Ontario and Erie. The only exception is the presence of live specimens of the marine amphipod *M. obtusatus*. Although some marine amphipods can tolerate exposure to freshwater for some time, there is no chance of *M. obtusatus* establishing a population in a freshwater environment (J. Thomas, pers. comm.).

The only freshwater species found that is new to the Great Lakes was the oligochaete *A. onegensis*. We detected only one dead individual from one ship. This species likely has a low invasion risk because of low inoculum and its poor condition upon collection. This species' only North American report is from Alaska (Holmquist, 1974); it was first described for Lake Onega, in Northwest Russia, which was visited 129 days earlier by the vessel on whose hull it was found in the Great Lakes (Hrabe, 1962). Lake Onega is part of the extensive shipping canal system that links the Caspian Sea with the Baltic Sea; many vessels entering the Great Lakes originate in the Baltic Sea region (Colautti *et al.*, 2003).

The presence of non-indigenous freshwater species on the hull of a transoceanic ship is probably limited by the stresses of transit across the ocean. In addition to the sheer forces from the ship's movement and scarcity of food (Ruiz & Smith, 2005), organisms attached to the exterior surfaces of ships have to endure high salinity and wide temperature fluctuations (Coutts, 1999; Davidson *et al.*, 2006). Nevertheless, there are

species that can tolerate a wide range of salinities. Several freshwater and brackish-water species, mainly amphipods, mysid shrimps and bivalves were found to survive high salinities for over 48 h in laboratory experiments (Santagata *et al.*, 2008; Ellis & MacIsaac, 2009). Some of these species were detected in our samples (e.g. *G. tigrinus* and *D. r. bugensis*); however, none of them are new to the Great Lakes. Whether dreissenid mussels could tolerate exposure to open-ocean water would likely depend on trip duration, water temperature and the animal's respiration requirements. Furthermore, almost all of the dreissenid mussels collected were < 2 mm, indicating that they were recent settlers on the hull and possibly produced in the Great Lakes. Notwithstanding this, the presence in our hull fouling samples of established NIS, whose presence in the Great Lakes has been attributed to ballast water (Ricciardi, 2006), indicates a possible role for hull fouling. Although freshwater invaders found in the present study were not detected in other hull fouling surveys in North America (Ruiz & Smith, 2005; Davidson *et al.*, 2006), hull fouling on transported pleasure craft has been attributed with colonization of Ireland by zebra mussels (Minchin *et al.*, 2006). Given available evidence, we are unable to determine if any of the aforementioned species entered the lakes attached to ship hulls, or whether they are contaminants that fouled the vessels as they moved through the Great Lakes.

An earlier hull fouling study raised an alarm regarding the risk for intercontinental transport of NIS to the Great Lakes (Drake & Lodge, 2007). Our results are not consistent with this possibility, but rather suggest that the ship sampled by Drake and Lodge represents an extreme case of hull fouling. The total propagule load carried by that ship, estimated at $\sim 1.17 \times 10^6$ organisms, was higher than all but our most densely fouled vessel (Table 1). The Drake and Lodge ship was relatively small (5200 m^2 underwater surface area) as compared with the vessels sampled here (mean 6756 m^2), thus it had a much higher density (225 individuals m^{-2}) of fouling organisms than even our most heavily fouled ship (215 individuals m^{-2}). On average, ships in our study carried a total potential inoculum, an order of magnitude lower than Drake & Lodge's (2007) vessel.

Our species richness patterns parallel the species abundance patterns. Drake & Lodge (2007) found more species than we observed on any single ship. Using pooled sample data for all ships, our Chao-1 richness estimates for samples with (133) and without (115) port water species considered were not significantly different from the Drake & Lodge (2007) value (122), which also would have included port water species. Our species accumulation curve for all ships considered together (Fig. 4) also was lower than that of Drake & Lodge (2007), perhaps reflecting that their vessel experienced two prolonged stays in port – including in a Peruvian port – before it entered the Great Lakes. Our PCA analysis identified vessels with histories of operation in Pacific waters of South America as being particularly vulnerable to fouling infestations (Fig. 6). We agree with Drake & Lodge (2007) that atypical cases pose the greatest invasion risk, and their ship likely represents one of these examples. With the exception of two copepod species, their study did not find any freshwater species new to the Great Lakes. That finding is consistent with our observations and indicates that the introduction risk posed to the Great Lakes by hull fouling on transoceanic ships is very limited.

Comparison with other introduction vectors

Mid-ocean ballast water exchange has been mandatory since 1993 for all ships entering the Great Lakes in ballast that seek to discharge water into the lakes (US Coast Guard, 1993). This regulation was extended in both Canada (2006) and the USA (2008) to include vessels entering the lakes with only residual water and sediments in their ballast tanks. These regulations appear to have dramatically reduced the scale of ballast water as a potential introduction vector (H.J. MacIsaac, unpublished data). Nevertheless, this vector still appears to pose a greater risk of introducing NIS than does hull fouling (Fig. 7), primarily owing to a higher degree of environmental matching.

Comparison with marine studies

A larger number of studies have addressed hull fouling in the marine environment. We observed an average of 1.5% of hull surfaces fouled, which is roughly comparable to 0.5% cover on container vessels (Ruiz & Smith, 2005), but much lower than that observed on recreational (up to 40%; Floerl *et al.*, 2005; Ashton *et al.*, 2006) and commercial vessels (up to 45%; Gollasch, 2002; Coutts & Taylor, 2004). Heavy fouling is not a

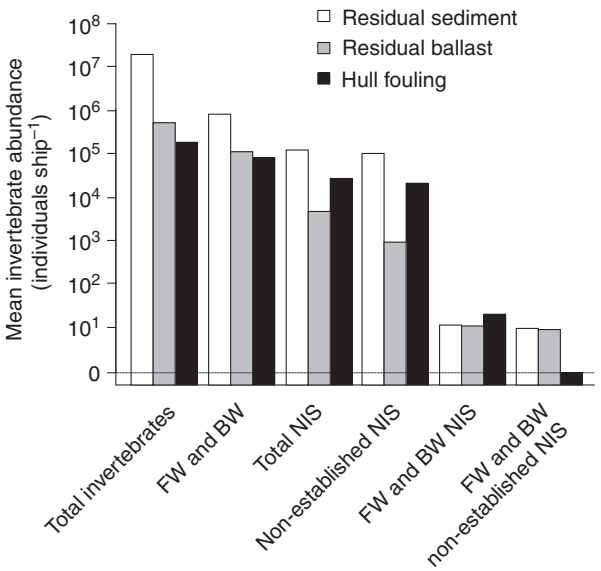

Figure 7 Estimated invertebrate propagule loads transported to the Great Lakes via hull fouling (solid bars), residual sediment (open bars) and residual ballast water (grey bars). Hull fouling data are from this study, whereas residual sediment and ballast data were derived from Duggan *et al.* (2005). FW = freshwater, BW = brackish-water, NIS = non-indigenous species.

prerequisite for high invasion risk, as large number of organisms can be transported into a busy port by vessels even if fouling is not extensive so long as the ships are large and numerous (Floerl *et al.*, 2009). Gollasch (2002) found that hull fouling was the top vector overall for introduction of species into the North Sea, and was much more important than ballast. The number of hull fouling species found per ship in that study was comparatively modest (maximum 15), as compared with up to 36 taxa in our study and 74 in Drake & Lodge's (2007) study. Furthermore, our study found that estimated richness on large commercial vessels can be several times higher than the observed richness, even with intensive sampling. These findings confirm the potential importance of hull fouling as an introduction vector in the marine environment (Gollasch, 2002).

It is still not clear why some vessels are more heavily fouled than others. Both total abundance and species richness were unrelated to time since last application of antifouling paint and time since dry-dock (Fig. 6), which is inconsistent with the work of Coutts (1999). Coutts (1999) also determined that typical sailing speed was inversely related to fouling, which is consistent with our observations (Fig. 6, equation 1) and with those of Ruiz & Smith (2005). Marine container ships achieve typical sailing speeds of 21–24 knots (Ruiz & Smith, 2005), far faster than the ships sampled by us (13–14.3 knots). All things being equal, faster speed should result in lower fouling intensity owing to increased hydrodynamic drag.

Most previous studies have shown weak relationships between hull fouling and voyage history. While this usually resulted from low sample size, our results indicate that management of biofouling risk cannot rest on antifouling paints alone, but must consider factors related to operational patterns such as time in port (Coutts, 1999; Ruiz & Smith, 2005) and sailing routes (Coutts & Taylor, 2004). Shipping routes seem to play a central role in the development of fouling communities. For example, we observed a distinct negative correlation between fouling intensity and time spent operating in high latitude waters, and a positive correlation with time spent in ports on the Pacific coast of South America (Fig. 6). Ice scouring has been shown to remove both fouling communities and antifouling coatings (Lewis *et al.*, 2004; Lee & Chown, 2007).

Time spent in port over the last year has been identified as an important determinant of fouling both in marine studies (Coutts, 1999; Ruiz & Smith, 2005) and in our study on the Great Lakes (Fig. 6, equations 1 and 2). Once species begin to accumulate on the hull, positive feedback may accelerate additions of new species. Dense biofouling cover of macroalgae, tunicates and large mussels may provide structural habitat complexity that can partially account for the relatively high diversity and abundance observed in some marine studies (e.g. Gollasch, 2002; Coutts & Taylor, 2004; Floerl *et al.*, 2005). We never observed this degree of fouling intensity on vessels visiting the Great lakes, possibly because of the negative effects of switching between different salinity regimes on biofouling communities (Davidson *et al.*, 2006). However, we did observe a strong relationship between barnacle cover and non-barnacle richness and abundance. Barnacles thus have the potential to facilitate transport of NIS through provision of complex habitat (van Overdijk *et al.*, 2003).

Fouling patterns on the hull

Previous studies have shown that hull fouling is not evenly distributed across the hull (Coutts & Taylor, 2004; Ruiz & Smith, 2005). Studies quantifying the amount of hull fouling in specific locations have found the existence of niche areas very heavily fouled (Coutts & Taylor, 2004). Our data indicate fouling intensity is greatest in the stern section of vessels (Davidson *et al.*, 2008), including both edges of the rudder and the rope guard. Our data indicate that the leading edge of the rudder and sea-chest grating have the potential to transport relatively large numbers of species (Fig. 5). In particular, sea-chest grating appears to be a hot-spot for hull fouling diversity even though average percent cover is low (Fig. 5). Sea-chests have been identified as protected locations posing a high biosecurity risk in terms of the potential to transfer NIS across countries (Coutts *et al.*, 2003; Coutts & Dodgshun, 2007). Coutts & Dodgshun (2007) found 150 different taxa, of which 10% were non-established NIS and 15% introduced NIS, inside the sea-chests of 42 vessels operating in New Zealand. We found 50 taxa on sea-chest grates of 20 vessels. Considering that we did not sample inside sea-chests, we predict that our survey has underestimated both the abundance and diversity of fouling organisms.

Conclusion and next steps

Hull fouling associated with international shipping does not seem to be a strong vector for NIS introduction into freshwater ecosystems, and is not a management priority in the Great Lakes. Hull fouling seems to pose a lesser risk than ballast water vectors in freshwater habitats mainly because of lack of environmental match, but still has a considerable potential for transport of large numbers of organisms and species (see Lockwood *et al.*, 2009). Factors associated with operational patterns and shipping routes may equal or exceed in importance hull husbandry as a determinant of the development of fouling communities on large, transoceanic vessels.

As a next step, the relationship between fouling extent and variables relating to the ship's travel and maintenance history should be explored further on a larger number of vessels and regions, to provide tools for large-scale risk assessment of this vector.

ACKNOWLEDGEMENTS

We thank the shipping companies, crews and the Shipping Federation of Canada that provided access to vessels, and Colin

van Overdijk, Capt. Phil Jenkins and divers for facilitating sampling. We are grateful for assistance with taxonomic identifications by Dale Calder, James Carlton, Jan Ciborowski, Anne Cohen, Jeffery Cordell, Stanley Dodson, Christer Erséus, Ryan Fisher, Beverly Hymes, Patrick Hudson, Harry Lee, Rosalie Maddocks, André Martel, Fábio Bettini Pitombo, Ian Smith, James Thomas, Paul Valentich-Scott and Herb Vandermeulen. Jim Muirhead, Anne Chao and Robert Colwell provided assistance with the statistical analyses, while Johanna Bradie provided edits and comments on the manuscript. Funding for this study was provided by NSERC's Canadian Aquatic Invasive Species Network, Ontario Ministry of Natural Resources, and by a DFO invasive species research chair and NSERC Discovery Grant to HJM. We are grateful to Oliver Floerl and two anonymous referees for helpful comments on the manuscript.

REFERENCES

Ashton, G.V., Boos, K., Shucksmith, R. & Cook, E.J. (2006) Risk assessment of hull fouling as a vector for marine non-natives in Scotland. *Aquatic Invasions*, **1**, 214–218.

Burnham, K.P. & Anderson, D.R. (2004) Multimodel inference. Understanding AIC and BIC in model selection. *Sociological Methods and Research*, **33**, 261–304.

Carlton, J.T. (1985) Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanography and Marine Biology: An Annual Review*, **23**, 313–371.

Carlton, J.T. & Hodder, J. (1995) Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th century sailing vessel. *Marine Biology*, **121**, 721–730.

Chao, A. (1987) Estimating the population size for capture-recapture data with unequal catchability. *Biometrics*, **43**, 783–791.

Chao, A. & Shen, T.J. (2003) *User's Guide for Program SPADE (species prediction and diversity estimation)*. Updated August 2008. Available at: http://chao.stat.nthu.edu.tw/.

Chao, A. & Shen, T.J. (2006) *SPADE version 3.1*. Available at: http://chao.stat.nthu.edu.tw/.

Colautti, R.I. & MacIsaac, H.J. (2004) A neutral terminology to define 'invasive' species. *Diversity and Distributions*, **10**, 135–141.

Colautti, R.I., Niimi, A.J., van Overdijk, C.D.A., Mills, E.L., Holeck, K. & MacIsaac, H. (2003) Spatial and temporal analysis of transoceanic shipping vectors to the Great Lakes. *Invasive species: vectors and management strategies* (ed. by G.M. Ruiz and J.T. Carlton), pp. 227–246, Island Press, Washington.

Coutts, A.D.M. (1999) *Hull fouling as a modern vector for marine biological invasions: investigation of merchant vessels visiting northern Tasmania*. MSc Thesis, Australian Maritime College, Launceston.

Coutts, A.D.M. & Dodgshun, T.J. (2007) The nature and extent of organisms in vessel sea-chests: a protected mechanism for marine bioinvasions. *Marine Pollution Bulletin*, **54**, 875–886.

Coutts, A.D.M. & Taylor, M.D. (2004) A preliminary investigation of biosecurity risks associated with biofouling on merchant vessels in New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **38**, 215–229.

Coutts, A.D.M., Moore, K.M. & Hewitt, C.L. (2003) Ships' sea-chests: an overlooked transfer mechanism for non-indigenous marine species? *Marine Pollution Bulletin*, **46**, 1504–1515.

Davidson, I.C., Sytsma, M.D. & Ruiz, G.M. (2006) *Preliminary investigations of biofouling of ships' hulls: nonindigenous species investigations in the Columbia River*. Final report April 2006. U.S. Coast Guard Research and Development Center, New London, CT.

Davidson, I.C., McCann, L.D., Fofonoff, P.W., Sytsma, M.D. & Ruiz, G.M. (2008) The potential for hull-mediated species transfers by obsolete ships on their final voyages. *Diversity and Distributions*, **14**, 518–529.

Drake, J.M. & Lodge, D.M. (2007) Hull fouling is a risk factor for intercontinental species exchange in aquatic ecosystems. *Aquatic Invasions*, **2**, 127–137.

Duggan, I.C., van Overdijk, C.D.A., Bailey, S.A., Jenkins, P.T., Limén, H. & MacIsaac, H.J. (2005) Invertebrates associated with residual ballast water and sediments of cargo-carrying ships entering the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 2463–2474.

Ellis, S. & MacIsaac, H.J. (2009) Salinity tolerance of Great Lakes invaders. *Freshwater Biology*, **54**, 77–89.

Evans, S.M., Birchenough, A.C. & Brancato, M.S. (2000) The TBT ban: out of the frying pan into the fire? *Marine Pollution Bulletin*, **40**, 204–211.

Farrapeira, C.M.R., Melo, A.V.O.M., Barbosa, D.F. & Silva, K.M.E. (2007) Ship hull fouling in the Port of Recife, Pernambuco. *Brazilian Journal of Oceanography*, **55**, 207–221.

Floerl, O., Inglis, G.J. & Hayden, B.J. (2005) Risk-based predictive tool to prevent accidental introductions of non-indigenous marine species. *Environmental Management*, **35**, 765–778.

Floerl, O., Inglis, G.J., Dey, K. & Smith, A. (2009) The importance of transport hubs in stepping-stone invasions. *Journal of Applied Ecology*, **46**, 37–45.

Fofonoff, P.W., Ruiz, G.M., Steves, B. & Carlton, J.T. (2003) In ships or on ships? Mechanisms of transfer and invasion for non-native species to the coasts of North America. *Invasive species: vectors and management strategies* (ed. by G.M. Ruiz and J.T. Carlton), pp. 152–182, Island Press, Washington, DC.

Godwin, L.S. (2003) Hull fouling of maritime vessels as a pathway for marine species invasions to the Hawaiian Islands. *Biofouling*, **19**, 123–131.

Gollasch, S. (2002) The importance of ship hull fouling as a vector of species introductions into the North Sea. *Biofouling*, **18**, 105–121.

Gollasch, S. (2007) Marine vs. freshwater invaders: is shipping the key vector for species introductions to Europe?. *Invasive species: vectors and management strategies* (ed. by G.M. Ruiz and J.T. Carlton), pp. 339–345, Island Press, Washington, DC.

Gotelli, N.J. & Colwell, R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.

Gotelli, N.J. & Entsminger, G.L. (2006) *Ecosim: null models software for ecology*, *Version 7*. Acquired Intelligence Inc. and Kesey-Bear, Jericho, VT 05465. Available at: http://gary entsminger.com/ecosim.htm.

Holeck, K., Mills, E.L., MacIsaac, H.J., Dochoda, M., Colautti, R.I. & Ricciardi, A. (2004) Bridging troubled waters: understanding links between biological invasions, transoceanic shipping, and other entry vectors in the Laurentian Great Lakes. *BioScience*, **10**, 919–929.

Holmquist, C. (1974) On *Alexandrovia onegensis* Hrabe from Alaska, with a revision of the Telmatodrilinae (Oligochaeta, Tubificidae). *Zoologisches Jahrbuch Systematic Okologie Geographie der Tiere*, **101**, 249–268.

Hrabe, S. (1962) Oligochaeta limicola from Onega Lake collected by Mr. B. M. Alexandrov. *Publications of the Faculty of Science, University J. E. Purkyne, Brno*, **485**, 277–333 (in Russian).

Kelly, D.W., Lamberti, G.A. & MacIsaac, H.J. (2009) The Laurentian Great Lakes as a case study of biological invasion. *Bioeconomics of invasive species: integrating ecology, economics, policy and management* (ed. by R.P. Keller, D.M. Lodge, M. Lewis and J.F. Shogren), pp. 205–225, Oxford University Press, Oxford.

Lawler, J.J., Aukema, J.E., Grant, J., Halpern, B., Kareiva, P., Nelson, C.R., Ohleth, K., Olden, J.D., Schlaepfer, M.A., Silliman, B. & Zaradic, P. (2006) Conservation science: a 20-year report card. *Frontiers in Ecology and the Environment*, **4**, 473–480.

Lee, J.E. & Chown, S.L. (2007) *Mytilus* on the move: transport of an invasive bivalve to the Antarctic. *Marine Ecology (Progress Series)*, **339**, 307–310.

Lewis, P.N., Riddle, M.J. & Hewitt, C.L. (2004) Management of exogenous threats to Antarctica and the sub-Antarctic islands: balancing risks from TBT and nonindigenous marine organisms. *Marine Pollution Bulletin*, **49**, 999–1005.

Lloyd's Register Fairplay (2008) *World Fleet Statistics 2007*. Lloyd's Register of Shipping, London.

Lockwood, J.L., Cassey, P. & Blackburn, T.M. (2009) The more you introduce the more you get: the ole of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions*, **15**, 904–910.

McEwen, G.F., Johnson, M.W. & Folsom, T.R. (1954) A statistical analysis of the performance of the Folsom plankton sample splitter, based upon test observations. *Archiv fűr Meteorologie, Geophysyk und Klimatologie*, **A7**, 502–527.

Minchin, D. & Gollasch, E. (2003) Fouling and ships' hulls: how changing circumstances and spawning events may result in the spread of exotic species. *Biofouling*, **19**(Suppl.), 111–122.

Minchin, D., Floerl, O., Savini, D. & Occhipinti-Ambrogi, A. (2006) Small craft and the spread of exotic species. *The Ecology of Transportation: Managing Mobility for the Environment* (ed. by J. Davenport and J.L. Davenport), pp. 99–118, Springer, Dordretch.

Molnar, J.L., Gamboa, R.L., Revenga, C. & Spalding, M.D. (2008) Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, **6**, 485–492.

van Overdijk, C.D.A., Grigorvich, I.A., Mabee, T., Ray, W.J., Ciborowski, J.J.H. & MacIsaac, H.J. (2003) Microhabitat selection by the invasive amphipod *Echinogammarus ischnus* and native *Gammarus fasciatus* in laboratory experiments and in Lake Erie. *Freshwater Biology*, **48**, 567–578.

Ricciardi, A. (2006) Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions*, **12**, 425–433.

Ruiz, G.M. & Carlton, J.T. (2003) Invasion vectors: a conceptual framework for management. *Invasive species: vectors and management strategies* (ed. by G.M. Ruiz and J.T. Carlton), pp. 459–504, Island Press, Washington, DC.

Ruiz, G.M. & Smith, G. (2005) *Biological study of container vessels at the port of Oakland*. Final report, 155 pages. Submitted to the Port of Oakland by G.M. Ruiz and G. Smith, Smithsonian Environmental Research Center, Edgewater. Available at: http://www.serc.si.edu.

Santagata, S., Gasiūnaite, Z.R., Verling, E., Cordell, J.R., Eason, K., Cohen, J.S., Bacela, K., Quilez-Badia, G., Johengen, T.H., Reid, D.F. & Ruiz, G.M. (2008) Effect of osmotic shock as a management strategy to reduce transfers of nonindigenous species among low-salinity ports by ships. *Aquatic Invasions*, **3**, 61–76.

US Coast Guard (1993) *Ballast water management for vessels entering the Great Lakes*. Code of federal regulations 33-CFR Part 151.1510. The U.S. Government Printing Office. Available at: http://www.gpoaccess.gov (accessed 6 November 2009).

World Trade Organization (2008) *International Trade Statistics 2008*. Available at: http://www.wto.org.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Hull fouling invertebrate specimens identified to species. Freshwater and brackish-water non-indigenous species (NIS) that have not been reported in the Great Lakes are indicated with an asterisk. Habitat codes: F = freshwater, M = marine, E = estuarine. Port water samples were collected directly adjacent to the ship at the time of hull surveys.

Appendix S2 Hull fouling specimens not identified to species. Codes as per Appendix S1. P = present, but not quantified.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

BIOSKETCHES

Francisco Sylvester. 1998: graduated in Biological Sciences from the Universidad Autónoma de Madrid. 1999–2002: worked at the Argentine National Environmental Agency and National Parks Service. 2006: PhD in Biology, University of Buenos Aires, on ecology of invasive golden mussel *Limnoperna fortunei* in the lower Paraná river. 2007-present: Postdoctoral fellowship at the Great Lakes Institute for Environmental Research, University of Windsor, ON, working on hull fouling as a vector for the introduction of aquatic invasive species.

Hugh MacIsaac is a professor and DFO Invasive Species Research Chair at the Great Lakes Institute for Environmental Research. He also directs the Canadian Aquatic Invasive Species Network, a consortium of professors and government researchers in Canada. Hugh is interested in vectors and pathways, by which invasive species are introduced, and on modelling secondary spread.

Editor: David Richardson