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Invasion of the southern Gulf of St. Lawrence by the clubbed tunicate (*Styela clava* Herdman): Potential mechanisms for invasions of Prince Edward Island estuaries

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Abstract

All but one of the nine non-native marine species that established populations in the southern Gulf of St. Lawrence (sGSL) in the past decade initially invaded the sGSL via coastal and estuarine waters of Prince Edward Island (PEI). Almost half of these species are tunicates, and all but one still occur only in PEI. Recent introductions include *Styela clava* Herdman in 1997, *Botryllus schlosseri* (Pallas) in 2001, *Botrylloides violaceus* Oka in 2002, and *Ciona intestinalis* (Linnaeus) in 2004. The goal of this paper was to investigate which characteristics of PEI estuaries may have resulted in their being more susceptible to tunicate invasions than estuaries elsewhere in the sGSL. At least one genus that recently established viable populations in PEI was previously introduced to the Gulf of St. Lawrence, apparently without establishing permanent populations. This implies that either propagule pressure has increased or environmental factors are more conducive to establishment now than they were previously. The fluctuating resource availability model predicts increased invasibility of environments that experience pulses of resources such as space or nutrients. Intense development of both agriculture and aquaculture in PEI, and high population density compared to other areas of the sGSL, are associated with high and fluctuating estuarine nutrient levels and a large surface area of artificial substrates (mussel socks) that is kept relatively free of competitors, and is replaced regularly. Changes in nutrient loading and the development of aquaculture have also occurred within the past two to three decades. The provision of artificial structure is likely a critical factor in the successful establishment of tunicates in PEI, because natural hard substrates are scarce. Facilitation by green crabs (*Carcinus maenas* L.) may be a contributing factor in the spread of *Styela*. Only one estuary lacking green crabs has an established population of *Styela*, and at least two known inoculations of *Styela* into estuaries without green crabs have failed. A likely mechanism for facilitation is the consumption by green crab of the snail *Astyris lunata*, a known *Styela* predator.
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1. Introduction

All but one of the nine non-native marine species that established populations in the southern Gulf of St. Lawrence (sGSL) in the past decade initially invaded the

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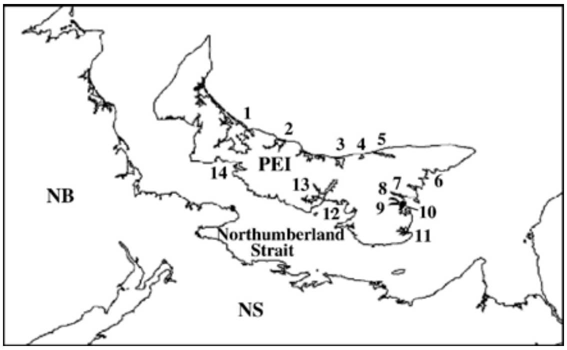

Fig. 1. Map of Prince Edward Island. 1 = Malpeque Bay, 2 = New London Bay, 3 = Tracadie Bay, 4 = Savage Harbour, 5 = St. Peters Bay, 6 = Souris River and town of Souris, 7 = Cardigan River, 8 = Brudenell River, 9 = Montague River, 10 = Georgetown, 11 = Murray River, 12 = Vernon River/Orwell Bay, 13 = Charlottetown, 14 = Summerside.

sGSL via coastal and estuarine waters of Prince Edward Island (PEI) (see Fig. 1). Almost half of these non-native species are tunicates, and all but one of the recent tunicate invaders of the sGSL still occur only in PEI. Recent introductions include clubbed tunicate (*Styela clava* Herdman) in 1997, golden star tunicate (*Botryllus schlosseri* (Pallas)) in 2001, violet tunicate (*Botrylloides violaceus* Oka) in 2002, and sea vase (*Ciona intestinalis* (L.)) in 2004. PEI is the only place in Atlantic Canada where *Styela* is reported. The other three species are found along the Atlantic coast of Nova Scotia (NS), and *Botryllus* and *Ciona* occur in New Brunswick (NB) waters of the Bay of Fundy (Carver et al., 2003; Locke and Hanson, unpublished data; S. Robinson, personal communication). An independent introduction of *Ciona* to NS waters of the sGSL was discovered in 2005 (Locke and Hanson, unpublished data).

Because they produce short-lived larvae, most long-distance dispersal of non-native tunicates has been attributed to shellfish transfers, or to shipping vectors such as ballast water and hull fouling (Buizer, 1980; Lambert and Lambert, 1998; Minchin and Duggan, 1988; Lützen, 1999). Details of international transfers of tunicates with shellfish are scarce, but would presumably involve the movement of attached juveniles or adults due to the short duration of the larval stage. Both larval and recently metamorphosed tunicates have been recorded in ballast tanks (Carlton and Geller, 1993). Most tunicates are rheophobic (Lützen, 1999), however, trans-Pacific dispersal on towed hulls has occurred recently (Lambert, 2002). Transport of tunicates on the hulls of faster moving vessels may be feasible in semi-enclosed areas on the ships (i.e., chain lockers, sea chests), but has not been reported.

Establishment of a species in a new area is affected by habitat and by the composition of the existing community. There is virtually no natural habitat for attachment of adult tunicates in sGSL estuaries. Bivalve aquaculture gear, docks, breakwaters, and channel markers are the substrates predominantly colonized by tunicates in the sGSL, although *Botrylloides* also grows on eelgrass (*Zostera marina* L.) and macroalgae. In PEI, aquaculture equipment provides far more substrate than any other marine structure; e.g., 16–19% of the surface area of two typical estuaries was occupied by mussel socks with an average length of 3 m (Grant et al., 2005). Associated lines, buoys, and anchoring systems, not included in this estimate, provide additional substrate.

Biotic diversity may serve as a proxy of both habitat and community effects on establishment. The effect of diversity on invasibility is currently thought to be scale-dependent (Stohlgren et al., 1999; Bruno et al., 2005; Davies et al., 2005). For tunicates, diversity of fouling organisms could reflect spatial competition and the proportion of suitable substrate available for settlement, consistent with the negative diversity–invasibility relationship reported in small-scale experiments (e.g., Stachowicz et al., 1999). At larger scales, species diversity may serve as a proxy of habitat diversity; if the presence of many habitat types increases the likelihood of a suitable habitat existing in the area, a positive diversity–invasibility relationship would result. The likelihood of encountering predators, competitors, or facilitators is also likely to increase with diversity, so that the net effect on species interactions could be positive, negative or neutral. One might predict that fouling organisms would be the community most relevant to opportunities for tunicate invasions, but this was likely not the case in our study area. Aquaculture management practices maintain very low biomass of most fouling organisms on mussel socks (LeBlanc et al., 2003); therefore, the relationship of tunicate invasions to species diversity in the sGSL may be more closely associated with the overall diversity of estuarine communities, reflecting the presence of predators, competitors that affect planktonic food concentrations, or potential facilitators.

Species diversity is affected by disturbance, productivity, resource availability and heterogeneity, as well as species interactions (Stohlgren et al., 1999; Bruno et al., 2005; Davies et al., 2005). Davis et al. (2000) integrated the effects of these factors into a “fluctuating resource availability” theory, which states that the susceptibility of a community to invasion increases during periods of pulsed resources (e.g., nutrients or space). Several studies support this theory. Stachowicz et al. (1999)

showed that holding diversity constant, but increasing space, dramatically increased the settlement of tunicates. Likewise, invasive plants respond positively both to increases in nutrients, and to heterogeneity in supply (Davies et al., 2005). According to the intermediate disturbance and subsidy-stress models, somewhat disturbed environments should support the highest levels of diversity and production (Connell, 1978; Odum, 1985), either of which may open up opportunities for invaders under the Davis et al. (2000) model.

Facilitation of an invader by a member of the existing community through mutualism, habitat modification, or suppression of a predator may also enhance establishment. Facilitation by previously established invaders has accelerated the rate of invasion in several well-studied estuaries (Carlton, 1996; Cohen and Carlton, 1998; Simberloff and Von Holle, 1999). We focused on the green crab, *Carcinus maenas* (L.), as a potential facilitator of *Styela*. Green crabs consume prey from at least 158 genera and have been widely documented to decrease the diversity and biomass of estuarine communities (Cohen et al., 1995; Grosholz and Ruiz, 1996). Green crabs could facilitate tunicate invasions by preying on tunicate predators. In the northeastern USA, four taxa are known to eat *Styela clava*: the lunar dovesnail *Astyris lunata* (Say), well-ribbed dovesnail *Anachis lafresnayi* (Fischer and Bernardi), greedy dovesnail *Anachis avara* (Say), and a fish, the cunner *Tautogolabrus adspersus* (Walbaum) (Osman and Whitlatch, 1995, 1999, 2004; Whitlatch et al., 1995). Of these, only *A. lunata* and *T. adspersus* occur in PEI waters (Brunel et al., 1998; Locke and Hanson, unpublished data).

The goal of this paper was to investigate which characteristics of PEI estuaries may have resulted in their being more susceptible to tunicate invasion than estuaries elsewhere in the sGSL, using *Styela* as a model. We examined vectors of dispersal, and factors that might affect establishment, including the potential for facilitation by an earlier invader.

2. Methods

2.1. Opportunities for invasion

We examined shipping and movements of materials in the shellfish industry as potential vectors. Because *Styela* is not found in Atlantic Canada outside of PEI, the initial introduction to the area must have taken place from an international source. Secondary dispersal could have occurred through local movements of boats and/or shellfish products.

The only international shipping traffic that we were able to evaluate was commercial shipping. Under the Canada Shipping Act, vessels entering Canadian waters must report their ballast water status. We obtained ballast water reports from 2001–2004 (M. Balaban, Transport Canada, Dartmouth, NS), and examined the international traffic to sGSL ports.

Most navigable estuaries in Atlantic Canada have wharves for fishing boats and/or marinas for recreational boats. As an index of relative boating activity, we divided the number of harbours used by fishing boats in each of the three provinces (Small Craft Harbours, Fisheries and Oceans Canada, Moncton, NB) by the coastline determined from a 1:20,000 map (B. Firth, personal communication). We also identified boat-related activities that occur in only a few areas, such as drydocks, cruise ship docks, etc.

In Canada, international and inter-provincial movements of bivalves are regulated by law, but there is limited information on intra-provincial movement. Aquaculturists wishing to import bivalves, or indeed any species, must obtain a permit from their provincial Introductions and Transfers Committee, administered by Fisheries and Oceans Canada. We contacted the committees (C. MacIsaac, PEI chair; D. Harris, NS chair; A. LeBlanc, NB secretariat) for records of importations since the mid-1990s.

2.2. Opportunities for establishment

We looked for differences between PEI and other areas of the sGSL in indices of disturbance, nutrient levels, and habitat as factors promoting establishment of invasive species. We examined indices of land-based activities, potentially associated with estuarine disturbance, by comparing population density (2005) and the proportion of land used for farming (2001) in the three provinces (Statistics Canada, www40.statcan.ca, accessed 6 April 2006). We compared estuarine nutrient levels by examining published values and our own data from a survey of estuaries in PEI, NS and NB in 2001. Landings of aquacultured bivalves (Aquaculture Production Statistics for 2003, Fisheries and Oceans Canada, www.dfo-mpo.gc.ca/communic/statistics/aqua/aqua03_e.htm, accessed 20 June 2005) were used as a proxy of the amount of habitat available to tunicates.

The diversity of the estuarine communities was based on sampling of eelgrass (*Zostera marina*) beds in 21 estuaries of the sGSL during 2001, which was part of a study examining the effects of green crabs on benthic community structure (Ellis, unpublished data). Epibenthic sled tows were used to sample invertebrates at three or four sites per estuary. Nearshore fishes were sampled at four to eight sites per estuary using a 30-m beach seine. Factorial analysis of variance was used to assess the effect of location (PEI or elsewhere in sGSL) and green crabs (present or absent) on species richness (total species per estuary summed across all samples) of nearshore fishes and epibenthic invertebrates. Green crabs had to be included as a factor in this analysis to account for its expected effect on diversity. In PEI, we surveyed seven estuaries with, and four without, green crab populations. Elsewhere in the sGSL, we surveyed four and six estuaries with and without green crab populations, respectively. All analyses were conducted using JMP statistical software, version 4.0 (SAS Institute Inc., Cary, NC).

2.3. Opportunities for facilitation

We thought that the likeliest facilitator for establishment of alien tunicates would be a species that had recently invaded PEI, arrived before the tunicates, had substantial overlap in distribution with tunicates, and had a plausible mechanism of facilitation. To identify potential facilitators, we examined the timing and spatial patterns of invasions in PEI estuaries, using data from the synoptic surveys, supplemented by distribution information obtained from N.G. MacNair (personal communication), J. Davidson (personal communication), and Audet et al. (2003). Six invasive species were included: the four tunicates, green crabs, and the Japanese skeleton shrimp *Caprella mutica* Schurin. Recent macroalgal (*Codium fragile* ssp. *tomentosoides* (van Goor) Silva) or planktonic (*Penilia avirostris* Dana, *Pseudo-nitzschia fraudulenta* (Cleve) Hasle) invaders of the sGSL were excluded from the analysis because their distribution overlapped very little with the six species listed above.

Our further analysis concentrated on the potential effects of green crabs on a known predator of tunicates: the snail *Astyris lunata.* Using data from the synoptic survey, factorial analysis of variance was conducted on log-transformed mean (by estuary) abundances of *Astyris* in relation to estuary location (PEI or elsewhere in sGSL) and green crab (present or absent). To evaluate the green crab–*Astyris* relationship independently of the spatial trends inherent to the synoptic survey, we examined data from an experimental study of the effects of green crabs on nearshore communities (Thompson, unpublished data). Subtidal (0.5 m at low tide) enclosures (4 m^2 enclosed with 0.7 mm mesh, four replicates in each of two sequential trials) were installed with 0, 1, and 5 green crabs m^{-2} in Caribou estuary, NS, from July to September 2002. At the end of the experiments, the cages were sampled using 10 benthic cores (0.05 m^2), and all benthic invertebrates larger than ≈ 1 mm were collected and counted. A factorial analysis of variance was used to assess the effects of green crab density on log-transformed *Astyris* sp. abundance during the two trials.

3. Results

3.1. Opportunities for invasion

Ten ports in the sGSL were visited by international commercial traffic from 2001 to 2004: four ports in PEI (Summerside, Charlottetown, Georgetown, Souris), three in NS (Pugwash, Pictou, Aulds Cove), and three in NB (Dalhousie, Belledune, Miramichi). Total arrivals (first port of arrival in Canada) over the four years were 325 vessels. Of these, only 50 vessels arrived at PEI ports, compared to 111 in NS and 164 in NB. Furthermore, only a single international vessel was recorded at Georgetown, the only port in southeastern PEI (the area where green crabs, *Styela* and *Ciona* were first reported). There are no international ports along the northern shore of PEI where *Botryllus*, *Botrylloides* and the alga *Codium* first became established. The northern shore of PEI might have received propagules released during deballasting of ships transiting major shipping routes in the Gulf (Gilbert et al., 2004), if those organisms were advected inshore. Ballast water transport was the only possible vector of the two holoplanktonic invasive species identified in PEI waters: the cladoceran *Penilia avirostris*, and the dinoflagellate *Pseudo-nitzschia fraudulenta* (Bernier and Locke, 2006; Locke and Hanson, unpublished data). The latter species, which was detected for the first time in the sGSL in estuaries of NS and PEI in 2001, had been sampled from a ballast tank of a freighter visiting a NS port less than a month earlier (Carver and Mallet, 2002; S. Bates, personal communication).

Fishing boats, recreational boats, dredgers, barges, coast guard ships, and other vessels visit all areas of the sGSL. There is one cruise ship port: Charlottetown, PEI, which has not been implicated as a primary site of invasion. Our index of local boating activity differed little among provinces: 76 harbours were used for fishing boats in PEI (2.9 harbours/100 km of coast), 44 in NS (2.6 harbours/100 km) and 72 in NB (2.6 harbours/100 km). Qualitatively, there may be some important differences in boating patterns that may explain the high frequency of introductions in southeastern PEI. We speculate, for example, that *Ciona* may have arrived in the vicinity of Georgetown, PEI, as a

Table 1
First year of discovery of six invasive species in PEI estuaries (numbers refer to estuary locations noted in Fig. 1)

Estuary	*Styela clava*	*Botryllus schlosseri*	*Botrylloides violaceus*	*Ciona intestinalis*	*Carcinus maenas*	*Caprella mutica*
Brudenell (8)	1998				1998	1998
Montague (9)	1998			2004	1998	1999
Murray (11)	1999				1998	by 2003
Vernon/Orwell (12)	2000				1998	
Malpeque (1)	2002				(2000)*	
Cardigan (7)	2002				1997	2002
Tracadie (3)	(2002)*					
St. Peters (5)		2001			2001	
Savage (4)		2002	2002		2001	
Souris (6)					1998	2000
Charlottetown (13)					2000	

**Styela clava* was found on a researcher's mussel sock in Tracadie Bay in 2002, but is apparently not established in the bay (D. Bourque, personal communication). *Carcinus maenas* was found by fishermen in Malpeque Bay in 2000 but there have been no subsequent observations (N. MacNair, personal communication).

result of boat traffic from southeastern NS, where a large infestation is associated with bivalve aquaculture (Carver et al., 2003). A possible vector for such a transfer could be the commercial tuna fishing boats that move seasonally between the waters of eastern PEI, and those of southeastern NS. Equally possible, southeastern PEI is a popular destination for recreational boats from the eastern seaboard of the United States, often stopping in the Lunenburg NS area (where *Ciona*, *Botryllus* and *Botrylloides* are common), and the Bras d'Or Lakes in NS (where *Botryllus* is common) en route. Georgetown, PEI, is also the site of one of the two drydocks in the sGSL: the other is located in Pictou, NS.

Slow-moving dredges and barges involved in harbour maintenance in the sGSL often carry a large biomass of fouling organisms (Locke, personal observation). In 2002, the discovery of unfamiliar tunicates on barges involved in breakwater construction in Savage Harbour, northern PEI, led to the barges being towed to sea for defouling to reduce the risk of introducing these taxa, which were subsequently identified as *Botryllus* and *Botrylloides*. Despite this precaution, both species are now established in Savage Harbour; whether due to an inoculation from the construction vessels or from some other vector is undetermined.

No permits for the international transfer of molluscs to the sGSL have been granted since 1997. In that year, Northern quahogs (*Mercenaria mercenaria* (L.) var. *notata*) were imported from Massachusetts, USA to PEI, held in quarantine for a year, then distributed to estuaries in PEI, NB and NS for growth trials. We did not detect any association between the distribution of the quahogs and the occurrence of tunicates. Malpeque disease of American oyster (*Crassostrea virginica* Gmelin), found throughout the sGSL, has prevented the importation of oysters since the 1950s (Drinnan, 1990). Aquacultured blue mussels (*Mytilus edulis* L.) were, until recently, commonly transferred into the sGSL from the Bras d'Or Lakes, NS. These transfers were discontinued following the discovery of parasitic *Haplosporidium nelsoni* in the Bras d'Or Lakes in 2002. However, many transfers continue between estuaries within the sGSL. Most PEI aquaculturists readily acknowledge that the local transport of tunicates with bivalve transfers has been a vector of secondary spread within the province. Indeed, the establishment of several populations of *Styela*, *Botrylloides* and *Botryllus* can be linked to known aquaculture transfers or processing of tunicate-infested mussel harvests.

3.2. Opportunities for establishment

Land is used more intensively in PEI than in NB or NS. Population density is the highest in Canada (24.0 indiv. km^{-2}), compared to 17.3 indiv. km^{-2} in NS and 10.3 indiv. km^{-2} in NB. PEI is far more agricultural (46% of land area) than NS (8%) or NB (5%). Meeuwig (1999) found a clear link between estuary eutrophication and watershed area used for agriculture in PEI. Nitrogen may be a limiting nutrient in sGSL estuaries (Meeuwig et al., 1998). Mean levels of nitrogen-based nutrients (nitrate, nitrite, ammonia) were 11–49% higher in PEI estuaries than elsewhere in the sGSL in summer, 2001 (Ellis et al., unpublished data). Because agriculture is the major nutrient source in PEI, large fluctuations in estuarine nutrients can accompany runoff events. Macroalgal blooms, followed by periods of anoxia and massive die-offs, have become common indicators of severe eutrophication in recent years whereas they were infrequent 20–30 years ago (Raymond et al., 2002). Few other areas in the sGSL experience such extreme events associated with nutrient loading.

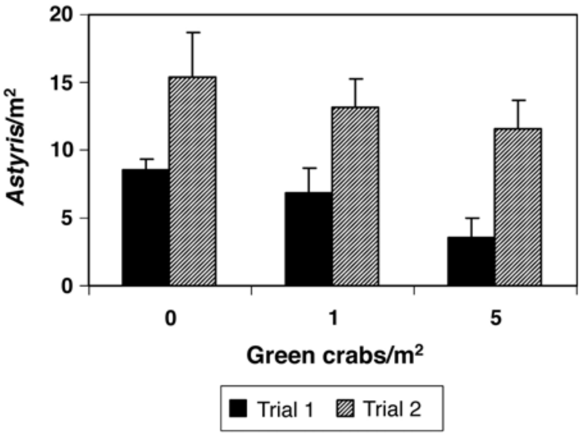

Fig. 2. Effects of green crab abundance on *Astyris* mean (SE) abundance in two experimental trials in enclosures in Caribou, NS.

There was much more molluscan aquaculture in PEI than the other provinces. In 2003, 19,862 tonnes of bivalves were produced in PEI, but only 2803 tonnes in New Brunswick (sGSL only) and 2303 tonnes in Nova Scotia (all coasts combined). Blue mussel accounted for 85% of the biomass harvested in PEI.

Diversity of epibenthic invertebrates and inshore fishes were similar in PEI and mainland waters, suggesting comparable levels of either biological resistance or enhancement of invasion success in the two areas. Species richness of epibenthic invertebrates did not vary significantly with either of the factors (location, green crab) investigated by ANOVA. Location was not a significant factor in the ANOVA of fish diversity. However, fish diversity was lower in estuaries with green crab (7.3 species ± SE 0.47) than those without green crab (9.8 species ± 0.39) ($P < 0.001$).

3.3. Opportunities for facilitation

Green crab was the only marine invader that arrived shortly before the tunicates, and had a consistently overlapping pattern of distribution with one or more tunicate species (Table 1). The greatest overlap was between the distribution of green crabs and *Styela*, presumably because the more recent invaders were not as widely distributed. The only PEI estuary with *Styela* but apparently lacking green crabs is Malpeque Bay.

The postulated mechanism of facilitation, predation of green crabs on *Astyris*, was not supported by analysis of synoptic survey data. *Astyris* was significantly ($P = 0.01$) more abundant in estuaries with green crabs (17.7 *Astyris* m^{-2} ± SE 6.25) than those without green crabs (5.5 *Astyris* m^{-2} ± 1.97). Location (PEI vs. mainland) was not a significant factor.

In contrast to the results of the synoptic survey, abundance of *Astyris* sp. was significantly ($P < 0.05$) reduced at green crab density of 5 crabs m^{-2} in a controlled experimental setting in Caribou, NS (Fig. 2), consistent with the hypothesis that green crab presence could facilitate tunicate invasions through suppression of a predator. The abundance of *Astyris* increased significantly from early (Trial 1) to late (Trial 2) summer ($P < 0.001$).

4. Discussion

Differences in the types or intensity of anthropogenic vectors (a proxy of propagule pressure) were unlikely to be responsible for the high rates of primary invasions to PEI compared to other parts of the sGSL. Indeed, there was less international shipping traffic to PEI than elsewhere. There were no bivalve aquaculture introductions from international sources, although intraprovincial aquaculture transfers have contributed to secondary spread of tunicates. Non-anthropogenic vectors such as rafting on vegetation, which may be an important means of dispersal for colonial species (Van Name, 1910; Worcester, 1994), are unlikely to have introduced tunicates to PEI from the nearest known populations on the Atlantic coast, given the distances involved and the direction of currents.

At least one genus that recently established viable populations in PEI was previously introduced to the Gulf of St. Lawrence, apparently without establishing permanent populations. A botryllid tunicate, either *Botryllus* or *Botrylloides*, was found at 90 m depth in the sGSL off Percé, Québec (QC), and in the St. Lawrence Estuary (170 m) near Baie-Comeau, QC (Whiteaves, 1901; Van Name, 1910). The absence of subsequent reports suggests that establishment was unsuccessful, perhaps because of adverse water depth and exposure. It is likely, however, that if shipping was introducing alien tunicates into deep waters of the Gulf a century ago, they were also being introduced into inshore waters where they evidently did not become established at that time. Following Lonsdale's (1999) argument that the invasibility of an environment is determined by propagule pressure, the characteristics of the invading species, and environmental factors, we have to conclude that either propagule pressure has increased or environmental factors are more conducive to establishment now than they were previously.

Invasion of tunicates into estuaries with high and fluctuating nutrient loads and a large surface area of artificial substrate available for settlement, such as those in PEI, is consistent with the concept that invasibility increases with unused resources (Davis et al., 2000). Further, the taxa that have invaded PEI may be indicators of disturbance. In Spain, *Ciona intestinalis* and *Styela plicata* (Lesueur) are considered opportunistic colonizers of disturbed environments such as harbours. In particular, they are indicators of "intense stress" from factors such as substrate alteration, anoxia and excessive siltation (Naranjo et al., 1996).

The provision of artificial structures is likely a critical factor in the successful establishment of tunicates in PEI, because natural hard substrate is scarce. Elsewhere, tunicates are often among the first organisms to colonize artificial structures placed in estuaries (Otsuka and Dauer, 1982). Indeed, non-indigenous tunicates often form the dominant component of the epifauna on many harbour and marina structures, including boats, floats, and aquaculture structures (Lambert and Lambert, 1998). That many of the aquaculture structures in PEI are mussel socks that rarely touch bottom enhances their suitability for tunicate colonization. Tunicate colonization is generally more successful on floating than on fixed structures (Connell, 2000; Holloway and Connell, 2002), perhaps because they are more difficult for benthic predators to access.

Interestingly, the presence of aquaculture structures appears to have altered the distribution of cunners, one of the potential predators of *Styela* in PEI estuaries. In NB estuaries without aquaculture, cunners primarily occur in the lowermost, lagoon, portions of estuaries (Hanson and Courtenay, 1995; Joseph et al., 2006). However, cunners are frequently associated with mussel aquaculture structures in PEI, including those located well up the tidal river component of the estuary (N.G. MacNair, personal communication; D. Bourque, personal communication). We speculate that *Styela* may be present in such abundance as to preclude any predatory control, or else the cunners may be preying preferentially on other epifauna on the socks. Cunners may in fact interfere with other predators of tunicates; *Astyris lunata* is often found in the guts of cunners from other sites in the sGSL (Hanson, unpublished data).

Facilitation by green crabs may be a contributing factor in the spread of *Styela*. We are aware of at least two failed potential inoculations: *Styela* was detected on a mussel sock in Tracadie Bay, and mussel socks from Malpeque Bay, carrying *Styela*, were processed at a plant discharging wastewater into New London Bay. Three years later, there have been no further indications of *Styela* in either location. Both bays have extensive aquaculture operations and receive agricultural runoff. The most obvious difference from the southeastern PEI estuaries where *Styela* are abundant is that green crabs are not present. *Astyris lunata*, the only known invertebrate predator of *Styela* that occurs in PEI, is commonly found on mussel socks (LeBlanc et al., 2003). *Astyris* was susceptible to predation by green crabs (Fig. 2). Even if the green crabs were not present on the socks, they could potentially reduce the recruitment of *Astyris* under this scenario. However, contrary to expectations, our synoptic survey indicated higher *Astyris* densities in estuaries occupied by green crabs. Unfortunately, the synoptic survey confounded the effects of location and green crab, which were found only in the eastern half of the sGSL. It is possible that there was a systematic difference in nutrient load, carrying capacity, or other environmental factor in estuaries that were invaded by green crabs. One intriguing possibility is that green crab-induced disturbance, at the relatively low population densities currently present in most sites in the sGSL, is in the "intermediate zone" *sensu* both Connell (1978) and Odum (1985). If that were the case, higher levels of ecosystem diversity and/or production would be supported and one would expect higher invasion rates of non-indigenous taxa including tunicates. This would be an interesting hypothesis to investigate through experiments.

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