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Risk Assessment for Two Solitary and Three Colonial Tunicates in Both Atlantic and Pacific Canadian Waters

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Évaluation des risques posés par deux tuniciers solitaires et trois tuniciers coloniaux dans les eaux canadiennes de l'Atlantique et du Pacifique

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ABSTRACT

Non-indigenous tunicates have become a global concern, especially for the aquaculture industry. Some of these tunicate species have rather extensive invasion histories dating back several decades while others are much more recent. In order to determine the potential risk posed by these non-indigenous tunicate species to Canadian waters, including both the Atlantic and Pacific coasts, a formal risk assessment was undertaken. This risk assessment included two solitary tunicates, the club tunicate, *Styela clava*, and the vase tunicate, *Ciona intestinalis*; and three colonial ones, the golden star tunicate, *Botryllus schlosseri*, the violet tunicate, *Botrylloides violaceus*, and *Didemnum* sp. Regardless of species, most global introductions of tunicates have been attributed to hull fouling or aquaculture-related activities suggesting these non-indigenous tunicates are good hitchhikers. Three of these tunicate species already exist in Canadian waters on both coasts (golden star tunicate, violet tunicate, and club tunicate), while vase tunicate is present on the Canadian Atlantic coast and *Didemnum* sp. is present on the Canadian Pacific coast. There is considerable concern about the potential ecological and genetic impacts if these tunicates spread in Canada. Life history characteristics largely preclude long-distance natural dispersal or dispersal via ballast water but a number of other potential vectors exist, especially the movement of recreational or small craft. The unlikelihood of natural long distance dispersal due to the very brief larval phase means that management of human-mediated transport vectors could effectively control future spread of these tunicates.

The Department of Fisheries & Oceans Canada conducted a national risk assessment, including a peer-review workshop, to determine the potential risk posed by non-indigenous tunicates in Canada. This assessment included evaluating the risk of arrival, survival, reproduction and spread of each species, as well as their pathogens, parasites or fellow travelers (e.g. other invasive species) should they be introduced as well. These components were assessed in an expert workshop using best available information on their biology, potential vectors of introduction, and impacts in both native and introduced ranges. The assessment concluded these non-indigenous tunicates posed a high ecological risk on both coasts with the exception of *C. intestinalis* where the ecological risk on the Pacific coast was deemed moderate. The genetic risk was moderate for each species on both coasts. For pathogens, parasites or fellow travelers the ecological and genetic risks were low for each of the colonial species. As *C. intestinalis* can harbor an amoebic parasite that could impact native and cultured salmon stocks the ecological risk was deemed moderate. The ecological risk posed by hitchhikers of *S. clava* was high as they include the colonial tunicates. However, as little is known about many potential pathogens, parasites and fellow travelers of these tunicate species there was considerable uncertainty.

RÉSUMÉ

Les tuniciers non indigènes constituent maintenant un problème mondial, surtout pour l’industrie aquacole. L’invasion par certaines de ces espèces remonte à plusieurs décennies, tandis que pour d’autres, elle est plus récente. Afin de déterminer le risque potentiel posé par ces espèces de tuniciers non indigènes dans les eaux canadiennes, sur les côtes aussi bien de l’Atlantique que du Pacifique, une évaluation du risque officielle a été entreprise. Elle visait deux tuniciers solitaires, soit l’ascidie plissée, Styela clava, et l’ascidie jaune, Ciona intestinalis, et trois tuniciers coloniaux, le botrylle étoilé, Botryllus schlosseri, le botrylloïde violet, Botrylloides violaceus,et l’espèce Didemnum. Quelle que soit l’espèce, la plupart des tuniciers s’introduisent un peu partout dans le monde sous forme de salissure sur les coques de bateaux ou dans le cadre d’activités liées à l’aquaculture, ce qui porte à croire qu’ils sont de bons voyageurs ‘opportunistes’. Trois de ces espèces existent déjà dans les eaux canadiennes sur les deux côtes, le botrylle étoilé, le botrylloïde violet et l’ascidie plissée), tandis que l’ascidie jaune est présente sur la côte canadienne de l’Atlantique et Didemnum, sur la côte canadienne du Pacifique. Leur propagation au Canada suscite des préoccupations considérables en raison de leurs répercussions écologiques et génétiques possibles. Les caractéristiques de leur cycle biologique écartent la possibilité de dispersion naturelle sur de longues distances ou de dispersion par l’eau de ballast, mais il existe un certain nombre d’autres vecteurs, notamment le mouvement des petits bateaux ou des bateaux de plaisance. L’invraisemblance d’une dispersion naturelle sur de longues distances, compte tenu de la brièveté du stade larvaire, fait en sorte qu’une bonne gestion des vecteurs de transport d’origine humaine pourrait effectivement limiter la dispersion future de ces tuniciers.

Le ministère des Pêches et des Océans du Canada a procédé à une évaluation du risque nationale qui comportait un atelier d’examen par des pairs, afin de déterminer le risque potentiel posé par les tuniciers non indigènes au Canada. L’exercice comprenait une évaluation du risque d’arrivée, de survie, de reproduction et de propagation de chaque espèce, ainsi de leurs pathogènes, parasites et compagnons de route (p. ex. d’autres espèces envahissantes), le cas échéant. Ces composantes ont été évaluées au cours d’un atelier d’experts au moyen des meilleures données disponibles sur leur biologie, les vecteurs potentiels d’introduction et les répercussions sur leurs aires indigène et d’introduction. L’évaluation a permis de conclure que ces tuniciers non indigènes posaient un risque écologique élevé sur les deux côtes, à l’exception de C. intestinalis dont le risque écologique sur la côte du Pacifique est jugé modéré. Le risque génétique est modéré dans le cas de chaque espèce sur les deux côtes. En ce qui concerne les pathogènes, les parasites et les compagnons de route, les risques écologique et génétique sont faibles pour chaque espèce coloniale. Puisque C. intestinalis peut abriter des parasites amibiens susceptibles d’avoir des conséquences sur les stocks de saumon indigènes et d’élevage, le risque écologique a été jugé modéré. Quant au risque écologique posé par les compagnons de route de S. clava, il est élevé car ils incluent les tuniciers coloniaux. Toutefois, puisqu’on dispose de peu de connaissances sur les nombreux pathogènes, parasites et compagnons de route potentiels de ces espèces de tuniciers, l’incertitude qui subsiste est considérable.

INTRODUCTION

Non-indigenous species (NIS) pose an enormous risk to native biodiversity and can compromise ecosystem function (e.g., Sala et al. 2000). Recently, for marine ecosystems, non-indigenous tunicate species have become a global concern and Canadian waters are no exception. As biofouling organisms, several tunicate species have spread dramatically in recent years, often with negative impacts on native species and aquaculture gear or product (e.g., Lambert and Lambert 2003, Carver et al. 2003). Whether or not a non-indigenous species becomes invasive depends on the impact of the non-indigenous species in its newly invaded habitat. The Government of Canada (2004) defines invasive alien species as "those harmful alien species whose introduction or spread threatens the environment, the economy or society, including human health". For biofouling organisms like tunicates that can displace native species and foul aquaculture gear and product the potential exists that they will become invasive in Canadian waters once established.

In order to characterize the potential risk posed by a new invader to Canadian waters or the spread of an existing invader to additional waters, a formal risk assessment is conducted. This risk assessment is adapted from the process outlined in the Canadian National Code on Introductions and Transfers of Aquatic Organisms and contains two-parts. Part I evaluates the probability of establishment and consequence of establishment of an aquatic organism while Part II evaluates the probability of establishment and consequence of establishment of a pathogen, parasite or fellow traveler of the aquatic organism. Within each Part of the national framework two component ratings are determined (the probability and consequences of establishment) and each are assigned ratings of high (risk is likely, or very likely, to occur), medium (there is probability of negative impact), or low (risk is considered to be insignificant). In addition, a level of certainty also is assigned as a gradient from very certain (scientific basis), reasonably certain, reasonably uncertain, to very uncertain ("best guess").

This document summarizes the results of a risk assessment conducted to evaluate the risk posed by five non-indigenous tunicate species if introduced into Canadian waters and contains information for both Atlantic and Pacific coastal waters. This assessment was undertaken collectively for five tunicate species including two solitary ones, the club tunicate, *Styela clava*, and the vase tunicate, *Ciona intestinalis*; and three colonial ones, the golden star tunicate, *Botryllus schlosseri*, the violet tunicate, *Botrylloides violaceus*, and *Didemnum* sp. The risk assessment process requires the best available information for the species of interest. The biological information was obtained from Clarke and Therriault (2007) for *Styela clava*, Carver et al. (2006a) for *Ciona intestinalis*, Carver et al. (2006b) for *Botryllus schlosseri* and *Botrylloides violaceus*, and Daniel and Therriault (2007) for *Didemnum* sp. A draft risk assessment document was prepared for all five species and peer-reviewed at a workshop attended by international aquatic invasive species and tunicate experts in March 2007 (see Appendix A for workshop participants). Reese *et al.* (2007) provides a synopsis of the workshop. This document is a synthesis of the draft document and input from the workshop participants. For each tunicate species, a summary of its basic biology, native, non-native and potential distribution in Canada, and the risk assessment is provided. In addition, in order to gain scientific knowledge with respect to vectors and pathways and potential impacts associated with non-indigenous tunicate species, a formal survey of both NIS and tunicate experts was conducted. The results from this survey help guide the level of risk or uncertainty associated with each of the five tunicate species' risk assessments.

METHODS AND MATERIALS

Predicting suitable environments

The potential future ranges for the five non-indigenous species of tunicates were predicted by environmental niche modeling based on their current North American distribution on each coast. This represents a conservative approach in that as a species' distribution increases over time additional suitable environments will be identified. The only exception was *Ciona intestinalis* where the east coast prediction was projected onto the west coast as no reports of this species were available on the Canadian Pacific coast. We applied the Genetic Algorithm for Rule-set Prediction (GARP) to predict suitable environments. Models are constructed using species presence and geo-referenced environmental data. We selected ten oceanographic environmental layers to predict the potential distribution; seasonal (winter, spring, summer and fall) salinity and temperature, annual chlorophyll and annual oxygen concentrations. The environmental layers were generated based on the National Oceanographic and Atmospheric Administration (NOAA) ocean database and predictions were limited to coastal areas to a depth of <200m in accordance with deepest recorded depths for the tunicate species studied here. On the east coast, predictions were made from 26°N (Florida) to 60°N (Hudson Strait) and on the west coast from 23°N (Baja California) to 60°N (Nunivak Island). These environmental layers were tested separately for their contribution to each species model using multiple linear regression (see Table 1), following Drake and Bossenbroek (2004). Environmental variables that contributed significantly to model prediction accuracy were then used to create 100 predictions using a 0.001 convergence limit and a maximum of 3000 iterations (per simulation), following the best subset procedure described by Anderson et al. (2003). The resulting predictions were converted into a map of percentage environmental match using the 'Raster Calculator' in ArcMAP 9.0.

Transport vectors

Potential transport vectors for dispersal of tunicates were identified through an expert web-based survey. An online questionnaire was designed and sent to 520 experts (participants at recent marine invasive and aquatic species conferences and the first invasive sea squirt conference) and three mailing lists (associated with either tunicates, invasive or alien species). Each respondent was asked to identify their level of expertise of tunicates. Based on their response, the questionnaire subsequently provided species-specific questions, group-specific (colonial vs. solitary tunicates) questions or questions on tunicates in general. A total of 132 experts answered the questionnaire, identifying the relative importance of ten potential vectors for dispersal: larval drift, dispersal of adults attached to flotsam, ballast water, movement of aquaculture gear and stock, fragments in fishing gear, aquarium releases, intentional release to establish a food source, hull fouling on slow moving barges, hull fouling of large (>50m) and small (<50m) vessels. Respondents were asked to provide an estimate of importance for each vector and the uncertainty associated with their reply. For both importance and uncertainty respondents had a choice of five answers: very high, high, medium, low, and very low. Definitions for each category were provided with each question (Appendix B). For both importance and uncertainty we calculated the mean for each set of responses.

Based on the results from the expert survey we gathered spatially explicit information on the distribution of transport vectors identified as important. Aquaculture site information was gathered for both coasts from government agencies, aquaculture associations, and scientists and was converted into point data that was then transformed into density maps using ArcMAP 9.1. Thus, it was possible to reflect the density of aquaculture sites in a particular area. Similarly, small craft vessel traffic was predicted based on the distribution of small craft harbors (east coast) or small craft harbors, anchorages, ports and marinas (west coast) depending on data availability. This information was converted into density maps. The frequency of different types of large commercial vessels, fishing boats, and barge movements on the west coast was determined based ship traffic data for 2003 (courtesy of the Canadian Marine Communications and Traffic Services [MCTS]). The frequency of vessel movements through a particular area was converted into density layers as above. Due to the unavailability of suitable data a similar analysis could not be conducted for the east coast.

Potential impacts

The magnitude of potential impacts and associated uncertainty was determined as part of the expert survey described above. Experts were asked for the relative impact non-indigenous tunicates can have on: biodiversity, marine protected areas, shellfish aquaculture, finfish aquaculture, commercial fisheries, vessels / moorings, recreational activities (boating, fishing, diving, etc.). In order to measure potential uncertainty of these impacts, respondents also were asked to identify the likelihood of these impacts occurring (Appendix B). For both impact level and uncertainty we calculated the mean for each set of responses.

RESULTS

Survey results

The survey of tunicate and aquatic invasive species experts identified several vectors potentially important for the introduction, or in most cases, secondary spread of each of the five non-indigenous species considered here. Although subtle differences exist, generally the trends are similar whether experts responded as species specialists, group specialists or tunicate generalists (Figure 1). Hull fouling on barges (slow moving vessels) and aquaculture-related transfers were identified as having the greatest potential to introduce or spread non-indigenous tunicates. In contrast, aquaria and intentional release were identified as having the lowest potential to introduce or spread non-indigenous tunicates. These findings are consistent with the literature on this subject. The survey also collected information on uncertainty. In general, respondents who identified themselves as being knowledgeable about tunicates in general had greater uncertainty than other respondents, regardless of the vector in question (Figure 2). Most vector uncertainty was low to moderate, with lower uncertainty for vectors that have been demonstrated to transport tunicates in the past, most notably aquaculture and hull fouling on barges (slow moving vessels) (Figure 2).

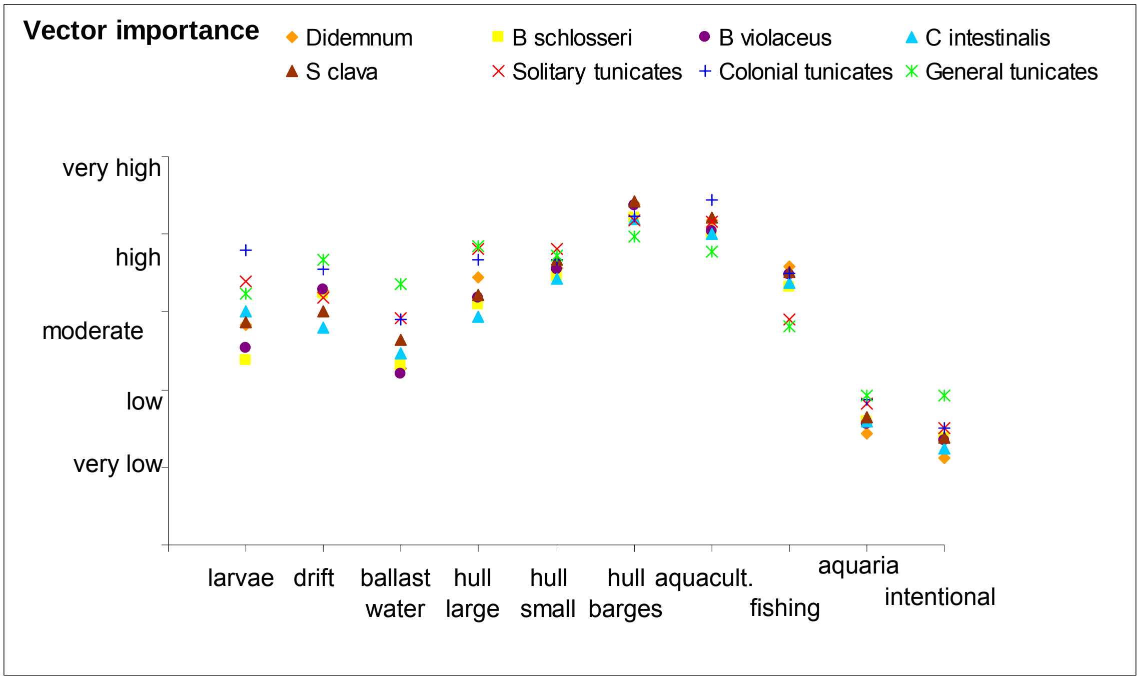


Figure 1: Mean vector importance as determined by experts via an online survey.

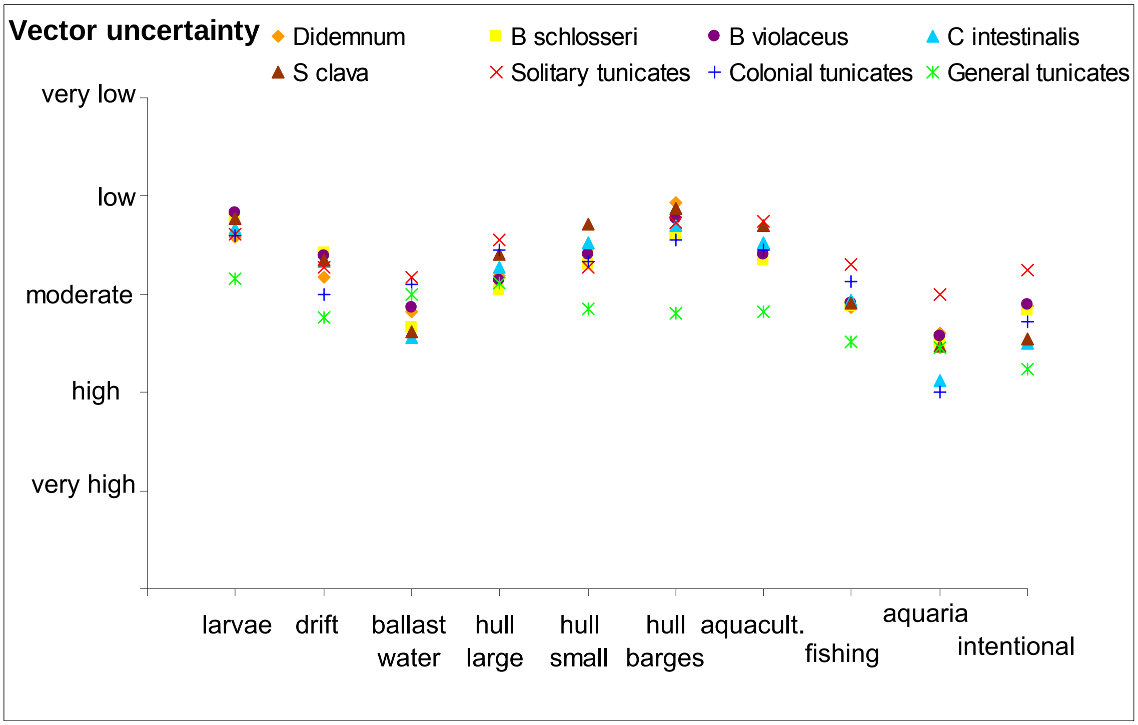


Figure 2: Mean vector uncertainty as determined by experts via an online survey.

The survey of tunicate and aquatic invasive species experts identified several potentially important impacts for each of the five non-indigenous species considered here. Although subtle differences exist, generally the trends are similar whether experts responded as species specialists, group specialists or tunicate generalists (Figure 3). For example, impacts tended to be highest for *Didemnum* sp. and lowest for *Botryllus* *schlosseri*. Although most impacts were considered to be moderate by most respondents, impacts on shellfish aquaculture were rated high. These findings are in general agreement with the literature and field observations where some of the most documented impacts of these tunicates are on mussel and oyster aquaculture. The survey also collected information on uncertainty associated with these impacts. In general, respondents identified most impacts as likely but were more certain each of the five tunicate species considered here would have impacts on shellfish aquaculture and fouling of recreational vessels (Figure 4).

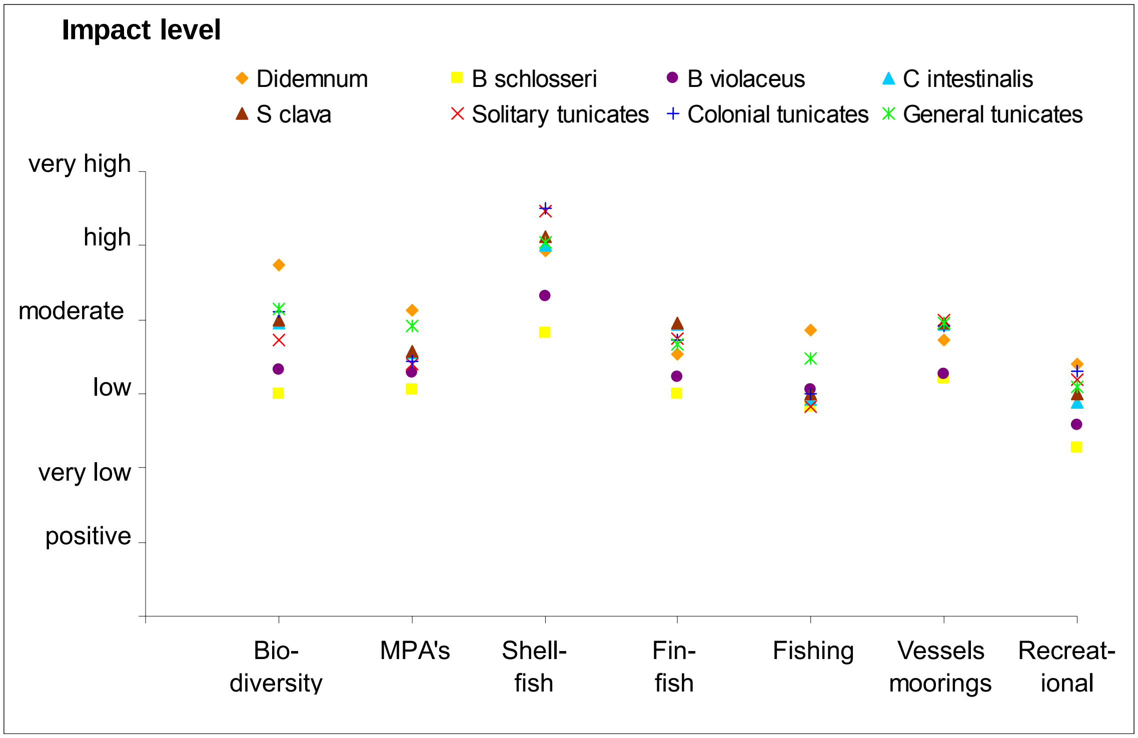


Figure 3: Mean impact level as determined by experts via an online survey.

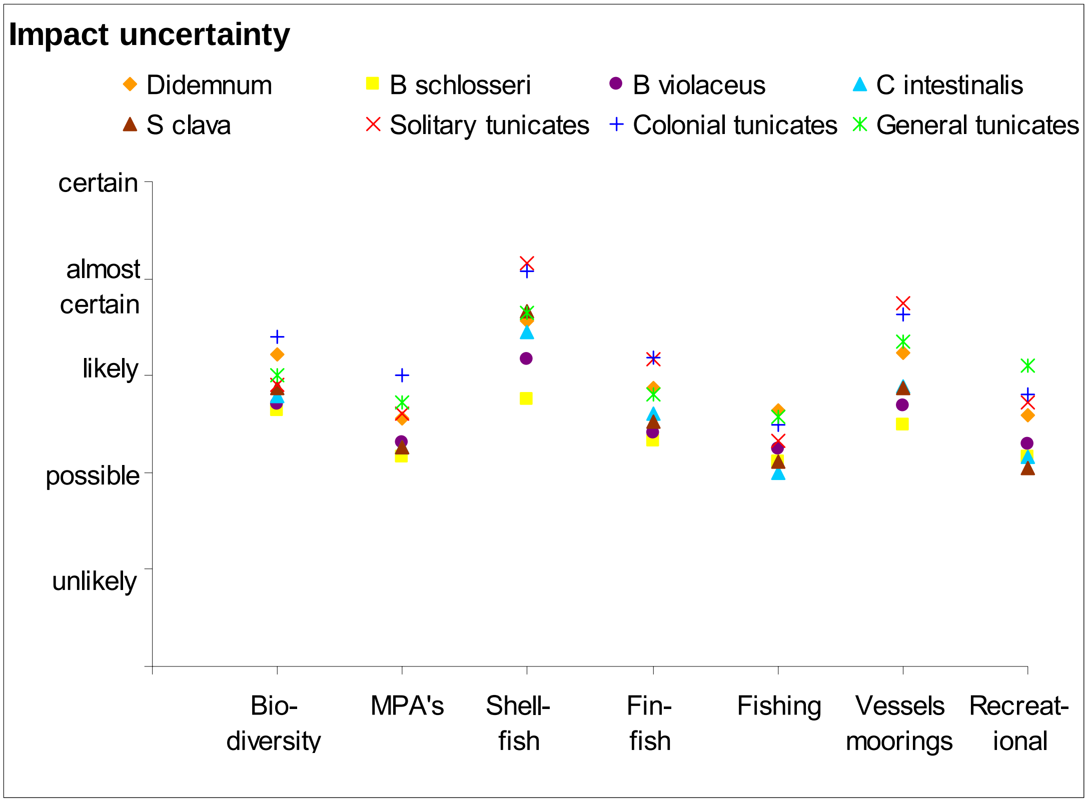


Figure 4: Mean impact uncertainty as determined by experts via an online survey.

Potential vectors: west coast

A number of potential vectors exist on the west coast including aquaculture-related transfers, fouling on recreational or small craft, and fouling on the hulls of larger vessels including container ships, tankers and commercial fishing vessels. Most aquaculture in British Columbia (BC) is around the Strait of Georgia, notably Baynes Sound and Oekover Inlet (Figure 5). Higher than average densities also occur in the major inlets along the west coast of Vancouver Island and in Johnstone Strait (Figure 5). The highest density of small craft moorings is around the Strait of Georgia, extending through Johnstone Strait and into the Central Coast of BC (Figure 6). Additional point-source densities occur near Prince Rupert and Skidegate Inlet in the Queen Charlotte Islands (Figure 6). Most container ship activity in BC is related to international trade. These foreign vessels enter Juan de Fuca Strait enroute to the ports of Vancouver or Delta to offload their cargo (Figure 7). Similar to container ships, most tanker traffic is into the port of Vancouver but additional tanker traffic in the north coast is destined to Prince Rupert and Kitimat, both ports recently scheduled for expansion (Figure 8). In BC, most fishing vessels are operating on inside waters. These include the Strait of Georgia, through Johnstone Strait, and along the mainland side of Queen Charlotte Sound and Hecate Strait right up to Dixon Entrance (adjacent to Prince Rupert) (Figure 9). Most of the tug and barge traffic in BC waters is within the Strait of Georgia (Figure 10). A similar level of traffic exists within Puget Sound, highlighting the interconnectedness of this inland sea. Additional tug and barge traffic exists within a relatively constrained band along the mainland coast extending from Vancouver in the south to Prince Rupert in the north (Figure 10).

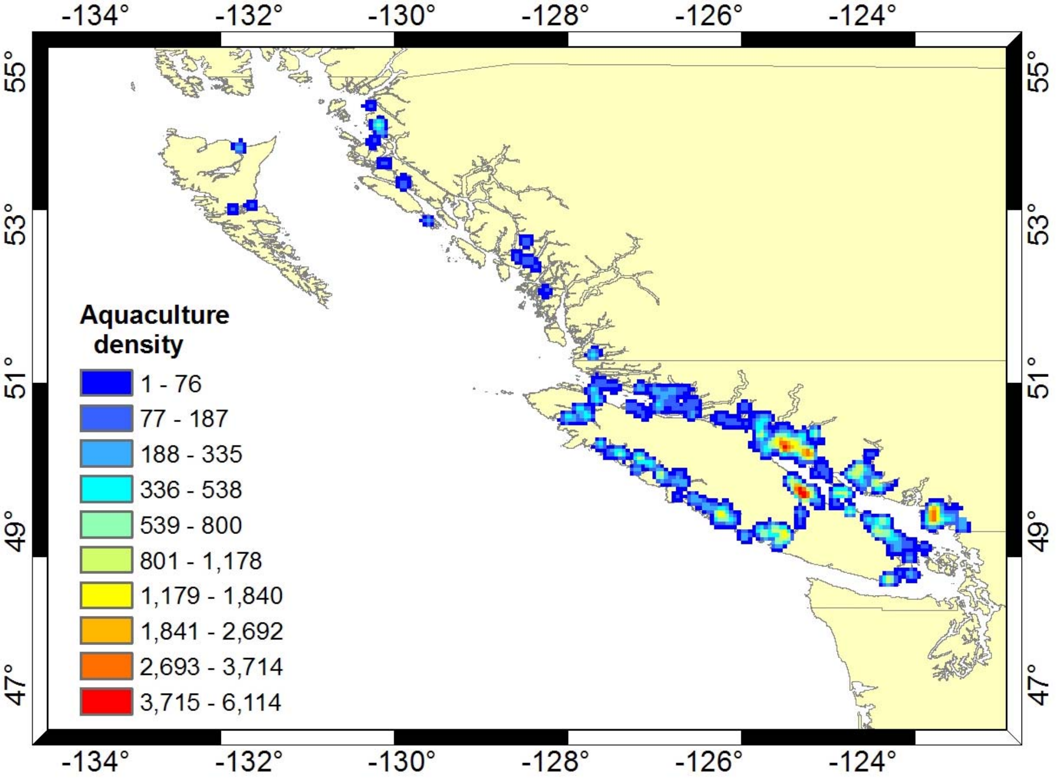


Figure 5: Density of aquaculture facilities in BC waters.

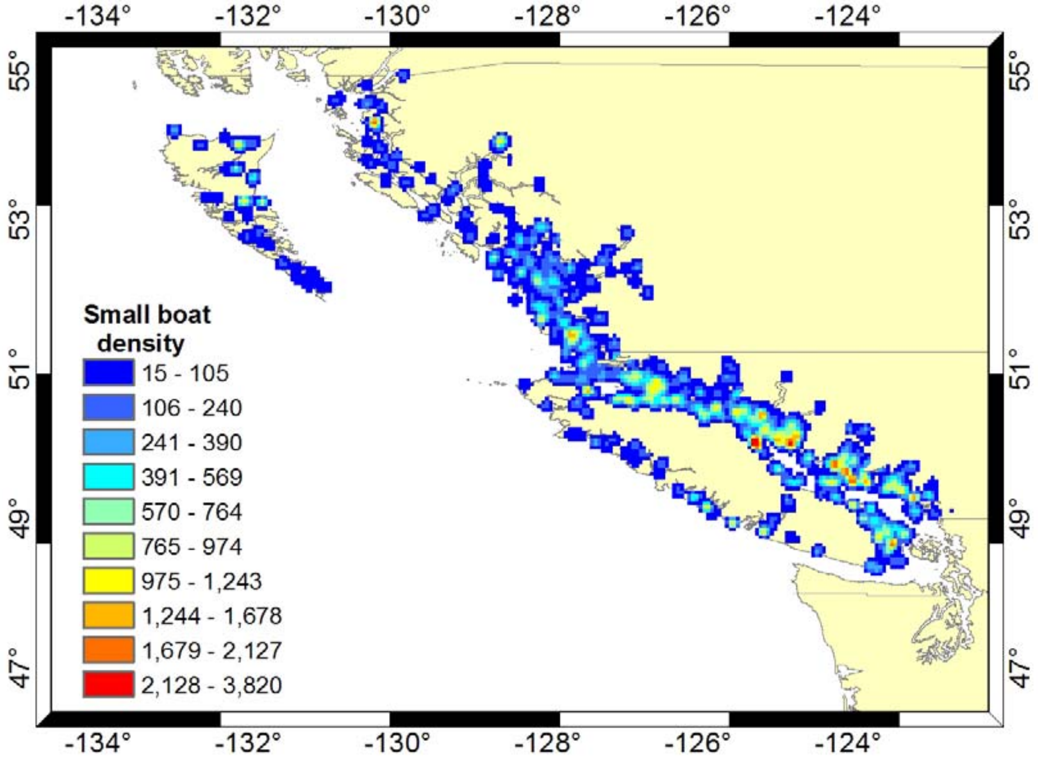


Figure 6: Density of small craft marinas, moorings and anchorages in BC waters.

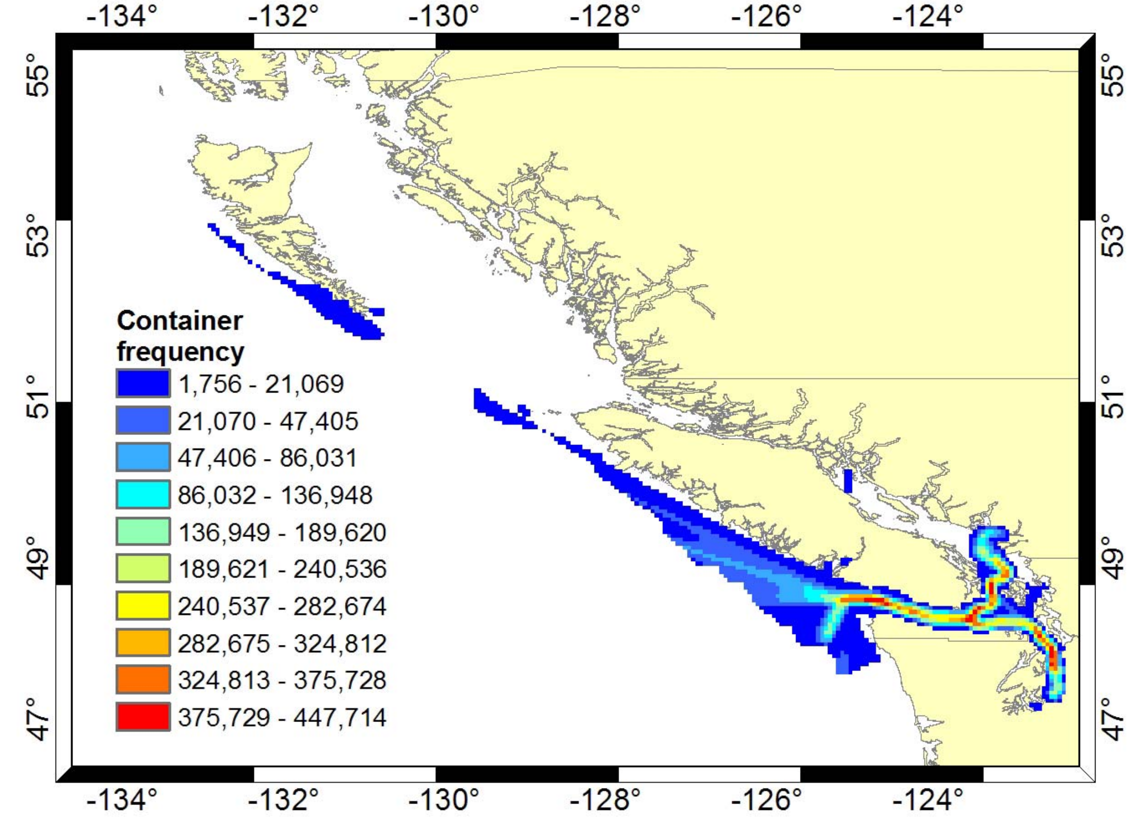


Figure 7: Density of container ship activity in BC waters.

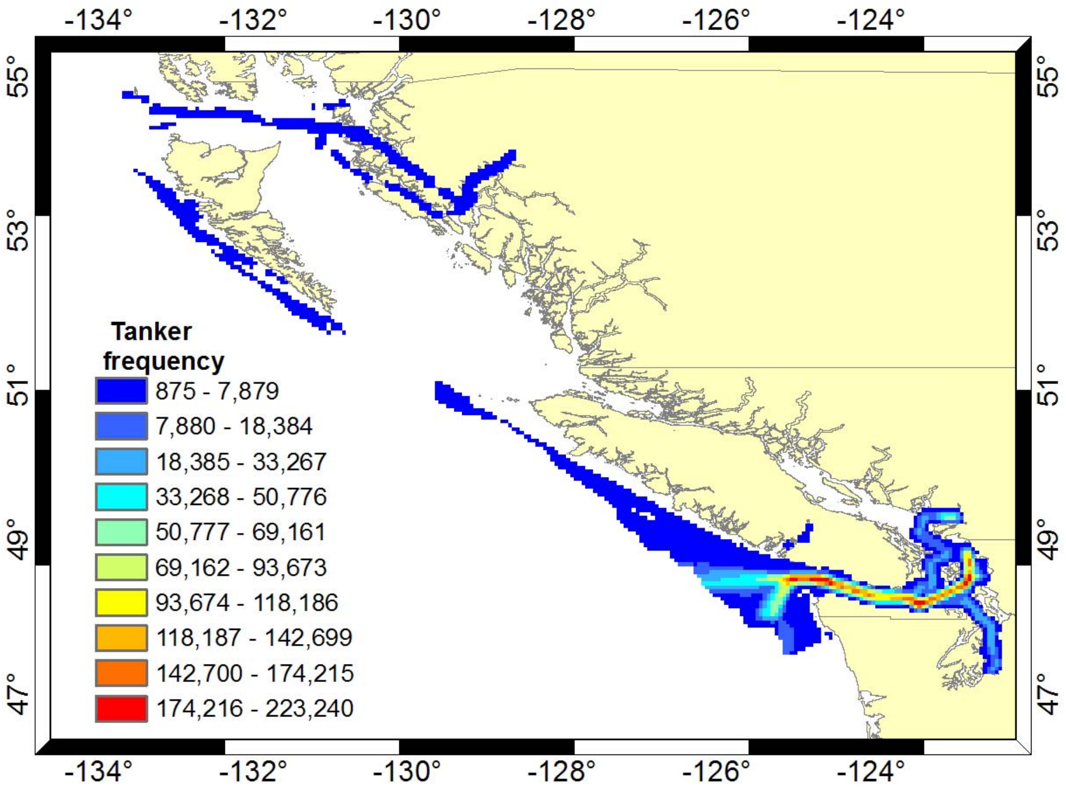


Figure 8: Density of tanker ship activity in BC waters.

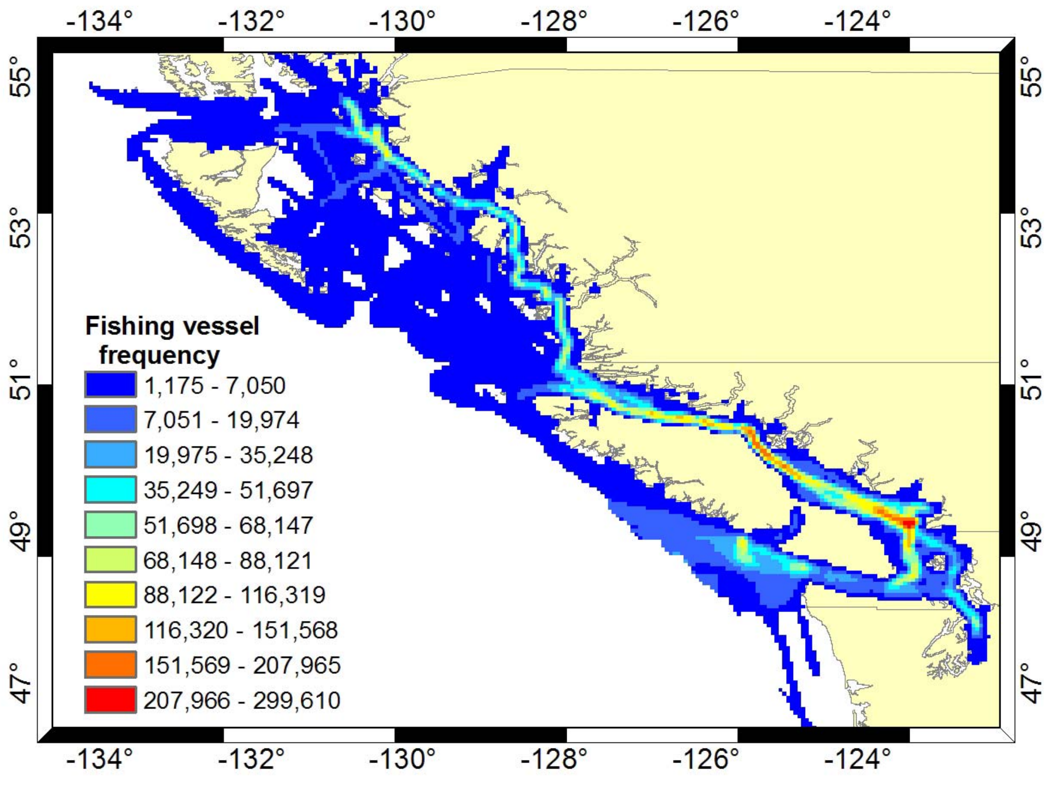


Figure 9: Density of fishing vessel activity in BC waters.

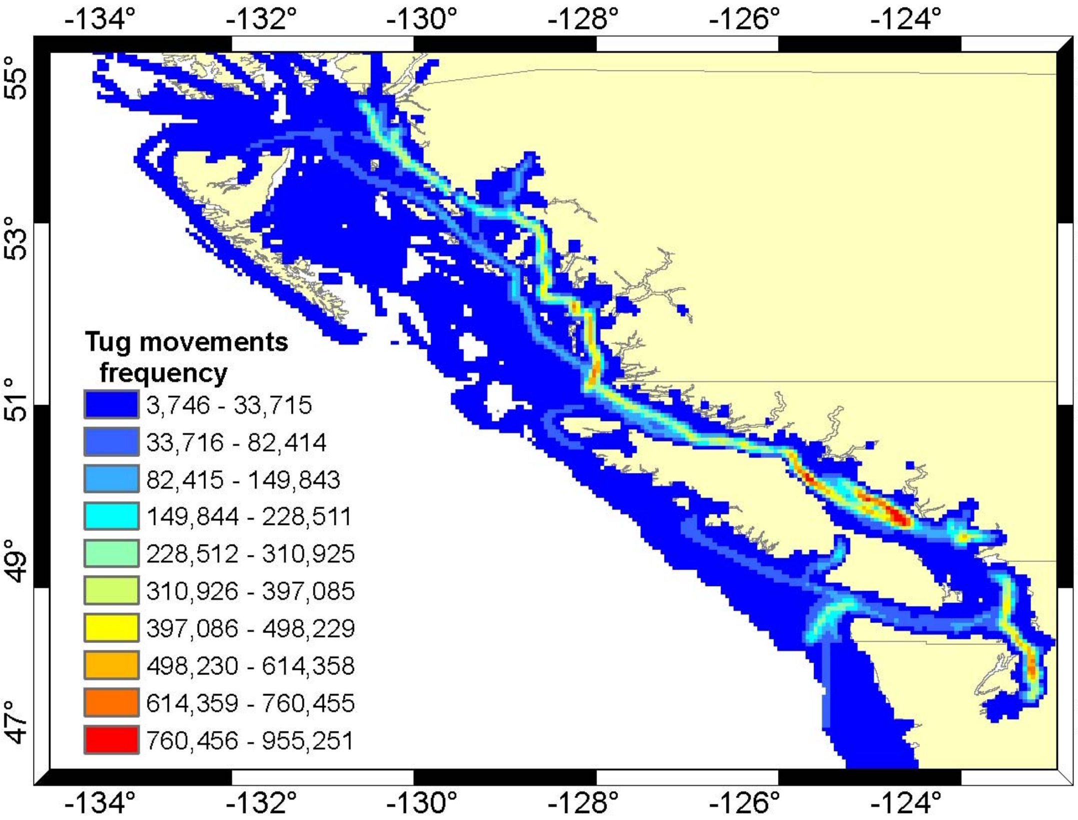


Figure 10: Density of tug/barge activity in BC waters.

Potential vectors: east coast

A number of potential vectors exist on the east coast including aquaculture-related transfers, fouling on recreational or small craft and fouling on commercial ships. However, we were unable to quantify this vector as we were for the west coast due to unavailable data. In Atlantic Canada, the greatest density of aquaculture facilities is in waters around PEI (Figure 11). Many lower density sites exist scattered around Newfoundland, Nova Scotia, New Brunswick (both near St. Andrews in the Bay of Fundy and on the Atlantic coast) and the Magdalen Islands (Figure 11). Most of the small craft harbors exist around the island of Newfoundland (Figure 12) but this could represent an artifact in the available data as other parts of the Maritimes have much higher population densities. Other higher density areas exist around southwestern Nova Scotia and New Brunswick (mouth of the Bay of Fundy), around Cape Breton Island, PEI and the Magdalen Islands (Figure 12).

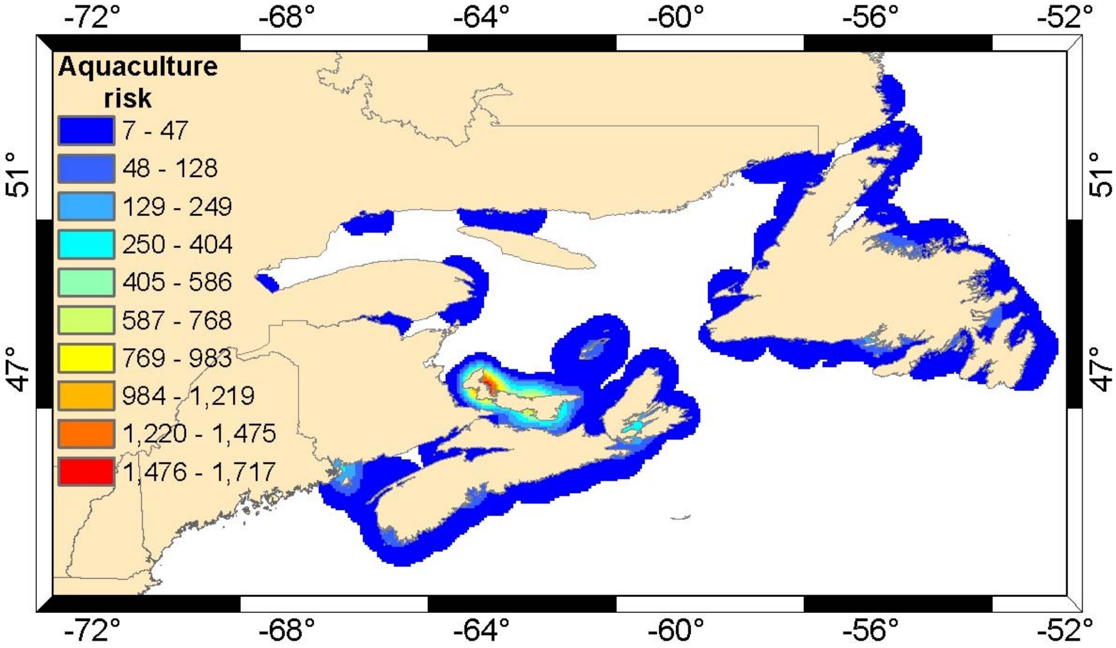


Figure 11: Density of aquaculture facilities around Atlantic Canada.

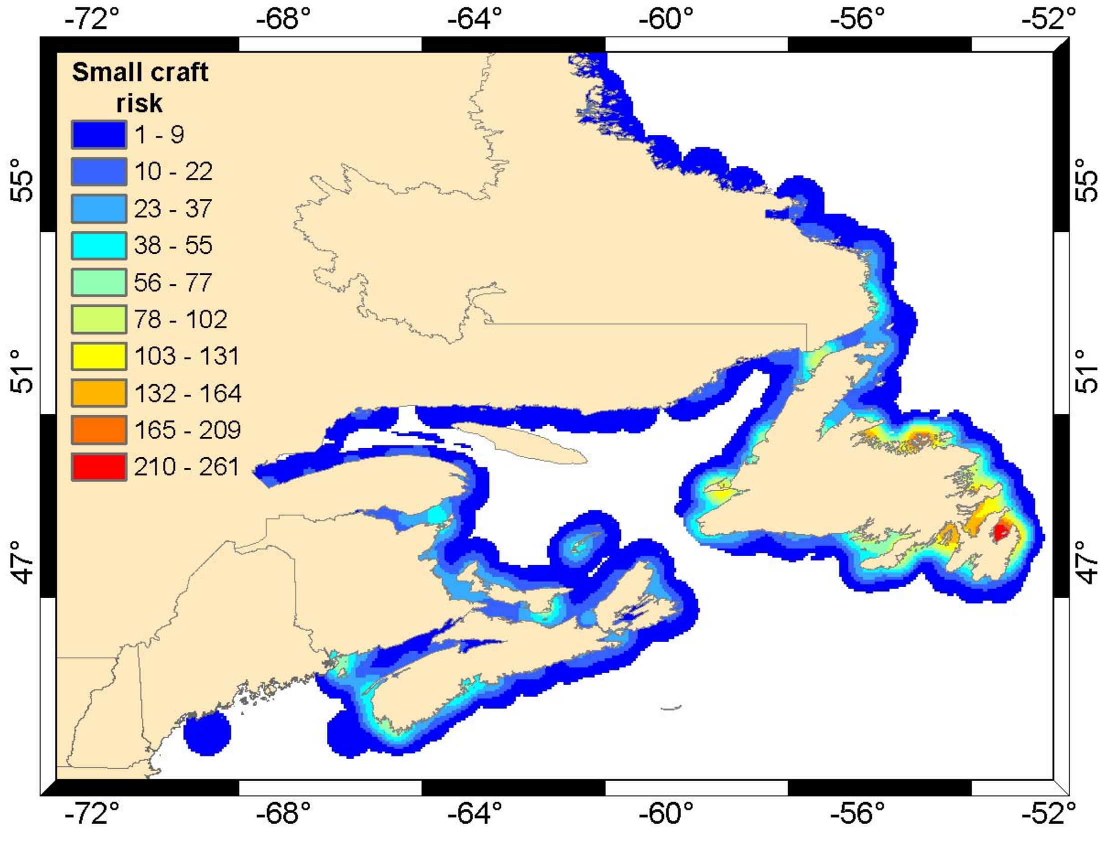


Figure 12: Density of small craft harbors around Atlantic Canada.

Club Tunicate (*Styela clava*)

Background and biology

Styela clava is a large, solitary tunicate with a cylindrical body resembling a club. The tunic is tough, brown, and leathery and tapers to a short stalk that terminates at a disc-like holdfast. Larger specimens often are covered with epibionts such as corals, algae, hydroids, sponges and smaller conspecifics (Lutzen 1999) and these hitchhikers could be transferred to new locations if adult specimens are moved. *Styela clava* is hermaphroditic but not self-fertile as this species has external fertilization (Wallace 1961, Kott 1985) with eggs and larvae that are lecithotrophic, remaining planktonic for 24-28 hours at 20°C before settling, attaching and metamorphosing on the substrate (Cohen 2005). Studies indicate that the maximum settlement distance of ascidians in general is 10m from the adult and most dispersal is much less (Osman and Whitlatch 1995; McHenry 2005). Upon maturity, this species has the ability to spawn every 24 hours (Biosecurity New Zealand 2005) with sexually mature adults ranging in size from 90 to 160mm (Kott 1985, Lutzen 1999) until attaining a maximum age of about three years (Morris et al. 1980; Lambert and Lambert 1998).

Styela clava is a low intertidal to subtidal fouling species. It has been recorded at 40m depth but more typically occurs between 15-25m (Lutzen 1999). This species can withstand tidal emersion in some microhabitats (e.g., under rocks) and is most common in sheltered habitats with low wave action, such as inlets, bays, harbours and marinas (Lutzen 1999). Also, it has been found on high energy outer coasts on the partially protected surfaces of rocks or pilings. *Styela clava* can be found attached to a range of artificial substrates, including pier pilings, jetty walls, concrete structures, submerged ropes, buoys, floating docks, and vessel hulls (Lutzen 1999, NIMPIS 2002). As a secondary settler, *S. clava* can settle on substrates already fouled by other species. It is found on natural substrates as well, including rocks and bivalve beds and epiphytically on *Crassostrea gigas*, *Mytilus edulis*, and *Sargassum muticum* (Lutzen 1999, NIMPIS 2002).

As an extremely efficient suspension feeder *S. clava* consumes phytoplankton, zooplankton and other suspended organic materials (NIMPIS 2002). Similar to other large solitary ascidians it is capable of high filtration rates and experiences ontogenetic and size-selective shifts in predation pressure (Lambert and Lambert 2003). Larvae are susceptible to predation by a range of planktivorous predators while newly settled ascidian juveniles become prey for mobile carnivorous or omnivorous invertebrates and fishes (reviewed in Lambert 2005b). However, once *S. clava* reaches the adult stage, the tough, leathery tunic provides considerable defense and no predators have been recorded for northwestern Europe (Lutzen 1999, NIMPIS 2002; Cohen 2005).

Globally, *S. clava* is such a successful invader because of its ability to tolerate of a wide range of environmental factors, a trait common among many invasive species (Clarke and Therriault 2007). For example, *S. clava* was able to survive temperatures as low as 2°C in the Netherlands (Lambert and Lambert 2003) and as high as 23°C in the United Kingdom (Holmes 1969). However, it is important to note that this species was unable to reproduce at temperatures less than 15°C (Eno et al. 1997). This species has behavioral adaptations to handle adverse conditions or environmental stressors (e.g. hyposalinity). *Styela clava* can close its siphons for extended periods of time, with adult specimens able to withstand temporary drops in salinity, to as low as 8‰ (Sims

1984, Lutzen 1999). However, Davis and Davis (2004) reported that this species was not found in areas where the salinity was consistently lower than 20‰ suggesting a lower threshold of salinity tolerance does exist. Sims (1984) described this species as possessing limited hyperosmotic capability, supporting its absence in highly estuarine conditions which would be less than 20‰ (Lutzen 1999). Larvae metamorphose in salinities between 20‰ and 32‰, while 18‰ is deleterious to the larvae (Kashenko 1996 IN Lutzen 1999). Therefore the salinity tolerance of *S. clava* is consistent with the distribution pattern described by Davis and Davis (2004).

This species occurs in lower densities on natural substrates, 50-100/m^2 and an order of magnitude higher on artificial surfaces, 500-1000/m^2 (see Lutzen 1999 for review). Floating artificial substrates form a unique habitat that may be highly suitable for invading *S. clava* populations, causing the significant difference in density compared to natural habitats (Lambert 2005a). The characteristics that promote invasion on these surfaces may include a lack of benthic predators and low structural complexity. In addition, the introduction of a new substrate free from the established communities of native species offers valuable new space to invaders. Once a foothold has been established, the short dispersal distance may allow *S. clava* to generate high density local populations.

A number of vectors have been proposed for the introduction of *S. clava* worldwide including ship fouling, live oyster transfer and fisheries gear fouling. Ballast water introduction has largely been ruled out for this species because of the short larval period. Circumstantial evidence for some vectors exist based on the timing and geographical distribution of reported sightings. Its introduction to Plymouth Harbour in 1953 coincided with the arrival of warships returning from the Korean War suggesting hull fouling was the vector. The introduction in Elkhorn Sound, California in the late 1920s was consistent with the timing of adult Japanese oyster import and the rapid spread of *S. clava* within PEI may be attributable to the movement of aquaculture and fisheries equipment between sites.

Known distribution

Styela clava is native to the northwest Pacific Ocean. It was originally described by Herdman (1881) from specimens derived from Kobe and the Inland Sea area of Japan (Abbott and Johnson 1972). Its native range extends from the Sea of Okhotsk, through southern Siberia, Japan, Korea and northern China, south to Shanghai (Cohen 2005). Now *S. clava* occurs worldwide in temperate marine waters including both western and eastern Canada, the western and eastern United States (US), the United Kingdom (UK), Europe, Asia, and Australia. The timing of reported introductions worldwide is summarized by Clarke and Therriault (2007).

Potential distribution in Canada

The potential distribution of *S. clava* in Canadian waters is based on environmental conditions at the Canadian sites where *S. clava* currently occurs on both the Atlantic and Pacific coasts. On the Atlantic coast, the waters in the southern Gulf of St. Lawrence, especially the waters around PEI and New Brunswick are highly favorable (Figure 13). Favorable conditions also exist around Cape Breton Island and several locations along the Atlantic coast of Nova Scotia, the head of the Bay of Fundy and near St. Andrew’s (Figure 13). A lower ecological suitability exists for the southwestern corner of

Newfoundland and waters near the tip of the Gaspe Bay peninsula (Figure 13). On the Pacific coast *S. clava* has a very high environmental match throughout much of BC (Figure 14). This includes the Strait of Georgia, Johnstone Strait, the major inlets along the West Coast of Vancouver Island (Barkley Sound, Claquot Sound, Nootka Sound and Esperanza Inlet), much of the Central Coast and North Coast of BC, and the Queen Charlotte Islands (Figure 14).

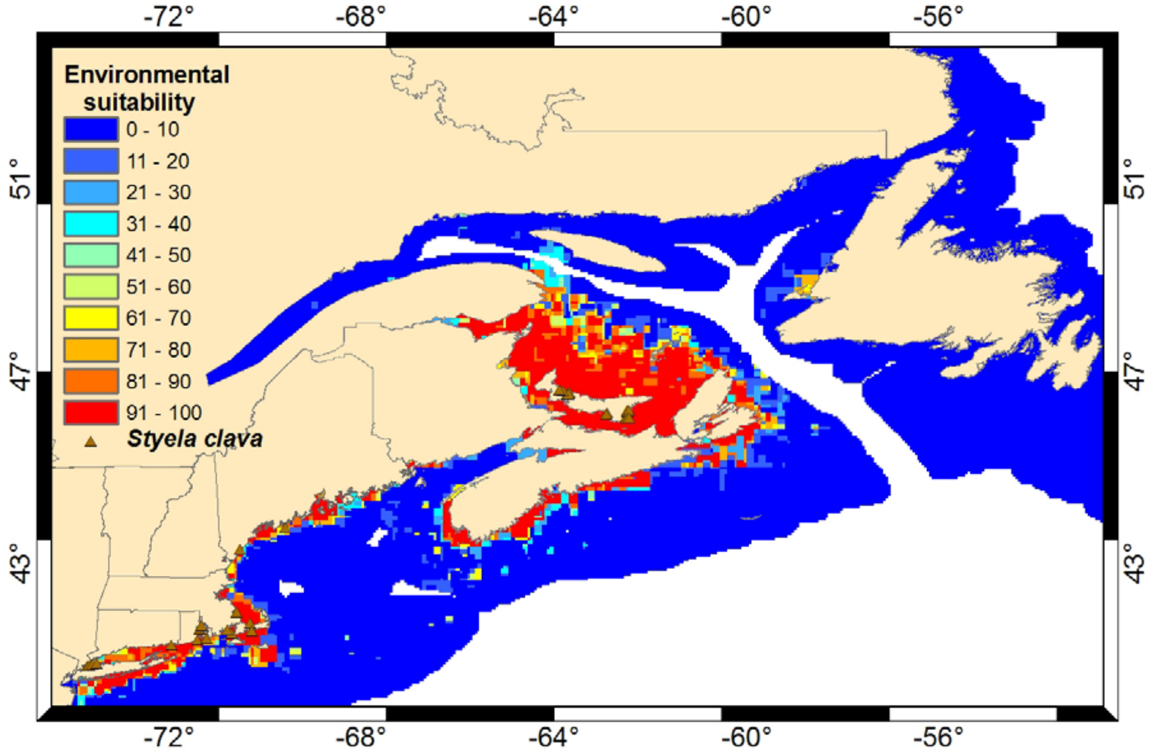


Figure 13: Potential distribution of *S. clava* on the Atlantic coast based on temperature and salinity tolerances.

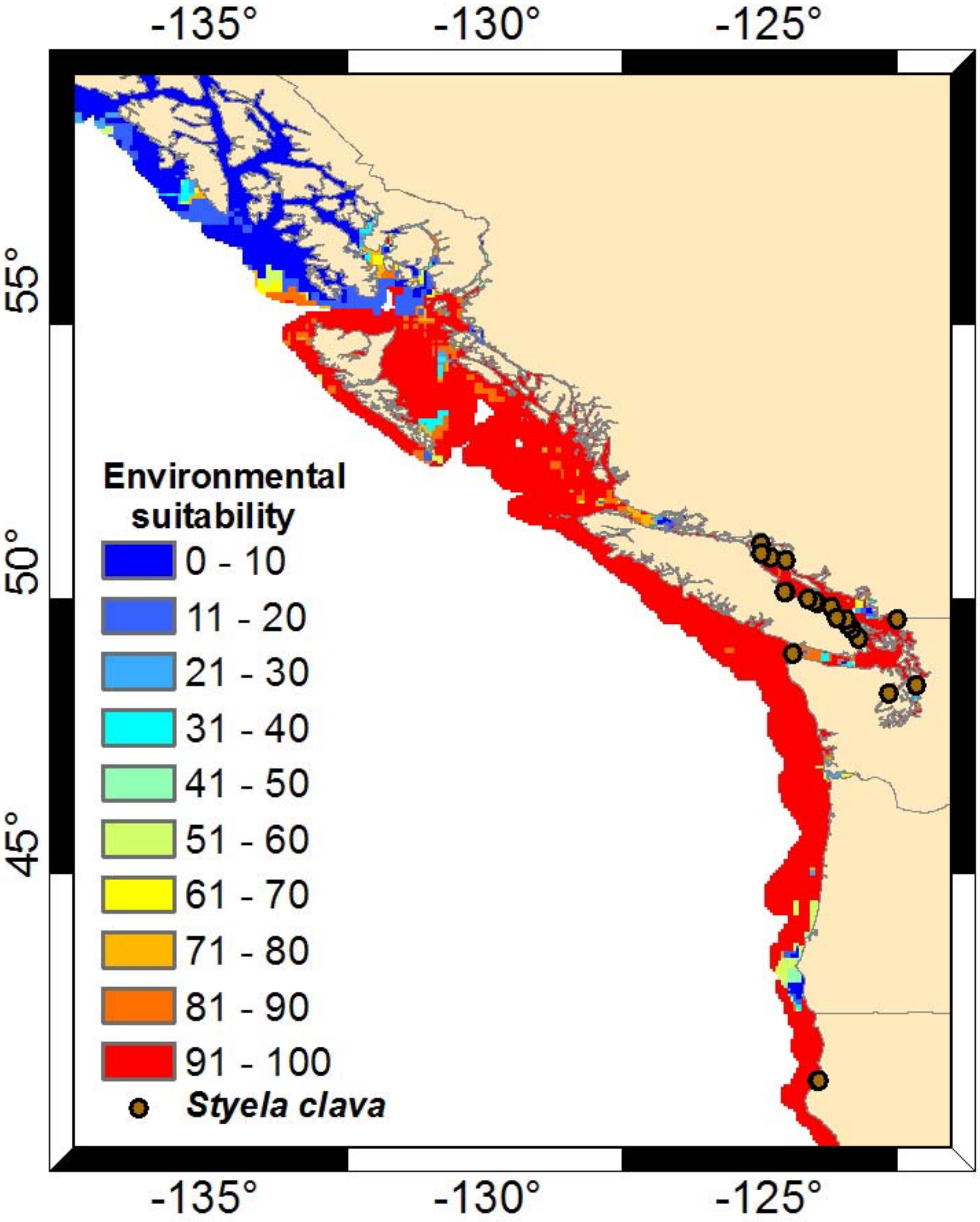


Figure 14: Potential distribution of *S. clava* on the Pacific coast based on temperature and salinity tolerances.

Risk Assessment Part I – Aquatic Organism Ecological and Genetic Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate of probability of the organism successfully colonizing and maintaining a population if introduced.

The primary vectors proposed for the introduction of *S. clava* worldwide include ship fouling, live oyster transfer and fisheries gear fouling. On the Pacific coast the most probable vector and pathway was historical aquaculture transfers directly from Japan. Prior to concerns about non-native species or their associated hitchhikers, adult Japanese oyster were introduced directly from Japan to Canada for aquaculture purposes (Quayle 1969, Bourne 1979). Similar practices existed along the American Pacific coast so secondary spread from these sites to Canadian waters can not be dismissed. In contrast, on the Atlantic coast, hull fouling on commercial or military vessels is more likely based on the timing of invasions. For Atlantic Canada, these vessels could have been of European or US origin given *S. clava* was established on both sides of the Atlantic Ocean prior to its arrival in the Canadian Maritimes. Natural vectors of dispersal appear unlikely for this species given the very short larval phase and the fact that adults are sedentary, biofouling organisms. Given that *S. clava* has established populations on both the Atlantic and Pacific coasts, it is certain the species can arrive, survive and reproduce in Canadian waters. In addition, considerable, potential suitable habitat exists on both coasts (Figures 13 and 14) suggesting further spread or additional introductions are possible.

(2) If the organism escapes from the area of introduction, estimate the probability of its spreading.

Given the presence of populations on both the Atlantic and Pacific coasts, there is a very high probability that *S. clava* could spread on both coasts. This biofouling species has an affinity for artificial structures on which it does very well (Lutzen 1999). Considerable such structure exists on both coasts, although potentially higher on the Pacific coast, especially structures related to aquaculture and recreational small craft harbors (marinas and anchorages) (Figures 5-6).

(3) Final Rating: *Styela clava*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Very High	Very Low	Very High	Very Low
Survival	Very High	Very Low	Very High	Very Low
Reproduction	Very High	Very Low	Very High	Very Low
Spread	Very High	Very Low	Very High	Very Low
Overall	**Very High**	**Very Low**	**Very High**	**Very Low**

Step 2: Determining the Consequences of Establishment of an Aquatic Organism

(1) Ecological impact on native ecosystems both locally and within each region.

Based on the results of its introduction throughout the world (Clarke and Therriault 2007), there is little doubt that *S. clava* would have significant negative impacts on habitat structure and potentially foodweb and trophic structure of aquatic ecosystems by inducing changes in plant, invertebrate and possibly fish communities. This species' ability to settle on a range of artificial surfaces causes dense fouling of fishing gear, moorings, and ropes and is difficult and time-consuming to remove. As a member of the biofouling community, this species competes for food and space with other filter feeding organisms and benthic species and can reach maturity earlier than competing species, especially cultured bivalves. Although *S. clava* has the ability to reduce settlement rates of co-occurring species locally it appears to have little effect on post-settlement community dynamics (Whitlatch et al. 1995). In Japan, Denmark and eastern Canada, *S. clava* has been reported as a major pest of oyster and mussel farms (Cohen 2005) and in many places, this species has replaced local ascidians as the dominant member of shallow, protected habitats worldwide (Whitlatch et al. 1995; Lutzen 1999; Lambert and Lambert 2003).

The results of the expert survey suggested *S. clava* would have moderate ecological consequences (Figure 3). Furthermore, this survey suggested higher consequences on industry, especially shellfish aquaculture (Figure 3) due to this species affinity for artificial structure and its ability to overgrow cultured species such as mussels and oysters.

(2) Genetic impacts on local self-sustaining stocks or populations.

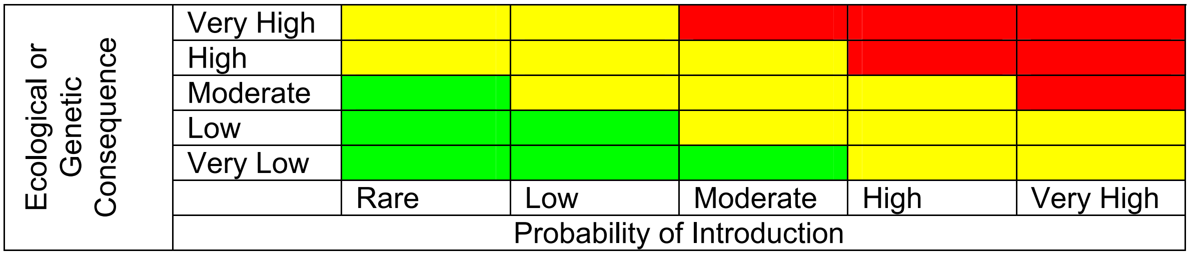
On the Pacific coast there are native members of the family Styelidae, including *S. montereyensis* and *S. gibbsii*. Similar *Styela* species do not exist on the Atlantic coast. However, given that *S. clava* is unable to self-fertilize, it appears this species has histocompatability complexes that would likely prevent hybridization between *S. clava* and native *Styela* species. It is highly unlikely *S. clava* would have any genetic impact on other native fauna on either coast.

(3) Final Rating: *Styela clava*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Moderate	Moderate	Moderate	Moderate
Genetic Consequence	Very Low	Low	Low	Low

Step 3: Estimating Aquatic Organism Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.



Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Aquatic Organism Overall Risk Potential for *Styela clava*.

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	High	Moderate	High	Moderate
Genetic	Moderate	Low	Moderate	Low

Risk Assessment Part II – Pathogen, Parasite or Fellow Traveler Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate the probability that a pathogen, parasite or fellow traveler may be introduced along with the potential invasive species.

Little is known about the parasites and pathogens of *S. clava* (Clarke and Therriault 2007). However, larger specimens often are covered with epibionts such as corals, algae, hydroids, sponges, other tunicates (including non-native species) and smaller conspecifics (Lutzen 1999). Since movement of adult individuals is the most probable method of secondary spread by *S. clava*, these epibionts could be moved at the same time, especially if transfer is by hull fouling or via movements associated with aquaculture activities. However, at this time, it is unclear if any of the epibionts associated with *S. clava* are non-indigenous or if they would impact other species, with the exception of some of the colonial tunicate species identified in this risk assessment.

(2) Estimate the probability that the pathogen, parasite or fellow traveler will encounter susceptible organisms or suitable habitat.

Given that at least some of the epibionts associated with *S. clava* would be species generalists able to exploit a variety of potential settling substrates, including other members of the biofouling community, some of these epibionts could encounter susceptible organisms. However, it is unclear if any of these would be pathogenic or parasitic to the organisms they encounter. Field observations have detected non-indigenous colonial tunicates (e.g., *Botryllus schlosseri* and *Botrylloides violaceus*) overgrowing *S. clava*.

(3) Final Rating: pathogen, parasite or fellow traveler for *Styela clava*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	High	Very High	High	Very High
Survival	High	Very High	High	Very High
Reproduction	High	Very High	High	Very High
Spread	High	Very High	High	Very High
Overall	**High**	**Very High**	**High**	**Very High**

Step 2: Determining the Consequences of Establishment of a Pathogen, Parasite or Fellow Traveler

(1) Ecological impacts on native ecosystems both locally and within the region including disease outbreak, reduction in reproductive capacity, habitat changes, etc.

Given that other non-indigenous colonial tunicate species have been identified as potential fellow travelers on *S. clava*, ecological impacts would be high (see species-specific risk assessments). Currently, no known pathogens or parasites of *S. clava* have been identified. This is consistent with species that are transported as fouling organisms as it is usually the progeny of these organisms that become established at new locations rather than dislodgement of the individual. This dramatically reduces the probability that a pathogen or parasite will be introduced at the same time as larval stages often are assumed to be relatively insusceptible to these agents. However, fellow travelers such as colonial tunicates could be introduced or spread either by dislodgement or larval release.

(2) Genetic impacts on local self-sustaining stocks or populations (i.e. whether the pathogen, parasite, or fellow traveler affects the genetic characteristics of native stocks or species).

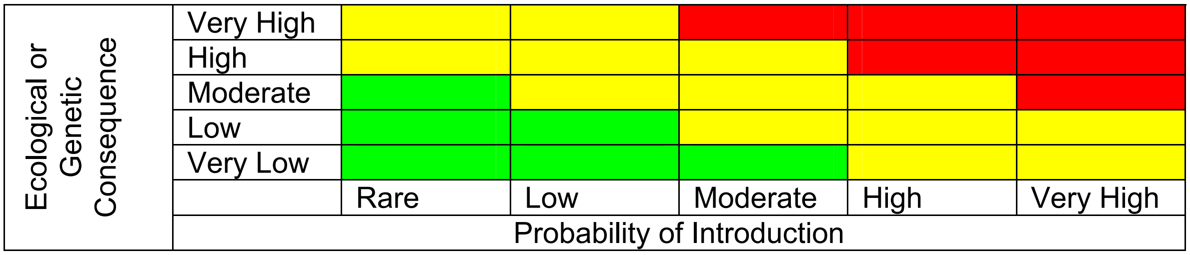
Nothing is known about the potential genetic impacts on local populations. However, there are species related to possible travelers native to Canada; therefore the potential for genetic contamination exists.

(3) Final Rating: pathogen, parasite or fellow traveler for *Styela clava*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	High	Low	High	Low
Genetic Consequence	Low	Low	Low	Low

Step 3: Estimating Pathogen, Parasite or Fellow Traveler Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.



Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Pathogen, Parasite or Fellow Traveler Overall Risk Potential for *Styela clava*.

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	High	Very High	High	Very High
Genetic	Moderate	Very High	Moderate	Very High

Vase Tunicate (*Ciona intestinalis*)

Background and biology

Ciona intestinalis is a solitary tunicate that can grow up to 15 cm in length and 3 cm in diameter. The cylindrical body is supported by a gelatinous tunic that starts soft and becomes more leathery with age. The tunic is translucent although color can vary from pale greenish/yellow to orange. Two siphons are located at one end of the body possessing yellow margins and in some cases orange/red pigment spots. *Ciona intestinalis* is a sessile filter feeder which is typically observed attached to hard natural or artificial substrates by short projections of the tunic. It may occur in dense aggregations, often as a dominant member of the biofouling community, in enclosed or semi-protected marine embayments.

Ciona intestinalis is an oviparous, simultaneous hermaphrodite with concurrent production of eggs and sperm with reproductive output that is size rather than age dependent (Millar 1952). Generally, *C. intestinalis* do not self-fertilize but Rosati and Santis (1978) reported that 15% of individuals were self-fertile in the Gulf of Naples (Italy). Although size at maturity varies among populations (Dybern 1965), once individuals reach maturity gametes are produced continually and released in broadcast spawning events as long as water temperatures are suitable. The duration of the tadpole larval phase is temperature dependent and estimates vary widely, from 6-36 hours (Millar 1952) to 2-10 days (Jackson 2005). Similarly, Dybern (1965) reported the duration of the larval phase as 4-5 days at 10-12°C or 24-36 hours at 18-20°C. Dispersal potential has been estimated at 100-1000 m (Jackson 2005), but this will depend on swimming speed, duration of the tadpole phase and most importantly on the local hydrographic regime. Petersen and Svane (1995) noted that Scandinavian populations tend to be highly localized which may be indicative of limited dispersal.

Ciona intestinalis is tolerant of a wide range of environmental conditions as evidenced by its cosmopolitan distribution. Although often considered a member of the subtidal, deepwater fauna where it lives on hard substrates (e.g., Brunel et al. 1998) many populations have been reported in shallow coastal waters, most notably as a member of the biofouling community on artificial substrates (Cohen et al. 2000a, Lambert and Lambert 1998; 2003) or aquaculture gear (Karayucel 1997, Hecht and Heasman 1999, Mazouni et al. 2001). Reported temperature tolerance varies among populations but *C. intestinalis* is generally considered a coldwater or temperate species, although temporary or transient populations have been reported from tropical harbors (Monniot and Monniot 1994). Populations that experience 25-28°C water are suspected of being near the upper threshold for this species (Carver et al. 2006). Generally, adult mortality increases below 10°C but in Atlantic Canada populations have survived for a couple of months at temperatures around –1°C (Carver et al. 2003). *Ciona intestinalis* also is tolerant of a wide range of salinities with reported tolerances between 12‰ and 40‰ making it a euryhaline species (Carver et al. 2006a), although most populations are found in full salinity waters. Further, this species can not withstand extended periods in waters with less than 11‰ salinity (Dybern, 1967) but may be able to withstand short term salinity fluctuations. *Ciona intestinalis* is typically found in areas of low flow or minimal wave exposure, although it can reportedly withstand flow rates up to 3 knots (Jackson 2005). Further, this species can tolerate very low flow rates which give it a competitive advantage in areas of minimal water exchange such as harbors, marinas and docks. Also, sporadic population outbreaks or irregular peaks in recruitment have been observed apparently independent of changes in environmental conditions (Keough 1983, Cayer et al. 1999) and some report a rather patchy distribution for this species both in its native range (Petersen and Svane 1995) and its invaded range where it occurs predominantly on artificial substrates (e.g., Monniot and Monniot 1994, Lambert and Lambert 2003).

One of the major factors in the establishment and composition of marine epifaunal communities is the availability of suitable substrate for recruitment. Typically natural habitats offer stable substrates which develop a complex highly diverse community which may be relatively resistant to invasion (Stachowicz et al. 1999, Stachowicz et al. 2002). By comparison, artificial substrates which are frequently disrupted due to seasonal maintenance or fluctuating environmental conditions provide ideal habitat for highly opportunistic species. Koechlin (1977) reported that *C. intestinalis* rapidly colonized floating metal tanks in a newly constructed harbor at Lezardrieux (France) at densities up to 2000 individuals per square meter. Small predatory gastropods (*Anachis* spp and *Mitrella lunata*) can play an important role in determining the survival of *C. intestinalis* but other potential predator species exist such as nudibranchs, flatworms, nemerteans, small crustaceans and certain polychaetes (Osman and Whitlatch 2004). Further, as *C. intestinalis* grows and reaches a size refuge from small gastropods; this species becomes increasingly vulnerable to larger invertebrate predators such as sea stars, crabs or even fish (Osman and Whitlatch 2004). However, the extent to which any of these potential predators can control *C. intestinalis* invasions remains uncertain.

Given that *C. intestinalis* has limited dispersion potential during its larval phase and remains attached for its adult phase, range extensions likely involve hitchhiking on natural or artificial substrates. Vercaemer (pers. obs.) found *C. intestinalis* rafting on clumps of the alga *Codium fragile* but hull fouling on slow-moving vessels (e.g., barges, small watercraft) is likely responsible for most intraregional dispersal. For example, Lambert and Lambert (2003) noted that although *C. intestinalis* probably was introduced into San Francisco Bay, California via commercial shipping, regional traffic likely was responsible for its subsequent dispersal along the California coast and south into Mexico. Similar observations have been made in Australia where recreational boating is believed to have contributed significantly to the spread of *C. intestinalis* there (Cohen et al. 2000b, MacDonald 2004) as has the movement of aquaculture product or fouled equipment among sites (Cohen et al. 2000b).

Known distribution

Ciona intestinalis is believed to have originated in the Northeast Atlantic. Early records are available for the coasts of Denmark, Norway, France and Britain in the late 1700s and early 1800s (Kott 1990). Carver et al. (2006a) summarize the current native distribution as the coastal waters of Britain, the Netherlands, Denmark, Sweden and Norway and colder water locations including the Faeroe Islands, east coast of Greenland and as far north as Spitsbergen and Bear Island. Also, native *C. intestinalis* populations exist in the Mediterranean including Spain, Italy, Greece, Turkey and Egypt (Millar 1966). Most would consider *C. intestinalis* to be a cosmopolitan invader who has been spread widely throughout all temperate regions by shipping activities, primarily as a biofouling organism on vessel hulls (Monniot and Monniot 1994, Lambert and Lambert 1998). In North America *C. intestinalis* has been documented in the Canadian Arctic (Atkinson and Wacasey 1976), but this may have been one of the coldwater subspecies, forma *gelatinosa* or forma *longissima*. Whether it occurs along the Labrador coast and up into Hudson Bay is not known. Huntsman (1912) lists *C. intestinalis* as a member of the benthic invertebrate community on the west coast of Canada, but it is possible that specimens of *C. savignyi* may have been misidentified. Further, *C. savignyi* is likely a cryptogenic species in BC, likely originally from the northwestern Pacific (Lambert 2003, Lamb and Hanby 2006).

In South America, *C. intestinalis* has been reported from Brazil, Argentina, Peru, and Chile while reports from southern waters include South Africa, Australia, New Zealand (see Carver et al. 2006a). *Ciona intestinalis* also has been reported throughout Indonesian waters and northwards along the coasts of Korea, China and Japan (NIMPIS 2002).

Potential distribution in Canada

The potential distribution of *C. intestinalis* in Canadian waters is based on environmental conditions at the Canadian sites where *C. intestinalis* currently occurs along the Atlantic coast. Although *C. savignyi* occurs along the Pacific coast it is unknown how closely related these species are and whether or not the environmental tolerances of *C. savignyi* would represent those of *C. intestinalis*. On the Atlantic coast, the waters along the Atlantic coast of Nova Scotia are highly favorable as are the waters around the Bay of Fundy and waters around Cape Breton Island (Figure 15). Highly favorable conditions also exist on the eastern side of PEI, the southwest corner of Newfoundland and the southern Gulf of St. Lawrence (Figure 15). A lower ecological suitability exists for deeper waters off the coast of Nova Scotia near Sable Island (Figure 15). On the Pacific coast, the highest environmental match exists throughout Johnstone Strait, the major inlets of the Central Coast and into the North Coast of BC (Figure 16). There are moderate matches along the east and north coast of the Queen Charlotte Islands (Figure 16). Unlike *S. clava*, *C. intestinalis* has a very low environmental match throughout the Strait of Georgia (Figure 16). Also, much of southeastern Alaska appears quite favorable for this species, possibly reflective of its coldwater preference.

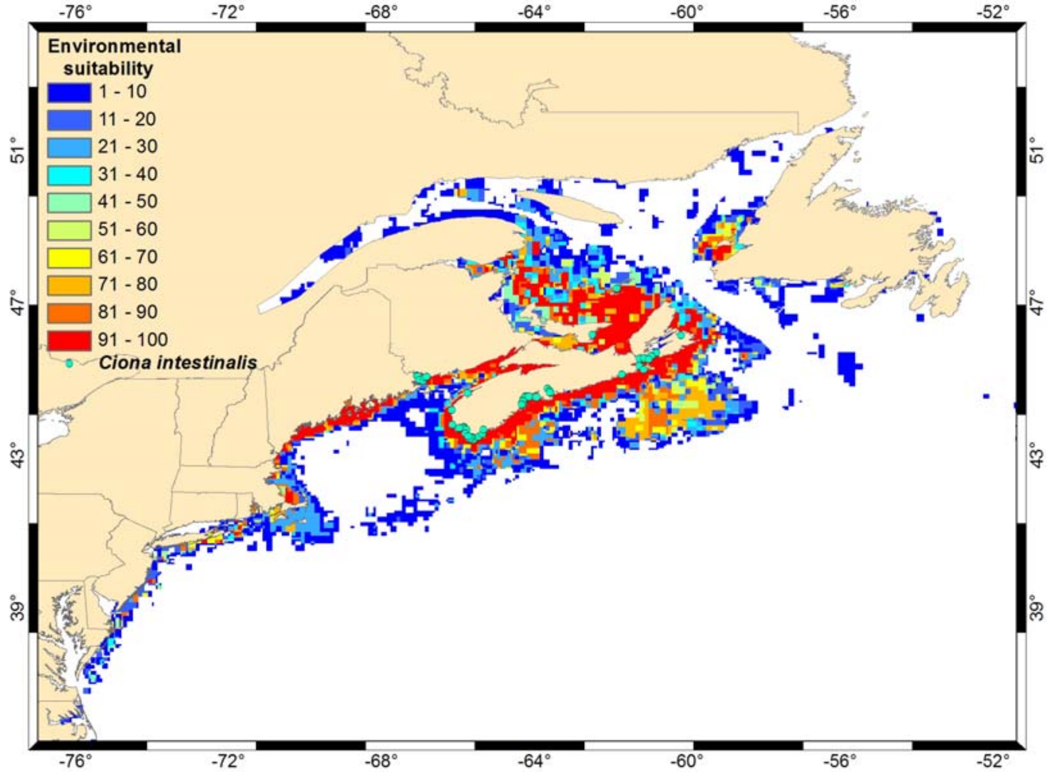


Figure 15: Potential distribution of *C. intestinalis* on the Atlantic coast based on temperature and salinity tolerances.

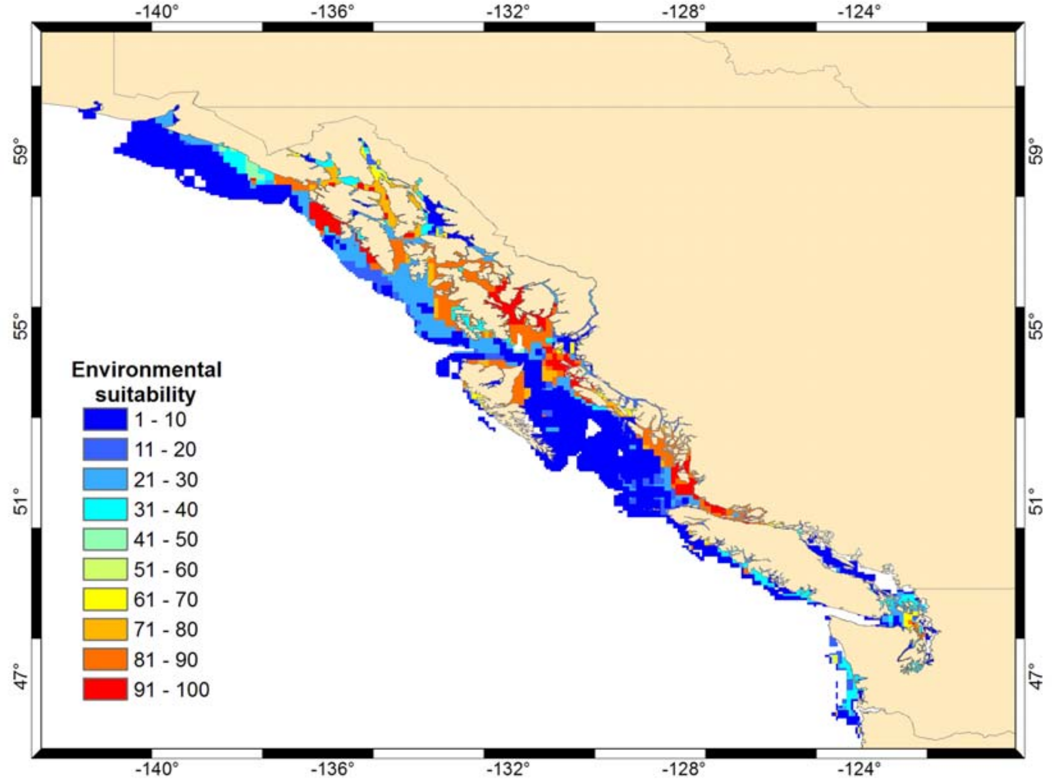


Figure 16: Potential distribution of *C. intestinalis* on the Pacific coast based on temperature and salinity tolerances determined from Atlantic Canada populations.

Risk Assessment Part I – Aquatic Organism Ecological and Genetic Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate of probability of the organism successfully colonizing and maintaining a population if introduced.

The primary vectors proposed for the introduction of *C. intestinalis* worldwide include ship fouling, and aquaculture and fisheries gear fouling. Attachment to the hull of slow moving vessels is a more likely vector compared to attachment to the hull of fast moving vessels (commercial container or bulk carriers) unless transfer was associated with sheltered locations (sea chests, anchor lockers) as *C. intestinalis* can only tolerate speeds less than three knots before falling off (Jackson 2005). Natural vectors of dispersal are less likely for this species given the very short larval phase and the fact that adults are sedentary, biofouling organisms with the possible exception of rafting which has been observed for this species (Vercameer, pers. obs.). Given that *C. intestinalis* has established populations on the Atlantic coast it is certain the species can arrive, survive and reproduce in Atlantic Canadian waters. In addition, considerable, potential suitable habitat exists along the Atlantic coast (Figure 14). For Pacific waters it is less clear. *Ciona intestinalis* has been reported from Puget Sound, Washington but not from adjacent Canadian waters. If tolerances are similar to *C. savignyi*, then *C. intestinalis* could have significant suitable habitat since *C. savignyi* is widely distributed along the Pacific coast, Alaska to southern California (Lamb and Hanby 2006).

Hull fouling, especially on slow moving vessels (barges) and aquaculture-related activities ranked high among the experts as a potential vector for *C. intestinalis* (Figure 2). This is in agreement with the literature, but it is more likely *C. intestinalis* would arrive to Canadian Pacific waters via hull fouling given increased restrictions on aquaculture imports in recent years.

(2) If the organism escapes from the area of introduction, estimate the probability of its spreading.

Given the presence of populations on the Atlantic coast, good environmental matches and available potential vectors, including slow moving commercial and recreational watercraft and various aquaculture activities, there is a very high probability that *C. intestinalis* will spread on the Atlantic coast. As with other non-indigenous tunicate species, this species has an affinity for artificial structures which exist mainly in waters around New Brunswick, Nova Scotia and PEI, notably structures related to aquaculture and recreational small craft harbors (marinas and anchorages) (Figures 11 and 12). Given the overall lower environmental match for Pacific coastal waters (Figure 16) the probability of spreading is lower compared to Atlantic coastal waters. However, there is an abundance of potential vectors within this region that could be used by *C. intestinalis* if it were to establish populations in Canadian waters on the west coast, notably recreational watercraft and transfer of aquaculture product or gear or tug and barge activity (Figures 5, 6, and 9).

(3) Final Rating: *Ciona intestinalis*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Very High	Very Low	High	High
Survival	Very High	Very Low	High	Moderate
Reproduction	Very High	Very Low	High	Moderate
Spread	Very High	Very Low	Moderate	High
Overall	**Very High**	**Very Low**	**High**	**High**

Step 2: Determining the Consequences of Establishment of an Aquatic Organism

(1) Ecological impact on native ecosystems both locally and within the region.

Based on the results of its introduction throughout the world (Carver et al. 2006a), there is little doubt that *C. intestinalis* would have significant negative impacts on habitat structure and potentially foodweb and trophic structure of aquatic ecosystems by inducing changes in plant, invertebrate and possibly fish communities. This species' ability to settle on a range of artificial surfaces causes dense fouling of fishing gear, moorings, and ropes and is difficult and time-consuming to remove. For example, in a fouling community dominated by *C. intestinalis* the overall biomass (wet weight) was 78 tons per raft compared to only 27 tons per raft for the cultured mussels (Stenton-Dozey et al. 2001). As a member of the biofouling community, this species competes for food and space with other filter feeding organisms and benthic species and can reach maturity earlier than competing species, especially cultured bivalves.

Modeling of dense populations of *C. intestinalis* in a shallow cove with restricted circulation indicated the potential for significant depletion of food resources (Petersen and Riisgaard 1992, Riisgard et al. 1996, 1998) and a concurrent increase in the deposition of faecal matter to the bottom that could lead to organic enrichment, the development of anoxic sediments, the accumulation of hydrogen sulfide, and the degradation of the benthic community. The filter-feeding activity of *C. intestinalis* may negatively impact the abundance of microzooplankton, such as ciliates which typically feed on bacteria, as well as the planktonic larvae of bivalves (Bingham and Walters 1989). In addition to preying directly on oyster larvae, *C. intestinalis* inhibits oyster settlement by reducing the amount of substrate available (Osman et al. 1989). Post-settlement survivorship and growth also are affected by the presence of *C. intestinalis* that can out compete or displace other sessile filter-feeders, effectively depriving them of food and/or space, especially when resources are limited. For example, Lambert and Lambert (1998) noted that since *C. intestinalis* appeared in southern California in 1917, there has been a steady decline in the abundance of native ascidian species which previously dominated, an observation that is more apparent on floating structures where predators are absent or have less impact on biofouling communities.

The results of the expert survey suggested *C. intestinalis* would have moderate ecological consequences (Figure 3). Furthermore, *C. intestinalis* could be expected to have higher ecological consequences on shellfish aquaculture (Figure 3) as heavy infestations reduce flow rates, thereby interfering with oxygen exchange and potentially jeopardizing fish health (Carver et al. 2006a).

(2) Genetic impacts on local self-sustaining stocks or populations.

On the Pacific coast there is another member of the family Cionidae, *C. savignyi*, a species that has generated considerable uncertainty as it has frequently been misidentified as *C. intestinalis* (Lambert 2003). Given that the two putative species are extremely similar with only minor morphological differences sometimes apparent between species, hybridization between the two species can not be discounted. There is no similar native species on the Atlantic coast. It is highly unlikely *C. intestinalis* would have any genetic impact on other native fauna on either coast.

(3) Final Rating: *Ciona intestinalis*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Moderate	Moderate	Moderate	Moderate
Genetic Consequence	Very Low	Low	Low	Low

Step 3: Estimating Aquatic Organism Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.

Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Aquatic Organism Overall Risk Potential for *Ciona intestinalis*.

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	High	Moderate	Moderate	High
Genetic	Moderate	Low	Moderate	High

Risk Assessment Part II – Pathogen, Parasite or Fellow Traveler Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate the probability that a pathogen, parasite or fellow traveler may be

introduced along with the potential invasive species.

Little is known about the parasites and pathogens of *C. intestinalis* (Carver et al. 2006a). No diseases have yet been documented in *C. intestinalis*, but the species is known to possess parasitic or commensal copepods belonging to the order Doropygidae (Millar 1971). Becheikh et al. (1996) documented the presence of the copepod *Pachypygus gibber* inside the branchial sac of *C. intestinalis* but it apparently does not impact survival or growth. Pastore (2001) isolated eight species of copepods from the branchial sac of *C. intestinalis* in the Mediterranean Sea; the three most common were *P. gibber*, *Hermannella rostrata* and *Lichomolgus canui* while Ooishi and O'Reilly (2004) isolated another copepod species, *Haplostoma eruca*, from the intestine of *C. intestinalis* in Scotland. Given that past introductions of *C. intestinalis* were likely due to transport of adult organisms via hull fouling it is possible that these commensal copepods also were transferred. Populations in Atlantic Canada have not been examined for these potential fellow travelers. Tan et al. (2002) reported that biofouling of nets by *C. intestinalis* increased the disease risk to farmed salmon in Tasmania as *C. intestinalis* serves as a repository for the amoeba that is responsible for Amoebic Gill Disease (AGD).

For tunicates that are transported as fouling organisms, it is usually the progeny of these organisms that become established at new locations rather than dislodgement of the individual or colony. This dramatically reduces the probability that a pathogen, parasite or fellow traveler will be introduced at the same time as larval stages often are assumed to be relatively insusceptible to these agents.

(2) Estimate the probability that the pathogen, parasite or fellow traveler will encounter susceptible organisms or suitable habitat.

It is unknown how species specific the reported commensal copepods of *C. intestinalis* are. However, there are few solitary tunicates in Canadian waters that attain sizes similar to *C. intestinalis* which might suggest the size of the branchial sac is limiting for these commensal copepods. The one notable exception is *S. clava*, another invader. In Pacific waters, *C. savignyi* is very similar to *C. intestinalis* and it is very likely that any commensal copepod that can survive in *C. intestinalis* also would survive in *C. savignyi*.

It is probable that the amoeba responsible for AGD could be transported with *C. intestinalis* to finfish aquaculture sites in Atlantic or Pacific Canada and could impact both cultured and wild fish stocks.

(3) Final Rating: pathogen, parasite or fellow traveler for *Ciona intestinalis*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Low	High	Low	High
Survival	Low	High	Low	High
Reproduction	Low	High	Low	High
Spread	Low	High	Low	High
Overall	**Low**	**High**	**Low**	**High**

Step 2: Determining the Consequences of Establishment of a Pathogen, Parasite or Fellow Traveler

(1) Ecological impacts on native ecosystems both locally and within the region including disease outbreak, reduction in reproductive capacity, habitat changes, etc.

Thus far the only pathogen, parasite or fellow travelers identified include a group of commensal copepods and a pathogenic amoeba. It is probable that the commensal species could be rather host-specific and that impacts on native ecosystems would be minimal. However, given considerable finfish aquaculture in some locations, infestation of *C. intestinalis* harboring this amoeba could have significant impacts on salmon production. Further, since wild salmon often come in close proximity to finfish aquaculture sites during at least part of their life cycle, the potential for transfer to wild salmon stocks exist. Given recent declines in many BC salmon populations, an additional stressor like AGD would be detrimental to overall stock health and productivity.

For tunicates that are transported as fouling organisms, it is usually the progeny of these organisms that become established at new locations rather than dislodgement of the individual or colony. This dramatically reduces the probability that a pathogen, parasite or fellow traveler will be introduced at the same time as larval stages often are assumed to be relatively insusceptible to these agents.

(2) Genetic impacts on local self-sustaining stocks or populations (i.e. whether the pathogen, parasite, or fellow traveler affects the genetic characteristics of native stocks or species).

Nothing is known about the potential genetic impacts on local populations. However, there are species related to possible travelers native to Canada; therefore the potential for genetic contamination exists.

(3) Final Rating: pathogen, parasite or fellow traveler for *Ciona intestinalis*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Moderate	High	Moderate	High
Genetic Consequence	Low	High	Low	High

Step 3: Estimating Pathogen, Parasite or Fellow Traveler Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.

Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Pathogen, Parasite or Fellow Traveler Overall Risk Potential for *Ciona intestinalis*.

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	Moderate	High	Moderate	High
Genetic	Low	High	Low	High

Golden Star Tunicate (*Botryllus schlosseri*)

Background and biology

Colonial tunicates belonging to the family Botryllidae are soft, smooth and fleshy in texture. They may take a variety of forms from thin flat encrusting mats to thick irregular lobes or projections depending on the shape of the substrate. The colonies are made up of small zooids (1-2 mm) or individuals arranged in a stellate or star-shaped pattern around a shared exhalent aperture (Van Name 1945). A wide range of color morphs are listed for *B. schlosseri* including purple, green and orange in New Zealand (Millar 1982) or blue, white and orange on the west coast (Pederson et al. 2005). Records for the east coast of the US indicate that *B. schlosseri* may have purple zooids with brown margins, red zooids with orange margins or appear purple-red throughout (Plough 1978). In Atlantic Canada, this species is typically bi-colored with yellow or white zooids and brown, grey or black margins.

B. schlosseri is a cyclical hermaphrodite with male and female gonads that are separate and mature brooded larvae can be seen through the translucent tunic wall (Millar 1966). Further, this species can undergo asexual reproduction (selfing or budding) that produces homozygous offspring (Sabbadin 1989). Tadpole larvae of *B. schlosseri* can swim freely for up to 36 hours settling just prior to metamorphosis (Berrill 1950, Berril 1975) but most larvae remain within a few meters of the parental colony (Grosberg 1987). Larvae can settle near another colony and fuse or can grow into a new colony (Rinkevich and Weissman 1987; Chadwick-Furman and Weissman 2003). *Botryllus schlosseri* can survive stressful environmental conditions by discontinuing sexual reproduction and reducing the rate of budding until there is only one bud per zooid (Millar 1967). If all zooids are lost and only the vascular ampullae in the matrix survive, then vascular budding can generate a new colony when conditions improve. As with other ascidians, *B. schlosseri* is a mucus filter feeder that can extract particles as small as 0.5 μm from the water column (Bone et al. 2003). A positive relationship exists been food availability and fecundity in *B. schlosseri* (Grosberg 1988) and more productive warmer waters favor egg production compared to less productive cooler waters that favor male production (Newlon et al. 2003).

Globally, botryllid tunicates are distributed from sub-arctic to temperate waters consistent with their tolerance to a wide range of environmental conditions. There is limited, detailed information on temperature or salinity tolerances. For example, Rasmussen (1997) documented an extensive population of *B. schlosseri* on the bottom of a Danish fjord system with high salinity and high summer temperatures. This species occurs on a variety of natural and artificial substrates such as rocks, algae, other organisms, floats, ship hulls and aquaculture equipment. *Botryllus schlosseri* may occur in areas with high sediment loads but is generally found on downward-facing or suspended surfaces which reduce the risk of smothering (Hiscock 2005). Also, *B. schlosseri* was one of the few species tolerant of extremely polluted conditions in Algeciras Harbour in southern Spain (Naranjo et al. 1996) and Lambert and Lambert (2003) noted that both *B. schlosseri* and *Botrylloides violaceus* were among a depleted ascidian community at a highly polluted site in Mexico (Ensenada). Although tolerant of pollution, colonies are susceptible to desiccation, and are rarely observed in intertidal areas unless damp and shaded (Rinkevich et al. 1993).

Natural dispersal can occur for both asexual buds and tadpole larvae. Under laboratory conditions Rabinowitz and Rinkevich (2004) found that unattached buds could survive for up to 150 days whereas attached buds survived for only 35 days leading them to speculate that *B. schlosseri* had a high capacity to disperse over great distances in the water column. In contrast, the dispersal potential of the larval tadpole stage which only swims freely for up to 36 hours is relatively minor with Hiscock (2005) estimating a larval setting time of less than one day with a dispersal potential of 1-10 km depending on local hydrodynamic conditions. Several population genetic studies also suggest that larval dispersal is quite limited. Other natural vectors for dispersal include rafting on eelgrass, algae or other forms of floating debris (Van Name 1910). Transport of free-swimming larvae in the ballast water of ships is unlikely because of their short larval cycle but hull fouling of recreational watercraft, coastal fishing fleets and dredging barges is very likely. Lambert and Lambert (2003) noted that large numbers of boats with fouled hulls, especially small pleasure craft. Further, slow-moving boats or towed barges may be more likely vectors than faster moving ships due to the reduced friction on the hull surface that could displace biofouling organisms such as tunicates. Aquaculture activities also have been responsible for the introduction of colonial tunicates, including *B. schlosseri* (Polk 1962, Lambert 2005b).

Known distribution

Botryllus schlosseri most likely originated from the Mediterranean Sea (Berrill 1950), but is now considered cosmopolitan as it can be found on all continents except Antarctica (Van Name 1945, Kott 1985). Current records are available for the Mediterranean Sea, the Black Sea, the Adriatic Sea (Italy and Greece), and the Suez Canal. The non-native distribution of *B. schlosseri* is detailed in Carver et al. (2006b) and lists populations outside of the Mediterranean Sea occurring in the North Sea and the North Atlantic Ocean including the coasts of Norway, Britain, France, Portugal, Spain, Denmark, Germany, and Sweden. In North America, this species has been reported from locations along both the Atlantic and Pacific coasts of both the United States and Canada. In 2007 this species was identified for the first time in coastal waters of Newfoundland (G. Perry, pers. comm.) and these reports were included to predict the potential distribution of this species in Canadian waters (see below). *Botryllus schlosseri* also has been reported from New Zealand and Australia and is believed to exist in Japan and China (Hong Kong).

Potential distribution in Canada

The potential distribution of *B. schlosseri* in Canadian waters is based on environmental conditions at the Canadian sites where *B. schlosseri* currently occurs on both the Atlantic and Pacific coasts. On the Atlantic coast considerable highly favorable habitat exists throughout much of the area (Figure 17). These highly favorable conditions exist around all of PEI, the coasts of New Brunswick and Nova Scotia, including the Bay of Fundy, the southern Gulf of St. Lawrence, including the Magdalen Islands and most coastal waters of Newfoundland (Figure 17). On the Pacific coast, a very high environmental match exists throughout the Strait of Georgia, the major inlets along the southwest coast of Vancouver Island (Barkley Sound, Claquot Sound, Nootka Sound and Esperanza Inlet) and much of the Central Coast of BC (Figure 18). A high environmental match exists for parts of the Queen Charlotte Islands, especially the eastern side along Dogfish Bank and parts of the north coast including Masset Inlet (Figure 18).

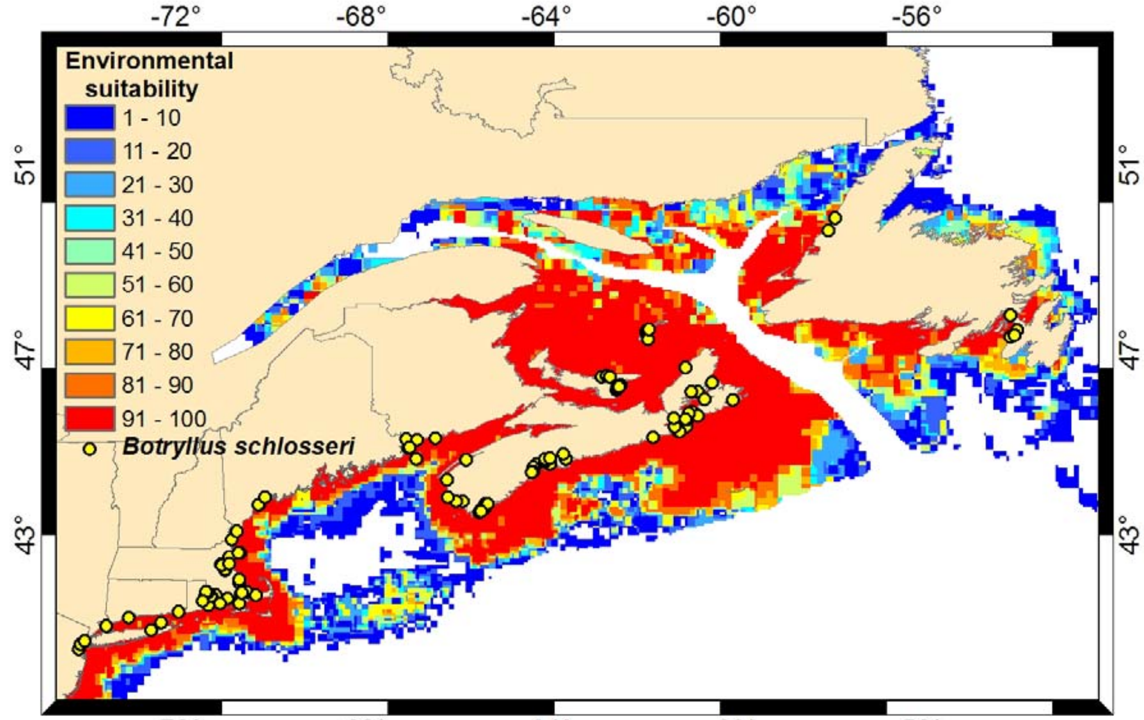


Figure 17: Potential distribution of *B. schlosseri* on the Atlantic coast based on temperature and salinity tolerances.

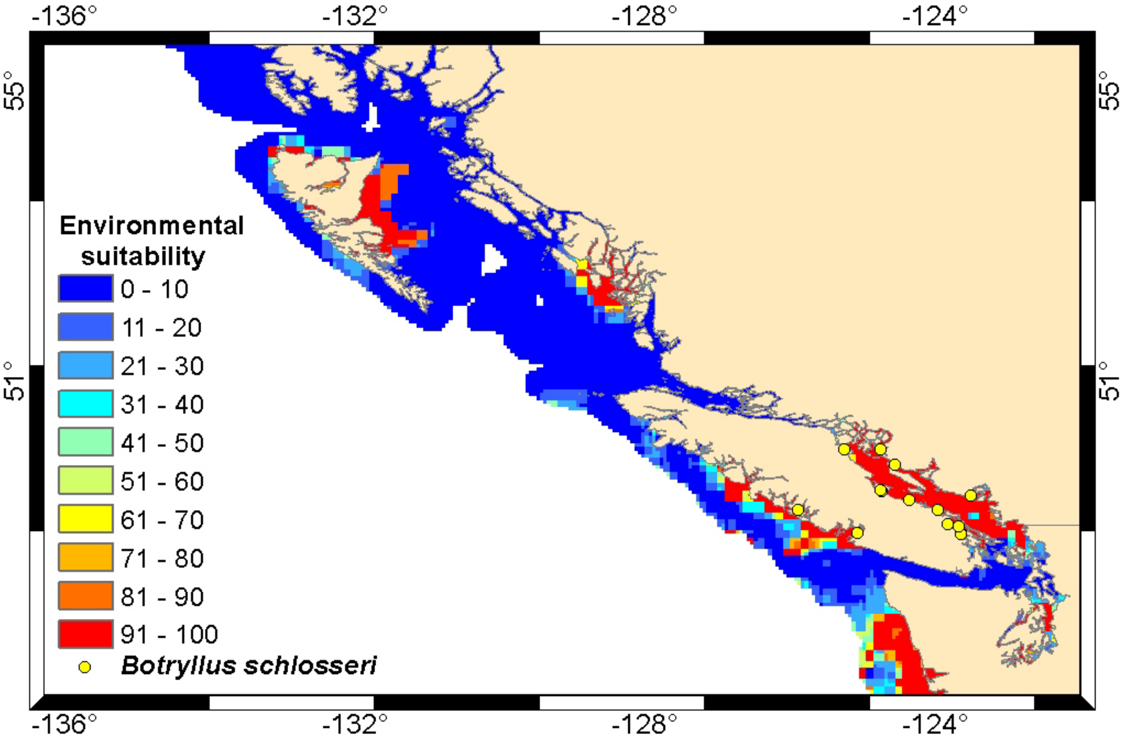


Figure 18: Potential distribution of *B. schlosseri* on the Pacific coast based on temperature and salinity tolerances.

Risk Assessment Part I – Aquatic Organism Ecological and Genetic Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate of probability of the organism successfully colonizing and maintaining a population if introduced.

The primary vectors proposed for the introduction of *B. schlosseri* worldwide include natural dispersal of buds or larvae, ship fouling, aquaculture and fisheries gear fouling. Natural dispersal can occur for both asexual buds and tadpole larvae. Other natural vectors for dispersal include rafting on eelgrass, algae or other forms of floating debris (Van Name 1910). Transport of free-swimming larvae in the ballast water of ships is unlikely because of their short larval cycle but hull fouling of recreational watercraft, coastal fishing fleets and dredging barges is very likely. Aquaculture-related activities also have been responsible for the introduction of colonial tunicates, including *B. schlosseri* (Polk 1962, Lambert 2005b). Given that this species has established populations on both the Atlantic and Pacific coasts it is certain *B. schlosseri* can arrive, survive and reproduce in Canadian waters. In addition, considerable, potential suitable habitat exists on both coasts (Figures 17 and 18) suggesting further spread or additional introductions are possible.

(2) If the organism escapes from the area of introduction, estimate the probability of its spreading.

Given the presence of populations on both the Atlantic and Pacific coasts, there is a very high probability that *B. schlosseri* could spread on both coasts. This biofouling species has an affinity for artificial structures on which it does very well (Lutzen 1999) and considerable such structure exists on both coasts, although potentially higher on the Pacific coast, especially structures related to aquaculture and recreational small craft harbors (marinas and anchorages) (Figures 5, 6 and 9).

(3) Final Rating: *Botryllus schlosseri*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Very High	Very Low	Very High	Very Low
Survival	Very High	Very Low	Very High	Very Low
Reproduction	Very High	Very Low	Very High	Very Low
Spread	Very High	Very Low	Very High	Very Low
Overall	**Very High**	**Very Low**	**Very High**	**Very Low**

Step 2: Determining the Consequences of Establishment of an Aquatic Organism

(1) Ecological impact on native ecosystems both locally and within each region.

Based on the results of its introduction throughout the world there is little doubt that *B. schlosseri* would have significant negative impacts on habitat structure and potentially foodweb and trophic structure of aquatic ecosystems by inducing changes in plant, invertebrate and possibly fish communities (Carver et al. 2006b). Also, Zajac et al. (1989) noted that *Botrylloides* sp. had a negative impact on the survival and growth of oyster spat probably as a result of localized food depletion and Arakawa (1990) noted that tunicates grew more rapidly than oyster spat, effectively reducing with their survival. This species' ability to settle on a range of artificial surfaces causes dense fouling of fishing gear, moorings, and ropes and is difficult and time-consuming to remove. In shellfish aquaculture operations, colonial tunicates may overgrow seed collectors thereby smothering young juveniles or excluding the settlement of the desired species. Finfish nets or shellfish cages also may become infested with mats of colonial tunicates which effectively eliminates the flow of oxygen and particles through the mesh (Cao et al. 1998). Finally, as a member of the biofouling community, colonial tunicates compete for space by overgrowing and smothering existing species; in some cases the net impact may be a reduction in community species diversity (Pederson et al. 2005).

The results of the expert survey suggested *B. schlosseri* would have moderate ecological consequences (Figure 3). The expert survey also indicated higher ecological consequences related to shellfish aquaculture (Figure 3).

(2) Genetic impacts on local self-sustaining stocks or populations.

Sabbadin and Graziani (1967) found that genetically distinct sub-populations of *B. schlosseri* existed under similar ecological conditions within the Lagoon of Venice.

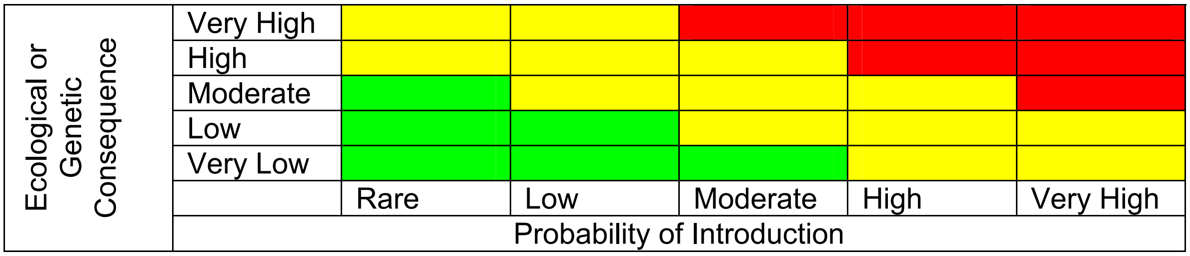
Further, Yund and O'Neil (2000) noted that genetic differentiation may occur over very short distances (8 to 21 m). Thus, it appears the most probable genetic impacts are on other *B. schlosseri* populations. It is highly unlikely *B. schlosseri* would have any genetic impact on other native fauna on either coast.

(3) Final Rating: *Botryllus schlosseri*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Moderate	Moderate	Moderate	Moderate
Genetic Consequence	Very Low	Low	Very Low	Low

Step 3: Estimating Aquatic Organism Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.



Ecological or Genetic Consequence						
	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Aquatic Organism Overall Risk Potential for *Botryllus schlosseri.*

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	High	Moderate	High	Moderate
Genetic	Moderate	Low	Moderate	Low

Risk Assessment Part II – Pathogen, Parasite or Fellow Traveler Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate the probability that a pathogen, parasite or fellow traveler may be introduced along with the potential invasive species.

Few studies are available on the disease agents that may affect colonial tunicates. Moiseeva et al. (2004) described a progressive fatal disease called 'cup cell disease' where the time course from first appearance to death was 30 to 45 days. The disease-causing agent was believed to be a haplosporidian protist. Levine (1981) examined 361 ascidian specimens of the 20 most common species in California and 14 species were parasitized. Oosihi (1999) described a copepod parasite *Botryllophilus ruber* on *B. schlosseri*, noting that an introduction of a species may actually come with an assemblage of parasites but no further quantitative or qualitative data were available.

For tunicates that are transported as fouling organisms, it is usually the progeny of these organisms that become established at new locations rather than dislodgement of the individual or colony. This dramatically reduces the probability that a pathogen, parasite or fellow traveler will be introduced at the same time as larval stages often are assumed to be relatively insusceptible to these agents.

(2) Estimate the probability that the pathogen, parasite or fellow traveler will encounter susceptible organisms or suitable habitat.

It is probable that the protist responsible for cup cell disease could infect native colonial tunicates. It is unclear if this protist was encountered by Levine (1981) but clearly many ascidian species have a complement of co-occurring species. The extent to which they are pathogenic or parasitic remains unknown.

(3) Final Rating: pathogen, parasite or fellow traveler for *Botryllus schlosseri*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Low	High	Low	High
Survival	Low	High	Low	High
Reproduction	Low	High	Low	High
Spread	Low	High	Low	High
Overall	**Low**	**High**	**Low**	**High**

Step 2: Determining the Consequences of Establishment of a Pathogen, Parasite or Fellow Traveler

(1) Ecological impacts on native ecosystems both locally and within the region including disease outbreak, reduction in reproductive capacity, habitat changes, etc.

Oosihi (1999) described a copepod parasite *Botryllophilus ruber* on *B. schlosseri* but it is unclear how species-specific this copepod is. However, given the many general similarities between *B. schlosseri* and *B. violaceus*, this copepod could be parasitic on *B. violaceus* as well.

(2) Genetic impacts on local self-sustaining stocks or populations (i.e. whether the pathogen, parasite, or fellow traveler affects the genetic characteristics of native stocks or species).

Nothing is known about the potential genetic impacts on local populations. However, there are species related to possible travelers native to Canada; therefore the potential for genetic contamination exists.

(3) Final Rating: pathogen, parasite or fellow traveler for *Botryllus schlosseri*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Low	High	Low	High
Genetic Consequence	Low	High	Low	High

Step 3: Estimating Pathogen, Parasite or Fellow Traveler Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.

Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Pathogen, Parasite or Fellow Traveler Overall Risk Potential for *Botryllus schlosseri*.

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	Low	High	Low	High
Genetic	Low	High	Low	High

Violet Tunicate (*Botrylloides violaceus*)

Background and biology

Botrylloides violaceus is a colonial tunicate that is soft, smooth and fleshy in texture. Similar to *B. schlosseri*, they may take a variety of forms from thin flat encrusting mats to thick irregular lobes or projections depending on the shape of the substrate. Colonies are made up of relatively large zooids (2-4 mm) distributed in elongated irregular rows around a common aperture (Van Name 1945). Colonies of *B. violaceus* are typically monocolored; the range of hues includes bright orange, burgundy, dull pink, lavender or purple (Lambert and Lambert 2003). This species occurs in sheltered areas on natural substrates such as algae or on artificial substrates such as floating docks or wharf pilings and is generally restricted to zones <50 m deep.

Relatively few studies have documented the life-history traits of *B. violaceus*, although Yamaguchi (1975) documented the life cycle of a botryllid in Japanese waters. The mean number of zooids exceeded 100 per colony within 2 weeks in the summer (20-25°C) and 4 weeks in the winter (14-20°C). Doubling time decreased by a factor of 3 with a 10°C increase in temperature. Mukai (1977) suggested that substrate or space limitation may have triggered the switch from asexual colony growth to sexual reproduction. Few data are available on the temperature and salinity tolerance of *B. violaceus*. Stachowicz et al. (2002) observed that elevated seawater temperatures favored the growth of *B. violaceus* over *B. schlosseri* while Lambert and Lambert (2003) noted that both species were among a depleted ascidian community at a highly polluted site in Mexico (Ensenada). Colonies are susceptible to desiccation, and are rarely observed in intertidal areas unless damp and shaded (Rinkevich et al. 1993).

Botryllid tunicates are cyclical hermaphrodites but unlike some colonial tunicates, these botryllid tunicates also can undergo asexual reproduction (selfing or budding) that produces homozygous offspring (Sabbadin 1989). Larvae can settle near another colony and fuse or can grow into a new colony (Rinkevich and Weissman 1987; Chadwick-Furman and Weissman 2003). *Botrylloides violaceus* is a viviparous species that has a gestation period of approximately one month during which time the embryo receives nutrients from blood flowing through the tunic. Towards the end of the cycle the body of the mother zooid fully disintegrates leaving only the brood pouch containing the surviving larva (Mukai et al. 1987). The tadpole larvae break through the body wall to reach the common atrial cavity (Berrill 1947). Unlike other members of this family, the larvae of *B. violaceus* are huge (1.7 mm) with 24-32 ampullae and swim for only a brief period before settling. Larvae generally require only 1-2 days to attach themselves, becoming a fully functional zooid (Takeuchi 1980).

Natural dispersal can occur for both asexual buds and tadpole larvae. However, given the short duration of the larval stage, dispersal via this vector should be limited. Other natural vectors for dispersal include rafting on eelgrass, algae or other forms of floating debris (Van Name 1910). Transport of free-swimming larvae in the ballast water of ships is unlikely because of their short larval cycle but hull fouling of recreational watercraft, coastal fishing fleets and dredging barges is very likely. Lambert and Lambert (2003) noted that large numbers of boats with fouled hulls, especially small pleasure craft. Further, slow-moving boats or towed barges may be more likely vectors than faster moving ships due to the reduced friction on the hull surface that could displace biofouling organisms such as tunicates. Aquaculture-related activities also have been responsible for the introduction of colonial tunicates (Lambert 2005b).

Known distribution

Botrylloides violaceus is believed to have originated from the northwest Pacific, most likely from Japan (Berrill 1950). This species is listed as one of the major biofouling species in Japanese waters (Tokioka 1953) and Yamaguchi (1975) described it as one of the most common fouling organisms on cultured pearl oysters in Japan. *Botrylloides violaceus* has been reported from both the Atlantic and Pacific coasts of North America in both the United States and Canada. This species also has been reported from Australia, Italy and the Netherlands.

Potential distribution in Canada

The potential distribution of *B. violaceus* in Canadian waters is based on environmental conditions at the Canadian sites where *B. violaceus* currently occurs on both the Atlantic and Pacific coasts. On the Atlantic coast, highly favorable habitat exists throughout the southern Gulf of St. Lawrence, especially in waters around PEI (Figure 19). Additional highly favorable habitat exists around Cape Breton Island, the head of the Bay of Fundy and along the Atlantic coast of Nova Scotia (Figure 19). On the Pacific coast a very high environmental match exists throughout much of BC (Figure 20). This favorable habitat stretches from the Strait of Georgia, through Johnstone Strait, the Central Coast, the North Coast and into southeastern Alaska (Figure 20). Also, the major inlets along the southwest coast of Vancouver Island (Barkley Sound, Claquot Sound, Nootka Sound and Esperanza Inlet) show a very high environmental match (Figure 20). A high environmental match exists for parts of the Queen Charlotte Islands, especially the eastern side along Dogfish Bank (Figure 20).

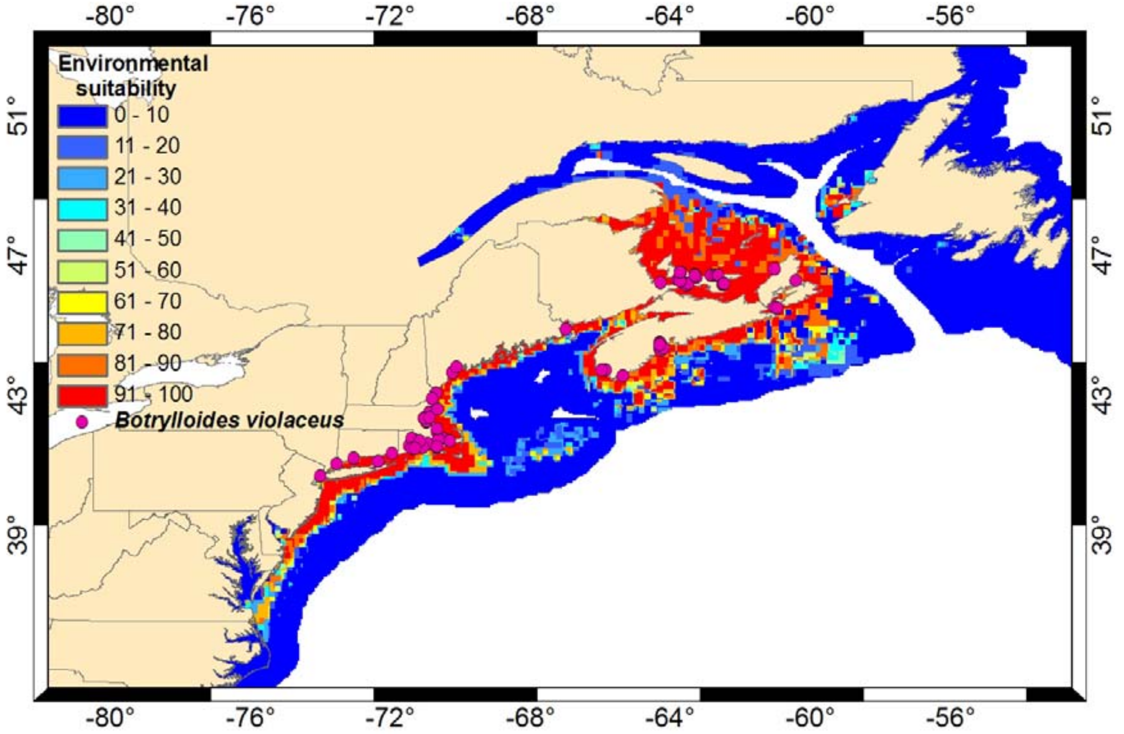


Figure 19: Potential distribution of *B. violaceus* on the Atlantic coast based on temperature and salinity tolerances.

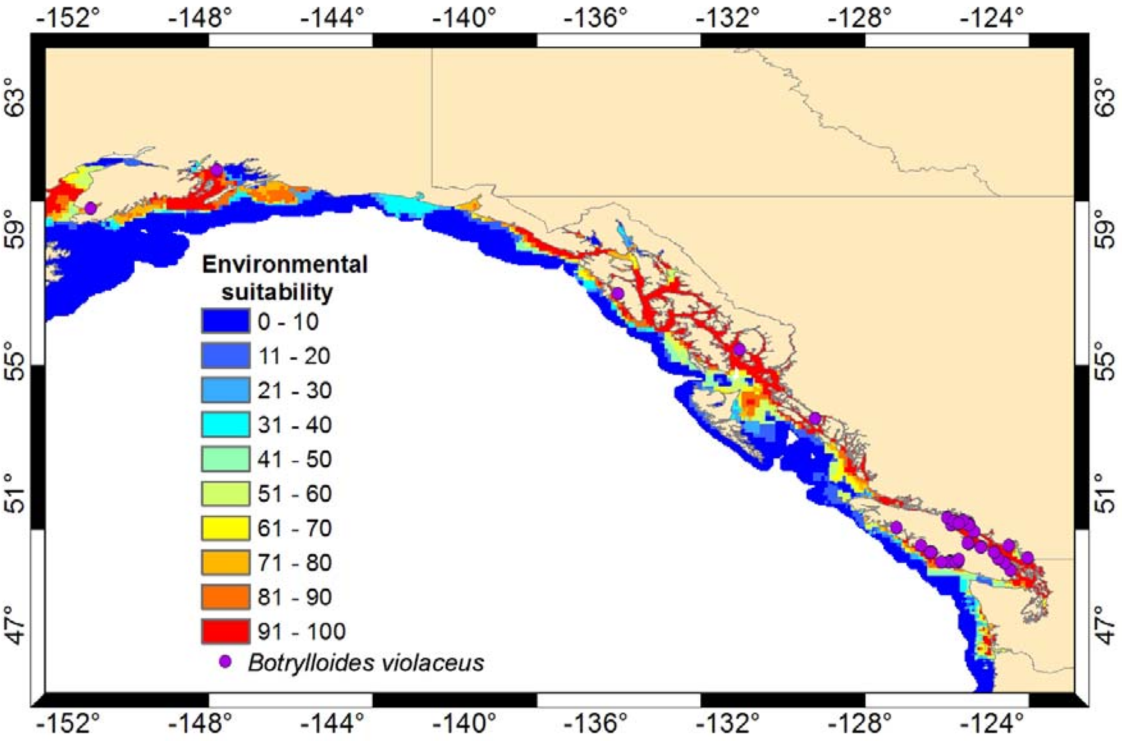


Figure 20: Potential distribution of *B. violaceus* on the Pacific coast based on temperature and salinity tolerances.

Risk Assessment Part I – Aquatic Organism Ecological and Genetic Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate of probability of the organism successfully colonizing and maintaining a population if introduced.

The primary vectors proposed for the introduction of *B. violaceus* worldwide include ship fouling, live oyster transfer and fisheries gear fouling. On the Pacific coast the most probable vector and pathway was historical aquaculture transfers directly from Japan. Prior to concerns about non-native species or their associated hitchhikers, adult Japanese oyster were introduced directly from Japan to Canada for aquaculture purposes (Quayle 1969, Bourne 1979). Similar practices existed along the American Pacific coast so secondary spread from these sites to Canadian waters can not be dismissed. In contrast, on the Atlantic coast, hull fouling on commercial or recreational vessels is more likely based on the timing of invasions. Fouling on a barge is suspected for introductions in PEI, most likely via the US but given established *B. violaceus* colonies on both sides of the Atlantic Ocean prior to its arrival in the Canadian Maritimes, the actual source population remains elusive. Natural vectors of dispersal appear unlikely for this species given the very short larval phase and the fact that adults are sedentary, biofouling organisms. Given that *B. violaceus* have established populations on both the Atlantic and Pacific coasts it is certain the species can arrive, survive and reproduce in Canadian waters. In addition, considerable, potential suitable habitat exists on both coasts (Figures 19 and 20) suggesting further spread or additional introductions are possible.

(2) If the organism escapes from the area of introduction, estimate the probability of its spreading.

Given the presence of populations on both the Atlantic and Pacific coasts, there is a very high probability that *B. violaceus* could spread on both coasts. This biofouling species has an affinity for artificial structures on which it does very well (Lutzen 1999) and considerable such structure exists on both coasts, although potentially higher on the Pacific coast, especially structures related to aquaculture and recreational small craft harbors (marinas and anchorages) (Figures 5, 6 and 9). Numerous potential vectors of dispersal also exist, especially those related to pleasure craft movements or movements associated with aquaculture-related activities.

(3) Final Rating: *Botrylloides violaceus*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Very High	Very Low	Very High	Very Low
Survival	Very High	Very Low	Very High	Very Low
Reproduction	Very High	Very Low	Very High	Very Low
Spread	Very High	Very Low	Very High	Very Low
Overall	**Very High**	**Very Low**	**Very High**	**Very Low**

Step 2: Determining the Consequences of Establishment of an Aquatic Organism

(1) Ecological impact on native ecosystems both locally and within each region.

Based on the results of its introduction throughout the world (Carver et al. 2006b), there is little doubt that *B. violaceus* would have significant negative impacts on habitat structure and potentially foodweb and trophic structure of aquatic ecosystems by inducing changes in plant, invertebrate and possibly fish communities. Zajac et al. (1989) noted that *Botrylloides* sp. had a negative impact on the survival and growth of oyster spat probably as a result of localized food depletion and Arakawa (1990) noted that tunicates grew more rapidly than oyster spat, effectively reducing with their survival. This species' ability to settle on a range of artificial surfaces causes dense fouling of fishing gear, moorings, and ropes and is difficult and time-consuming to remove. Also, in shellfish aquaculture operations, colonial tunicates may overgrow seed collectors thereby smothering young juveniles or excluding the settlement of the desired species. Finfish nets or shellfish cages also may become infested with mats of colonial tunicates which effectively eliminates the flow of oxygen and particles through the mesh (Cao et al. 1998). Finally, as a member of the biofouling community, colonial tunicates compete for space by overgrowing and smothering existing species; in some cases the net impact may be a reduction in community species diversity (Pederson et al. 2005). Also, Sebens (1997) noted that the combined presence of sea urchins and *B. violaceus* severely impacted the indigenous assemblage, and this tunicate seemed immune to urchin grazing. It was argued that, even in the absence of the urchins, the capacity of *B.*

violaceus to overgrow and outcompete indigenous species may have compromised the natural habitat.

The results of the expert survey suggested *B. violaceus* would have moderate ecological consequences (Figure 3). Again, based on the expert survey and literature accounts, higher ecological consequences are expected for shellfish aquaculture (Figure 3).

(2) Genetic impacts on local self-sustaining stocks or populations.

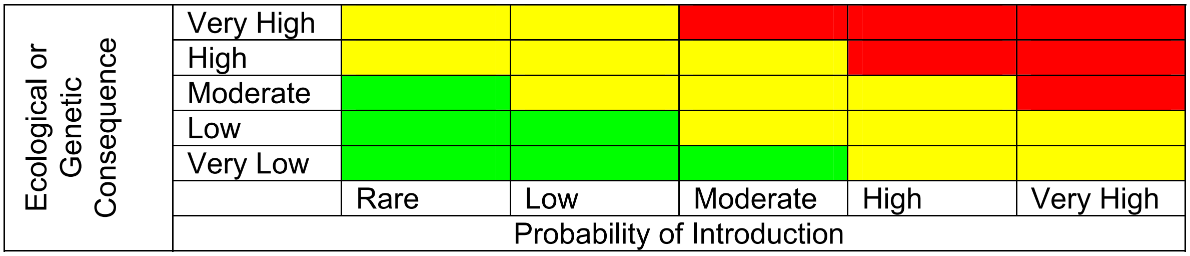
It is highly unlikely *B. violaceus* would have any genetic impact on native fauna on either coast.

(3) Final Rating: *Botrylloides violaceus*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Moderate	Moderate	Moderate	Moderate
Genetic Consequence	Very Low	Low	Very Low	Low

Step 3: Estimating Aquatic Organism Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.



Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Aquatic Organism Overall Risk Potential for *Botrylloides violaceus.*

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	High	Moderate	High	Moderate
Genetic	Moderate	Low	Moderate	Low

Risk Assessment Part II – Pathogen, Parasite or Fellow Traveler Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate the probability that a pathogen, parasite or fellow traveler may be introduced along with the potential invasive species.

Little is known about the parasites and pathogens of *B. violaceus* (Carver et al. 2006b) and few studies are available on the disease agents that may affect colonial tunicates in general. Moiseeva et al. (2004) described a progressive fatal disease called cup cell disease where the disease-causing agent was believed to be a haplosporidian protist. Also, Oosihi (1999) described a copepod parasite *Botryllophilus ruber* on *B. schlosseri* that might parasitize *B. violaceus* as well given the number of similarities between species.

(2) Estimate the probability that the pathogen, parasite or fellow traveler will encounter susceptible organisms or suitable habitat.

It is unclear if *B. violaceus* is susceptible to this protist or if it could serve as a host or repository that would eventually cause disease in other species.

(3) Final Rating: pathogens, parasites or fellow travelers for *Botrylloides violaceus*.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Low	Very High	Low	Very High
Survival	Low	Very High	Low	Very High
Reproduction	Low	Very High	Low	Very High
Spread	Low	Very High	Low	Very High
Overall	**Low**	**Very High**	**Low**	**Very High**

Step 2: Determining the Consequences of Establishment of a Pathogen, Parasite or Fellow Traveler

(1) Ecological impacts on native ecosystems both locally and within the region including disease outbreak, reduction in reproductive capacity, habitat changes, etc.

Given that no known pathogens, parasites or fellow travelers have been identified specifically for *B. violaceus*, impacts are likely negligible, if they exist. Given the invasion history of this tunicate, which may include several decades on the west coast of North America following introduction associated with Japanese oyster transfers, if other species were being transported with *B. violaceus*, these species should have been identified by now.

For tunicates that are transported as fouling organisms, it is usually the progeny of these organisms that become established at new locations rather than dislodgement of the individual or colony. This dramatically reduces the probability that a pathogen, parasite or fellow traveler will be introduced at the same time as larval stages often are assumed to be relatively insusceptible to these agents.

(2) Genetic impacts on local self-sustaining stocks or populations (i.e. whether the pathogen, parasite, or fellow traveler affects the genetic characteristics of native stocks or species).

Nothing is known about the potential genetic impacts on local populations.

(3) Final Rating: pathogens, parasites or fellow travelers for *Botrylloides violaceus*.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Very Low	Very High	Very Low	Very High
Genetic Consequence	Very Low	Very High	Very Low	Very High

Step 3: Estimating Pathogen, Parasite or Fellow Traveler Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.

Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Pathogen, Parasite or Fellow Traveler Overall Risk Potential for *Botrylloides violaceus.*

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	Low	Very High	Low	Very High
Genetic	Low	Very High	Low	Very High

***Didemnum* sp.**

Background and biology

It is important to note that the Family Didemnidae remains taxonomically complex and controversial and no general consensus exists on what species of *Didemnum* is invading coastal waters worldwide (e.g., Valentine 2003, Cohen 2005, Daniel and Therriault 2007). For this report we adopt the view that *Didemnum* sp. is native to Japan, with one known type-location at Ise Bay, near Nagoya (Gretchen Lambert, pers. comm.).

Didemnum sp. is characterized by many small zooids (1-2 mm) embedded in a sheet-like, gelatinous matrix called a tunic (Kott 1989, Monniot et al. 1991, Kott 2001, Tyree 2001, Pederson 2004, Cohen 2005, Lambert 2005b, Lambert and Lambert 2005). Embedded within the tunic are white, calcareous stellate spicules that give the colony a white dot appearance (Kott 2004, Cohen 2005, Lambert and Lambert 2005, Geerlofs and Gordon 2005). These colonies form thin encrusting sheets or irregularly lobed encrusting mounds depending on colony location and age (Valentine 2003, Cohen 2005, Geerlofs and Gordon 2005).

Colonial ascidians typically live from 1 to 3 years (Berrill 1950, O'Clair and O'Clair 1998) but age determination is difficult due to the periodic regeneration and reduction colonies undergo (Millar 1971, Tyree 2001). Colonial tunicates can grow and spread both by sexual reproduction via tadpole larvae and asexual reproduction via propagative budding, making them good invaders. Also, unlike other ascidians, didemnids can bud while the gonads are maturing (Monniot et al. 1991) and can undergo precocious budding where blastozooids are produced in the larvae within the tunic (Kott 2001) further enhancing their invasion potential. Factors such as season, temperature, and habitat type each affect the extent of *Didemnum* sp. colony growth. *Didemnum* sp. has been reported to grow rapidly during summer months (Valentine et al. 2005a) but slowly during winter months as the colony often reduces to a dormant bud (Millar 1971, Nakauchi and Kawamura 1990, Monniot et al. 1991). Habitat type also affects *Didemnum* sp. colony growth with faster growth reported from open coastal habitats due to an apparent competitive advantage over other co-occurring species that can become overgrown by the colony (Osman and Whitlatch 2005).

Didemnum sp. is hermaphroditic and ovoviviparous producing tadpole larvae via sexual reproduction (Van Name 1945, Monniot et al. 1991, Tyree 2001). Ascidian gonad development and spawning are controlled by several factors with light and temperature, which change seasonally, apparently most important (Millar 1971, Berrill 1975, Svane and Young 1989, Forward et al. 2000, Bates 2005, Lambert 2005a). For ascidians in temperate waters spawning generally occurs during summer months (Cohen 2005, Lambert 2005b).

Released larvae only swim in the water column for minutes to hours before settling (Monniot et al. 1991, Cohen 2005, Lambert and Lambert 2005, Valentine et al. 2005b) thereby lowering their ability to spread quickly naturally. Larval dispersal away from the parental colony is affected by currents, wave action, wind exposure, angle of the sun, and water temperature (Hurlbut 1992, Stoner 1992, Forward et al. 2000) but the short time ascidian larvae spend in the water column may limit the impact such factors exert on larval dispersal thereby limiting spread.

As a sessile marine invertebrate, movement of the adult colony of *Didemnum* sp. is limited, although the colony can expand through larval dispersal, fragmentation or moving along with its habitat (e.g., rafting). Modes of transport, and possible introduction, include hull fouling of ships and boats, fouling of fishing gear, fishing trawls, dredges, colony fragments in ballast water, movement of oyster and other shellfish stock or gear, and natural process like currents (Millar 1971, Monniot et al. 1991, Lambert 2002, Cohen 2005, Lambert 2005b, Valentine et al. 2005b).

Didemnum sp. tolerates a wide range of environmental conditions including temperature, salinity, and water quality (Millar 1971, Lambert and Lambert 1998, Lambert 2002, Lambert 2005b). *Didemnum* sp. colonies are found in water temperatures ranging from –2°C to 24°C with 4°C possibly representing a critical temperature where cooler temperatures limit spread or growth (Cohen 2005, Lambert 2005b, Valentine et al. 2005a). Ascidians tend to hibernate, die off, or go dormant when temperatures are not favorable and resume growth and reproduction when favorable conditions return (Millar 1971, Nakauchi and Kawamura 1990, Monniot et al. 1991). Colonies on Georges Bank are found in 4°C to 15°C water and apparently do not die off during the winter months, possibly because temperatures don't become cold enough to initiate the dormancy period (Valentine et al. 2005a).

Didemnum sp., like most ascidians, is rarely found in salinities less than 25‰ but it can tolerate wide fluctuations in salinity (Millar 1971, Vázquez and Young 2000, Lambert 2005b). However, at salinities lower than 20‰ ascidians tend to close their siphons which can lead to zooid death (Stoner 1992, Tyree 2001). Ascidian larvae also are affected by salinity as larvae remain below a halocline (Vázquez and Young 1996). In lower salinity waters, colonial tunicate larvae do not metamorphose or metamorphose slowly, resulting in reduced survival (Vázquez and Young 2000).

Generally, ascidians can not withstand extended periods of air exposure (desiccation) but can tolerate a wide range of water quality. For example, when exposed to air for more than 3 hours per day for 28 consecutive days *Didemnum* sp. died (Valentine et al. 2005a). Many ascidians are tolerant of high water pollution, high particulate and dissolved organic matter, and may even consume organic pollutants like heavy metals and hydrocarbons (Monniot et al. 1991, Tyree 2001, Lambert 2005b).

Didemnum sp. has the ability to colonize a variety of natural and artificial hard structures but not muddy or sandy substrates from the intertidal zone to about 65m as on Georges Bank (Coutts 2002, Valentine 2003, Cohen 2005, Valentine et al. 2005b). *Didemnum* sp. grow on natural substrates such as rock outcrops, gravel seabeds, pebbles, cobble, boulders, tunicates, sponges, macroalgae, hydroids, anemones, bryozoans, polychaetes, scallops, mussels, oysters, limpets, barnacles, other ascidians, shell, and hard clay with stones (Berrill 1950 and 1955, Monniot et al. 1991, Lambert 2002, Valentine 2003, Cohen 2005, Gittenberger 2005, Valentine et al. 2005a and 2005b). *Didemnum* sp. can colonize artificial structures including docks, floats, wood and metal pilings, moorings, rope, steel chain, automobile tires, plastic, ship hulls, buoys, jetties, concrete, iron, and wood (Millar 1971, Monniot et al. 1991, Tyree 2001, Lambert 2002, Valentine 2003, Cohen 2005, Geerlofs and Gordon 2005a and 2005b).

Prior to settlement, the tadpole larvae do not actively feed but following settlement they become sessile marine filter feeders (Berrill 1955, Jeffery 1997, Monniot et al. 1991, Tyree 2001, Lambert and Lambert 2005). Ascidian diets consist primarily of phytoplankton, suspended particulate matter, diatoms, invertebrate larvae, and suspended bacteria (Millar 1971, Monniot et al. 1991, Bak et al. 1998, O'Clair and O'Clair 1998, Tyree 2001). *Didemnum* sp. has few known predators possibly due to the low nutritive value of the tunic (Tarjuelo et al. 2002, Lambert 2005b). The common periwinkle, *Littorina littorea*, preyed upon a weakened *Didemnum* sp. colony (Valentine 2003, Carman 2005, Valentine et al. 2005a) and chitons, sea urchins and sea stars are reported predators of *D. vexillum* (Valentine 2003). Also, little is known about the diseases and parasites specific to *Didemnum* sp. In general, ascidians have communal, parasitic and symbiotic organisms living in their tunics, branchial chambers, gut tracts, or atrial chambers (Millar 1971, Monniot 1990, Monniot et al. 1991, O'Clair and O'Clair 1998, Kott 2001, Tyree 2001). Carman (2005) found 18 species of benthic foraminifera in *Didemnum* sp. samples but all were dead. Copepods, amphipods, shrimps, polychaetes, molluscs, decapods, hydroids, algae, nematodes, ciliates, protozoans (gregarines), suctorian ciliates, and pea crab have been found living on or in ascidians (Millar 1971, Monniot 1990, Monniot et al. 1991, O'Clair and O'Clair 1998, Kott 2001, Tyree 2001, Coutts 2002). These organisms are thought to do relatively little harm to the organism but some parasitic copepods, nemertean worms and decapod crabs could cause minor damage to the tunicate (Monniot 1990, Monniot et al. 1991, O'Clair and O'Clair 1998).

Known distribution

Given the ongoing taxonomic debate concerning species identity, we have assumed that *Didemnum* sp. is native to Japan (Cohen 2005, Daniel and Therriault 2006). Further, we consider the type location for *Didemnum* sp. to be Ise Bay, near Nagoya (Gretchen Lambert, pers. comm.), but it is probable this species is more widely distributed. *Didemnum* sp. has been introduced to six countries, including Canada (BC) worldwide: Ireland, France, the Netherlands, New Zealand, and the United States with offshore colonies identified on the US side of Georges Bank (Valentine et al. 2005b).

Potential distribution in Canada

The potential distribution of *Didemnum* sp. in Canadian waters is based on environmental conditions at the Canadian sites where *Didemnum* sp. currently occurs on the Pacific coast. On the Atlantic coast, highly favorable habitat exists around the southeastern waters of Nova Scotia and locations around Cape Breton Island (Figure 21). Additional favorable habitat exists in offshore waters, including Georges Bank where this species is known to occur (Figure 21). On the Pacific coast a very high environmental match exists through the Strait of Georgia, within the major inlets along the west coast of Vancouver Island (Barkley Sound, Claquot Sound, Nootka Sound and Esperanza Inlet) and around much of the Queen Charlotte Islands (Figure 22). Additional favorable habitat exists in deeper waters in Hecate Strait including those around Goose Bank in Queen Charlotte Sound (Figure 22).

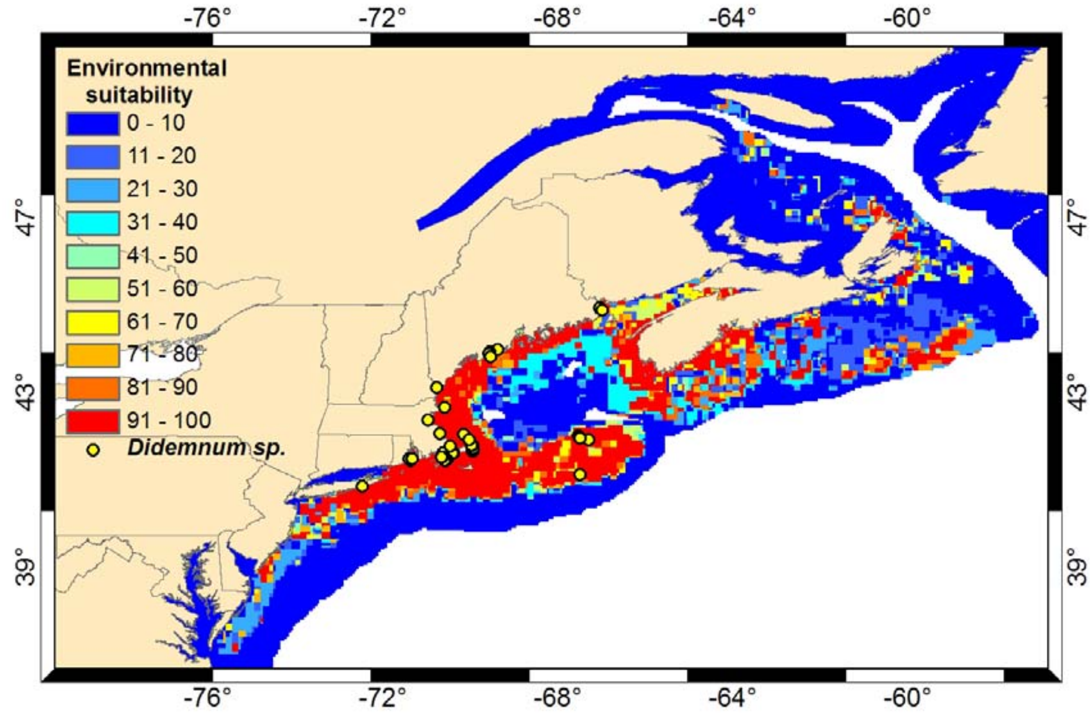


Figure 21: Potential distribution of *Didemnum* sp. on the Atlantic coast based on temperature and salinity tolerances.

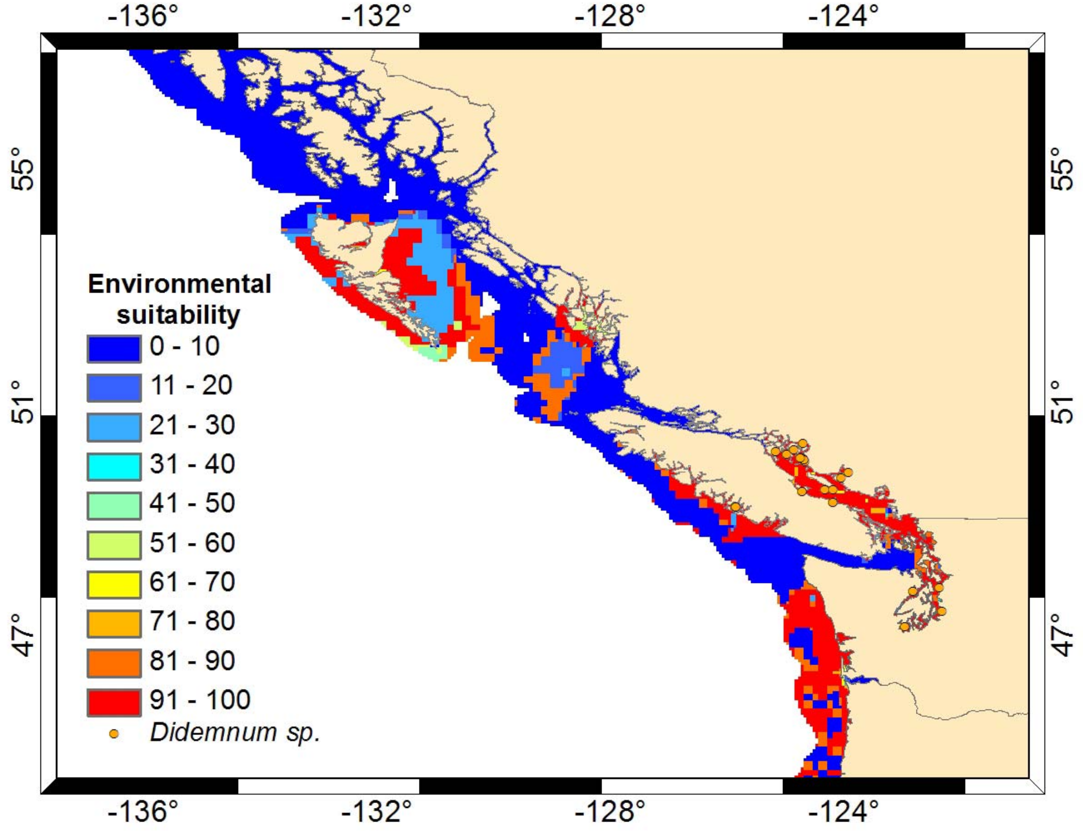


Figure 22: Potential distribution of *Didemnum* sp. on the Pacific coast based on temperature and salinity tolerances.

Risk Assessment Part I – Aquatic Organism Ecological and Genetic Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate of probability of the organism successfully colonizing and maintaining a population if introduced.

The primary vectors proposed for the introduction of *Didemnum* sp. worldwide are ship fouling and historical live oyster transfer. On the Pacific coast the most probable vector and pathway was historical aquaculture transfers directly from Japan. Prior to concerns about non-native species or their associated hitchhikers, adult Japanese oyster were introduced directly from Japan to Canada for aquaculture purposes (Quayle 1969, Bourne 1979). Similar practices existed along the American Pacific coast so secondary spread from these sites to Canadian waters can not be dismissed. On the Atlantic coast, considerable shellfish aquaculture took place in the northeast, some of which was supplemented with oysters imported from Japan. However, hull fouling on commercial vessels also is probable based on the timing of invasions. Natural vectors of dispersal appear unlikely for this species given the very short larval phase and the fact that adults are sedentary, biofouling organisms. Given that *Didemnum* sp. has established populations on the Pacific coast and has an offshore population on Georges Bank adjacent to Canadian waters (in addition to coastal populations in the US northeast) it is certain the species can arrive, survive and reproduce in Canadian waters. In addition, considerable, potential suitable habitat exists on both coasts (Figures 21 and 22) suggesting further spread or additional introductions are possible, especially in Atlantic Canada.

The results of the expert survey suggested *Didemnum* sp. would have high ecological consequences, especially on biodiversity and marine protected areas and could have very high consequences for shellfish aquaculture (Figure 3).

(2) If the organism escapes from the area of introduction, estimate the probability of its spreading.

Given the presence of populations on both the Atlantic and Pacific coasts, there is a very high probability that *Didemnum* sp. could spread on both coasts. In addition to broad environmental matches on both coasts there are a number of vectors previously responsible for spreading *Didemnum* sp. in close proximity to known *Didemnum* sp. colonies. For example, on the Pacific coast most populations are near aquaculture facilities and on Georges Bank there are considerable commercial fisheries, especially the offshore scallop fishery. Also, as with other non-indigenous tunicate species considered here, vectors that have dispersed *Didemnum* in the past, most notably hull fouling and aquaculture, continued to rank high in our expert survey (Figure 2).

(3) Final Rating: *Didemnum* sp.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Very High	Very Low	Very High	Very Low
Survival	Very High	Very Low	Very High	Very Low
Reproduction	Very High	Very Low	Very High	Very Low
Spread	Very High	Very Low	Very High	Very Low
Overall	**Very High**	**Very Low**	**Very High**	**Very Low**

Step 2: Determining the Consequences of Establishment of an Aquatic Organism

(1) Ecological impact on native ecosystems both locally and within each region.

Based on the results of its introduction throughout the world (Daniel and Therriault 2007), there is little doubt that *Didemnum* sp. would have significant negative impacts on habitat structure and potentially foodweb and trophic structure of aquatic ecosystems by inducing changes in plant, invertebrate and possibly fish communities. This species' ability to settle on a range of artificial surfaces causes dense fouling of fishing gear, moorings, and ropes and is difficult and time-consuming to remove. As a member of the biofouling community, this species competes for food and space with other filter feeding organisms and benthic species and can reach maturity earlier than competing species, especially cultured bivalves. As *Didemnum* sp. spreads over large areas, like Georges Bank, native populations are likely to be displaced or lost. Of the non-indigenous tunicates considered here, *Didemnum* sp. ranks among the highest in terms of ecological impacts, including those related to aquaculture (Figure 3).

(2) Genetic impacts on local self-sustaining stocks or populations.

On both the Pacific and Atlantic coasts there are native members of the family Didemnidae including *Didemnum carnulentum*, *Didemnum albidum*, *Trididemnum opacum*, and *Didemnum candidum*. However, given the ongoing taxonomic debate over *Didemnum* sp. it is difficult to ascertain whether hybridization between this and other Didemnids is possible. It is highly unlikely *Didemnum* sp. would have any genetic impact on other native fauna on either coast.

(3) Final Rating: *Didemnum* sp.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	High	Low	High	Low
Genetic Consequence	Low	Low	Low	Low

Step 3: Estimating Aquatic Organism Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.

Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Aquatic Organism Overall Risk Potential for *Didemnum* sp.

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	High	Low	High	Low
Genetic	Moderate	Low	Moderate	Low

Risk Assessment Part II – Pathogen, Parasite or Fellow Traveler Risk Assessment Process

Step 1: Determining the Probability of Establishment

(1) Estimate the probability that a pathogen, parasite or fellow traveler may be introduced along with the potential invasive species.

Little is known about the diseases and parasites specific to *Didemnum* sp. (Daniel and Therriault 2007). Carman (2005) found 18 species of benthic foraminifera on New Hampshire *Didemnum* sp. samples. All of the benthic foraminifera were dead, possibly due to the tunicate's acidic tunic (Carman 2005).

(2) Estimate the probability that the pathogen, parasite or fellow traveler will encounter susceptible organisms or suitable habitat.

Given that the benthic foraminifera were dead it is unknown if *Didemnum* sp. was a suitable host or if these species attempted to utilize *Didemnum* sp. as a secondary choice.

(3) Final Rating: pathogen, parasite or fellow traveler for *Didemnum* sp.

	Atlantic Coast		Pacific Coast	
Element	Rank	Uncertainty	Rank	Uncertainty
Arrival	Low	High	Low	High
Survival	Low	High	Low	High
Reproduction	Low	High	Low	High
Spread	Low	High	Low	High
Overall	**Low**	**High**	**Low**	**High**

Step 2: Determining the Consequences of Establishment of a Pathogen, Parasite or Fellow Traveler

(1) Ecological impacts on native ecosystems both locally and within the region including disease outbreak, reduction in reproductive capacity, habitat changes, etc.

Given only dead species of benthic foraminifera associated with *Didemnum* sp. the impacts are negligible, if they exist. Even if these species had been alive, their impact would be very difficult to determine.

For tunicates that are transported as fouling organisms, it is usually the progeny of these organisms that become established at new locations rather than dislodgement of the individual or colony. This dramatically reduces the probability that a pathogen, parasite or fellow traveler will be introduced at the same time as larval stages often are assumed to be relatively insusceptible to these agents.

(2) Genetic impacts on local self-sustaining stocks or populations (i.e. whether the pathogen, parasite, or fellow traveler affects the genetic characteristics of native stocks or species).

Nothing is known about the potential genetic impacts on local populations. However, there are species related to possible travelers native to Canada; therefore the potential for genetic contamination exists.

(3) Final Rating: pathogen, parasite or fellow traveler for *Didemnum* sp.

	Atlantic Coast		Pacific Coast	
Element	Magnitude	Uncertainty	Magnitude	Uncertainty
Ecological Consequence	Low	Very High	Low	Very High
Genetic Consequence	Low	Very High	Low	Very High

Step 3: Estimating Pathogen, Parasite or Fellow Traveler Risk Potential

The following summary table was used to determine the overall risk potential by combining the probability of establishment estimate determined in Step 1 with the three consequences of establishment determined in Step 2. In the table Green = Low Risk, Yellow = Moderate Risk and Red = High Risk.

Ecological or Genetic Consequence	Very High	🟨	🟨	🟥	🟥	🟥
	High	🟨	🟨	🟨	🟥	🟥
	Moderate	🟩	🟨	🟨	🟨	🟥
	Low	🟩	🟩	🟨	🟨	🟨
	Very Low	🟩	🟩	🟩	🟨	🟨
		Rare	Low	Moderate	High	Very High
	Probability of Introduction					

Summary of the Pathogen, Parasite or Fellow Traveler Overall Risk Potential for *Didemnum* sp.

	Atlantic Coast		Pacific Coast	
Risk Component	Rating	Uncertainty	Rating	Uncertainty
Ecological	Low	Very High	Low	Very High
Genetic	Low	Very High	Low	Very High

SUMMARY

The ecological and genetic risk posed by each of the non-indigenous tunicate species analyzed here are similar. On the Atlantic coast, each of the five species poses a high ecological risk with moderate uncertainty, except for *Didemnum* sp. where uncertainty was low (Table 1). On the Pacific coast the ecological risk was high for all species with low to moderate uncertainty except *C. intestinalis* for which the ecological risk was moderate with high uncertainty (Table 1). The slightly lower ecological risk rating for *C. intestinalis* on the Pacific coast was due to increased uncertainty on the arrival of this species to this region. The genetic risk posed by these non-indigenous tunicates on each coast is moderate (Table 1). However, in regions where similar native species exist, *Styela* on the west coast and *Didemnum* on both the Atlantic and Pacific coasts, the genetic consequences of an introduction could be higher.

The ecological risk posed by pathogens, parasites or fellow travelers associated with each of the three colonial non-indigenous tunicate species is low (Table 2). Since colonial tunicates are potential hitchhikers associated with *S. clava*, the ecological risk is high for this species (Table 2). Similarly, since *C. intestinalis* has been shown to harbor an amoebic parasite that is responsible for Amoebic Gill Disease (AGD) that could impact both cultured and wild salmon, the ecological risk was deemed moderate (Table 2). There is an inverse relationship between the level of risk and its associated uncertainty when it comes to pathogens, parasites or fellow travelers of non-indigenous tunicate species. These fellow organisms have not been well studied thereby increasing the uncertainty of arrival, survival, reproduction, spread and potential impacts including ecological and genetic consequences.

Table 1: Summary of the Aquatic Organism Overall Risk Potential for each of the five non-indigenous tunicate species.

Species	Atlantic Coast		Pacific Coast	
	Rating	Uncertainty	Rating	Uncertainty
	Ecological Risk			
Styela clava	High	Moderate	High	Moderate
Ciona intestinalis	High	Moderate	Moderate	High
Botryllus schlosseri	High	Moderate	High	Moderate
Botrylloides violaceus	High	Moderate	High	Moderate
Didemnum sp.	High	Low	High	Low
	Genetic			
Styela clava	Moderate	Low	Moderate	Low
Ciona intestinalis	Moderate	Low	Moderate	High
Botryllus schlosseri	Moderate	Low	Moderate	Low
Botrylloides violaceus	Moderate	Low	Moderate	Low
Didemnum sp.	Moderate	Low	Moderate	Low

Table 2: Summary of the Parasite, Pathogen or Fellow Traveler Overall Risk Potential for each of the five non-indigenous tunicate species.

Species	Atlantic Coast		Pacific Coast	
	Rating	Uncertainty	Rating	Uncertainty
	Ecological Risk			
Styela clava	High	Very High	High	Very High
Ciona intestinalis	Moderate	High	Moderate	High
Botryllus schlosseri	Low	High	Low	High
Botrylloides violaceus	Low	Very High	Low	Very High
Didemnum sp.	Low	Very High	Low	Very High
	Genetic			
Styela clava	Moderate	Very High	Moderate	Very High
Ciona intestinalis	Low	High	Low	High
Botryllus schlosseri	Low	High	Low	High
Botrylloides violaceus	Low	Very High	Low	Very High
Didemnum sp.	Low	Very High	Low	Very High

DISCUSSION

This national risk assessment for two solitary and three colonial non-indigenous tunicates clearly highlights the threat these invasive species pose to Canadian waters on both the Atlantic and Pacific coasts. The overall ecological risk posed by each of these species was classified as ‘high’, generally with ‘moderate’ uncertainty. This combination of high impact with moderate uncertainty suggests further actions will be required within the risk analysis framework, including management actions that could limit further spread or impact of these species. The only exception was *C. intestinalis* for Pacific waters which was classified as having a `moderate` ecological risk. The slightly lower level of risk associated with its potential introduction into Pacific waters, combined with a moderate level of risk suggested through the expert survey, resulted in the final rating for *C. intestinalis*. However, the `moderate` impact level identified by the expert survey still consists of a measurable widespread impact, but which is reversible, or of limited severity or duration (see Appendix B for details).

The risk assessments for each of the five non-indigenous tunicate species presented here represents a starting point. It is meant to provide scientific advice for decision makers, managers and policy makers who have to manage, control or mitigate the potential impact of these non-indigenous species. The scientific advice contained in this document is meant to inform the overall risk analysis framework that includes socio-economic considerations and should not be considered independent of this framework. The overall risk analysis framework for the Canadian Department of Fisheries and Oceans is currently under development but a draft framework has been adopted by the Centre of Expertise for Aquatic Risk Assessment (CEARA) (Figure 23; Mandrak and Cudmore 2006). The information contained within this document, and ancillary accompanying documents, may need to be re-visited as more information is gained and identified data gaps filled. For example, the predictions of environmental suitability were based on the current tunicate distributions, therefore highlighting areas having the same environmental conditions as those the species has already been found in. One potential limitation to this approach is that it could underestimate the potential habitat available for each species if the current invaded range on each coast is not fully representative of its potential range on each coast. As these species spread to new areas it is possible the predicted suitable habitats also will increase as the true habitat limitations become more apparent.

The risk assessment presented here focused on the risk posed to either the Canadian Atlantic or Pacific region. If one wanted to look at the risk posed within a region, each province within the Atlantic region for example, an additional risk assessment should be undertaken. Although we used the best available data for the ecological niche modeling, it was at a larger spatial scale (i.e., Pacific Ocean, Atlantic Ocean). Further, additional smaller-scale data could be available that might better identify micro-scale habitats. For example, high resolution nutrient data exists for bays around PEI (Somers et al. 1999) that could refine the environmental suitability for each of the tunicate species studied here.

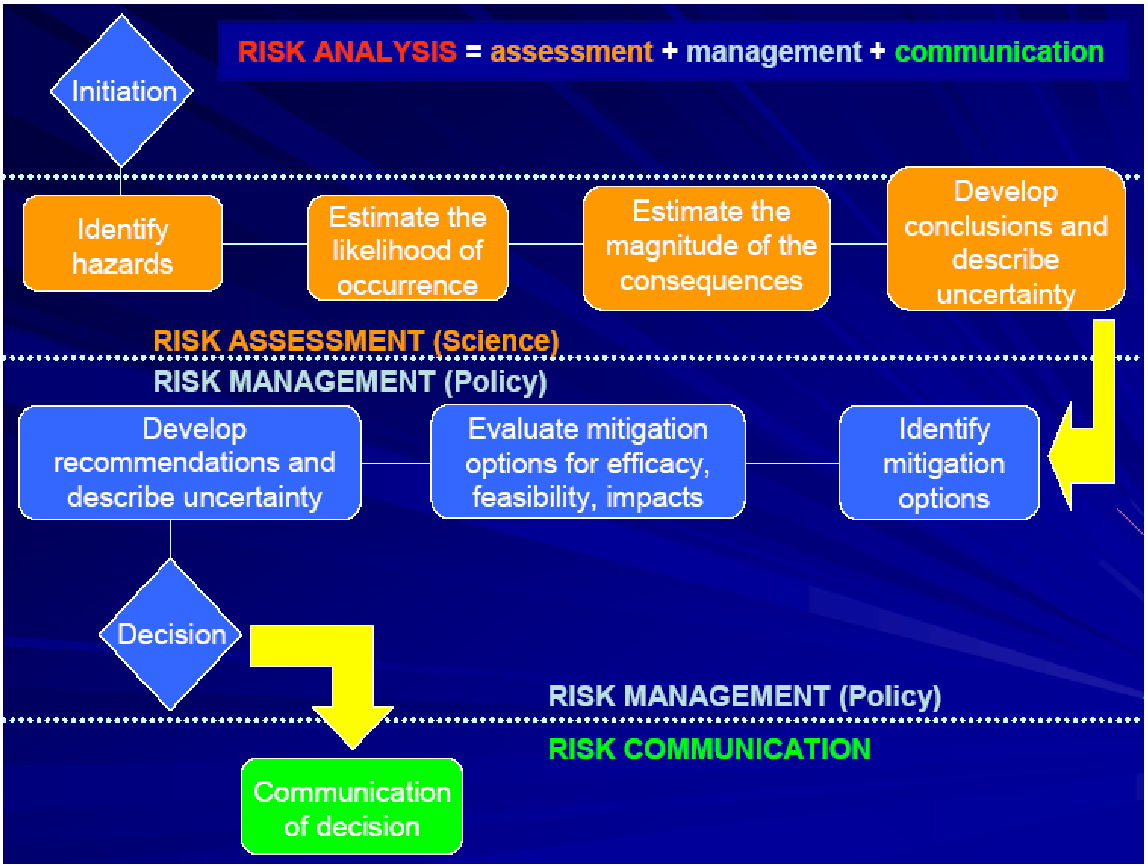


Figure 23: Schematic of the Risk Analysis Framework adopted by DFO (CEARA).

RECOMMENDATIONS

Since natural long distance dispersal of invasive tunicates appears very limited, increased management of human-mediated dispersal vectors could substantially reduce potential future spread of these non-indigenous tunicate species. Thus, best practice advice should be developed in collaboration with aquaculture groups, small craft operators and other stakeholders. These efforts should be aimed at educating marine users on these species, including how they can spread to determine suitable practices to limit further spread and associated ecological and genetic consequences.

In order to increase the level of resolution for these risk assessments, higher resolution data on environmental conditions and the patterns of human transport vectors (e.g., recreational boating, tug and barge or aquaculture related activities) in nearshore waters where most of these non-indigenous species first arrive is required. For example, little information on the potential for spread of tunicate species via small craft traffic currently exists. Additional research into the distribution pattern of this vector and associated level of risk would allow a more informed evaluation. Further, increased resolution may allow additional refinement to proposed or existing monitoring programs for non-indigenous species.

There is relatively little known on pathogens, parasites and fellow travelers of non-indigenous tunicate species. Thus, the level of uncertainty for potential pathogens, parasites and fellow travelers of non-indigenous tunicate species is high even though the risk is currently assumed to be low. The extent to which these organisms could affect native and commercially important species is largely unknown. Additional research on these species would refine the current assessment by lowering the uncertainty.

Finally, we recommend a central register for invasive species risk assessments, reports and monitoring findings be developed. This would ensure up-to-date information, including recommendations, are readily available to all involved parties including scientists, managers, and stakeholders. This register could house marine use data that would allow more timely access to critical data for rapid risk assessments.

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APPENDIX A: Participants at the National Peer-review Workshop for the Risk Assessment for Two Solitary and Three Colonial Tunicates in Both Atlantic and Pacific Canadian Waters

Participant	Organization
Jerry Amirault	Lobster Science Centre AVC of UPEI
Garth Arsenault	
Jay Baker	
Carla Barkhouse	DFO Gulf Region
Renee Bernier	DFO Gulf Region
Franck Berthe	AVC of UPEI
Mac Campbell	DFO Gulf Region
Ken Campbell	PEI Fishermen's Association
Mary Carman	Woods Hole Oceanographic
Becky Cudmore	DFO Ontario Region
Jeff Davidson	AVC of UPEI
Jennifer Dijkstra	University of New Hampshire
Kim (Swan) Gill	PEI AA
Mark Hanson	DFO Gulf Region
Larry Harris	University of New Hampshire
Matthias Herborg	DFO Pacific Region
Jonathan Hill	DFO Gulf Region
Elaine Hoagland	US National Oceanic & Atmospheric Administration
Russel Kerr	AVC of UPEI
Francoise Labonte	DFO Ottawa HQ
Gretchen Lambert	
Thomas Landry	DFO Gulf Region
Jean Lavallee	Lobster Science Centre
Julie Lavallee	
Andrea Locke	DFO Gulf Region
Jean MacDonald	PEI Aquaculture Alliance
Neil MacNair	PEI DAFA
Marie-Josee Maillet	Province of NB
Nick Mandrak	DFO Ontario Region
Jennifer Martin	DFO Maritimes Region
Cynthia McKenzie	DFO Newfoundland Region
Chris McKindsey	DFO Quebec Region
Allan Morrison	PEI DAFA
Jason Mullen	Aquaculture Association of NS
Lea Murphy	DFO Gulf Region
Gilles Olivier	DFO Gulf Region
Judy Pederson	MIT Sea Grant College Program
Selma Pereira	DFO Quebec Region
Geoff Perry	DFO Newfoundland Region
Pedro Quijon	UPEI Biology
Erica Reese	DFO Gulf Region
Bob Reid	National Marine Fisheries Service Rhode Island
Art Smith	DFO Gulf Region
Janet Smith	
Tom Therriault	DFO Pacific Region
Page Valentine	US Geological Surveys Woods Hole
Peter Warris	PEI AA

APPENDIX B: Definitions for Vector and Impact Questions used in the Expert Survey

Vector Importance

Very low: Tunicates have not been demonstrated or believed to utilize this vector, does not require management.
Low: Tunicates are unlikely to spread by this vector. May require efforts to minimize spread.
Moderate: Tunicates can spread by this vector in favorable circumstances. Management could provide a reduction of spread.
High: Tunicates have extensively used this vector. Management would be important for a reduction of spread, but none has been attempted.
Very high: Tunicates have extensively used this vector despite extensive management efforts.

Vector Uncertainty Levels

Very high uncertainty: Little to no information; expert opinion based on general species knowledge.
High uncertainty: Limited information; third party observational information or circumstantial evidence.
Moderate uncertainty: Moderate level of information; first hand, unsystematic observations.
Low uncertainty: Substantial scientific information; non peer-reviewed information.
Very low uncertainty: Extensive scientific information; peer-reviewed information.

Impact Level

Positive A positive impact. Improvement of the factor in question.
Very low negative No measurable impact. Consequences can be absorbed without additional management action.
Low negative A measurable limited impact. Disruption to the factor in question, but reversible or limited in time, space, or severity. May require management effort to minimize.
Moderate negative A measurable widespread impact. A widespread disruption to the factor in question, but reversible, or of limited severity, or duration. Can be managed under normal circumstances.
High negative A significant impact. A widespread disruption to the factor in question that persists over time, or is likely not reversible. Will require effective management or adaptation of procedures.
Very high negative A critical impact. Extensive disruption to the factor in question, that is irreversible. May already be unmanageable or will become so unless effective management is immediately put in place.

Estimated Probability if Impact

Unlikely Impact will only occur in exceptions or is not expected
Possible Impact could occur in some circumstances
Likely Impact will probably occur in most circumstances
Almost certain Impact is expected to occur in most circumstances
Certain Impact has been observed to occur