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Research Article

# Fouling around: vessel sea-chests as a vector for the introduction and spread of aquatic invasive species

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## Abstract

Sea-chests, recesses built into the hull of a vessel, have been recently identified as hotspots for fouling organisms. In this study, we examined the types and abundances of taxa found in sea-chests of commercial vessels, and investigated whether vessel specifications and voyage histories influenced the nature and extent of sea-chest fouling. Eighty-two sea-chests were sampled from 39 commercial vessels while in dry dock on the West or East Coast of Canada. Overall, 80% of the vessels showed evidence of sea-chest fouling, and 46% harboured at least one non-indigenous species. In total, 299 unique taxa were recorded, including a number of non-indigenous and cryptogenic organisms that collectively made up 20.5% and 14.4% of the taxa sampled from West and East Coast vessels, respectively. Additional results suggested that in-service period (i.e., duration since last sea-chest cleaning) and vessel origin (i.e., domestic versus international) may, in part, determine the nature and extent of sea-chest fouling. By contrast, vessel size and port duration were unable to explain taxonomic richness or abundance of fouling organisms in sea-chests. Taken together, these findings highlight the role of sea-chests as an important vector responsible for the introduction and spread of a variety of taxa, including aquatic invasive species, but also suggest that the factors that influence sea-chest fouling in commercial vessels are complex. Further research, aimed at better understanding the determinants of sea-chest fouling and the efficacy of anti-fouling systems, would help further refine management strategies and reduce the risks associated with sea-chest fouling.

**Key words:** biofouling, biological invasions, dispersal, non-indigenous, seachests, shipping, vessels

## Introduction

For centuries, shipping has served as the principal vector for the introduction and spread of aquatic invasive species worldwide (e.g., Cohen and Carlton 1998; Ruiz et al. 2000; Hewitt et al. 2004). Historical, slow ocean-going vessels transported aquatic species that fouled or bored into the hulls of vessels, likely extending the distributions of numerous “fouling” organisms across oceans and along coastlines (Carlton and Hodder 1995). Today, contemporary shipping is generally characterized by relatively faster vessel speeds, shorter port residence times, and routine hull husbandry, which in turn, may minimize the extent of biofouling on vessel hulls. However, given increased levels of global trade, associated shipping traffic, and new regulations for anti-fouling agents (e.g., a worldwide ban of tributyltin (TBT) compounds), biofouling continues to pose serious invasion risks, particularly if aquatic organisms are still able to readily hitchhike from port to port on vessels (Carlton 1996; Gollasch 2002; Minchin and Gollasch 2003).

In addition to the flat surfaces of a vessel’s hull, there are several specialized ‘niche’ areas on which organisms can attach, including bow thrusters, rudders, propellers, intakes, and sea-chests. Sea-chests are protected, cavity-like structures, built into the hull of a vessel and typically covered with metal grates (Coutts et al. 2003). Despite housing water intakes used for engine cooling, ballast water operations, and emergency fire-fighting, sea-chests are typically characterized by relatively low water flows compared to higher velocities and shear stresses experienced on the exposed, flat surfaces of the hull. Such low-flow environments provide a relatively protected refuge for many fouling organisms, leading to increased survivorship and thriving communities (Coutts and Dodgshun 2007). Indeed, recent studies suggest that sea-chests and sea-chest grates are hotspots for biofouling, and by extension, may serve as an important vector for the introduction and spread of non-indigenous species (Coutts and Taylor 2004; Coutts and Dodgshun 2007; Sylvester and MacIsaac 2010).

Anecdotally, sea-chests have been implicated in the transport and potential spread of several non-indigenous species, including *Mytilus galloprovincialis*, a well-known invasive mussel that was found in large numbers in the sea-chests of an Antarctic supply vessel (Lee and Chown 2007); *Caprella mutica*, another widely introduced species that has spread throughout the North Atlantic, Northeast Pacific and South Pacific, possibly as a hitch-hiker in the sea-chests of commercial vessels (Ashton et al. 2007; Frey et al. 2009); and *Molgula citrina*, a North Atlantic ascidian that recently has been discovered in the North Pacific, and potentially introduced to the region via sea-chests (Lambert et al. 2010). However to date, only a few detailed studies have quantified the magnitude of biofouling within sea-chests (Coutts et al. 2003; Coutts and Dodgshun 2007), thus providing only some of the information needed to make sound policies on vector regulation and invasive species management. The objective of this current study is to further assess the role of sea-chests as a potential vector for the introduction and spread of fouling organisms, including aquatic invasive species. Specifically, we examined the types and abundances of taxa found in sea-chests of commercial vessels visiting or operating in Canadian waters, and whether certain vessel specifications and voyage histories influenced the nature and extent of sea-chest fouling.

## Methods

### *Vessel sampling and characteristics*

Between 2006 and 2009, we sampled sea-chests from 39 commercial vessels in dry dock on the West (n = 25) and East (n = 14) Coasts of Canada (sampling locations: Victoria, British Columbia; Les Méchins, Québec; Halifax, Nova Scotia). For each vessel, we obtained available data related to vessel specifications and voyage history by interviewing ship personnel; vessels with specific data gaps (e.g., unreported port durations) were sampled but excluded from respective statistical analyses (see Appendix 1). Sampled vessels represented a variety of types and size classes, including barge (n = 2), general cargo (n = 9), passenger (cruise) (n = 2), ferry (n = 13), fishing (n = 3), research (n = 9), and tug (n = 1), and ranged from 139 to 109,000 gross tonnage (gt). Port duration was estimated as the average number of days spent in the last five ports of call (mean = 4.3 days, sd = 4.4, min. = 0, max. = 16.6, n = 30). In-service period was measured as the number of months since last inspection and cleaning of sea-chests, including the complete removal of all fouling organisms. While the majority of these vessels were in dry dock for routine maintenance following several consecutive years at sea, a few ships were docked for emergency repairs, providing the opportunity to sample vessels over a broad range of in-service periods (mean = 29.6 months, sd = 15.5, min. = 0.5, max. = 59.1, n = 28). We classified vessel origin as 'domestic' (n = 23) if travel occurred exclusively in Canadian waters (i.e., along each coast), or 'international' (n = 16) if the vessel sailed to a foreign port within the previous five ports visited. Based on interview responses and visual inspections, all sampled vessels and associated sea-chests appeared to have been fitted with cathodic protection systems (e.g., Cathelco®) and/or treated with anti-fouling coatings (e.g., Interspeed 640 Red A/F) to control biofouling. Unfortunately detailed records for these anti-fouling systems (e.g., type, location, and age) were unavailable for most vessels, and therefore not suitable for further analysis.

### *Sea-chest sampling and characteristics*

Shortly after the arrival of each vessel, we sampled between one and four sea-chests (mean = 2.1 sea-chests per ship, sd = 0.8), depending on permitted access and time; in total 82 sea-chests were examined. Preliminary sea-chest surveys conducted prior to this study (Couture and Simard 2007) suggested that, in general, fouling organisms are not evenly distributed within sea-chests, but rather in patches. To account for spatial heterogeneity, we implemented a sampling design that consisted of quadrat sampling, timed searches, and visual estimates. Within each sea-chest, three $0.01m^2$ quadrats were placed on surfaces with the greatest amount of biofouling, and all organisms within each quadrat were collected using a putty scraper (Ardisson et al. 1990). We then conducted timed inspections (5 minutes) to search for rarer taxa potentially not sampled within the quadrats. To assess whether the sampled organisms were alive, specimens were examined prior to preservation in 70% ethanol. Within each entire sea-chest, we also relied on visual estimates of percentage cover to quantify the overall extent of biofouling. To minimize observer biases on different coasts, estimates made in the field were later verified by a single investigator using photos. Although sea-chests varied in size as characterized by total surface area (mean = 7.6 $m^2$, sd = 9.5, min. = 0.2, max. = 53.5), this standardized sampling approach ensured equal sampling effort among sea-chests. Sea-chest grates were occasionally sampled by taking scrapings or bulk collections (taxonomic data available upon request), but ultimately these samples were not included in the following analyses.

*Classification of taxa*

Individuals larger than 1 mm were examined with dissecting and compound microscopes and subsequently identified to species or the lowest taxonomic level possible using available taxonomic keys and species descriptions (e.g., Carlton 2007). Taxa were further categorized as either ‘indigenous’ (taxa that are native to each respective coast), ‘non-indigenous (non-established)’ (non-native taxa that have not been reported previously in the region), ‘non-indigenous (established)’ (non-native taxa that are presently established in the region), ‘cryptogenic’ (taxa of unknown origin), or ‘unknown’ (taxa that have been identified to genus or higher, and whose origin remains unclear). For certain groups, we consulted with additional taxonomic experts to confirm the identity and origin of each species (see acknowledgements). Specimens that appeared dead upon collection and already represented by live individuals (e.g., empty shells of mussels (*Mytilus* sp.) and tests of barnacles (Balanomorpha)) were excluded from further analyses.

*Data analysis*

To quantify the extent of biofouling, we calculated both taxonomic richness (i.e., number of unique taxa) and abundance (i.e., average percentage cover) of organisms for each vessel. We then examined whether either of these measures were influenced by vessel specifications or voyage histories. Linear models were implemented to separately test the effects of each factor: regression was used to examine vessel size, port duration, and in-service period, while an analysis of variance (ANOVA) was used to evaluate vessel origin. For the purposes of testing the effect of these factors on taxonomic richness, we randomly selected a single sea-chest per vessel to avoid biases that may have been introduced by uneven sampling of multiple sea-chests among vessels. To test their effect on abundance, we used the average percentage cover of all sea-chests sampled within each vessel. Taxonomic richness and abundance data were square-root and arcsine transformed, respectively, to meet statistical assumptions. We recognize that these factors could be confounded by complex interactions; however, data gaps reduced the usefulness of a full-factorial multiple regression. Analysis of similarity (ANOSIM) tests, based on Bray-Curtis similarity matrices calculated from presence/absence of taxa, were also performed to evaluate similarities of taxonomic composition between and within vessels. Separate tests were performed for each coast, and rather than creating zero-adjusted coefficients, samples that contained no species were removed from the analyses (Clarke and Gorley 2006). Analyses were carried out using JMP v4.0.2 (SAS Institute) and PRIMER v6.1.9 (PRIMER-E Ltd.).

## Results

*Extent of biofouling in sea-chests*

Overall, 80% of sampled vessels showed evidence of sea-chest fouling. The number of unique taxa found in each sea-chest ranged from 0 to 47 with an average of 8.9 ± 12.1 taxa (mean ± sd); within each vessel, taxonomic richness ranged widely from 0 to 61 (Figure 1A) with an average of 14.9 ± 16.8 taxa (mean ± sd). The extent of biofouling as measured by surface area

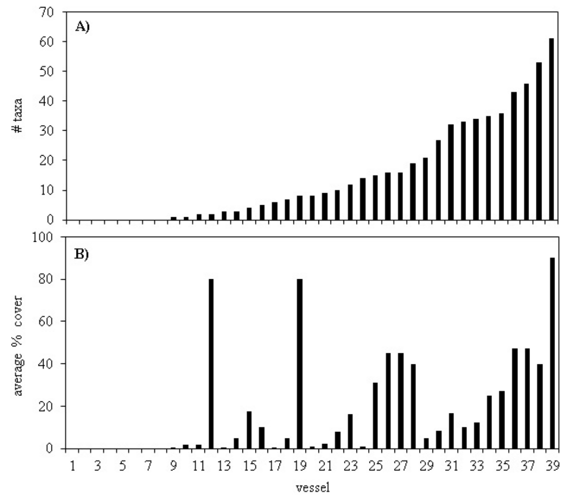


**Figure 1.** Distribution of A) taxonomic richness (number of taxa) shown with B) corresponding abundance (average percentage cover) in sea-chests for sampled vessels. Vessels ordered by increasing taxonomic richness, and do not correspond to codes in Appendix 1.

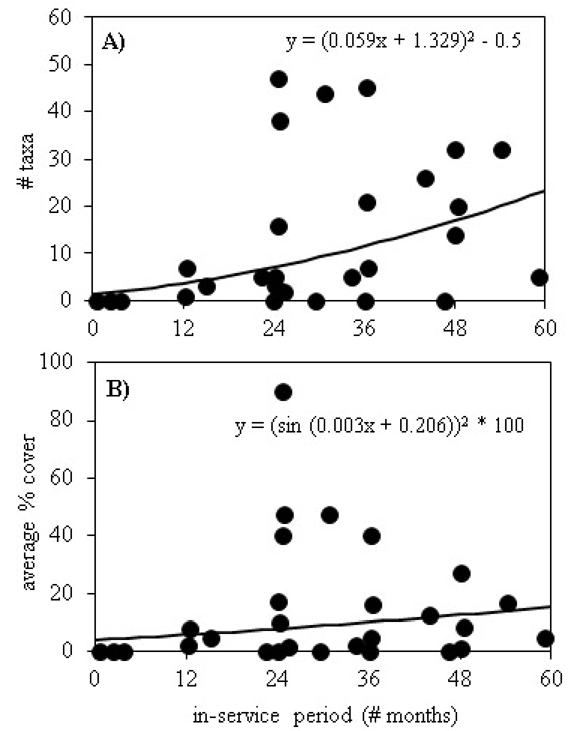


**Figure 2.** Relationship between in-service period (i.e., duration since last sea-chest cleaning) and extent of fouling as measured by: A) taxonomic richness (number of taxa), and B) abundance (average percentage cover) in sea-chests for each sampled vessel. Regression line shows back-transformed model fit.

coverage varied from 0 to 90% (Figure 1B) and, across all vessels, averaged 17.8 ± 24.6% (mean ± sd). Interestingly, vessels with heavily fouled sea-chests (i.e., high average percentage cover) did not necessarily equate to those with elevated taxonomic richness (i.e., large number of taxa) (Figure 1).

In total, we collected 299 distinct taxa (see Appendix 2), representing a broad spectrum of invertebrates, algae, and sea-grass, and including 54 non-indigenous (both non-established and established) and cryptogenic species. Communities were dominated by arthropods (primarily barnacles and amphipods, found in 63% of sea-chests), molluscs (bivalves and gastropods, 55%), cnidarians (primarily hydrozoans, 45%), polychaetes (45%), and bryozoans (30%). While the majority of these taxa were recognized as indigenous, we also identified a substantial number of non-indigenous and cryptogenic organisms that collectively, comprised 20.5% and 14.4% of the taxa sampled from West and East Coast vessels, respectively (Table 1). Non-indigenous species were found more frequently on international vessels (63% of those sampled) than on domestic vessels (35%). Overall, 46% of all vessels sampled (43% of West Coast vessels; 48% of East Coast vessels) harboured at least one non-indigenous species within a sea-chest.

*Effects of vessel specification and voyage history on biofouling*

Only a few of the factors related to vessel specification or voyage history, when analyzed separately, explained taxonomic richness or abundance in sea-chests (Table 2). Vessel size appeared to have a marginally significant effect on biofouling ($p = 0.08$); however, when the two largest vessels (both cruise ships) were excluded from the analysis this weak effect was not statistically significant (taxonomic richness: $R^2 = 0.042$, $F_{[1, 34]} = 1.480$, $p = 0.23$; abundance: $R^2 = 0.030$, $F_{[1, 34]} = 1.043$, $p = 0.31$). Port duration also had no significant effect on biofouling. By contrast, in-service period, defined as the number of months since previous cleaning of sea-chests, showed a significant positive relationship with taxonomic richness, but not abundance (Figure 2, Table 2). For both measures, however, sea-chest fouling appeared to significantly increase on vessels with in-service periods greater than 24 months (taxonomic richness: $t = -2.509$, $df = 26$, $p = 0.02$; abundance: $t = -2.110$, $df = 26$, $p = 0.04$).

**Table 1.** Number and percentage of indigenous, non-indigenous (non-established), non-indigenous (established), cryptogenic, and unknown taxa sampled from the sea-chests of West and East Coast vessels.

| | West Coast Vessels | | East Coast Vessels | |
|---|---|---|---|---|
| Origin of Taxa | # of Taxa | % of Taxa | # of Taxa | % of Taxa |
| Indigenous | 82 | 42.1 | 51 | 36.7 |
| Non-indigenous (non-established) | 26 | 13.3 | 14 | 10.1 |
| Non-indigenous (established) | 9 | 4.6 | 2 | 1.4 |
| Cryptogenic | 5 | 2.6 | 4 | 2.9 |
| Unknown | 73 | 37.4 | 68 | 48.9 |
| Total | 201 | | 139 | |

**Table 2.** Summary of linear model results of the effects of vessel specifications and voyage histories on: A) taxonomic richness (number of taxa, square-root transformed), and B) abundance (average percentage cover, arcsine transformed) in sea-chests. Effect size for vessel size (gross tonnage), port duration (average number of days in port), and in-service period (number of months since last sea-chest cleaning) is the slope (linear regression); effect size for vessel origin (domestic vs. international) is the mean difference (ANOVA). Significance level, α = 0.05.

| Factor | $R^2$ | Effect Size | F | df (n-2) | p |
|---|---|---|---|---|---|
| **A) Taxonomic richness** | | | | | |
| Vessel size | 0.080 | $2.61 \times 10^{-5}$ | 3.149 | 36 | 0.08 |
| Port duration | 0.003 | 0.025 | 0.079 | 28 | 0.78 |
| In-service period | 0.183 | 0.059 | 5.840 | 26 | **0.02** |
| Vessel origin | 0.050 | 0.886 | 1.946 | 37 | 0.17 |
| **B) Abundance** | | | | | |
| Vessel size | 0.083 | $4.55 \times 10^{-6}$ | 3.268 | 36 | 0.08 |
| Port duration | 0.007 | -0.006 | 0.186 | 28 | 0.67 |
| In-service period | 0.027 | 0.003 | 0.724 | 26 | 0.40 |
| Vessel origin | 0.062 | 0.172 | 2.360 | 36 | 0.13 |

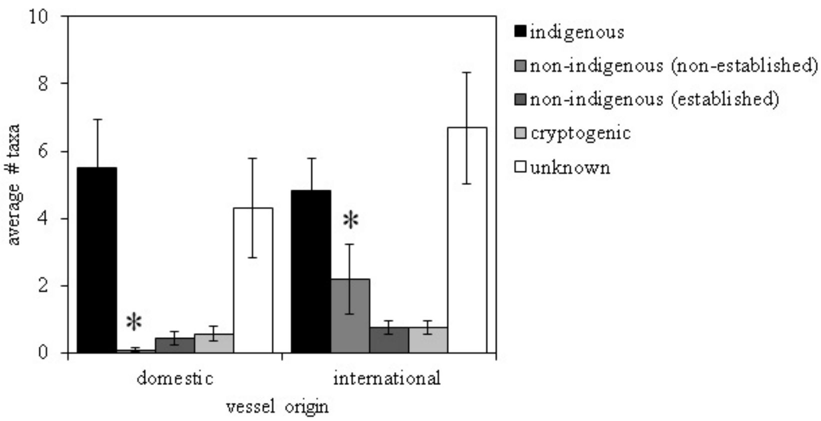


**Figure 3.** Average (± standard error) number of indigenous, non-indigenous (non-established), non-indigenous (established), cryptogenic, and unknown taxa sampled from a single sea-chest in domestic and international vessels. Asterisk highlights significant difference in average number of taxa between domestic and international vessels (p = 0.01).

The overall effect of vessel origin was not statistically significant, although international vessels harboured more taxa (15.2 ± 3.4, mean ± se) and had a higher average percentage cover (25.0 ± 8.5) relative to domestic vessels (10.9 ± 3.1 taxa, 13.1 ± 3.4 % cover). Moreover, non-indigenous (both non-established and established), cryptogenic, and unknown taxa were on average more prevalent on international vessels (Figure 3), albeit this difference was only statistically significant for non-indigenous species ($F_{[1, 37]} = 6.74$, $p = 0.01$). By contrast, indigenous taxa were found in similar numbers on both international and domestic vessels. Further analysis (ANOSIM) revealed that taxonomic composition was more similar within a vessel relative to among vessels (West Coast: R = 0.59, p = 0.001; East Coast: R = 0.72, p = 0.001). The same analyses for vessels grouped by origin suggested that sea-chests of domestic and international vessels did not significantly differ in overall taxonomic assemblage (West Coast: $R = 0.04$, $p = 0.22$; East Coast: $R = 0.04$, $p = 0.34$).

## Discussion

Similar to earlier investigations (Coutts et al. 2003; Coutts and Dodgshun 2007), our findings confirm that commercial vessels can harbour both an abundance and a diversity of fouling organisms, including many non-indigenous and cryptogenic taxa, within their sea-chests. While the above results indicate that the extent of biofouling, as measured by both percentage cover and taxonomic richness, is quite variable across vessels, the frequency of biofouling, as estimated by the percentage of vessels with sea-chest fouling, is relatively high with 80% of the sampled vessels exhibiting some level of biofouling. In general, these findings are consistent with the few other studies that have quantified fouling in sea-chests or on sea-chest gratings, and highlight the role of this niche area as a hotspot for biofouling (Coutts and Taylor 2004; Coutts and Dodgshun 2007; Sylvester and MacIsaac 2010).

The number of non-indigenous species found in this study further underscores the notion that sea-chests pose a serious risk for the introduction and spread of aquatic invasive species (Coutts and Dodgshun 2007). On both coasts a sizeable proportion (~15–20%) of the sea-chest community was identified as non-indigenous or cryptogenic. These results are lower than those reported in a recent hull fouling study that also sampled vessels on both coasts of Canada (~40-46%) (Sylvester et al. 2011); however, it is important to note that this latter investigation focused on international vessels only. By contrast, our estimates are comparable to those found in a similar sea-chest study (~25%), which surveyed both international and domestic vessels (Coutts and Dodgshun 2007). In addition to these findings, nearly half of all vessels sampled here carried at least one non-indigenous species within their sea-chests. We documented a number of non-indigenous taxa that have already successfully invaded other areas of the world, and may be well-adapted to the temperate waters of Canada. For example, the gammarid amphipod *Elasmopus rapax* was found in significant numbers (>1,500 individuals/$m^2$) in the sea-chests of an international vessel arriving from Hawaii to the West Coast of Canada. This small amphipod has been reportedly introduced in Hawaii (Coles et al. 1999; but see Hughes and Lowry 2010), California (Chapman in Carlton 2007), and in temperate ports throughout southern Australia (Hughes and Lowry 2010). Similarly the bryozoan *Bugula neritina,* a well-known fouling organism widely introduced and expanding on both coasts of North America (e.g., Cohen and Carlton 1995; Pederson et al. 2005), was discovered in the sea-chests of an international vessel that had traveled extensively throughout the Atlantic prior to arrival on the East Coast of Canada. Given these invasion histories, such fouling species seem primed for establishing populations in the temperate waters of Canada; and based on our observations, sea-chests could serve as the primary vector of introduction for these non-indigenous species.

Sea-chests may also play an important role in the secondary spread of already established non-indigenous or cryptogenic species. During this study, we discovered large numbers (100–4,300 individuals/$m^2$) of the invasive caprellid amphipod *Caprella mutica* in the sea-chests of several domestic vessels, each exclusively operating in the West Coast or in the East Coast of Canada. While we proposed that sea-chests may have facilitated the spread of this species, at least along the West Coast (Frey et al. 2009), a similar argument may hold for many of the other non-indigenous and cryptogenic species found in the sea-chests of domestic vessels, including the invasive tunicate *Ciona intestinalis* – a species already present on both coasts of North America and whose spread is of concern to managers and policy-makers (Therriault and Herborg 2008; Locke et al. 2009). The above results confirm that the sea-chests of international vessels are more likely to harbour non-indigenous species (and more of them). However once established, domestic vessels may play an equally significant role by spreading these species via intra-coastal voyages (Simkanin et al. 2009; for examples from recreational boats, see Clarke Murray et al. 2011; Lacoursière-Roussel et al. 2012).

Previous investigations have demonstrated that certain factors, related to vessel specifications and voyage histories, likely play a major role in contributing to the nature and extent of biofouling (e.g., Coutts and Dodgshun 2007; Davidson et al. 2009; Sylvester and MacIsaac 2010). Accordingly, we expected vessels with longer port durations and longer in-service periods to show increased taxonomic richness and abundance. However, we found no significant relationship between port duration and the extent of biofouling in this study, and contrary to prediction, observed relatively high levels of sea-chest fouling in some vessels that had brief port residency times (e.g., averaging one day or less). Admittedly the available data was limited to the last five ports, and may not accurately reflect typical port durations since last dry docking. As an additional caveat, it is important to note that anti-fouling systems were not evaluated in our analyses, and may have confounded some of our findings. By contrast, in-service period did appear as an important determinant for sea-chest fouling, which is consistent with other biofouling studies (Coutts 1999; Davidson et al. 2009; but see Sylvester and MacIsaac 2010). On average, vessels operating for more than approximately 24 months since last dry docking had significantly higher taxonomic richness and abundance. These results, coupled with comparable findings from recent hull fouling investigations (Davidson et al. 2009; Sylvester et al. 2011), demonstrate that current cleaning and maintenance practices may not sufficiently control biofouling. They also suggest that shorter periods between scheduled maintenance may be an effective management strategy to prevent excessive levels of biofouling (Sylvester et al. 2011). Although we were unable to examine other factors, including sailing speed, voyage routes, extensive port history, sea-chest environmental conditions, and anti-fouling systems due to data limitations, such factors may be important determinants of sea-chest fouling (Coutts et al. 2010; Sylvester et al. 2011), and represent vital areas for further research.

Indeed, a comprehensive understanding of the factors that significantly influence sea-chest fouling would likely improve our ability to identify and manage associated invasion risks. For example, although vessel origin may not account for overall taxonomic richness or abundance, our results show that origin does influence the type of species that are associated with sea-chests. International vessels are more likely to harbour non-indigenous (both non-established and established) species, and on average, carry significantly more non-indigenous (non-established) species than domestic vessels. In Canada, more than half of recent commercial shipping traffic is international (Statistics Canada 2012), with the majority of West Coast arrivals originating in Asia or the West Coast of the United States, and East Coast arrivals coming from the East Coast of the United States or Europe (Lo et al. 2012). Lo et al. (2012) also showed that wetted (immerged) surface area, used as a proxy for potential propagule pressure of biofouling, was significantly correlated with vessel arrivals. However it is important to consider that, as found with ballast water (Verling et al. 2005), the large variation of sea-chest fouling among vessels observed in this study suggests that invasion risk may not be a simple function of total vessel arrivals, but rather dependent on a complexity of factors involving various vessel specifications and voyage histories. Understanding which factors contribute most to sea-chest fouling remains essential for developing effective invasion management strategies.

Collectively, our findings support the notion that sea-chests represent a greater source of non-indigenous species than previously thought (Coutts et al. 2003). Vessel biofouling is the oldest, most important vector contributing to the introduction and spread of aquatic invasive species, accounting for more than 40% of all marine invasions (Hewitt and Campbell 2010). Indeed, hull fouling has been directly attributed to a large number of non-indigenous species in different regions of the world (e.g., Gollasch 2002; Simkanin et al. 2009; Rocha Farrapeira et al. 2011), and may pose a greater invasion risk than all other vectors, including ballast water (Gollasch 2002; but for freshwater environments, see Sylvester and MacIsaac 2010). Among the various niche areas along the hull of a vessel, sea-chests have been identified as a major hotspot for biofouling (Coutts and Taylor 2004; Sylvester and MacIsaac 2010), suggesting that this vector alone may present a significant invasion risk. In the present study, we found 47 distinct taxa in a single sea-chest, comparable to the maximum taxonomic richness reported in a similar investigation (Coutts and Dodgshun 2007). By sampling a second sea-chest in the same vessel, richness increased to 61 distinct taxa, a level comparable to that found in more general hull fouling studies (Drake and Lodge 2007; Sylvester and MacIsaac 2010). Admittedly, these numbers represent maximum levels recorded; however, it has been argued that such extreme cases likely pose the greatest invasion risk (Drake and Lodge 2007; Sylvester and MacIsaac 2010). We note that sampling additional sea-chests and vessels would likely result in increased taxonomic richness, as species accumulation curves (not presented here) have yet to reach an asymptote. Moreover, the ANOSIM results showed that biotic communities in sea-chests are more similar within vessels than among vessels, suggesting that each vessel may deliver a relatively unique assemblage of organisms to recipient ports. Vessels with such rich fouling communities can rival those found in ballast water (Drake and Lodge 2007), further underscoring the relative importance of sea-chests as a major vector for aquatic invasive species.

Despite the associated invasion risks, relatively few policies and management strategies are currently in place to regulate sea-chests and other vectors of biofouling. Certain regional guidelines have been developed by some governments (Hewitt and Campbell 2007), but a more global approach that controls biofouling across all regions and all vessels has not been implemented yet. While mandatory regulations for ballast water management may have reduced invasion risk (albeit not completely, Bailey et al. 2011), a lack of comparable strategies for hull and sea-chest fouling allows for continued transport of biota and potential shipping-mediated biological invasions (Davidson and Simkanin 2012). Developing effective vessel fouling management strategies is essential, particularly given emergent trends in shipping activity (e.g., increased port residency for vessels during global economic downturns) and recent changes to regulations of anti-fouling coatings (e.g., ban of TBT), each of which may ultimately lead to increased levels of vessel fouling across the globe (Floerl and Coutts 2009; Piola and Hopkins 2012). In this study, all vessels reportedly employed an anti-fouling system to control biofouling within sea-chests (e.g., cathodic protection systems and/or anti-fouling coatings). Yet similar to other investigations (Coutts and Taylor 2004; Coutts and Dodgshun 2007; Davidson et al. 2009), biofouling was still substantial in some cases, suggesting that current treatments are not always effective. Even the most commonly employed marine growth prevention systems (i.e., sacrificial anodic copper dosing (Cathelco®) and electrochlorination (Chloropac®)) have operational limitations that in turn can influence efficacy (Grandison et al. 2011). Thermal treatment that uses heated seawater offers promise as an alternative method to control biofouling within sea-chests; however, this technology needs further refinement before being implemented under actual conditions (Piola and Hopkins 2012).

In conclusion, sea-chests serve as an important vector for the introduction and spread of aquatic invasive species. Indeed, sea-chests can rival other major transfer mechanisms such as ballast water, and similarly, would benefit from the development of effective biofouling management strategies. To promote a comprehensive approach to the control of vessel fouling, the International Maritime Organization recently outlined voluntary guidelines centered on management plans, documentation, inspections, maintenance, anti-fouling systems, and new design and construction (IMO 2011). These include practical recommendations for managing sea-chests and other niche areas, including the installation and upkeep of anti-fouling systems (e.g., marine growth prevention systems or thermal treatment systems). Following the adoption of these guidelines, it will be important to assess whether the voluntary measures translate into increased prevention of vessel-mediated biological invasions (Baily et al. 2011). For example, just prior to mandatory regulations in the United States, voluntary compliance with ballast water management guidelines was relatively high among vessels that reported; but overall compliance remained unknown due to vast under-reporting (Miller et al. 2005). Fortunately, education and inspection programs appear to result in increased compliance and improved management practices (Baily et al. 2011). Additional research, aimed at better understanding the factors that influence sea-chest fouling and testing the efficacy of anti-fouling systems, will help further refine management strategies and reduce the invasion risks associated with sea-chest fouling.

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## References

Ardisson P-L, Bourget E, Legendre P (1990) Multivariate approach to study species assemblages at large spatio-temporal scales: the community structure of the epibenthic fauna of the Estuary and Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 1364–1377, http://dx.doi.org/10.1139/f90-156

Ashton GV, Willis KJ, Cook EJ, Burrow M (2007) Distribution of the introduced amphipod *Caprella mutica* Shurin, 1935 (Amphipoda: Caprellida: Caprellidae) on the west coast of

Scotland and a review of its global distribution. *Hydrobiologia* 590: 31–41, http://dx.doi.org/10.1007/s10750-007-0754-y
Baily SA, Deneau MG, Jean L, Wiley CJ, Leung B, MacIsaac HJ (2011) Evaluating efficacy of an environmental policy to prevent biological invasions. *Environmental Science and Technology* 45: 2554–2561, http://dx.doi.org/10.1021/es102655j
Carlton JT (1996) Pattern, process, and prediction in marine invasion ecology. *Biological Conservation* 78: 97–106, http://dx.doi.org/10.1016/0006-3207(96)00020-1
Carlton JT (ed) (2007) The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon. 4th ed. The University of California Press, Berkeley, 1019 pp
Carlton JT, 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, http://dx.doi.org/10.1007/BF00349308
Chapman JW (2007) Gammaridea. In: Carlton JT (ed), The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon. 4th ed. The University of California Press, Berkeley, pp 545–618
Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth UK.
Clarke Murray C, Pakhomov EA, Therriault TW (2011) Recreational boating: a large unregulated vector transporting marine invasive species. *Diversity and Distributions* 17: 1161–1172, http://dx.doi.org/10.1111/j.1472-4642.2011.00798.x
Cohen AN, Carlton JT (1995) Nonindigenous aquatic species in a United States estuary: a case study of the biological invasions of the San Francisco Bay and Delta. US Fish and Wildlife Service, Washington DC
Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. *Science* 279: 555–558, http://dx.doi.org/10.1126/science.279.5350.555
Coles SL, DeFelice RC, Eldridge LG, Carlton JT (1999) Historical and recent introductions of non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. *Marine Biology* 135: 147–158, http://dx.doi.org/10.1007/s002270050612
Coutre J-Y, Simard N (2007) Évaluation préliminaire des risques potentiels d'introduction d'espèces non indigènes dans les eaux de la côte est canadienne par l'intermédiaire des caissons de prise d'eau des navires. Rapport manuscrit Canadien des sciences halieutiques et aquatiques 2824: 25 pp
Coutts, ADM (1999) Hull fouling as a modern vector for marine biological invasions: investigation of merchant vessels visiting northern Tasmania. MSc Thesis, Australian Maritime College, Tasmania, 283 pp
Coutts ADM, Moore KM, Hewitt CL (2003) Ships' sea-chests: an overlooked transfer mechanism for non-indigenous marine species? *Marine Pollution Bulletin* 46: 1504–1515, http://dx.doi.org/10.1016/S0025-326X(03)00292-3
Coutts ADM, Taylor MD (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, http://dx.doi.org/10.1080/00288330.2004.9517232
Coutts ADM, Dodgshun TJ (2007) The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. *Marine Pollution Bulletin* 54: 875–886, http://dx.doi.org/10.1016/j.marpolbul.2007.03.011
Coutts ADM, Piola RF, Hewitt CL, Connell SD, Gardner JP (2010) Effect of vessel voyage speed on survival of biofouling organisms: implications for translocation of non-indigenous marine species. *Biofouling* 26: 1–13, http://dx.doi.org/10.1080/08927010903174599
Davidson IC, Brown CW, Sytsma MD, Ruiz GM (2009) The role of containerships as transfer mechanisms of marine biofouling species. *Biofouling* 25: 645–655, http://dx.doi.org/10.1080/08927010903046268
Davidson IC, Simkanin C (2012) The biology of ballast water 25 years later. *Biological Invasions* 14: 9–13, http://dx.doi.org/10.1007/s10530-011-0056-1
Drake JM, Lodge DM (2007) Hull fouling is a risk factor for intercontinental species exchange in aquatic ecosystems. *Aquatic Invasions* 2: 121–131, http://dx.doi.org/10.3391/ai.2007.2.2.7
Floerl O, Coutts A (2009) Potential ramifications of the global economic crisis on human-mediated dispersal of marine non-indigenous species. *Marine Pollution Bulletin* 58: 1595–1598, http://dx.doi.org/10.1016/j.marpolbul.2009.08.003
Frey MA, Gartner HN, Clarke Murray C, Therriault TW (2009) First confirmed records of the non-native amphipod *Caprella mutica* (Schurin 1935) along the coast of British Columbia, Canada, and the potential for secondary spread via hull fouling. *Aquatic Invasions* 4: 495–499, http://dx.doi.org/10.3391/ai.2009.4.3.10
Gollasch S (2002) The importance of ship hull fouling as a vector of species introductions into the North Sea. *Biofouling* 18: 105–121, http://dx.doi.org/10.1080/08927010290011361
Grandison C, Piola R, Fletcher L (2011) A review of Marine Growth Prevention System (MGPS) options for the Royal Australian Navy. Commissioned by the Department of Defence, Marine Platforms Division, Defence Science and Technology Organisation, Victoria, 28 pp
Hewitt CL, Campbell ML, Thresher RE, Martin RB, Boyd S, Cohen BF, Currie DR, Gomon MF, Keough MJ, Lewis JA, Lockett MM, Mays N, McArthur MA, O'Hara TD, Poore GCB, Ross DJ, Storey MJ, Watson JE, Wilson RS (2004) Introduced and cryptogenic species in Port Phillip Bay, Victoria, Australia. *Marine Biology* 144: 183–202, http://dx.doi.org/10.1007/s00227-003-1173-x
Hewitt CL, Campbell ML (2007) Mechanisms for the prevention of marine bioinvasions for better biosecurity. *Marine Pollution Bulletin* 55: 395–401, http://dx.doi.org/10.1016/j.marpolbul.2007.01.005
Hewitt CL, Campbell ML (2010) The relative contribution of vectors to the introduction and translocation of invasive marine species. Commissioned by the Department of Agriculture, Fisheries and Forestry, Canberra, 56 pp
Hughes LE, Lowry JK (2010) Establishing a neotype for *Elasmopus rapax* Costa, 1853 and its presence as an invasive species in temperate Australian waters. *Journal of Crustacean Biology* 30: 699–709, http://dx.doi.org/10.1651/10-3290.1
IMO (International Maritime Organization) (2011) Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species. Annex 26, Resolution MEPC.207(62), London
Lacoursière-Roussel A, Forrest BM, Guichard F, Piola RF, McKindsey CW (2012) Modeling biofouling from boat and source characteristics: a comparative study between Canada and New Zealand. *Biological Invasions* 14: 2301–2314, http://dx.doi.org/10.1007/s10530-012-0230-0
Lambert G, Shenkar N, Swalla B (2010) First Pacific record of the north Atlantic ascidian *Molgula* citrina – bioinvasion or circumpolar distribution? *Aquatic Invasions* 5: 369–378, http://dx.doi.org/10.3391/ai.2010.5.4.06
Lee JE, Chown SL (2007) *Mytilus* on the move: transport of an invasive bivalve to the Antarctic. *Marine Ecology Progress Series* 339: 307–310, http://dx.doi.org/10.3354/meps339307
Lo VB, Levings CD, Chan KMA (2012) Quantifying potential propagule pressure of aquatic invasive species from the commercial shipping industry in Canada. *Marine Pollution Bulletin* 64: 295–302, http://dx.doi.org/10.1016/j.marpolbul.2011.11.016
Locke A, Hanson JM, MacNair NG, Smith AH (2009) Rapid response to non-indigenous species. 2. Case studies of invasive tunicates in Prince Edward Island. *Aquatic Invasions* 4: 249–258, http://dx.doi.org/10.3391/ai.2009.4.1.25

Miller AW, Ruiz GM, Lion K (2005) Status and trends of ballast water management in the United States. Second biennial report of the National Ballast Information Clearinghouse, Submitted to the United States Coast Guard, 2004. Smithsonian Environmental Research Center, Edgewater, Maryland, 31 pp

Minchin D, Gollasch S (2003) Fouling and ships' hulls: How changing circumstances and spawning events may result in the spread of exotic species. *Biofouling* 19: 111–122, http://dx.doi.org/10.1080/0892701021000057891

Pederson J, Bullock R, Carlton J, Dijkstra J, Dobroski N, Dyrynda P, Fisher R, Harris L, Hobbs N, Lambert G, Lazo-Wasem E, Mathieson A, Miglietta M-P, Smith J, Smith III J, Tyrrell M (2005) Marine Invaders in the Northeast: Rapid Assessment Survey of Non-native and Native Marine Species of Floating Dock Communities, August 2003 MIT SeaGrant, 40 pp

Piola RF, Hopkins GA (2012) Thermal treatment as a method to control transfers of invasive biofouling species via vessel sea chests. *Marine Pollution Bulletin* 64: 1620–1630, http://dx.doi.org/10.1016/j.marpolbul.2012.05.028

Rocha Farrapeira CM, de Oliveira Tenório D, Duarte do Amaral F (2011) Vessel biofouling as an inadvertent vector of benthic invertebrates occurring Brazil. *Marine Pollution Bulletin* 62: 832–839, http://dx.doi.org/10.1016/j.marpolbul.2010.12.014

Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Hines AH (2000) Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics* 31: 481–531, http://dx.doi.org/10.1146/annurev.ecolsys.31.1.481

Simkanin C, Davidson I, Falkner M, Sytsma M, Ruiz G (2009) Intra-coastal ballast water flux and the potential for secondary spread of non-native species on the US West Coast. *Marine Pollution Bulletin* 58: 366–374, http://dx.doi.org/10.1016/j.marpolbul.2008.10.013

Statistics Canada (2012) Shipping in Canada 2011. Ottawa, 191 pp

Sylvester F, MacIsaac HJ (2010) Is vessel hull fouling an invasion threat to the Great Lakes? *Diversity and Distributions* 16: 132–143, http://dx.doi.org/10.1111/j.1472-4642.2009.00622.x

Sylvester F, Kalaci O, Leung B, Lacoursière-Roussel A, Clarke Murray C, Choi FM, Bravo MA, Therriault TW, MacIsaac HJ (2011) Hull fouling as an invasion vector: can simple models explain a complex problem? *Journal of Applied Ecology* 48: 415–423, http://dx.doi.org/10.1111/j.1365-2664.2011.01957.x

Therriault TW, Herborg L-M (2008) A qualitative biological risk assessment for vase tunicate *Ciona intestinalis* in Canadian waters: using expert knowledge. *ICES Journal of Marine Science* 65: 781–787, http://dx.doi.org/10.1093/icesjms/fsn059

Verling E, Ruiz GM, Smith LD, Galil B, Miller AW, Murphy KR (2005) Supply-side invasion ecology: characterizing propagule pressure in coastal ecosystems. *Proceedings of the Royal Society B* 272: 1249–1257, http://dx.doi.org/10.1098/rspb.2005.3090

**Supplementary material**

The following supplementary material is available for this article:

**Appendix 1.** Supplementary data for vessels and sea-chests sampled.

**Appendix 2.** Non-indigenous (including non-established and established), cryptogenic, indigenous, and unknown taxa found in the sea-chests of vessels sampled while in dry dock on the West Coast and East Coast of Canada.

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