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# Process of invasiveness among exotic tunicates in Prince Edward Island, Canada

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**Abstract** Over the past decade, four exotic tunicates (*Styela clava*, *Ciona intestinalis*, *Botrylloides violaceus* and *Botryllus schlosseri*) have been reported in the Brudenell estuary in Prince Edward Island (PEI), Canada. *Styela clava* was the first exotic tunicate to arrive in 1997, rapidly establishing, spreading, invading, and eventually becoming a nuisance in several estuaries of PEI. In the Brudenell estuary, *S. clava* remained the only exotic nuisance tunicate until 2003. In the fall of 2004, the vase tunicate *C. intestinalis*, was reported in low abundance, followed by the two colonial species, *B. schlosseri* and *B. violaceus*, reported in the spring of 2005. The abundance of *C. intestinalis* rapidly increased post-introduction, eventually replacing *S. clava* as the foremost nuisance species on mussel farms in the estuary. To date, *C. intestinalis* continues to colonize this estuary at epidemic proportions, resulting in the continuing drop of *S. clava* abundance. The current abundance of *C. intestinalis* is estimated at 5 $cm^{-2}$, which is similar to *S. clava* abundance at its height in 2003. The 2006 abundance of *S. clava* is estimated to have fallen to near 0 $cm^{-2}$. The dominance of *C. intestinalis* as a fouling organism on mussel farms is considered a serious threat to this aquaculture industry, mainly due to its unmanageable weight. The process of the detection, establishment, invasiveness, and eventual rise to nuisance level of exotic tunicates in the Brudenell River is presented.

**Keywords** Aquatic invasive species · *Botrylloides violaceus* · *Botryllus schlosseri* · *Ciona intestinalis* · Exotic species · Nuisance species · *Styela clava*

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## Introduction

The introduction and establishment of an exotic species, defined as a species deliberately or accidentally released into an area in which it has not previously occurred, has become an increasing problem as the world becomes a more global community (Carlton and Geller 1993). An exotic species is invading or invasive when its population becomes self-generating, and typically expanding and competing with indigenous species, in a free living state in the wild. Both indigenous and exotic species can become nuisance or pest species, by interfering with the objectives or requirements of people (Binggeli 1994).

Non-indigenous species have numerous opportunities to "hitch a ride" to exotic places through several vectors. The main vectors identified in the marine and freshwater environments include: attachment to ship hulls, transfer through ballast water, the movement of aquaculture gear and product, and recreational boating (Buizer 1980; Minchin and Duggan 1988; Lambert and Lambert 1998; Lützen 1999). The local conditions for an introduced species will determine its success or failure in establishing, invading or becoming a nuisance in its new host area (Stachowicz et al. 1999; Locke et al. 2007). Prince Edward Island (PEI) appears to have optimal conditions for the successful introduction and establishment of exotic tunicates, which are sessile filter-feeding organisms that grow as solitary individuals or colonies depending on species. Since 1997, four introduced tunicate species have been identified in the waters surrounding PEI (MacNair 2005; Locke et al. 2007): *Styela clava* Herdman in 1997, *Botryllus schlosseri* Pallas in 2001, *Botrylloides violaceus* Oka in 2002 and *Ciona intestinalis* (L.) in 2004 (clubbed, golden star, violet, and vase tunicate, respectively). Locke et al. (2007) suggest that PEI is highly susceptible to invasion by tunicates because the island estuaries are highly productive areas with high nutrient loads and a large surface area of artificial substrate available for settlement, which is consistent with the concept that invasibility increases with unused resources (Davis et al. 2000). The provision of artificial structures is likely the critical factor in the successful establishment of tunicates on PEI, providing the hard substrate required of tunicates for settlement (Locke et al. 2007).

When there are multiple species of tunicate present, there is an expectation that inter-specific competition will play a role in the succession between species. Resident adults control successful settlement and recruitment of new species onto their substrate by: (1) preying on newly settling larvae, (2) removing available space for settling larvae to colonize, (3) stimulating or prohibiting larvae from settling on nearby substrate, and (4) increasing post settlement mortality by overgrowing newly attached individuals (Osman and Whitlatch 1995a, b). Established individuals and colonies interact with other tunicates post-settlement. Osman and Whitlatch (1995b) identified three factors affecting survivorship including (1) overgrowth by residents, (2) added physical structure for firmer attachment and (3) camouflage from motile predators. It has been observed that some species of adult organisms, such as the colonial tunicates *Diplosoma* and *Botryllus*, have the ability to overgrow weaker spatial competitors (Osman and Whitlatch 1995b; Hunt and Scheibling 1997)

The four non-native tunicates have been classified as aquatic invasive species (MacNair 2005) because their populations continue to self-generate and are expanding, both temporally and geographically. They can also be classified as nuisance species because they are significant foulers of the cultured mussel, *Mytilus edulis* (L.). Production and processing costs are increasing and the tunicates are competing for food and space with the mussel (Thompson and MacNair 2004), thus impacting the economics in the infested waters.

The goal of this study was to document the invasion process of an exotic species, *C. intestinalis*, from its successful establishment to its eventual categorization of nuisance species in an environment already successfully invaded by another exotic tunicate. Information on the successful establishment and spread of an exotic species is essential for policy makers when determining rapid response and management scenarios for new introductions.

## Materials and methods

### Study area

The Brudenell estuary, located at the eastern end of PEI, was chosen for this study due to its particular site characteristics (Fig. 1). Georgetown Harbour is a deep water port, located on the north side of the estuary and is considered a vector for introduced species through shipping traffic. This estuary was the site for the first identification of both *S. clava* and *C. intestinalis* in PEI. This estuary is also highly productive and economically important for the cultured mussel *M. edulis*, yielding growth rates of 2.25–2.32 mm $month^{-1}$ in 2004 (Department of Fisheries and Oceans 2005) and contributing 10.4% (2004) of total mussel production for PEI (Department of Fisheries and Oceans Canada 2006).

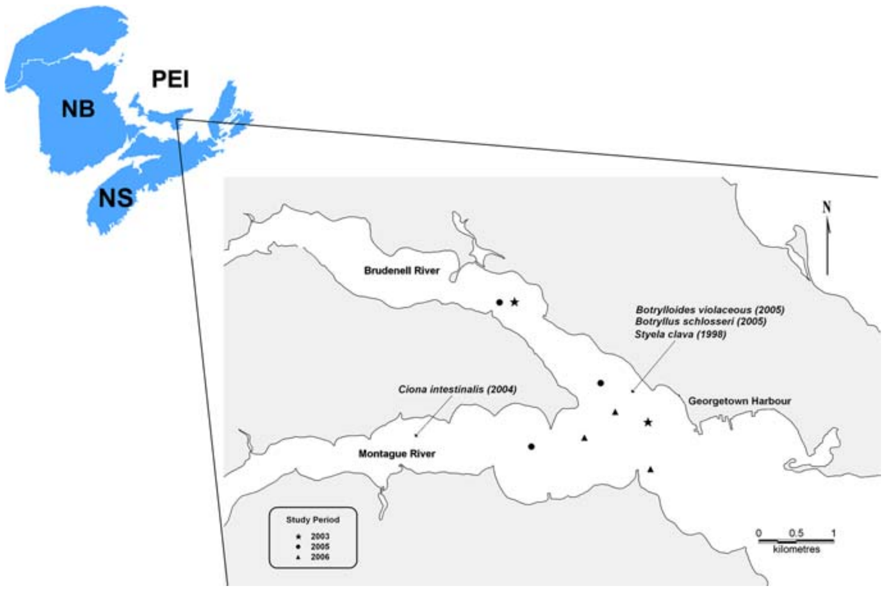


**Fig. 1** Map of the Brudenell estuary with sample sites and the areas of initial tunicate species detection identified, in PEI

### Experimental design

A series of collector plates, 10 × 10 cm pieces of PVC, were used as the primary substrate for examination. In 2003, two sites were selected in the estuary to determine the distribution and abundance of *S. clava*. At each site a collector rope with five collector plates, spaced 0.5 m apart, and having a 2 kg cinder block tied to the bottom of each of the collector ropes was deployed vertically 2 m below the surface of the water. Collectors were deployed at the beginning of June and retrieved at the end of October. In 2005 and 2006, collector ropes of a similar design were deployed at three sites; however, only three plates were tied to each of the collector ropes in 2006 and they were tied to mussel longlines, weighted with a 20 cm spike (approximately 120 g).

### Laboratory analysis

Tunicates were removed from the collector plates and sorted by species. The total tunicate weight, by species, was measured within 36 h of collection for the entire collector plate; hydroids, caprellid amphipods, and other species were removed prior to weighing. Tunicate weight can be variable within this 36 h, but, nevertheless, the data provides gross trends between species over time. In 2003 and 2005, the samples were frozen and then later thawed to determine mean tunicate abundance. All individuals larger than 5 mm were measured and counted. In 2006, mean abundance was determined by counting individuals from a ¼ subsample of the collector plate.

### Statistical methods

Mean tunicate abundance and weight per collector plate, by species, were compared between sample years using two-sample *t*-tests with unequal variances. In 2003, *C. intestinalis* data was omitted from the analysis as the species had not been detected and in 2006, *S. clava* data was omitted from the analysis because the species abundance had been reduced to zero on the collector plates. All data were analyzed using Minitab 15 (© 2006 Minitab Inc.). All estimates are reported as mean ± standard error.

## Results

In 2003, at the beginning of this study, the estuary was only occupied by one species of exotic tunicate, *S. clava*. Mean abundance of *S. clava* per collector plate in the fall of 2003, was estimated at 351.8 ± 44.5 (Fig. 2). The abundance and distribution of *S. clava* in that system was not evaluated in 2004. However, in the fall 2004, *C. intestinalis* was identified in the upper reaches of the Montague River in limited abundance

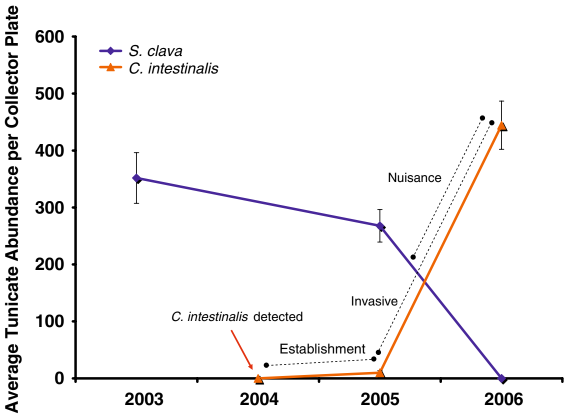


**Fig. 2** Mean abundance of *S. clava* and *C. intestinalis* by sampling year. Stages of establishment, invasiveness, and nuisance are indicated for *C. intestinalis*

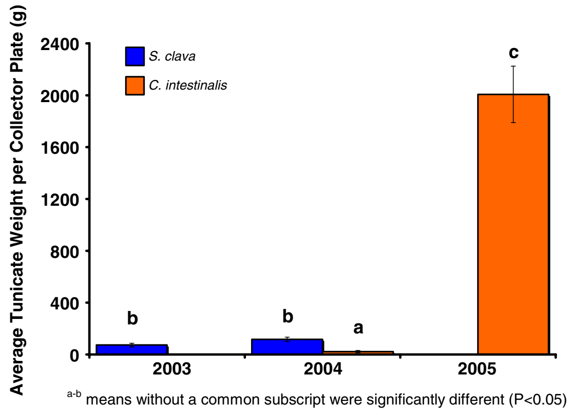


**Fig. 3** Mean weight of *S. clava* and *C. intestinalis* by sampling year

through the use of dive surveys (Fig. 1). Reports from MacNair (2005) indicated that *C. intestinalis* remained in a small area of Montague River, with *S. clava* greatly exceeding (>100×) the number of *C. intestinalis* on mussel socks. One year later, in 2005, the distribution of *C. intestinalis* had spread throughout the Montague River and abundance was increasing with an average of 9.9 ± 3.5 individuals per collector plate, though not significant compared to *S. clava* abundance in 2003 ($P < 0.001$). Abundance of *C. intestinalis* on collector plates ranged from 23.2 ± 7.5 in Montague River to 3.2 ± 1.6 and 3.4 ± 1.5 at the two sites in the Brudenell estuary. The abundance of *S. clava* had declined from 2003 ($P = 0.131$), with an average of 267.8 ± 28.4 individuals per collector plate. In 2006, 2 years after *C. intestinalis* was first identified, it had spread throughout the estuary. Its abundance had risen to epidemic proportions, averaging 444.4 ± 42.3 individuals per collector plate, significantly greater ($P < 0.001$) from 2005 abundance. The mean weight of *C. intestinalis* per collector plate had greatly increased from 2005; weight increasing from 22.5 ± 8.4 to 2007 ± 218 g ($P < 0.001$, Fig. 3). The number of *S. clava* had declined to near eradication with sporadic observations of older animal (>1 year old) on mussel culture gear, and 0 abundance on the collectors (Fig. 2). By the end of this study, *C. intestinalis* abundance had risen above *S. clava* abundance at its height in 2003 ($P = 0.151$). The weight of *C. intestinalis* on the collector plates was far greater ($P < 0.001$) than *S. clava* had ever been, averaging 2007 ± 218 g in 2006 compared to a maximum of 117.0 ± 17.0 g for *S. clava* in 2005.

## Discussion

This study documents the introduction of an exotic species, *S. clava*, its invasive success and nuisance to mussel aquaculture, followed by its decline with the introduction of a more successful spatial competitor, *C. intestinalis*. Both *S. clava* and *C. intestinalis* are considered exotic nuisance species to PEI, but *C. intestinalis* is the greater threat, especially to the well established mussel aquaculture industry. Within 2 years of being identified, *C. intestinalis* had established as the dominant fouling species in the estuary. Despite being reported in 2005, the two colonial species, *B. schlosseri* and *B. violaceus* were not detected in this study. The primary question raised is how has *C. intestinalis* taken over as the dominant species.

Recruitment of *C. intestinalis* was observed to start one month earlier (June) compared to *S. clava* (July). The reproduction cycle of *C. intestinalis* starts when water temperature reach 8°C (Carver et al. 2003; Ramsay et al. submitted), while the reproductive cycle of *S. clava* only starts when water temperatures exceeds 12°C (Bourque et al. 2007; Ramsay et al. submitted). This 4°C gap (approximately one month) provides *C. intestinalis* a significant recruitment advantage over *S. clava*. Presumably, the early recruitment and growth of *C. intestinalis* on clean substrate in the estuarine system is sufficient to inhibit subsequent settlement of *S. clava* (see Osman and Whitlatch 1995a).

Observations indicate *S. clava* is unable to settle on *C. intestinalis* because of its soft tunic and mucoidal surface. During the study by Ramsay et al. (submitted) only one observation was made of *S. clava* settling on *C. intestinalis*. This was late in the field season and it appeared that the tunic walls of *C. intestinalis* were thick enough for *S. clava* settlement. At this time, however, recruitment of *S. clava* is already approaching its end (Bourque et al. 2007).

Millar (1952) reported that the reproductive cycle of tunicates was confined to a few months in the summer and usually only occurred with animals over 1 year old. However, he was referring to native species, not exotic species. Exotic species appear to have a competitive edge compared to native species and quickly become invasive in new areas in which there are unused resources, such as space. This is exemplified in PEI, with *C. intestinalis* exhibiting a long and continuous breeding season (Ramsay et al. submitted). New recruits can reproduce in their first year and there is even a possibility of a third generation within one season. Several important factors contributing to *C. intestinalis* success in these rivers are (1) a longer reproductive season than other detected tunicates, (2) the presence of a mucoidal tunic to hinder settlement of other species, (3) faster growth, and (4) tolerance of tight crowding due to the long tubular flaccid structure resulting in more per square cm.

Previously established criteria to define exotic, invasive and nuisance species can be used here to illustrate the dynamics between the four detected exotic tunicates, *B. schlosseri*, *B. violaceus*, *S. clava* and *C. intestinalis*. As clearly illustrated in Fig. 2, the abundance of *S. clava* peaked between 2003 and 2005, while *C. intestinalis* was still in its establishment phase in 2005, building from its first occurrence (presence) in 2004. *Ciona intestinalis* only entered its invasive phase after 2005, when its population reached self regeneration and expansion. At this point, the invasion of *C. intestinalis* was clearly starting to dominate habitat niches for other species, including *S. clava*. In 2006, *C. intestinalis* abundance increased well above *S. clava*, replacing it as the main nuisance species for mussel aquaculture in the Brudenell estuary. The status of being "invasive" and/or a "nuisance" can differ over time and space for each exotic tunicate. *Styela clava* is no longer considered a nuisance species in this area, and could eventually be re-classified from invader to exotic. *Botryllus schlosseri* and *B. violaceus* failed to establish in the estuary and are not considered invasive or a nuisance. *Ciona intestinalis* has out-competed *S. clava* and is now invasive and a nuisance in the Brudenell estuary.

Other areas in which *S. clava* is invasive and a nuisance in PEI such as Malpeque Bay and Murray River, where *C. intestinalis* has not yet become established, continue to show a stable or increasing population of *S. clava*, both temporally and geographically. This provides evidence that the declining population of *S. clava* in Brudenell is a direct result of the invasive success of *C. intestinalis*. In 2006, *C. intestinalis* was detected in Murray River in low abundance. Given that Murray River and the Brudenell estuary have similar environmental conditions, it will be of interest whether the process of invasiveness for *C. intestinalis* is similar.

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