A screening procedure for potential tunicate invaders of Atlantic Canada

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Abstract
The conditions for a successful invasion involve the intersection of a species, its vector, and an appropriate receiving environment. Species distribution (biogeography) and availability of a shipping vector were used as filters to reduce a list of 57 tunicates with a history of invasion in marine or estuarine waters worldwide, to a more manageable basis for a “watch list” or “trigger list” for non-indigenous invasive tunicates in Atlantic Canada. Seven species from the worldwide invasives list were already present in Atlantic Canada: the non-indigenous Styela clava, Ciona intestinalis (cryptogenic in southern Nova Scotia but non-indigenous in northern Atlantic Canada), Botryllus schlosseri, Botrylloides violaceus and Molgula manhattensis, and the native Aplidium glabrum and Didemnum candidum. Nine species, not currently present in Atlantic Canada, were removed from the analysis due to insufficient distribution data. All of the remaining 41 species co-occurred in one or more bioregions with species presently found in Atlantic Canada. Examination of distributions relative to shipping patterns eliminated eight species not present in the areas with the most shipping traffic to Atlantic Canada: the eastern seaboard of the USA, the Caribbean Sea, northern Europe and the Mediterranean Sea. A climate zone filter to remove species found only in subtropical or tropical waters eliminated 21 species. Applying both the shipping and climate zone filters resulted in a “watch list” of 17 tunicate species considered the most likely to successfully invade Atlantic Canada: Ascidia sydneiensis, Ascidella aspersa, Botrylloides leachi, Clavelina lepadiformis, Cnemidocarpa irene, Corella eumyota, Cystodytes dellechisaei, Didemnum vexillum, Diplosoma listerianum, Perophora japonica, Perophora multiclathrata, Phallusia mammillata, Polyandrocarpa zorritensis, Polyclinum constellatum, Styela canopus, Styela plicata, and Symplegma brakenhielmi.

Key words: tunicate, ascidian, biogeography, prediction, invasion, Atlantic Canada

Introduction
Prediction of species invasions that are likely to occur in a particular bioregion or ecosystem is considered a prerequisite for developing a “watch list” for early detection (to determine where and how to monitor species of concern) or “trigger list” for rapid response (to identify what risk assessments and control methods should be prepared ahead of time) (Ricciardi and Rasmussen 1998; Locke and Hanson 2009). In recent years, there have been numerous attempts to model and predict species invasions (e.g., Williamson and Fitter 1996; Ricciardi and Rasmussen 1998; Smith et al. 1999; Kolar and Lodge 2001; Peterson and Viegals 2001; Peterson 2003; Reussner and Lee 2005; Nyberg and Wallentinus 2005; Miller et al. 2007). These models have been based either on species attributes associated with invasion success, or on the niche requirements of species. While invasion has been regarded as an enormously complex process, some phases of the process are highly predictable; for example, modeling the ecological niche characteristics of a species can predict its potential geographic distribution at a
level sufficient for management (Peterson 2003). One problem with ecological niche modeling, from the viewpoint of developing a list of likely invaders to a region, is the time required to obtain sufficient point distribution and environmental information for a large number of potential invasive species (Peterson and Vieglais 2001). The effort required to obtain this detailed information may be justified in the study of a few high-risk species, but there is also a need for a rapid screening tool that can narrow down a list of potential invaders from an extensive list of candidates (e.g., Ricciardi and Rasmussen 1998).

The conditions for a successful invasion involve the intersection of a species, its vector, and an appropriate receiving environment. In terrestrial environments, for example, the probability of successful introduction is positively correlated with the climate/habitat similarity between donor and recipient regions as well as propagule supply (Hayes et al. 2004). Marine biogeographical classification systems have been based on climate, ocean basins, oceanography, bathymetry and biotic distributions (Kelleher et al. 1995; Lourie and Vincent 2004), and can be used as a surrogate of climate/habitat similarity. Here, I use the biogeographic classification adopted by the International Union for the Conservation of Nature (IUCN) (Kelleher et al. 1995) which has been used to compile ballast water transit data by the Global Ballast Water Programme and Lloyds Maritime Intelligence Unit, and to match potential invaders with shipping from their donor regions in previous AIS hazard analyses (Hayes et al. 2004; Reusser and Lee 2005). Carlton (1996) suggested that an analysis of shipping traffic from regions containing known invaders could help predict where those species would next invade. Hull fouling (including sea chests and other structures) has been proposed as a vector for many invasive tunicates (e.g., Coutts et al. 2003; Minchin and Sides 2006).

In this paper I propose using species distributions (biogeography) and vector traffic patterns to develop a screening tool for potential tunicate invaders of Atlantic Canada. The starting point was to update and expand upon an existing list of tunicates with a history of invasion of marine and estuarine waters (Hayes et al. 2004) and remove species already present in Atlantic Canada. This is consistent with the approach of Ricciardi and Rasmussen (1998), who recommended a focus on species with a known invasion history when predicting future invaders. To these known invaders, I then applied a biogeographic filter (based on co-occurrence with invasive tunicates presently found in Atlantic Canada) followed by a shipping vector filter to screen potential invaders of Atlantic Canada from the worldwide invasive tunicate biota. Conceptually, this process is comparable to Stages I (transport vector survival and release filter) and II (environment survival and reproduction filter) in an invasion framework developed by Colautti and Maclsaac (2004). Finally, I applied a second environmental filter to eliminate species currently found only in tropical and subtropical waters.

Methods

The starting point for this analysis was a list of 57 tunicate species with a history of invasion somewhere in the world; distribution records were supplemented for the 40 species listed in the database of Hayes et al. (2004) and records of another 17 species were obtained from a survey of the scientific literature and invasive species websites (Table 1). A regional code corresponding to one of the 208 IUCN bioregions (Kelleher et al. 1995) was assigned to each location where each tunicate occurred.

Five non-indigenous species are presently found in Atlantic Canada (Botrylloides violaceus Oka, 1927, Botryllus schlosseri (Pallas, 1766), Ciona intestinalis (Linnaeus, 1767), Molgula manhattensis (DeKay, 1843) and Styela clava (Herdman, 1882). Ciona intestinalis is cryptogenic in southern Nova Scotia but non-indigenous in the Gulf of St. Lawrence. Two other species present in Atlantic Canada, Aplidium glabrum (Verrill, 1871) and Didemnum candidum (Savigny, 1816) are native, but have a history of invasion in other regions. These seven species occur in numerous regions worldwide. The assumption on which this analysis is based is that environmental conditions in those regions were similar enough to those of Atlantic Canada for components of the biological community to survive in both areas. Thus, the regions outside of Atlantic Canada where these seven species occurred were treated as potential donor regions for new tunicate invasions. The other invasive tunicates occurring in those regions were treated as species with the potential to survive in Atlantic Canada. This assumption is graphically represented by Venn diagrams illustrating the range of possibilities from complete or near-
Table 1. List of invasive tunicate species, screened by vector and climate filters

<table>
<thead>
<tr>
<th>Species</th>
<th>In bioregion(s) with heavy ship traffic to Atlantic Canada</th>
<th>Found in temperate or colder bioregions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aplidium phortax (Michaelsen, 1924)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ascidia nigra (Savigny, 1816)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ascidia sydneiensis Stimpson, 1855</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Ascidiola aspersa (O.F. Müller, 1776)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Asterocarpa humilis (Heller, 1878)</td>
<td>X</td>
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<tr>
<td>Botryllodes aniceps (Herdman, 1891)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Botryllodes leachi (Savigny, 1816)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Botryllodes nigrum Herdman, 1886</td>
<td>X</td>
<td></td>
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<tr>
<td>Botryllodes perspicuus Herdman, 1886</td>
<td>X</td>
<td></td>
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<tr>
<td>Botryllodes simodensis Saito &amp; Watanabe, 1981</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Ciona savignyi Herdman, 1882</td>
<td>X</td>
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<tr>
<td>Clavelina lepadiiformis (Müller, 1776)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Clavelina oblonga Herdman, 1880</td>
<td>X</td>
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<tr>
<td>Cnemidocarpa rarensis Hartmeyer, 1906</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Corella eumyoia Traustedt, 1882</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Cystodytes dellechiangi (Della Valle, 1877)</td>
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<tr>
<td>Didemnum perlicidum Monniot, 1983</td>
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<tr>
<td>Didemnum vexillum Kott, 2002</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Diplosoma listerianum (Milne-Edwards, 1841)</td>
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<td>X</td>
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<tr>
<td>Distaplia bermeudensis Van Name, 1902</td>
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<tr>
<td>Ecineiscidium tubinata Herdman, 1880</td>
<td>X</td>
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<tr>
<td>Eudistoma olivaceum (Van Name, 1902)</td>
<td>X</td>
<td></td>
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<tr>
<td>Eusynystyla hartmeyeri Michaelsen, 1904</td>
<td>X</td>
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<tr>
<td>Eusynystyla tincta (Van Name, 1902)</td>
<td>X</td>
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<tr>
<td>Herdmania momus (Savigny, 1816)</td>
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<tr>
<td>Lissoclinum fragile (Van Name, 1902)</td>
<td>X</td>
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<tr>
<td>Microcosmus exasperatus Heller, 1878</td>
<td>X</td>
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<tr>
<td>Microcosmus squamiger Michaelsen, 1927</td>
<td>X</td>
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<tr>
<td>Perophora japonica Oka, 1927</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Perophora multilaterata Sluiter, 1904</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Phallusia mammilata (Cuvier, 1815)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Polyanthocarpa zorritensis (Van Name, 1931)</td>
<td>X</td>
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<tr>
<td>Polyctenaser paupertatum Savyigny, 1816</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Rhodosoma turcicum (Savigny, 1816)</td>
<td>X</td>
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<tr>
<td>Styela bicolor (Sluiter, 1887)</td>
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<tr>
<td>Styela canopus (Savigny, 1816)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Styela plicata (Lesueur, 1823)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Symplogena brahenhielmi Michaelsen, 1904</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Symplogena reptans (Oka, 1927)</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Symplogena rubra Monniot, 1972</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Trididemnum sibogae (Hartmeyer, 1910)</td>
<td></td>
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</tbody>
</table>

Sources: Abdul and Sivakumar (2007); Barros et al. (2009); Berrill (1932); Blum et al. (2007); Brunel et al. (1998); Cohen (2005); Colombra (1974); Davis and Davis (2007); Eldredge and Smith (2001); Gollasch and Nehring (2006); Goodbody (1962); Goodbody (2004); Hansson (1998); Hayes et al. (2004); Hernández-Zanuy and Carballo (2001); Izquierdo-Muñoz et al. (2009); Kott (2005); Kott (2005); Lambert (2009); Mastrototaro and Brunetti (2006); Monniot (2002); Monniot and Monniot (2001); Nagabushananam and Thompson (1997); Naranjo et al. (1996); Primo and Vásquez (2004); Rius et al. (2007); Rocha and Kremer (2005); Rocha et al. (2005a); Rocha et al. (2005b); Rocha et al. (2009); Russ (1982); Sanamyan and Schories (2004); Shenkar et al. (2008); Shenkar and Loya (2008); Stoner et al. (2002); Svensson et al. (2007); Van Name (1945); Vásquez and Young (2000); Zvyagintsev et al. (2007). Web sites: Encyclopedia of Marine Life of Britain and Ireland; Global Invasive Species Database; Marine Life Information Network for Britain and Ireland; Marine Species Database for Eastern Africa; Marine Species Registers for the Northwest North Atlantic Ocean; Museum Victoria Natural Sciences Collection; New Zealand National Aquatic Biodiversity Information System; United States Geological Survey Nonindigenous Aquatic Species; World Register of Marine Species.
complete overlap of species tolerance (Figure 1(a)) to species for which this assumption represents the region of overlap despite largely different habitat requirements (Figure 1(b)). The strength of the predictions developed here will be greater if the situation corresponds to Figure 1(a), although this assumption has not been tested.

The availability of shipping vectors was used to further refine the list of potential invaders. Vessel movement tracks were obtained from Kelly (2004), and patterns in international shipping traffic to Atlantic Canada were examined in order to identify likely sources of primary invasion. Tunicates from regions with heaviest shipping traffic to Atlantic Canada were considered to be the most likely future invaders.

Lastly, the list of tunicates generated from these two filters was re-examined, and tunicate species presently distributed only in subtropical and tropical regions were removed.

Results

Of the 57 tunicate species with a history of invasion somewhere in the world, seven already occur in Atlantic Canada and there were insufficient data to analyze the distributions of nine species. Therefore the remainder of the analysis was based on 41 species (Table 1). Sixty-five bioregions outside of Atlantic Canada contained one or more of the seven invasive tunicate species found in Atlantic Canada (Figure 2). For the purposes of this analysis, non-indigenous, cryptogenic or native distributions were treated equally. These bioregions are distributed in coastal waters throughout the world; Antarctica was the only continent not represented. All tunicate species examined co-occurred in one or more of these regions with the tunicates presently found in Atlantic Canada, so the “co-occurrence filter” did not remove any species.

Vessel ballast water tracks indicated high concentrations of vessels coming to eastern Canada from the eastern seaboard of the USA, the Caribbean Sea, northern Europe and the Mediterranean Sea (Kelly 2004). Thirty-three of the 41 invasive tunicate species not currently present in Atlantic Canada were found in these water bodies. While one or more of the seven Atlantic Canadian invasive tunicates had distributions in the Caribbean and Mediterranean Seas, it seemed reasonable to assume that tunicates found only in subtropical or tropical regions would be unlikely to survive in the cold winter conditions of Atlantic Canada. Presumably, if those species were able to survive in temperate conditions, they would already be found somewhere in the temperate zone, assuming there were vectors available to transport them to temperate regions. In other words, the environmental tolerances of these species relative to those currently living in Atlantic Canada may be represented by the non-overlapping area of the Venn diagram (Figure 1B). Alternatively, if the reason that a species has not occurred in a temperate region is the absence of vectors to transport it into temperate waters, it may be reasonable to assume that the species has a low risk of invasion of temperate regions. Either reason is probably sufficient to eliminate these species as high-risk invaders of Atlantic Canada. The climate filter alone eliminated 21 species that were found only in subtropical or tropical waters. Combining the climate and vectors filters eliminated a total 24 of the 41 species on the original list, leaving a “watch list” of 17 tunicates: Ascidia sydneiensis, Ascidiella aspersa, Botrylloides leachi, Clavelina lepadiformis, Ctenocarpa irene, Corella eumyota, Cystodytes dellechiajei, Didemnum vexillum, Diplosoma listerianum, Perophora japonica, Perophora multicirrata, Phallusia mammillata, Polyandrocarpa zorritensis, Polyclinum constellatum, Styela canopus, Styela plicata, and Symplegma brakenhielmi (Figure 3).
Figure 3. Worldwide distribution of 17 tunicate species considered potential invaders of Atlantic Canada: A - Ascidia sydnetensis, B - Asciidiella asperses, C - Botryooides leachi, D - Clavelina lepadiformis, E - Cnemidocarpa irene, F - Corella eumyota, G - Cystodytes dellachiaiei, H - Didemnum vexillum, I - Diplosoma listerianum, J - Perophora japonica, K - Perophora multiclavatira, L - Phallusia mammillata, M - Polyandrocarpa zorritensis, N - Polyclinum constellatum, O - Styela canopus, P - Styela plicata, Q - Symplegma brakenhielmi. Bioregion base map after Kelleher et al. (1995)
Discussion

Species invasions incorporate a strong element of chance, but one of the limiting factors for successful establishment is the environmental tolerance of the invader (Colautti and MacIsaac 2004). Thus, environmental compatibility of the donor and receiving regions can be used as a filter to reduce an extensive list of potential invaders to a more manageable-sized “watch list” of organisms whose distribution suggests they could survive conditions in the receiving environment. Matching distributions to determine invasion risk is not a new concept; it has been used in the Global Ballast Water Programme’s Ballast Water Risk Assessment protocol (e.g., Clarke et al. 2003); to evaluate the risk of marine pests in Australia (Hayes and Sliwa 2003; Hayes et al. 2004); and to examine large-scale patterns of invasion risk (Reusser and Lee 2005), for example.

There are limitations to the biogeographic approach: for example, the number of donor regions may be underestimated if the seven species found in Atlantic Canada have not invaded all the bioregions in which they could survive. This could easily happen for reasons unrelated to environment, e.g., the absence of a vector or the presence of a predator or competitor that prevented establishment. The environmental tolerances of members of a community are unlikely to be identical, so some species from a donor region may be able to tolerate the receiving environment whereas others may not. Genetic variability between tunicate populations is not taken into account; however, determinants of invasion success act at the population rather than the species level (Colautti and MacIsaac 2004) and climatic niche shifts can occur during invasion (Broennimann et al. 2007). For example, inoculations from a northern European source population may explain the recent rapid expansion of European green crab, Carcinus maenas (Linnaeus, 1758), into northern portions of Atlantic Canada that were apparently unavailable to the genotypes previously established on the east coast of North America (Roman 2006). The available distribution data are also confounded by sampling effort, influenced by publication venue and language, and require two major assumptions: that the alpha-level taxonomy is correct, and that specimens are correctly identified. On the other hand, the same restrictions apply to more data-intensive methods such as environmental niche modeling (e.g., Peterson and Vieglais 2001).

The approach presented here is intentionally “approximate” in the interest of speed, but could be made more powerful by incorporating additional data. The bioregion-matching step could be made more quantitative by examining the proportion of overlapping species, as was done by Lee and Reusser (2005). The same authors suggested that combining analyses of global patterns of invasion with the invasion history of species and a better understanding of vectors could increase the predictive power of their approach. The recent development of a Canadian Ballast Water Database has simplified the incorporation of shipping vectors data into an invasive species screening protocol for Canadian waters. Increased predictive power could be obtained by the incorporation of habitat/niche requirements (Lee and Reusser 2005) but that would most likely be too data-intensive for an initial screening.

As demonstrated here, this method can be used to reduce a long list of possible invaders, based solely on the invasion history of species, to a more manageable list for which, if desired, a more detailed risk analysis can be conducted. Here, the two-level screening (environmental matching at the bioregion level, then vectors) reduced a list of 41 species to either 33 potential species (including the Caribbean and Mediterranean Seas based on the availability of shipping vectors) or 17 higher-likelihood species (including only the eastern USA and northeastern Europe, based on a second climate-related filter). In Australia, the application of four levels of screening criteria (including assessments of environmental and economic impacts) to a list of 1088 marine and estuarine invaders not currently found in Australia resulted in a list of 37 “next pest species” (Hayes et al. 2004). One difference is the absence of an environmental screening step in the Australian approach; environmental conditions would be a much less effective filter of potential invaders in Australia than in Canada, where cold temperatures can be a limiting factor in marine invasions (DeRivera et al. 2007).

Development of methods to predict the “next one” can make a major contribution to invasive species management by allowing improvements in early detection capability (how and where should we monitor?) and rapid response planning
(can we carry out risk assessments, or develop protocols for control methods for individual species or groups of organisms in advance of their arrival?). This paper is the first to attempt at prediction of which tunicate species might appear next in Atlantic Canadian waters. The most likely candidates are: *Ascidia sydneiensis*, *Ascidia aspersa*, *Botryllus leachi*, *Clavelina lepadiformis*, *Cnemidocarpa irene*, *Corella eumyota*, *Cystodytes dellichiaeji*, *Didemnum vexillum*, *Diplosoma listerianum*, *Perophora japonica*, *Perophora multicilathrata*, *Phallusia mammilata*, *Polyandrocarpa zorritensis*, *Polyclinum constellatum*, *Styela canopus*, *Styela plicata*, and *Symplegma brakenhielmi*.

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