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Effect of shipping traffic on biofouling invasion success at population and community levels

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Abstract The relationship between shipping and invasion success in marine waters has rarely been demonstrated empirically. In commercial ports, greater shipping activity is expected to increase invasion success at both the community and population levels by altering the diversity of exotic species discharged (colonization pressure) and the number of introduction events (propagule number). This study sought large-scale correlations between metrics of shipping activities and exotic fouling species diversity at the community (species richness evaluated using standardized port surveys) and population (genetic diversity) levels. The richness of exotic fouling species was evaluated at the community level by sampling 15 commercial ports in marine temperate Canadian waters. At the population level, we investigated genetic diversity of the model fouling species *Botryllus schlosseri* in five commercial ports, by identifying 262 individuals at the mitochondrial cytochrome c oxidase subunit I gene and eight polymorphic nuclear microsatellites. Measures of community- and population-level richness were related to various measures of propagule number and colonization pressure related to ship ballast and hull biofouling. Patterns of exotic fouling diversity are consistent with the hypothesis that increased shipping arrivals increase establishment success at both the community and population levels. As expected for fouling exotic species, ballast water was a less important vector; introduction success was best correlated to the number of arrivals of non-merchant ships from close regions, suggesting that this vector is important for successful invasion of this group of organisms.

Keywords Maritime transport · Shipping · Biodiversity · Propagule pressure · Molecular

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Introduction

Predictive invasion models suggest that increasing maritime activities (i.e. shipping) increase the probability of successful biological invasion (Lockwood

et al. 2005; Ricciardi 2006). However, despite great efforts to overcome the lack of empirical data (Bossenbroek et al. 2007; Buchan and Padilla 1999; Leung et al. 2006; Reusser et al. 2013) and the development of estimates of propagule pressure related to shipping activities (Herborg et al. 2007; National Research Council 2011), empirical evidence for the relationship between invasion success and maritime activities is largely lacking.

All else being equal, the number of exotic species in a given area is logically a function of the number and abundance of exotic species that have been introduced to it (Lockwood et al. 2009). In other words, invasion success is a function of colonization pressure (i.e., total number of species released in a single recipient region, Lockwood et al. 2009), and propagule pressure, the latter being a function of propagule number (i.e., number of discrete introduction events) and propagule size (i.e., number of individuals released in an introduction event) (Lockwood et al. 2005, 2009). At the community level, the likelihood that an exotic species establishes in a region is a function of complex interactions among environmental conditions (Wonham et al. 2013) and species characteristics (e.g. biotic resistance and invasion meltdown; Elton 1958; Simberloff and Von Holle 1999; Von Holle and Simberloff 2005). At the population level, greater propagule number may affect invasiveness by altering intra-population diversity (Lee 2002; Roman 2011). In short, genetic diversity is expected to decrease from native to invasive ranges (Allendorf and Lundquist 2003), although propagules from multiple genetically distinct source populations may increase genetic diversity in the latter (Novak and Mack 1993; Zalewski et al. 2010; Tonione et al. 2011). However, as shown in a growing number of studies (Kelager et al. 2013; Novak 2007; Roman and Darling 2007; Sakai et al. 2001), single introductions may occasionally lead to successful invasions (Dlugosch and Parker 2008; Puillandre et al. 2008; Zayed et al. 2007), Although a growing number of studies have used genetic markers to study exotic species dispersal (Darling et al. 2012; Ordóñez et al. 2013), how genetic diversity affects invasion processes through repeated introductions remains debated (Reem et al. 2013a; Roman and Darling 2007; Suarez and Tsutsui 2008). Determining the extent to which shipping increases invasion success at the community and population levels may help elucidate ecological mechanism that

drive invasion success and potentially advance management of invasive species.

In the marine realm, exotic fouling macroinvertebrates are increasingly a nuisance for human-made structures (Lambert and Lambert 2003) and have severely impacted aquaculture (Carver et al. 2006a; Mckindsey et al. 2007). Reducing the spread and avoiding future introductions of fouling species is thus a priority for marine conservation. However, the uncertainty about the relative importance of human vectors and pathways for the introduction and spread of marine fouling species impedes our ability to develop effective management strategies (Ruiz et al. 2015; Scianni et al. 2013). Exotic biofouling species often have short larval periods (e.g., tunicates) and are thus unlikely to be translocated by ballast water (Lambert 2005; Carver et al. 2006a). The biology and ecology of fouling species may favor their spread on vessel hulls, but, except for New Zealand, Australia and the Hawaiian Islands (Hewitt et al. 2009; State of Hawaii 2011), no regulations currently exist to reduce introductions from ship hull fouling.

Although most initial management efforts to reduce invasion by ship-borne species have focused on the importance of ballast-related vectors (IMO 2004), management of which has greatly reduced the rate of exotic species establishment in the Great Lakes in North America (Pagnucco et al. 2015), recent efforts have also addressed the importance of ship hulls and related external structures as vectors for biofouling species (Coutts and Taylor 2004; Davidson et al. 2009; Katsanevakis et al. 2013; Minchin et al. 2006; Sylvester et al. 2011). The abundance and richness of vessel fouling communities varies with ship size, trip history, source, voyage time, distance and speed (Davidson et al. 2008; Ruiz and Smith 2005). Ballast water regulations require international vessels to exchange water of filled ballast tanks or to flush empty ones prior to discharging ballast water within recipient ports. However, while propagule survivorship may be elevated for short voyages (MacIsaac et al. 2002), ballast water regulations do not target ships arriving from neighboring waters (Government of Canada 2006). Moreover, a recent Canadian shipping study demonstrated that mid-ocean exchange does not successfully reduce the abundance of macroinvertebrates in ballast water tanks, but it could further reduce risk of invasions by declining propagule pressure and enhancing demographic constraints for

invaders (Briski et al. 2012; Paolucci et al. 2015). Investigating correlations between shipping traffic and fouling invasion success may clarify the role of this pathway in exotic species introductions and inform management of this species group (Ojaveer et al. 2014).

In this study, we used the Canadian shipping database with standardized port sampling on the Atlantic and Pacific coasts of Canada to evaluate the influence of various aspects of propagule pressure due to shipping on exotic species establishment success. At the community level, we evaluate how exotic fouling community species richness is related to shipping traffic. At the population level, we examine how the genetic diversity of a dominant colonial fouling tunicate, *Botryllus schlosseri*, is related to shipping traffic. *B. schlosseri* is a cosmopolitan invader of coastal marine communities (Ben-Shlomo et al. 2001, 2006, 2008, 2010; Berrill 1950; Paz et al. 2003; Rinkevich et al. 2001; Stoner et al. 2002) and was used in this study as a model species because of (1) its rapid recent invasion success in Canadian waters (Sephton et al. 2011), (2) its ability to be transported through hull biofouling pathways (Lacoursière-Roussel et al. 2012a, b), (3) its short-term planktonic larvae (Ayre et al. 1997; Carver et al. 2006a), and (4) the well-developed genetic markers for this species.

Materials and methods

Sampling was done in 15 commercial ports on the east and west coasts of Canada. Western ports include seven ports in British Columbia: Port Alberni, Nanaimo, Esquimalt, Cowichan Bay, Campbell River, Vancouver, and Kitimat (Fig. 1). Eastern ports include eight ports in Nova Scotia: Yarmouth, Shelburne, Liverpool, Halifax, Sheet Harbour, Point Tupper, Little Narrows, and Sydney (Fig. 2).

Shipping data

To integrate vessel histories and maximize shipping data quantity and quality, we obtained detailed Canadian shipping data cross-referenced with multiple government databases: Canadian Coast Guard's Information System on Marine Navigation (INNAV), Transport Canada Ballast Water Database (TCBWD),
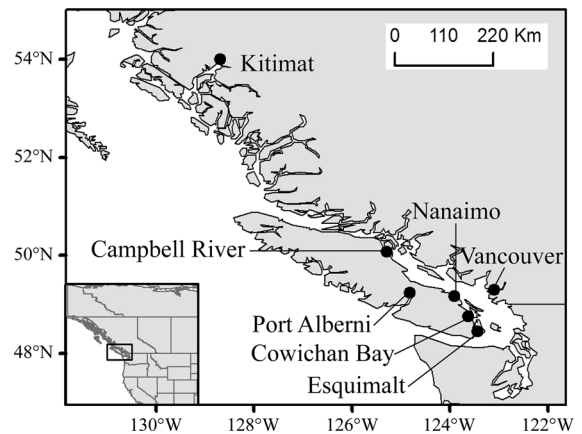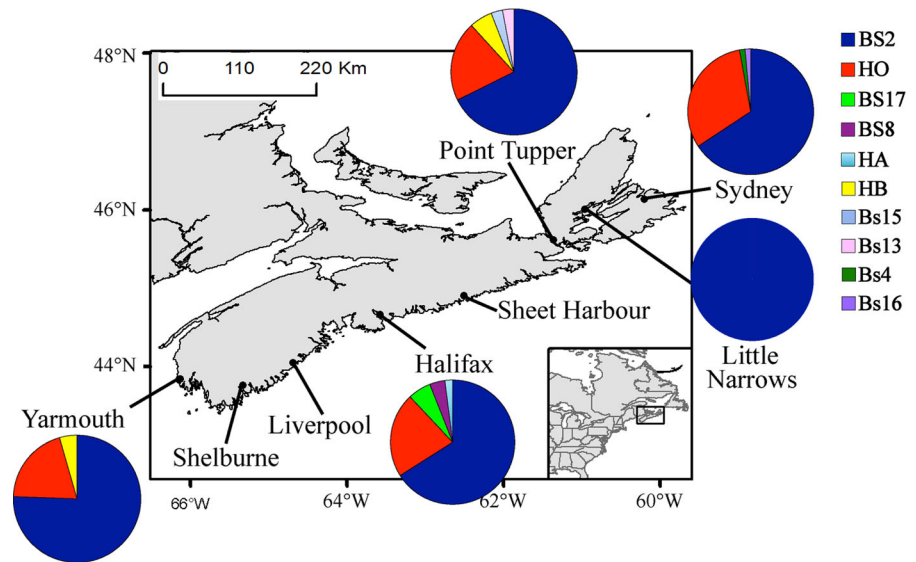

Fig. 1 Sampled site locations in western Canada

Canadian Coast Guard's Vessel Traffic Operations Support System (VTOSS), United Stated' National Ballast Information Clearinghouse (NBIC). Please refer to Adams et al. (2013) and Linley et al. (2014) for details about the Canadian shipping databases. Based on Statistics Canada (2016), the relative (i.e. port-specific) level of shipping is constant over time ($R^2 \geq 0.99$ for international cargo arrivals between 2002 and 2006 for both coasts (Statistics Canada 2016): see Figure S1). Trends related to relative levels of shipping among ports within coasts should thus remain unchanged due to inter-annual variability in shipping levels. We therefore used the single year available of the Canadian shipping database; 2006 for eastern Canada and 2008 for western Canada. Further, given that most exotic species in Canadian ports (at least most of those observed in the present study) were first noted fairly recently (Carlton 1996), we believe that recent shipping data reflect historical records that are most relevant to the arrival of these species in the examined ports.

Shipping data was obtained for both ballast water discharges and ship arrivals. Ballast water discharge was estimated as both (1) the number of ballast water discharge events and (2) the total volume of ballast water discharged. These pathways were highly correlated on the west coast (arrivals merchant foreign vs. ballast event foreign, merchant foreign vs. ballast volume foreign and ballast event foreign vs. ballast volume foreign: Pearson correlation coefficient (r) > 0.999) but less so on the east coast such that ship arrivals was strongly correlated with ballast water discharge events (r = 0.95) but weakly with the total

Fig. 2 Sampled site locations and relative frequencies of *Botryllus schlosseri* COI haplotypes from 5 ports in Nova Scotia, eastern Canada

volume of ballast water discharged (r = 0.61) and the relationship between the number of ballast water discharge events and the total volume of ballast water discharged was intermediate in strength (r = 0.77). Based on shipping data availability, we evaluated if the total number of ship arrivals, which logically gives a better indication of the strength of the hull biofouling pathway than do ballast-related metrics, are better predictors of invasive species diversity than are ballast water-related indicators of propagule pressure.

Vessels that discharge less than 8 m^3 of ballast water per ballast tank are not recorded and are thus not included in the analyses. Ballast water tanks are considered independently (i.e., a ship may discharge from multiple ballast water tanks and each such tank is considered as a discharge event as ballast sources often differ among ballast tanks within a single ship). Because the species density discharged alive at the end of a vessel's voyage in recipient ports is a function of voyage history, arrivals of merchant (i.e., commercial goods carriers) and non-merchant ships were evaluated separately, when applicable. Data for non-merchant vessels were only available for eastern Canada and include the following categories: commercial fishing vessels, cruise ships, government boats, ferries, tugs, barges, special purpose (i.e., dredgers, cable vessels, crane ships, drill rigs, offshore supply vessels), research vessels (i.e., non-government vessels), large pleasure craft (i.e., large craft that are required to submit a voyage plan prior to leaving a port). In

addition, as source region may alter trip histories (e.g., time underway and exposure to coastal vs. oceanic environments) and ballast water management requirements (DiBacco et al. 2012), foreign and domestic ships were first evaluated separately. Data for domestic vessels were also only available for eastern Canada.

The Food and Agriculture Organization of the United Nations (FAO) regions have similar environmental conditions. Thus, sources were pooled within FAO regions to describe general patterns of propagule pressure between coasts and estimate propagule pressure among ports for both eastern and western Canada (see Harvey et al. 1999). Vessels from nearby areas on each coast are exempt from ballast water regulations (Casas-Monroy et al. 2014) and thus shipping traffic from these closer FAO regions was also evaluated separately (i.e. North-east Pacific for the west coast and north-west Atlantic for the east coast).

Exotic fouling communities

Exotic macroinvertebrate fouling species and associated communities in ports were evaluated based on a standardized scuba diving survey on the west and east coasts of Canada. Dive surveys in commercial ports are often limited due to the risk associated with sampling, cost, and time. Furthermore, identifying a large number of exotic species groups is a significant taxonomic challenge and the invasion status of species

are often unknown or debated. Invasion studies that target the total number of exotic species diversity within ports may thus be somewhat biased as the determination of what is actually "exotic" is not always clear. Moreover, due to their functional traits, species groups may be transported by contrasting vectors and pathways. The present study therefore used a standardized sampling effort to target a specific species group (i.e. biofouling) to compare among ports and regions to evaluate links with potential shipping-related vectors.

The standardized sampling was done over a short time period and most often on a single substrate type (pilings) to limit seasonal and substrate-related (i.e., floating vs. static wharf substrates) variations. Divers targeted macrobenthos and collected samples of all taxa that could not be identified in the field for subsequent lab identification by local or international experts. The target list, based on knowledge of local experts, included species already known to have invaded the target region, or that could reasonably be expected to invade it (i.e., invasive species that have invaded other countries that are linked to Canada through potential vectors). At least one sample of each macro-fouling species identified in each port was collected to confirm field identifications and, when a species was not listed, a sample from each port was kept as a taxonomic reference. Except for tunicate species, which were conserved in 99 % ethanol, specimens collected were conserved in 7 % formaldehyde buffered with borax. Our survey did not target algae and species that could not be readily identified underwater, such as sponges, worms, amphipods (except caprellids) and the exotic *Mytilus galloprovincialis* as it is morphologically indistinguishable from the native *Mytilus edulis*. Smaller species were identified in the laboratory from subsamples taken from piling scrapings. To ensure consistency among sites and ports, underwater identifications were done by the same diver (i.e., Anaïs Lacoursière-Roussel) who was trained before starting the survey.

West coast ports were surveyed from July to August 2007 and East coast ports from June to July 2008. Sampling sites within a port were randomly distributed, other than for safety regulations, ship traffic and company agreements. Within each of six sites in each port, four adjacent pilings at wharf edges were sampled from low tide level to 8 m depth (at low tide);

surveying four pilings in a single dive (i.e. no need to change scuba tanks) was found to be the most effective method to rapidly survey ports and limit seasonal variability among ports. A pair of scuba divers did a visual survey of each piling for 10 min. Each identified species was checked on location-specific lists of likely fouling species printed on waterproof sheets. For the few wharfs not constructed using pilings, sampling was done within vertical transects of a similar size (same depth range, ca. 2 m wide). In each site, including pilings and vertical walls, a square 60 × 60 cm quadrat was surveyed intensively for 5 min, photographed using a high resolution camera, and all organisms within a 10 × 10 cm quadrat collected by scraping for subsequent lab identification to detect less obvious species on each of two pilings at a depth of three meters below low water. In addition, 10 settlement collectors were deployed as far as possible from each other in each port studied in eastern Canada for a period of approximately 2.5 months to survey recruiting fouling species. Plates were deployed between June 19 and July 19 and were collected from October 3 to 11, 2008. All collectors were suspended in the water column at about 1.5 m depth and were made of an upside-down 20 cm diameter plastic flowerpot base on top of three 10 × 10 cm PVC recruitment plates (see Sephton et al. (2011) for a similar design). Taxonomic identification included exotic species cited in the literature (Canadian Wildlife Federation 2008; Carlton 1985; NISbase 2008; Williams 2007) and all species of uncertain origin. In addition to morphological identifications on the east coast, the 18S gene was amplified to confirm species identifications for tunicates (method given in detail by Stewart-Clark, University of Prince Edward Island: Supporting Information S1).

Genetic diversity

Intra-population diversity was evaluated as the genetic diversity of the exotic colonial tunicate, *Botryllus schlosseri*, using mitochondrial and nuclear markers. Specimens were collected in 2008 and 2009 on different sections of the settlement collectors described above, as well as additional collectors attached to floating structures, in invaded commercial ports in Nova Scotia (i.e., Yarmouth, Halifax, Point Tupper, Little Narrows, and Sydney). All samples were preserved in 95–100 % ethanol prior to genetic

analyses. Genomic DNA (gDNA) was extracted from 4 to 6 zooids from each sampled colony following Elphinstone et al. (2003). A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified and sequenced in all individuals. All samples were also genotyped for eight polymorphic microsatellite markers: BS321 (Pancer et al. 1994), PB29, PB49, PBC1 (Stoner et al. 1997), Bsm1, Bsm2, Bsm4 and Bsm6 (Molecular Ecology Resources Primer Development Consortium et al. 2010). Amplification, sequencing and genotyping were done according to the protocols described in Lacoursière-Roussel et al. (2012a). COI haplotypes were identified using COLLAPSE v. 1.2 (Posada 2004). Relationships among haplotypes were investigated using a parsimony haplotype network in TCS v. 1.21 (Clement et al. 2000). The best-fit model of nucleotide substitution (TrN + I), selected using MODELTEST v. 3.7 (Posada and Crandall 1998) under the Akaike Information Criterion (AIC; Posada and Buckley 2004), was used to calculate sequence divergences between haplotypes in MEGA v.4.0 (Tamura et al. 2007). For the COI data, genetic diversity in each sampling location was calculated using the standard indices of haplotype (Θ) and nucleotide (π) diversity in DNASP v.5 (Rozas et al. 2003). Genetic diversity for microsatellite data was calculated as the total number of alleles (N_A), private alleles (N_P), allelic richness (A_r), and expected heterozygosiy (H_E) in FSTAT v. 2.9.3.2 (Goudet 2002).

Data analysis

Relationships between shipping predictors (i.e., ship arrivals, ballast water discharge events and volume of ballast water discharged) and exotic species richness and genetic diversity were evaluated using multiple regressions. The factor Coast (East or West) was included as a dummy variable. To create the most parsimonious regression model, co-linearity among explanatory variables was first evaluated using forward selection and less important co-linear variables removed from subsequent analyses [see method in Blanchet et al. (2008)]. Multiple regression was done using the *lm()* function of the R stats package and forward selection was performed using 9999 permutations in the FORWARD.SEL() function of the R packfor package. Assumptions of homoscedasticity, normality, and the presence of outliers were evaluated by visual examination of diagnostic plots and a log(x + 1) transformation used to normalize the data for predictor variables.

Theories about ecological mechanisms driving invasion success were evaluated by fitting several models using maximum likelihood estimation: linear, Michaelis-Menton (Levine and D'Antonio 2003; Tilman 2004), logistic (Ruiz and Carlton 2003), and exponential (see potential ecological mechanisms in Table 1; Allee 1931; Cohen and Carlton 1998; Drake and Lodge 2006; Simberloff and Von Holle 1999). The fit of each was assessed independently using Aikake's information criterion corrected for finite sample sizes (AIC_c). Comparison of AIC scores evaluates which model 'best' predicts the observed data (i.e., that model having the minimum AIC score), thus providing insight into ecological processes that may explain the observed patterns (Table 1; Johnson and Omland 2004). Additionally, Δi ($\Delta i = (AIC_c)i - (AIC_c)min$) was used to contrast the relative significance among models ($0 \leq \Delta i \leq 2$: substantial support; $4 \leq \Delta i \leq 7$: some support; $\Delta i \geq 10$: essentially no support and can be omitted from further consideration; Burnham and Anderson 2002).

Table 1 Candidate dose–response models with potential ecological mechanism. *R* is the diversity in a specific commercial port and *P* is the level of shipping in this port

Model type	Model equation	Ecological mechanism
Linear	$R = aP + b$	No threshold, no saturation, and no accelerating or decelerating cumulative rate
Michaelis-Menton	$R = aP/(P + b)$	Saturation: import recruit limitation or community saturation
Logistic	$R = 1/(1 + e^{(-P)})$	Multiphasic relationship:
		(1) Threshold from invasion resistance and/or stochasticity and/or Allee effect
		(2) Consistent marginal benefit
		(3) Saturation: import limitation or community saturation
Exponential	$R = a + e^P$	Intra- and inter-specific interactions: inoculation frequency or/and Invasional Meltdown

Results

Shipping traffic

Overall, shipping traffic in the ports examined is greater on the west coast than on the east coast of Canada (Table 2). Eastern and western Canada received foreign merchant vessels discharging ballast water from a similar number of FAO source areas (i.e., $N = 13$ FAO regions on the west coast and $N = 11$ FAO regions on the east coast). However, a greater proportion of ships and ballast in western Canada originated from trans-oceanic voyages (33 % of ship arrivals, 61 % of ballast water discharge events and

Table 2 Shipping activity within the commercial ports of British Columbia (western Canada; including only foreign merchant vessels) and Nova Scotia (eastern Canada; including both foreign merchant and foreign and domestic non-merchant vessels) and the number and identity of exotic species within each location

Ports	SA	EBW	VBW (MT)	Non-merchant SA		Number of exotic species	Exotic species
				Foreign	Domestic		
West coast							
Kitimat	92	22	87,158	–	–	0	–
Campbell River	18	10	11,806	–	–	3	*S. japonica, E. crocea, D. listerianum*
Port Alberni	24	34	120,410	–	–	2	*A. improvisus, C. caspia*
Nanaimo	44	20	67,244	–	–	3	*S. japonica, B. violaceus, B. schlosseri*
Vancouver	2422	1428	14,239,961	–	–	4	*S. japonica, E. crocea, B. violaceus, C. drepanochir*
Cowichan Bay	1	5	13,783	–	–	2	*S. japonica, B. violaceus*
Esquimalt	35	2	45,297	–	–	2	*S. japonica, B. violaceus*
Total	2636	1521	14,585,659			8	
East coast							
Yarmouth	0	0	0	178	128	4	*B. schlosseri, B. violaceus, C. intestinalis, C. mutica*
Shelburne	52	1	1,409	4	119	3	*B. schlosseri, B. violaceus, C. intestinalis*
Liverpool	11	14	9,875	0	14	1	*C. mutica*
Halifax	1014	270	2232,635	168	611	4	*B. schlosseri, C. intestinalis, M. membranacea, C. mutica*
Sheet Harbour	8	6	153,970	0	32	1	*C. intestinalis*
Point Tupper	381	231	6657,258	86	470	5	*B. schlosseri, B. violaceus, C. intestinalis, M. membranacea, C. mutica*
Little Narrows	27	28	316,081	0	1	2	*T. navilis, B. schlosseri*
Sydney	69	6	35,738	43	36	5	*B. schlosseri, B. violaceus, C. intestinalis, M. membranacea, C. mutica*
Total	1562	556	9406,966	479	1411	6	

SA is the number of ship arrivals, EBW the number of ballast water discharge events and VBW the volume of ballast water discharged. Horizontal lines indicate that no exotic species were found. Exotic tunicates included *Ciona intestinalis* [see Carver et al. (2006b) and Bouchemousse et al. (2016)], *Botryllus schlosseri*, *Botrylloides violaceus* [see Carver et al. (2006a)] and *Diplosoma listerianum* [see Lambert and Lambert (1998)], arthropods included *Caprella mutica* [see Ashton et al. (2008)], *Caprella drepanochir* [see Ruiz et al. (2011)] and *Amphibalanus improvisus* [*Balanus improvisus*; see Lu et al. (2007)], bryozoan *Schizoporella japonica* [*S. unicornis*; see Wasson et al. (2001)] and *Membranipora membranacea* [see Berman et al. (1992) and Scheibling et al. (1999)], cnidarians *Ectopleura crocea* and *Cordylophora caspia* [see Carlton (1979)] and molluscs [only *Teredo navalis*, see Carlton (2003)]. Note that *B. schlosseri* was not used for subsequent molecular analysis when its abundance was too low to provide an adequate sample size ($N = 30$)

80 % of the volume of ballast water discharged were from vessels originating from the north-west Pacific and 19 % of ship arrivals originated from unknown regions). Foreign ships and discharged ballast in eastern Canada originated mainly from the north-west Atlantic (54 % of ship arrivals, 65 % of ballast water discharge events and 82 % of the volume of ballast water discharged and 17 % of ship arrivals originated from unknown regions).

On the west coast, shipping activity is concentrated in a single port (Vancouver: 92 % of total ship arrivals, 94 % of total ballast water discharge events and 98 % volume of ballast water discharged, Table 2). On the east coast, the maritime traffic in Nova Scotia ports is more geographically diffuse (Fig. 2) with shipping activities concentrated in two ports (Halifax and Point Tupper; Table 2). The relative importance of ship arrivals and ballast water differed between these two major ports (i.e., Halifax: 65 % of total ship arrivals, 49 % of the total ballast water discharge events and 24 % of the total volume of ballast water discharged; and Point Tupper: 24 % of total ship arrivals, 42 % of the total ballast water discharge events and 71 % of the total volume of ballast water discharged). Please refer to Figure S2 for the proportion of non-merchant vessels.

Fouling community diversity and shipping traffic

A total of eight and six fouling exotic species was found in west and east coast ports, respectively (Table 2). The multiple linear model (i.e. data from both coasts included, ship arrivals, ballast water discharge events, volume of ballast water discharged) including the foreign merchant vessels of the 15 sampled commercial ports, was not statistically significant ($P = 0.3$, $R^2 = 0.30$; foreign ship arrivals: $P = 0.2$, number of ballast water events: $P = 0.6$; volume of ballast water discharged: $P = 0.1$). The model becomes significant when only ships from neighboring FAO regions were considered (i.e. $P = 0.04$, $R^2 = 0.51$). Ballast water metrics for ships from the neighboring FAO regions were poor predictors of exotic species richness in ports (ballast water discharge events: $P = 0.04$, $R^2 = 0.28$; volume of ballast water discharged: $P = 0.03$, $R^2 = 0.32$) and only ship arrivals was included in the final model ($P < 0.01$, $R^2 = 0.43$; Fig. 3). The relationship between ship arrivals from neighboring FAO regions
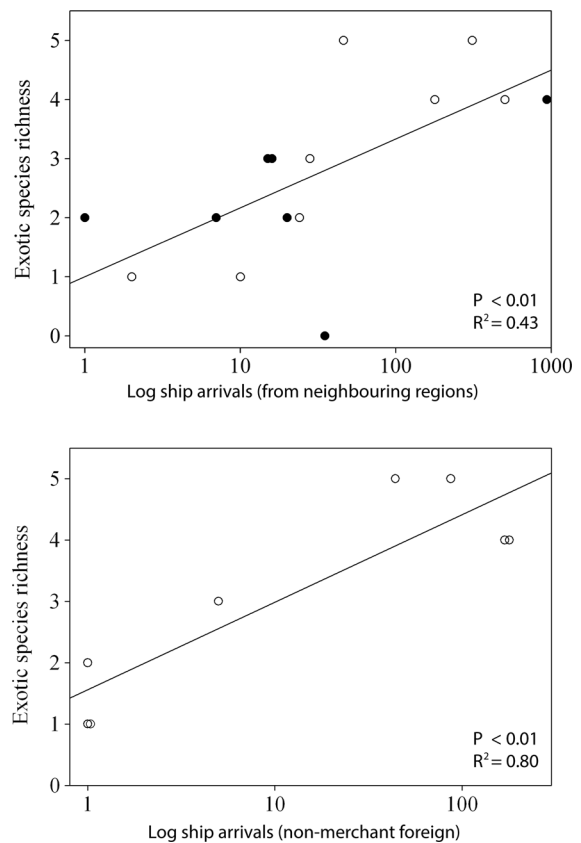

Fig. 3 Relationship between observed exotic species richness and the number of merchant ship arrivals originating from neighboring FAO regions in 15 Canadian commercial ports (*top*; *black dots*: western ports and *white dots*: eastern ports) and the number of arrivals of non-merchant foreigner vessels in eight eastern Canadian ports (*bottom*)

and exotic species richness was best described by a logistic model, although performance did not differ substantially among models ($\Delta i \leq 5.4$; AIC_c logistic = 53.2; AIC_c linear = 57.3; AIC_c Michaelis-Menton = 57.5; AIC_c exponential = 58.6). When each coast is considered independently, ship arrivals from neighboring FAO regions was not a significant predictor of community richness for the west coast ($P = 0.4$, $R^2 = 0.16$) but was significant for the east coast ($P = 0.01$, $R^2 = 0.68$).

For eastern Canada, when domestic/foreign and merchant/non-merchant vessels were examined separately, domestic ballast water metrics did not explain a significant portion of the variation in exotic species richness (discharge events: $P = 0.9$, $R^2 = 0.05$; ballast water volume discharged: $P = 0.8$, $R^2 = 0.08$) but forward selection retained non-merchant foreign

vessels, by itself, as a highly significant predictor of exotic species richness ($P < 0.01$, $R^2 = 0.80$; Fig. 3). The best fit between non-merchant foreign vessels and exotic species richness was described by a Michaelis-Menton function (linear $\Delta i = 14.3$; logistic $\Delta i = 5.4$; exponential $\Delta i = 15.5$).

Population genetic diversity and shipping traffic

Partial COI sequences were obtained for 262 *B. schlosseri* individuals in eastern Canada (Table 3). After alignment and trimming, a final length of 524 bp was used and ten haplotypes were observed (Fig. 2; Figure S3). Only haplotype Bs17 is new relative to those observed by Lacoursière-Roussel et al. (2012a) in Nova Scotia. Sequence divergence between haplotypes ranged from 0.2 % to 4.7 %. Microsatellite genotype data was obtained for 279 *B. schlosseri* individuals. In total, we recovered 57 alleles, of which 12 were private (Table 3). Ship arrivals better predicted genetic diversity than did ballast water (Fig. 4; ship arrivals: Θ, $P = 0.04$; π: $P = 0.05$; A_r: $P = 0.1$; H_E: $P = 0.09$; ballast water discharge events: Θ, $P = 0.09$; π: $P = 0.09$; A_r: $P = 0.2$; H_E: $P = 0.2$, and ballast water volume discharged $P > 0.1$ for all genetic indices; Table 3) and was retained by forward selection for haplotype diversity. Non-merchant vessels had greater power to predict the genetic diversity of *B. schlosseri* than did merchant vessels for both mitochondrial and nuclear markers. Of the non-merchant arrivals, foreign vessels had better predictive power than did domestic vessels (Fig. 4). However, for both merchant and non-merchant ships, ship arrivals from closer FAO regions were a poor predictor of genetic diversity (linear regression: $P > 0.05$).

For all significant relationships (see Fig. 4), the best fits between non-merchant ship arrivals and genetic diversity were best described by Michaelis–Menton functions ($\Delta i = 2.7$ and 2.5 for logistic relationships between domestic and foreign ship arrivals and haplotype diversity, respectively; $\Delta i \geq 10$ for
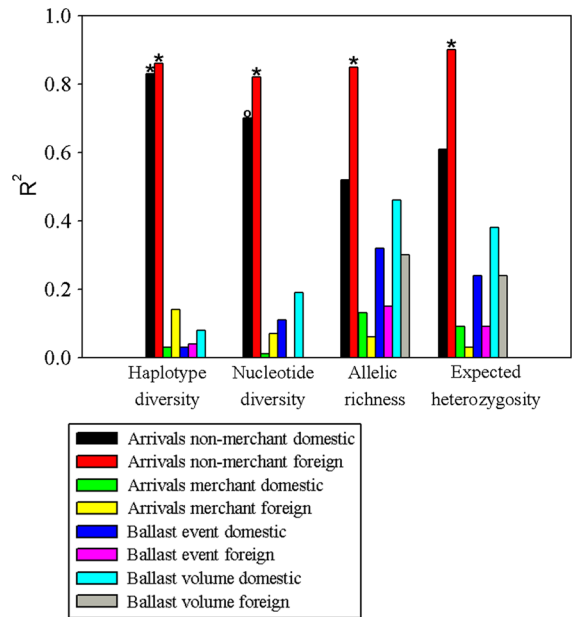

Fig. 4 Coefficient of determination (R^2) from linear regressions between the log of propagule pressure and genetic diversity. Propagule pressure estimated by ship arrivals and ballast water discharge events and water volumes discharged for domestic and foreigner vessels (see color legend) and genetic diversity obtained from haplotype diversity (Θ), nucleotide diversity (π), allelic richness (A_r), expected heterozygosity (H_E), for *B. schlosseri* in eastern Canada. The symbol *asterisk* indicates significant linear regressions ($P < 0.05$) and *circle* indicates a linear relationship with $P < 0.1$

Table 3 Population genetic diversity of *Botryllus schlosseri* for each sampled locations in which species was present in eastern Canada (Fig. 2)

Location	MtDNA					Microsatellite				
	N	N_h	Haplotypes	Θ	π	N	N_A	N_P	A_r	H_E
Yarmouth	45	3	Bs2, HO, HB	0.396	0.01199	54	46	4	4.092	0.6252
Point tupper	34	5	Bs2, HO, HB, BS13, BS15	0.510	0.01408	54	39	1	3.656	0.5733
Little narrows	63	1	Bs2	0.000	0.00000	61	34	1	2.803	0.4514
Sydney	70	4	Bs2, HO, BS4, BS16	0.476	0.01512	69	46	6	3.737	0.5763
Halifax harbour	50	5	Bs2, HO, B17, Bs8, HA	0.521	0.01351	41	39	0	3.609	0.5726

N, sample size; N_h, number of haplotypes; Θ, haplotype diversity; π, nucleotide diversity; N_A, number of alleles; N_P, number of private alleles; A_r, allelic richness; H_E, expected heterozygosity

exponential relationships between foreign ship arrivals and haplotype diversity, foreign ship arrivals and nucleotide diversity, and domestic ship arrivals and expected heterozygosity and for linear relationships between foreign ship arrivals and nucleotide diversity and domestic ship arrivals and expected heterozygosity; for all other relationships, $4 \leq \Delta i \leq 10$).

Discussion

Shipping activity was correlated with both community- and population-level diversity of exotic fouling species in Canadian ports, suggesting that this vector is important in the establishment of exotic species. Increased genetic diversity of populations of exotic species may reflect multiple introductions (Kelager et al. 2013; Novak 2007; Roman and Darling 2007; Sakai et al. 2001), as may be the case in the present study where genetic diversity was positively correlated with the level of shipping traffic.

Ship arrival was a better predictor of exotic species diversity than was ballast water, suggesting either that the observed exotic fouling species were introduced mostly through hull biofouling and/or that hull fouling is a relatively more efficient pathway for successful establishment of exotic fouling species. The fact that ballast was less well correlated to exotic fouling richness is likely due to fouling functional traits and ballast regulations. Even over larger scales, the greater number of exotic fouling species in western ports is consistent with the result of Lo et al. (2012) who calculated that, overall, the west coast of Canada receives more ships (but a lower total wetted hull surface area) than do eastern ports, but that the latter receives a greater volume of ballast water [see also Sylvester et al. (2011) and DiBacco et al. (2012)].

Legislation to require international vessels to exchange filled ballast tanks or discharge ballast water prior to arriving in ports substantially reduces the risk of introducing exotic species (Bailey et al. 2011). While this may be true for planktonic species and those that have extended planktonic stages, given the results of the present study, it seems that ballast water is likely a less important pathway for the introduction of fouling macroinvertebrates, suggesting that existing ballast water regulations may not be effective in limiting the introduction of these species. Except for New Zealand, Australia (Hewitt et al. 2009), and the

Hawaiian Islands (State of Hawaii 2011), which have regulations and requirements for inspection, no regulations currently target the hull biofouling pathway to reduce exotic species introductions.

A number of mechanisms may explain why biofouling richness and genetic diversity were best correlated with arrivals of non-merchant ships. Non-merchant vessels are typically slower and likely to receive less maintenance than merchant ships, thus increasing the probability of them transporting biofouling species (Davidson et al. 2008). Additionally, while Sylvester et al. (2011) showed that propagule size and colonization pressure decrease with increasing latitude of ports, our results suggest that ships from neighboring ecosystems have a greater probability of establishing populations of exotic species than do ships from more distant areas, thus highlighting the importance of intra-coastal spread due to shipping. Whereas source origin influences propagule nature (Hillebrand 2004), intracoastal trips limit transit times and exposure to harsher non-coastal (i.e., oceanic) conditions, both of which likely increase the probability of survivorship during transit. Moreover, greater environmental similarity between source and recipient regions of nearby ecoregions likely increases the probability of successful establishment (MacIsaac et al. 2002; Smith et al. 1999).

As compared to some other regions (e.g., Hewitt et al. 2004; Simkanin et al. 2009), the exotic fouling species richness reported in the present study was not great. This result is consistent with the fact that Canada has a small population and imports are largely from the United-States across a terrestrial border and thus is likely to have lower maritime transport relative to other countries with a greater population size or where imports and exports occur mainly through maritime transport (e.g., New Zealand, Japan). The best fitted models for community and genetic diversity (i.e., logistic and Michaelis-Menton) suggests that, despite greater levels of shipping traffic, exotic fouling species richness and genetic diversity may be limited in major ports by the arrival and establishment of new recruits (i.e., genetic diversity or species richness arriving from vectors), saturation of settling sites, and/or density-dependent mortality (Hedge et al. 2012; Ruiz and Carlton 2003; Stachowicz et al. 1999). The present study also found some support (i.e. logistic model) for the notion that a threshold of shipping traffic is necessary for successful invasion to occur. A

high level of shipping arrivals may therefore be necessary to overcome invasion resistance, the Allee effect, or stochasticity (e.g., environmental or demographic variation) effects for successful invasion to occur (Table 1). A similar saturation-response may also occur in regions with greater shipping pressure. Further studies are needed to identify thresholds in the relationship between shipping levels and invasion success.

A higher level of ship traffic was not exponentially related to exotic population and community diversities. The latter effect would have supported the Invasional Meltdown Hypothesis (Simberloff and Von Holle 1999; Table 1). However, the effect of invasional meltdowns on dose–response curves is unclear. New exotic species are still being introduced and establishing in Nova Scotia waters (e.g., *Diplosoma listerianum*, *Styela clava*, *Ascidiella aspersa*, *Didemnum vexillum*, *Diadumene lineata*; Moore et al. 2014) and thus the maximum exotic species richness is expected to increase over time (i.e., upward shift of the saturation curve). The observed maximum species richness is therefore likely due to either synergistic similar independent introductions (e.g., invasion from similar sources) or secondary spread among them.

Frequency and timing of propagule arrivals have been suggested as key factors that determine invasion success (Drake and Lodge 2004), as has been shown through field experiments (Hedge et al. 2012). The present study provides evidence that greater ship arrivals increase the probability of successful marine invasions by increasing colonization pressure and propagule number. In Nova Scotia, *B. schlosseri* has displayed a typical invasion pattern insomuch as its abundance and distribution have increased throughout the region over a very short time period following its initial observation (ca. 10 years) and the species is now fairly ubiquitous in the region (Carver et al. 2006a). Interestingly, the exotic *B. schlosseri* seems to have first invaded the United States (Dijkstra et al. 2007) and thereafter, likely through secondary spread, invaded Canadian ports (Ben-Shlomo et al. 2010; Lacoursière-Roussel et al. 2012a; Dr Gregory M. Ruiz personal communication). The patterns observed in the present study may be relevant to a number of species that show northward spread, which may be caused by increasing temperatures due to global climate change or change in shipping frequency and

maintenance (e.g., Frisch et al. 2013; Reem et al. 2013b).

Investigation on vectors and pathways and standardizing evaluations of the numbers of exotic species may greatly inform management strategies (Ojaveer et al. 2014). The inclusion of data from recruitment panel-focused monitoring programs for the east coast to increase the sample size did not increase the total richness of exotic fouling species observed at the coast-scale but decreased the strength of the relationship between shipping data and invasion success (i.e. relationship between exotic fouling richness and ships arrivals from neighboring FAO regions decreases from $R^2 = 0.81$ to $R^2 = 0.68$ when the additional data is included in the analyses; the relationship between exotic fouling richness and arrivals of non-merchant foreign vessels shows the relationship to decrease from $R^2 = 0.88$ to $R^2 = 0.80$ when the additional survey data is included). Therefore, despite lower sampling effort, the standardization of the port surveys may greatly help of better understanding pattern of invasion success. Further, the inclusion and analysis of data from multiple surveys that used differing methodologies (e.g. community and genetic) makes understanding patterns elusive.

This study stresses the importance of considering specific ship attributes such as ship type and origin to understand the risk of shipping to the introduction and establishment of exotic species. Inaccuracies and inconsistencies among shipping databases are challenges to understanding the ecology and management of exotic fouling species (Lo et al. 2012). This study used shipping data for the year for which the best data was available. The use of multi-year shipping data would assess the consistency of shipping traffic levels between ports across years, and may improve our understanding of the ecological mechanisms related to invasion success. Likewise, we suggest that a better understanding of fouling species invasions will depend on the integration of key information such as ship surface area (Davidson et al. 2009), domestic and non-merchant vessel arrivals (e.g., missing data for western Canada), and of recent approaches to estimate patterns of invasion risks such as combining information on environmental heterogeneity and biogeographic distributions (Seebens et al. 2013) or clustering approaches based on the global shipping network (Xu et al. 2014).

References

Adams JK, Ellis SM, Chan FT, Bronnenhuber JE, Doolittle AG, Simard N, McKenzie CH, Martin JL, Bailey SA (2013) Relative risk assessment for ship-mediated introductions of aquatic nonindigenous species to the Atlantic Region of Canada. Can Sci Advis Secr Res Doc 2012/116:vi + 404

Allee WC (1931) Animal aggregations: a study in general sociology. University of Chicago Press, Chicago

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. Conserv Biol 17:24–30

Ashton GV, Stevens MI, Hart MC, Green DH, Burrows MT et al (2008) Mitochondrial DNA reveals multiple Northern Hemisphere introductions of *Caprella mutica* (Crustacea, Amphipoda). Mol Ecol 17:1293–1303

Ayre DJ, Davis AR, Billingham M, Llorens T, Styan C (1997) Genetic evidence for contrasting patterns of dispersal in solitary and colonial ascidians. Mar Biol 130:51–61

Bailey SA, Deneau MG, Jean L, Wiley CJ, Leung B et al (2011) Evaluating efficacy of an environmental policy to prevent biological invasions. Environ Sci Technol 45:2554–2561

Ben-Shlomo R, Douek J, Rinkevich B (2001) Heterozygote deficiency and chimerism in remote populations of a colonial ascidian from New Zealand. Mar Ecol Prog Ser 209:109–117

Ben-Shlomo R, Paz G, Rinkevich B (2006) Postglacial-period and recent invasions shape population genetics of botryllid ascidians along European Atlantic coasts. Ecosystems 9:1118–1127

Ben-Shlomo R, Motro U, Paz G, Rinkevich B (2008) Pattern of settlement and natural chimerism in the colonial urochordate *Botryllus schlosseri*. Genetica 132:51–58

Ben-Shlomo R, Reem E, Douek J, Rinkevich B (2010) Population genetics of the invasive ascidian *Botryllus schlosseri* from South American coasts. Mar Ecol Prog Ser 412:85–92

Berman J, Harris L, Lambert W, Buttrick M, Dufresne M (1992) Recent invasions of the Gulf of Maine: three contrasting ecological histories. Conserv Biol 6:435–442

Berrill NJ (1950) The Tunicata with an account of the British species. Ray Society, London

Blanchet FG, Legendre P, Borcard D (2008) Forward selection of explanatory variables. Ecology 89:2623–2632

Bossenbroek JM, Johnson LE, Peters B, Lodge DM (2007) Forecasting the expansion of zebra mussels in the United States. Conserv Biol 21:800–810

Bouchemousse S, Bishop JDD, Viard F (2016) Contrasting global genetic patterns in two biologically similar, widespread and invasive *Ciona* species (Tunicata, Ascidiacea). Sci Rep 6:24875

Briski E, Ghabooli S, Bailey SA, MacIsaac HJ (2012) Invasion risk posed by macroinvertebates transported in ship's ballast tanks. Biol Invasions 14:1843–1850

Buchan LAJ, Padilla DK (1999) Estimating the probability of long-distance overland dispersal of invading aquatic species. Ecol Appl 9:254–265

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York

Canadian wildlife federation (2008) Invasive species database in Canada. http://www.cwf-fcf.org/invasive/chooseSC.asp. Accessed 22 Feb 2009

Carlton JT (1979) History, biogeography, and ecology of the introduced marine and estuarine invertebrates of the Pacific coast of North America. Ph.D. thesis, University of California

Carlton JT (1985) Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. Oceanogr Mar Biol 23:313–371

Carlton JT (1996) Biological invasions and cryptogenic species. Ecology 77:1653–1655

Carlton JT (2003) A checklist of the introduced and cryptogenic marine and estuarine organisms from Nova Scotia to Long Island Sound, 2nd edn. Williams College—Mystic Seaport Maritime Studies Program, Seaport

Carver CE, Mallet AL, Vercaemer B (2006a) Biological synopsis of the colonial tunicates, *Botryllus schlosseri* and *Botrylloides violaceus*. Can Man Rep Fish Aquat Sci 2747:v + 42

Carver CE, Mallet AL, Vercaemer B (2006b) Biological synopsis of the solitary tunicate Ciona intestinalis. Can Man Rep Fish Aquat Sci 2746:v + 55

Casas-Monroy O, Linley RD, Adams JK, Chan FT, Drake DAR, Bailey SA (2014) National risk assessment for introduction of aquatic nonindigenous species to Canada by Ballast water. Can Sci Adv Sec Res Doc 2014/128:vi + 73

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Cohen AN, Carlton JT (1998) Accelerating invasion rate in a highly invaded estuary. Science 279:555–558

Coutts ADM, Taylor MD (2004) A preliminary investigation of biosecurity risks associated with biofouling on merchant vessels in New Zealand. N Z J Mar Fresh Res 38:215–229

Darling JA, Herborg LM, Davidson IC (2012) Intracoastal shipping drives patterns of regional population expansion by an invasive marine invertebrate. Ecol Evol 2:2557–2566

Davidson IC, McCann LD, Fofonoff PW, Sytsma MD, Ruiz GM (2008) The potential for hull-mediated species transfers by obsolete ships on their final voyages. Divers Distrib 14:518–529

Davidson IC, Brown CW, Sytsma MD, Ruiz GM (2009) The role of containerships as transfer mechanisms of marine biofouling species. Biofouling 25:645–655

DiBacco C, Humphrey DB, Nasmith LE, Levings CD (2012) Ballast water transport of non-indigenous zooplankton to Canadian ports. ICES J Mar Sci 69:483–491

Dijkstra J, Harris LG, Westerman E (2007) Distribution and long-term temporal patterns of four invasive colonial ascidians in the gulf of Maine. J Exp Mar Biol Ecol 342:61–68

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449

Drake JM, Lodge DM (2004) Global hot spots of biological invasions: evaluating options for ballast-water management. Proc R Soc B Biol Sci 271:575–580

Drake JM, Lodge DM (2006) Allee effects, propagule pressure and the probability of establishment: risk analysis for biological invasions. Biol Invasions 8:365–375

Elphinstone MS, Hinten GN, Anderson MJ, Nock CJ (2003) An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. Mol Ecol Notes 3:317–320

Elton CS (1958) The ecology of invasions by animals and plants. Methuen, London **181p**

Frisch D, Havel JE, Weider LJ (2013) The invasion history of the exotic freshwater zooplankter *Daphnia lumholtzi* (Cladocera, Crustacea) in North America: a genetic analysis. Biol Invasions 15:817–828

Goudet J (2002) FSTAT, a program to estimate and test gene diversities and fixation indices version 2.9.3.2

Government of Canada (2006) Ballast water control and management regulations. Can Gazette 140(13). http://gazette.gc.ca/rp-pr/p2/2011/2011-11-09/html/sor-dors237-fra.html. Accessed 12 Sept 2014

Harvey M, Gilbert M, Gauthier D, Ried DM (1999) A preliminary assessment of risks for the ballast water-mediated introduction of nonindigenous marine organisms in the Estuary and Gulf of St. Lawrence. Can Tech Rep Fish Aquat Sci 2268:x + 56p

Hedge LH, Connor WA, Johston E (2012) Manipulating the intrinsic parameters of propagule pressure: implication for bio-invasion. Ecosphere 3:art48

Herborg LM, Jerde CL, Lodge DM, Ruiz GM, MacIsaac HJ (2007) Predicting invasion risk using measures of introduction effort and environmental niche models. Ecol Appl 17:663–674

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. Mar Biol 144:183–202

Hewitt CL, Everett RA, Parker N (2009) Examples of current international, regional and national regulatory frameworks for preventing and managing marine. In: Rilov G, Crooks JA (eds) Biological invasions in marine ecosystems: ecological, management, and geographic perspectives. Springer, Heidelberg, pp 335–352

Hillebrand H (2004) On the generality of the latitudinal diversity gradient. Am Nat 163:192–211

International maritime organization (2004) Alien invaders in ballast water—new Convention adopted at IMO. http://www.imo.org/blast/mainframe.asp?topic_id=848&doc_id=3475. Accessed Mar 14 2013

Johnson JB, Omland KS (2004) Model selection in ecology and evolution. Trends Ecol Evol 19:101–108

Katsanevakis S, Zenetos A, Belchior C, Cardoso AC (2013) Invading European seas: assessing pathways of introduction of marine aliens. Ocean Coast Manag 76:64–74

Kelager A, Pedersen JS, Bruun HH (2013) Multiple introductions and no loss of genetic diversity: invasion history of Japanese Rose, *Rosa rugosa*, in Europe. Biol Invasions 15:1125–1141

Lacoursière-Roussel A, Bock DG, Cristescu ME, Guichard F, Girard P et al (2012a) Disentangling invasion processes in a dynamic shipping-boating network. Mol Ecol 21:4227–4241

Lacoursière-Roussel A, Forrest BM, Guichard F, Piola RF, McKindsey CW (2012b) Modeling biofouling from boat and source characteristics: a comparative study between Canada and New Zealand. Biol Invasions 14:2301–2314

Lambert G (2005) Ecology and natural history of the protochordates. Can J Zool 83:34–50

Lambert CC, Lambert G (1998) Non-indigenous ascidians in southern California harbors and marinas. Mar Biol 130:675–688

Lambert CC, Lambert G (2003) Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Mar Ecol Prog Ser 259:145–161

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391

Leung B, Bossenbroek JM, Lodge DM (2006) Boats, pathways, and aquatic biological invasions: estimating dispersal potential with gravity models. Biol Invasions 8:241–254

Levine JM, D'Antonio CM (2003) Forecasting biological invasions with increasing international trade. Conserv Biol 17:322–326

Linley RD, Doolittle AG, Chan FT, O'Neill J, Sutherland T, Bailey SA (2014) Relative risk assessment for ship-mediated introductions of aquatic nonindigenous species to the Pacific Region of Canada. DFO Can Sci Advis Secr Res Doc 2013/043:v + 208

Lo VB, Levings CD, Chan KMA (2012) Quantifying potential propagule pressure of aquatic invasive species from the commercial shipping industry in Canada. Mar Pollut Bull 64:295–302

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. Trends Ecol Evol 20:223–228

Lockwood JL, Cassey P, Blackburn TM (2009) The more you introduce the more you get: the role of colonization

pressure and propagule pressure in invasion ecology. Divers Distrib 15:904–910

Lu L, Levings CD, Piercy GE (2007) Preliminary investigation on aquatic invasive species of marine and estuarine macroinvertebrates on floating structures in five British Columbia harbours. Can Man Rep Fish Aquat Sci 2814:iii + 30

MacIsaac HJ, Robbins TC, Lewis MA (2002) Modeling ships' ballast water as invasion threats to the Great Lakes. Can J Fish Aquat Sci 59:1245–1256

Mckindsey CW, Landry T, O'Beirn FX, Davies IN (2007) Bivalve aquaculture and exotic species: a review of ecological considerations and management issues. J Shellfish Res 26:281–294

Minchin D, Floerl O, Savigny D, Occhipinti-Ambrogi A (2006) Small craft and the spread of exotic species. In: Davenberg J, Davenport JL (eds) The ecology of transportation: managing mobility for the environment. Springer, Dordrecht, pp 99–118

Molecular Ecology Resources Primer Development Consortium, Abdoullaye D, Acevedo I et al (2010) Permanent genetic resources added to molecular ecology resources database 1 August 2009–30 September 2009. Mol Ecol Resour 10:232–236

Moore AM, Vercaemer B, DiBacco C, Sephton D, Ma KCK (2014) Invading Nova Scotia: first records of *Didemnum vexillum* Kott, 2002 and four more non-indigenous invertebrates in 2012 and 2013. BioInvasions Rec 3:225–234

National Research Council (2011) Assessing the relationship between propagule pressure and invasion risk in ballast water. National Academy of Sciences, Washington, p vi + 144

NISbase (2008) Generate a species list from the NISbase distributed database system. http://www.nisbase.org/nisbase/index.jsp. Accessed 15 Feb 2009

Novak SJ (2007) The role of evolution in the invasion process. Proc Natl Acad Sci USA 104:3671–3672

Novak SJ, Mack RN (1993) Genetic variation in *Bromus tectorum* (*Poaceae*): comparison between native and introduced populations. Heredity 71:167–176

Ojaveer H, Galil BS, Minchin D, Olenin S, Amorim A, Canning-Clode J, Chainho P, Copp GH, Gollasch S, Jelmert A, Lehtiniemi M, McKenzie C, Mikuš J, Miossec L, Occhipinti-Ambrogi A, Pećarević M, Pederson J, Quilez-Badia G, Wijsman JWM, Zenetos A (2014) Ten recommendations for advancing the assessment and management of non-indigenous species in marine ecosystems. Mar Policy 44:160–165

Ordóñez V, Pascual M, Rius M, Turon X (2013) Mixed but not admixed: a spatial analysis of genetic variation of an invasive ascidian on natural and artificial substrates. Mar Biol 160:1645–1660

Pagnucco KS, Maynard GA, Fera SA, Yan ND, Nalepa TF, Ricciardi A (2015) The future of species invasions in the Great Lakes–St. Lawrence River basin. J Great Lakes Res 41:96–107

Pancer Z, Gershon Z, Rinkevich B (1994) Direct typing of polymorphic microsatellites in the colonial ascidian *Botryllus schlosseri*. Biochem Biophys Res Commun 203:646–651

Paolucci EM, Hernandez MR, Potapov A, Lewis MA, MacIsaac HJ (2015) Hybrid system increases efficiency of ballast water treatment. J Appl Ecol 52:348–357

Paz G, Douek J, Caiqing M, Goren M, Rinkevich B (2003) Genetic structure of *Botryllus schlosseri* (Tunicata) populations from the Mediterranean coast of Israel. Mar Ecol Prog Ser 250:153–162

Posada D (2004) COLLAPSE (Version 1.2). A tool for collapsing sequences to haplotypes. http://darwin.uvigo.es/. Accessed 15 May 2012

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst Biol 53:793–808

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Puillandre N, Dupas S, Dangles O, Zeddam J-L, Capdevielle-Dulac C et al (2008) Genetic bottleneck in invasive species: the potato tuber moth adds to the list. Biol Invasions 10:319–333

Reem E, Douek J, Katzir G, Rinkevich B (2013a) Long-term population genetic structure of an invasive urochordate: the ascidian *Botryllus schlosseri*. Biol Invasions 15:225–241

Reem E, Mohanty I, Katzir G, Rinkevich B (2013b) Population genetic structure and modes of dispersal for the colonial ascidian *Botryllus schlosseri* along the Scandinavian Atlantic coasts. Mar Ecol Prog Ser 485:143–154

Reusser DA, Lee H, Frazier M, Ruiz GM, Fofonoff PW et al (2013) Per capita invasion probabilities: an empirical model to predict rates of invasion via ballast water. Ecol Appl 23:321–330

Ricciardi A (2006) Patterns of invasions in the Laurentian Great Lakes in the relation to changes in vector activity. Divers Distrib 12:425–433

Rinkevich B, Paz G, Douek J, Ben-Shlomo R (2001) Allorecognition and microsatellite allele polymorphism of *Botryllus schlosseri* from the Adriatic Sea. In: Sawada H, Yokosawa H, Lambert CC (eds) The biology of Ascidians. Springer, Tokyo, pp 426–435

Roman J (2011) Diluting the founder effect: cryptic invasions expand a marine invader's range. Proc R Soc B Biol Sci 273:2453–2459

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Ruiz GM, Carlton JT (2003) Invasion vectors: a conceptual framework for management. In: Ruiz GM, Carlton JT (eds) Invasive species: vectors and management strategies. Island Press, Washington, DC, pp 459–504

Ruiz GM, Smith G (2005) Biological study of container vessels arriving to the port of Oakland. Smithsonian Environmental Research Center, Edgewater. http://www.serc.si.edu/labs/marine_invasions/publications/PortOakfinalrep.pdf. Accessed 14 Mar 2013

Ruiz GM, Fofonoff P, Steves B, Dalhstrom A (2011) Marine crustacean invasions in North America: a synthesis of historical records and documented impacts. In: Galil BS, Clark PF, Carlton JT (eds) In the wrong place—Alien marine crustaceans: distribution, biology and impacts. Series in Invasion Ecology. Springer, New York, pp 215–250

Ruiz GM, Fofonoff P, Steves B, Carlton JT (2015) Invasion history and vector dynamics in coastal marine ecosystems: a North American perspective. Aquat Ecosyst Health 18:299–311

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J et al (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Scheibling RE, Hennigar AW, Balch T (1999) Destructive grazing, epiphytism, and disease: the dynamics of sea urchin–kelp interactions in Nova Scotia. Can J Fish Aquat Sci 56:2300–2314

Scianni C, Brown C, Newsom A, Nedelcheva R, Falkner M, Dobroski N (2013) Biennial report on the California marine invasive species program. Sacramento

Seebens H, Gastner MT, Blasius B (2013) The risk of marine bioinvasion caused by global shipping. Ecol Lett 16:782–790

Sephton D, Vercaemer B, Nicolas JM, Keays J (2011) Monitoring for invasive tunicates in Nova Scotia, Canada (2006–2009). Aquat Invasion 6:391–403

Simberloff D, Von Holle B (1999) Positive interaction of non-indigenous species: invasional meltdown. Biol Invasions 1:31–32

Simkanin C, Davidson I, Falkner M, Systsma M, Ruiz GM (2009) Intra-coastal ballast water flux and the potential for secondary spread of non-native species on the U.S. West Coast. Mar Pollut Bull 58:366–374

Smith DL, Wonham MJ, McCann LD, Ruiz GM, Hines AH, et al. (1999) Invasion pressure to a ballast-flooded estuary and an assessment of inoculants survival. Biol Invasions 1:67–87

Stachowicz JJ, Whitlatch RB, Osman RW (1999) Species diversity and invasion resistance in a marine ecosystem. Science 286:1577–1579

State of Hawaii (2011) Budgetary and other issues regarding invasive species. http://archive.jan2013.hawaii.gov/dlnr/reports-to-the-legislature/2012/FW11-Invasive-Species-Report-Chapter-194-Act-213-SLH08.pdf. Accessed Mar 14 2013

Statistics Canada (2016) Invasive species database in Canada. http://www5.statcan.gc.ca/olc-cel/olc.action?ObjId=54-205-X&ObjType=2&lang=en&Limit=1. Accessed 5 July 2016

Stoner DS, Quattro JM, Weissman IL (1997) Highly polymorphic microsatellite loci in the colonial ascidian *Botryllus schlosseri*. Mol Mar Biol Biotech 6:163–171

Stoner DS, Ben-Shlomo R, Rinkevich B, Weissman IL (2002) Genetic variability of *Botryllus schlosseri* invasions to the east and west coasts of the USA. Mar Ecol Prog Ser 243:93–100

Suarez AV, Tsutsui ND (2008) The evolutionary consequences of biological invasions. Mol Ecol 17:351–360

Sylvester F, Kalaci O, Leung B, Lacoursière-Roussel A, Clarke Murray C et al (2011) Hull fouling as an invasion vector: can simple models explain a complex problem? J Appl Ecol 48:415–423

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tilman D (2004) Niche tradeoffs, neutrality, and community structure: a stochastic theory of resource competition, invasion, and community assembly. Proc Natl Acad Sci USA 101:10854–10861

Tonione MA, Reeder N, Moritz CC (2011) High genetic diversity despite the potential for stepping-stone colonizations in an invasive species of gecko on Moorea, French Polynesia. PLoS One 6:e26874

Von Holle B, Simberloff D (2005) Ecological resistance to biological invasion overwhelmed by propagule pressure. Ecology 86:3212–3218

Wasson K, Zabin CJ, Bedinger L, Diaz MC, Pearse JS (2001) Biological invasions of estuaries without international shipping: the importance of intraregional transport. Biol Conserv 102:143–153

Williams SL (2007) Introduced species in seagrass ecosystems: status and concerns. J Exp Mar Biol Ecol 350:89–110

Wonham M, Byers J, Grosholz ED, Leung B (2013) Modeling the relationship between propagule pressure and invasion risk to inform policy and management. Ecol Appl 23:1691–1706

Xu J, Wickramarathne TL, Grey EK, Steinhaeuser K, Keller R, et al. (2014) Patterns of ship-borne species spread: a clustering approach for risk assessment and management of non-indigenous species spread. arXiv preprint arXiv:1401.5407

Zalewski A, Michalska-Parda A, Bartoszewicz M, Kozakiewicz M, Brzeziński M (2010) Multiple introductions determine the genetic structure of an invasive species population: American mink Neovison vison in Poland. Biol Conserv 143:1355–1363

Zayed A, Constantin SA, L Packer (2007) Successful Biological invasion despite a severe genetic load. PLoS One 2:e868