VASE TUNICATE, *Ciona intestinalis*:

ECOLOGY AND MITIGATION STRATEGIES

BY

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iii - iv

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ABSTRACT

The mussel aquaculture industry on Prince Edward Island (PEI) has grown over the last 20 years to now produce over 17 000 Mt, valued at $107 M to the economy and supplies approximately 80 % of the North American market (Department of Fisheries and Oceans, 2006). Recently, several exotic tunicates (*Styela clava*, *Ciona intestinalis*, *Botrylloides violaceus* and *Botryllus schlosseri*) have been detected throughout PEI which is cause for concern for the industry. Tunicates compete with mussels for space and food, potentially decreasing the growth rates and meat yields of cultured mussels. *Ciona intestinalis* is presently the dominant tunicate on mussel farms and is considered a serious threat to this aquaculture industry, mainly due to its unmanageable biomass.

Reproductive biology and development of *C. intestinalis* in a single season was investigated to assist aquaculturalists in developing control strategies and to understand the risk of spread to other aquaculture sites. The study included *C. intestinalis* gonad development, larval abundance and recruitment, and subsequent development after settlement. Histology showed gonads are ripe from May to December. Larvae were observed in water samples from early August through November, with a distinct peak in early October. Recruitment on experimental collectors, however, occurred from mid June until early December, with a peak in late-August. A rapid increase in biomass was documented in late July, six weeks after the initial recruitment. No substantial increase in *C. intestinalis* biomass was observed after mid August.

Anecdotal reports from mussel growers suggest that increased stocking density decreases the recruitment of the fouling organisms. To evaluate this hypothesis, 15 mussel socks of low (90 mussels per 30 cm), medium (250 mussels per 30 cm) and high

(500 mussels per 30 cm) densities were placed on three longlines in the Brudenell estuary in the fall 2005 and in the spring 2006. Samples were taken in June, August and October to determine the effect of different stocking densities and socking time on mussel productivity and *C. intestinalis* recruitment and growth. Mussel condition and shell length were not significantly different between the three stocking densities by the end of the field trial in October. In August, the mean length of *C. intestinalis* was significantly lower on the medium and high density socks and *C. intestinalis* weight was less on medium and high density socks from the fall socking period (no difference within spring socked densities); however, by the October sampling there was no difference in the weight of *C. intestinalis* between stocking density treatments. Time of socking did not appear to have any significant effect on the results, although the abundance of *C. intestinalis* was marginally higher in August and the biomass was higher in October, on the socks deployed in December, 2005. Mussel loss was between 50-60 % for all treatments, with no clear pattern being evident.

Ciona intestinalis has proven to be a significant invasive nuisance species to the mussel aquaculture industry. Multiple farm management practices utilizing optimal husbandry practices and knowledge of *C. intestinalis* reproduction and development are required to mitigate the impacts of this species.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AVC	Atlantic Veterinary College
BN	biomass-density relationship
°C	degree Celsius
CI	condition index
DFO	Department of Fisheries and Oceans
L.	Linnaeus
M	million
MMP	Mussel Monitoring Program
Mt	metric tonnes
n	sample size
OSD	optimal stocking density
P	P-value
PEI	Prince Edward Island
pers. comm.	personal communication
SE	standard error
SMN	Shellfish Monitoring Network

LIST OF TABLES

LIST OF FIGURES

Chapter 1 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The mussel aquaculture industry on Prince Edward Island (PEI) has grown over the last 20 years and now produces over 17 000 Mt, valued at $107 M for the economy (Department of Fisheries and Oceans, 2006). However, over the past decade, four major invasive species have been introduced into PEI waters: the clubbed tunicate, *Styela clava*; vase tunicate, *Ciona intestinalis*; golden star tunicate, *Botryllus schlosseri*; and the violet tunicate, *Botrylloides violaceus*. These four tunicates have been shown to be significant fouling organisms to the cultured mussel, *Mytilus edulis*, increasing production and processing costs. Fouling organisms also serve as competitors for food and space. As such, they have the potential to decrease growth rates and meat yields of the cultured mussel. Treatment to kill tunicates on mussels and aquaculture gear has had inconsistent results and is costly. A long-term solution for the mitigation of tunicates would include farm management practices that result in decreased biomass of tunicates on mussel gear and mussels. This study was developed to understand the development of the tunicates within a single breeding season and to assess optimal stocking density in mussels to be used as a part of a broader management strategy.

1.2. The cultured mussel, *Mytilus edulis*, on Prince Edward Island

1.2.1. Biology of the mussel

1.2.1.1. Environment

The blue mussel, *M. edulis*, is a sessile filter-feeding organism which occupies the littoral and sublittoral waters (Bayne, 1976). Mussel culture on PEI utilizes the sublittoral zone exclusively (Bayne, 1976). *Mytilus edulis* can survive temperatures ranging from below freezing to as high as 25 °C (Bayne, 1976; Brylinsky, 1989; Stewart, 1994; Mallet and Myrand, 1995), with optimal temperature for growth being 10-20 °C (Seed, 1976). Salinity can range from 0-35 ‰ for survival of *M. edulis* (Mallet, 1989; Stewart, 1994), but 26 ‰ is ideal (Department of Fisheries and Oceans, 2003).

1.2.1.2. Feeding

The main activity of the mussel is to feed through filtration. The mussel utilizes small organisms in the water column, primarily phytoplankton, as a food source (Bayne, 1976; Jørgensen, 1990). According to Bayne (1976), food particles 2-100 μm can be utilized, but particles 3-7 μm in diameter are retained with 100% efficiency. Bayne (1976) has described the process of feeding in mussels, beginning with water entering the mussel's pallial cavity through the inhalant siphon where food particles are picked up by the gills. The gills consist of four pairs of demibranchs which are comprised of two lamellae (an ascending and descending). Cilia on the lamellae are responsible for transporting the food particles. The particles are bound into mucous strings and are carried to the labial palps which regulate the amount of food entering the mouth and direct surplus material onto the rejection tracts of the mantle surface. From the mouth, food particles pass to the esophagus and into the stomach where particles are directed

either towards the digestive tubule duct openings or the intestine. Intracellular and extracellular digestion occurs in the digestive gland which also stores nutrient reserves and regulates their transfer to other tissues. Over a one hr period, under optimal growing conditions, a mussel filters between 30 and 60 times its own volume of water, amounting to 15 to 30 litres of water a day (Brylinsky, 1989).

1.2.1.3. Reproduction

Mytilus edulis reproduces by sexual means, producing gametes which are released into the water column (spawning) where fertilization takes place (Bayne, 1976). Reproduction occurs from mid May until late September, being initiated by temperature, food abundance or physical disturbance cues (Mallet, 1989). Mussel larvae require between 15 to 35 days to grow from fertilization to the stage when metamorphosis and settlement first becomes possible, the pediveliger (Bayne, 1976). After settlement the mussel larvae are known as spat. Bayne (1976) states that there is approximately a 1 % survivorship of all mussel larvae to the stage of substrate attachment. Time to maturity depends mostly on growing conditions such as water temperature, salinity, dissolved oxygen, and food availability (Bayne, 1976).

Mytilus edulis is capable of attaching to hard or gravelly surfaces by producing byssal threads which are threads of tanned protein secreted by an associated series of glands in the foot (Bayne, 1976). Through the use of byssal threads, mussels are able to withstand water movements in any direction, although they generally align parallel to the major direction of water flow (Bayne, 1976). It is because of this byssal apparatus that mussels are able to attach to socking material and be cultured using the longline technique in the subtidal zone of the water column.

1.2.2. Husbandry Practices

Mussel aquaculture can be done in various ways, including suspended culture (using rafts or longlines), bottom culture (by seeding intertidal or subtidal beds), and on "bouchots" (mussels attached to vertical wooden stakes planted in the intertidal area) (Hickman, 1992). In Atlantic Canada, the majority of mussel production is through suspended culture using longlines.

The industry is dependent on successful seed collection which is accomplished by the use of spat collectors. Spat collectors are approximately 1.5 m long pieces of frayed polypropylene rope that are attached to a longline approximately 40 cm apart (Swan, 2006). Collectors are deployed in the spring of the year, before the larvae recruit, where they provide adequate substrate for mussel larvae to attach. The timing of collector deployment is based on information from the Mussel Monitoring Program (MMP) which examines the number and size of mussel larvae in the water column to predict the optimal time for collector deployment (Swan, 2006).

Newly settled larvae, now known as spat, continue to grow reaching a size between 10 to 25 mm by the fall of the same year, but sometimes mussel seed is over-wintered on the collector ropes. At this time, mussel seed is stripped off the collector ropes, put through a declumping machine and graded according to size (MacDonald *et al.*, 2002). These mussels are placed in plastic mesh sleeves, called socks, at a uniform density ranging from 500 to 1000 mussels m^{-1} (MacDonald *et al.*, 2002) for each sock. Each sock is approximately 2 m long, but varies with water depth, current, and general growing conditions (Drapeau *et al.*, 2006).

The advantages of using suspended culture over bottom culture are faster growth rates of mussels and a higher tissue-to-shell ratio (Lauzon-Guay *et al.*, 2005a), as well as an end product free from sediment in the tissue of the mussel. Since mussel socks are suspended in the water column, held up by a series of buoys, additional bouys are periodically added throughout the growing season to counter the additional mass due to growth of mussels.

Depending on where the mussels are being grown a typical production cycle from start to finish is approximately 18 to 24 months when mussels reach a market size of 50-60 mm (Swan, 2006). Variability in time to marketable product depends on site characteristics (tidal flushing, food availability, water temperature, salinity, etc) and husbandry practices (sock spacing, seeding density, seed size, longline spacing, etc) (Drapeau *et al.*, 2006).

1.3. Epifauna associated with mussel production

Epifauna are organisms which attach to other surfaces, organisms, and substrates. These organisms can help with the culturing of mussels, as well as hinder the production cycle. Historically, there have been a set group of organisms associated with mussel production which are either relatively benign in nature or industry has learned to deal with them. However, in recent years, new epifauna which are particularly harmful to mussel production, have been identified.

Bayne (1976) places the natural enemies of the mussel into four categories: predators (*e.g.* crabs, starfish, birds); competitors for food and space (*e.g.* barnacles, tunicates); forms which attack the shell (*e.g. Cliona*, *Polydora*); and parasites (*e.g.*

Mytilicola, Pinnotheres). On PEI, predators and competitors for food and space are of the most concern to commercial populations of mussels.

1.3.1. Indigenous Epifauna to PEI

Crabs, starfish, the dogwhelk *Thais lapillus*, and oystercatchers were identified by Bayne (1976) as the main predators worldwide of mussel populations. In PEI ecosystems, starfish are viewed as the primary predator of mussels due to the absence of dogwhelks and oystercatchers. Crabs are not seen as predators, but as a beneficial organism in mussel production. Crabs serve as antifoulers on mussel socks by grazing on epifauna living on the sock, thereby keeping the sock free from fouling and by possibly strengthening byssal attachment.

The other concern to producers on PEI is the mussel's natural competitors for food and space. Large field studies to identify and quantify the species assemblages associated with mussel socks have been conducted to determine baseline information on which species of organisms were present in the mussel culture systems and to assess the possible impact of these organisms to the aquaculture industry (Ellis *et al.*, 2002). Lutz (2007) conducted a study in Brudenell estuary, PEI, to determine if there was a difference in species composition between three differing mussel stocking densities and also collected valuable information on the species present in that production system. The most concerning competitors for space and food are exotic tunicate species.

1.3.2. Exotic Epifauna to PEI

The introduction and establishment of 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 over the last century (Binggeli, 1994). Non-

indigenous species have greater opportunity of "hitching 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 waters, 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 host area (Stachowicz *et al.*, 1999; Locke *et al.*, 2007). An exotic species is considered invasive when its population becomes self-generating, and typically expands and competes 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 producers (Binggeli, 1994).

Prince Edward Island appears to have optimal conditions for the successful introduction and establishment of exotic tunicates (phylum: Chordata, subphylum: Urochordata) which are sessile filter-feeding organisms that grow as solitary individuals or colonies, depending on species. They have a free-swimming larval stage and an adult sedentary life stage. Since 1997, four tunicate species have been identified in the waters surrounding PEI (MacNair, 2005; Locke *et al.*, 2007). The introduced species are: the clubbed tunicate, *Styela clava* (Herdman) in 1997; golden star tunicate, *Botryllus schlosseri* (Pallas) in 2001; violet tunicate, *Botrylloides violaceus* (Oka) in 2002; and the vase tunicate, *Ciona intestinalis* (L.) in 2004. Locke *et al.* (2007) suggested that PEI is highly susceptible to invasion of tunicates because the island estuaries are highly productive areas with high nutrient loads. As well, a large surface area of artificial

substrate is 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 for tunicate settlement (Locke *et al.*, 2007).

Regarding mussel production, tunicates represent competitors for food and space on the mussel socks. Typically, natural habitats offer stable substrates which develop a complex highly diverse community and may be relatively resistant to invasion (Stachowicz *et al.*, 1999; Stachowicz *et al.*, 2002). Artificial substrates, in comparison, which are typically not stable, offer ideal habitat to opportunistic species. The mussel socks serve as an ideal substrate for the free swimming larvae to attach to and grow on. Tunicates also attach to mussel gear such as longlines, buoys, and anchors which increase the amount of effort required for growing mussels to market size.

To summarize, tunicates are competing for food and space with mussels and they are increasing the labour costs associated with growing and processing mussels. They thrive on artificial substrates where there is limited space available. Reaching lengths of up to 15 cm, they have the capability to out-compete other species that cannot reach out as far into the water (Lambert and Lambert, 1998; Lützen, 1999; Lambert and Lambert, 2003). Settlement generally occurs on structures that are already fouled, making it a secondary settler (Swan, 2006).

The four tunicates; *S. clava*, *B. schlosseri*, *B. violaceus*, and *C. intestinalis* have been classified as aquatic invasive species (MacNair, 2005) because their populations continue to self-generate and are expanding geographically. They have also proven to be a significant fouling organism to the cultured mussel, *M. edulis* (L.), thereby classifying

them as nuisance species. Production and processing costs are increasing and the tunicates are competing for food and space with the mussel (Thompson and MacNair, 2004). In addition to affecting the economics of the infested waters, these species have the potential to alter the local fauna and flora, displacing native species (Berman *et al.*, 1992).

1.3.2.1. Styela clava

Styela clava, commonly known as the clubbed tunicate, is a native to Asian waters, ranging from the Kurile Strait, Sea of Okhotsk, to southern Siberia, Japan, Korea, and at least as far south as Shanghai on the coast of China (Abbott and Johnston, 1972). By way of hull fouling and ballast water in ships, along with transfer of shellfish and seaweeds, tunicates have travelled over great distances (Lambert, 2005). Currently, *S. clava* is found on the coasts of California, eastern North America, Southern Australia, northwest Europe (Lützen, 1999), Washington State, Vancouver Island, PEI (Lambert and Lambert, 2003) and New Zealand (Biosecurity New Zealand, 2005).

The clubbed tunicate was introduced to the Atlantic Coast of North America from Japan via Europe sometime in the late 1960's, presumably by foreign ships coming from infested areas. *Styela clava* was first identified in PEI waters in the Brudenell River in 1998 but was known to exist prior to that time, at least as early as 1997 (Thompson and MacNair, 2004). Subsequently, *S. clava* has spread to several other PEI locations through boat traffic, movement of shellfish, and effluent from processing plants (Davidson *et al.*, 2005). Since first identification, *S. clava* has established populations in other eastern PEI waters including Montague River (1999), Murray River (2000), St. Mary's Bay (2001), Vernon/Orwell Rivers (2001), Cardigan River (2002), Marchwater

(2002), Darnley Basin (2005) and New London Bay (2006) (J. Hill, pers. comm.; Thompson and MacNair, 2004). A documented study of *S. clava's* spread in Marchwater, PEI by Davidson *et al.* (2005) showed the population establishing itself and now spreading among the leases with increasing biomass.

Lützen (1999) described this tunicate as being a long medieval club shape, growing up to 120 mm in length; however, Thompson and MacNair (2004) found tunicates as large as 180 mm in Murray River and Brudenell River, PEI. Swan (2006) described the external surface, known as the tunic, as being wart covered and brown in adults while wartless and yellowish-brown in younger tunicates. The tunic protects against desiccation allowing *S. clava* to survive out of the water for several days (Thompson and MacNair, 2004).

Styela clava has been shown to feed on planktonic food particles from 2 - 40 μm, although it is possible that larger sizes could be ingested as 40 μm was the largest particle size used (D. Bourque, Department of Fisheries and Oceans, Moncton, pers. comm. in Swan, 2006). Larvae of other aquatic species can be ingested by *S. clava*. Osman *et al.* (1989) found that *S. clava* consumed larvae of the American oyster *Crassostrea virginica*. This could affect oyster settlement rates and reduce population sizes near and around *S. clava*. Presumably, mussel larvae could also be consumed and viable spat settlement reduced. The filtering capacity of large solitary tunicates is outstanding and could have an effect on the nutrient content of the water (Petersen and Riisgård, 1992; Lambert and Lambert, 1998).

Styela clava reproduces exclusively by sexual means, producing larvae which are suspended in the water column for approximately 24 hrs before settling (Davidson *et al.*,

2005). Clubbed tunicates between 90 and 160 mm are considered sexually mature (Kott, 1985; Lützen, 1999); however, Davidson *et al.* (2005) found individuals greater than 25 mm to be sexually mature and capable of having larvae in the water column from the third week of June (when the temperature reaches approximately 15 °C) until late October. According to Davidson *et al.* (2005), the larvae cannot survive water temperatures less than 10 °C. A wide range of temperatures, from -2 °C to at least 23 °C (Holmes, 1976) and salinities as low as 8 ‰ (Sims, 1984) can be tolerated by *S. clava.* Recruitment is typically observed from late June to late October, with larvae showing a preference for dark or shaded areas and increased recruitment at lower depths (Davidson *et al.*, 2005). Other recent studies by Thompson and MacNair (2004) and Bourque *et al.* (2007) showed similar life histories for *S. clava.* Thompson and MacNair (2004) observed up to 6,000 tunicates m^{-1} on mussel socks in Murray River, PEI. Significant fouling of buoys, ropes, concrete blocks, scope lines, back lines, and floating oyster bags was also observed. It appears to prefer artificial substrates, such as those created by mussel culturing, over natural substrates.

1.3.2.2. Ciona intestinalis

Ciona intestinalis was probably native to northern Europe (Lambert and Lambert, 1998), but is now distributed worldwide (Lambert and Lambert, 1998; Millar, 1952) from subarctic to tropical regions (Dybern, 1965). It was only recently introduced into PEI waters, being first identified during the summer of 2004 in the Montague River. Since that time it has spread to Brudenell River (2005), St. Mary's Bay (2005), Cardigan (2006), and Murray River (2006) (J. Hill, pers. comm.).

Ciona intestinalis is a solitary urochordate composed of 95 % water (Carver *et al.*, 2003) and is transparent whitish or yellowish with five to seven conspicuous muscle bands on either side. Adult specimens may grow up to 150 mm in length and 30 mm in diameter (Carver *et al.*, 2006), having growth rates from 10 to 20 mm per month (Dybern, 1965; Carver *et al.*, 2003). The internal organs are clearly visible through the outer covering and the siphons have a yellow ring around the leading edge (MacNair, 2005). In older specimens, the tunic becomes more leathery and often develops a brownish colour as a result of algal or bacterial fouling (Carver *et al.*, 2006). *Ciona intestinalis* has the potential to establish itself in very dense populations with up to several thousand individuals per square metre (Millar, 1971; Svane, 1983; Carver *et al.*, 2003). This tunicate species has been observed rafting on the eelgrass *Zostera marina* (Petersen and Riisgård, 1992), which has not been seen with *S. clava.*

Tunicates feed by pumping the surrounding water through a continuously produced mucous net in which the suspended particles are trapped and the net is subsequently ingested (Millar, 1971). The mucous net of *C. intestinalis* retains particles as small as about 2 to 3 μm (Randløv and Riisgård, 1979; Jørgensen *et al.*, 1984) and is 70 % efficient at retaining 1 μm particles, feeding predominantly on zooplankton and phytoplankton. Petersen and Riisgård (1992) found that between 4 and 21 °C the maximum filtration rate (ml $min.^{-1}$ $ind.^{-1}$) increased linearly with increasing temperature, but above 21 °C the filtration rate declined rapidly. Petersen and Riisgård (1992) estimate that the huge late summer populations of *C. intestinalis* are capable of filtering a volume of water equal to the entire volume of a shallow Danish fjord every day.

As with the clubbed tunicate, *C. intestinalis* reproduces solely by sexual means. Studies by Carver *et al.* (2003) in Nova Scotia show the life cycle to be 12-18 months in duration, with larvae settling in summer, spawning the following spring, and eventually dying during the winter. In some instances, individuals that settle in May/June are capable of initiating egg production and spawning by August of the same year. Carver *et al.* (2003) found that spawning begins in mid to late May and continues until mid August; however, in PEI the season may be extended due to higher water temperatures. The general consensus for cold water populations along the Scandinavian coast and the Atlantic coast of North America is that individuals must reach 50-80 mm in length before being capable of producing viable gametes (Dybern, 1965; Cirino *et al.*, 2002; Carver *et al.*, 2003). Mature eggs are produced from early June onward, are fertilized, and typically spend 24-36 hrs in the water column in the larval stage before settling (Carver *et al.*, 2003). An individual 100 mm long is capable of producing more than 12000 eggs in one season (Carver *et al.*, 2003). In Nova Scotia, Carver *et al.* (2003) found that larvae could not survive temperatures less than 8 °C, which corresponds to a study by Dybern (1965). This species has a reputation for sporadic outbreaks or irregular intense peaks of recruitment (number of surviving individuals after settlement within a pre-determined amount of time) which are not linked to changes in environmental conditions (Keough, 1983; Cayer *et al.*, 1999).

1.3.2.3. Botrylloides violaceus

Botrylloides violaceus, the violet tunicate, is originally from Japan and now occurs along the west coast of North America from Alaska to California, on the east coast from the Gulf of Maine to Virginia (Mills *et al.*, 2000; Lambert and Lambert, 2003;

MacNair, 2005), as well as in Italy (Zaniolo *et al.*, 1998) and the Netherlands (Lambert and Lambert, 2003). The violet tunicate was first identified in Savage Harbour in 2004. Since that time these colonial tunicates have been found in Cardigan River (2005), St. Peter's Bay (2005), Brudenell River (2005), Tracadie Bay (2006), Marchwater (2005) and Cape Borden (2004) (J. Hill, pers. comm.).

Unlike the vase and clubbed tunicate, *B. violaceus* grows in colonies comprised of individuals (zooids) arranged in loose circles, rows, or dense clusters. According to Berrill (1941), individual tunicates are 1 to 2.5 mm in diameter and each group of zooids is comprised of five to 20 individuals with colonies consisting of thousands of zooids (Chadwick-Furman and Weissman, 1995). It grows in a jelly-like mass and is variable in colour, from bright orange to reddish or dull purple. Zooids are embedded in a transparent tunic and are all connected to one another by a network of blood vessels that terminate in sausage-shape ampullae (small sac-like structures) at the periphery of the colony. All zooids have an individual inhalant siphon, but a common exhalent siphon is shared by a group of zooids. The colonial vascular system plays an essential part in the integration of the colony as a whole. In young tunicates, the ampullae are situated along the edges, but as colonies grow they move to the interior.

Botrylloides violaceus reproduces sexually by producing larvae, and asexually by budding, as is the case with all colonial tunicates. Colonial tunicates are hermaphroditic; however, the male and female gonads develop asynchronously (Mukai *et al.*, 1987). Zooids of the female stage of the colony ovulate their eggs into the brood pouch and then sperm is released by the male phase of the colony. The sperm is collected through the inhalant siphon and fertilizes the egg which is approximately 60 μm in diameter (Mukai

et al., 1987). The larvae of the violet tunicate develop to a late stage within the brood pouch inside the colony, growing up to 2 to 3 mm long (Worcester, 1994). This is in contrast to solitary tunicates which have the egg fertilized by the sperm in the water column, external to the adult. Because of this, larvae only spend, on average, four min in the water column before settling (Worcester, 1994). As is the case with *C. intestinalis*, colonies of *B. violaceus* are capable of traveling large distances (*e.g.* 1800 m) by rafting on *Z. marina* (Worcester, 1994). Yamaguchi (1975) conducted a study showing how growth rates of *B. violaceus* vary with water temperature where colony size doubled in 7 days at 15 °C, 3.75 days at 20 °C and 2 days at 25 °C.

In a field study in Savage Harbour, PEI, in 2005, larvae were first noted in the water column on July 14th, the same day recruitment was observed on deployed collector plates (N. MacNair, pers. comm.). Larvae were last observed on October 14th with recruitment not observed after November 15th. Water temperature corresponding to first presence of larvae in the water column was between 10-12 °C. The end of larvae observed in the water column was also at this same temperature. Peak recruitment on collector plates occurred around August 18th, when summer water temperatures were high at approximately 20 °C (N. MacNair, pers. comm.).

This study also examined the effect of the tunicate on mussel condition and growth. The conclusion is that there was no significant difference in mussel productivity between tunicate-fouled and non-fouled mussel socks. The optimal mitigation strategy found was to spray vinegar (5% acetic acid) on the mussel socks to eliminate the tunicate, although this was probably not necessary based on the findings that mussel productivity

was not being affected by the violet tunicate. De-fouling at the time of processing is not considered an issue.

1.3.2.4. Botryllus schlosseri

The golden star tunicate is native to Europe and has been found on the east coast of North America, abundant in the Bay of Fundy and Bras D'or Lakes (MacNair, 2005). As for its local distribution on PEI, it was first identified in 2001 in St. Peter's Bay, but there were no additional reports of this tunicate until December, 2004, when this species and the violet tunicate were identified in Savage Harbour. Since identification in Savage Harbour, *B. schlosseri* has been found in Cardigan River (2005), St Mary's Bay (2005), Brudenell River(2005), Murray River (2006), Vernon/Orwell (2006), Hillsborough (2006), and West Point (2006) (J. Hill, pers. comm.)

The golden star tunicate is commonly grey in colour, being comprised of individual cells (zooids) arranged in clusters and embedded in a jelly-like matrix, similar to the violet tunicate. Each cluster consists of an excurrent siphon and is surrounded by several (often five) incurrent siphons (MacNair, 2005). The most identifiable feature of the golden star tunicate is the star-shaped markings on the surface of the colony (MacNair, 2005). Other than superficial differences, this species of tunicate is quite similar to *B. violaceus*. Yund and Stires (2002) found the minimum temperature required for sexual reproduction in the golden star tunicate to be 12 °C, again similar to *B. violaceus*. Presumably, the rest of *B. schlosseri*'s life history parallels *B. violaceus*. Presently, *B. schlosseri* is not thought to be a significant threat to the culturing of *M. edulis*.

1.3.3. Mitigation strategies to control tunicate fouling

Various treatments and production practices have been applied to *M. edulis* in an attempt to reduce recruitment and cause death of tunicates on the mussel socks. Systematic replacement of buoys on the mussel longlines and treatment of entire crops using acetic acid, salt brine solution and hydrated lime are used. However, these treatments are showing inconsistent results in reduction of tunicates on the mussel socks and are typically quite expensive due to the manpower required. Other treatments tried on the clubbed tunicate with little or no success include NaOH (caustic soda), hot water, steam, paraffin wax, lanolin, molasses, mild detergents (*e.g.* Mr. Clean, Palmolive, Sunlight), citric acid, formalin, hydrogen peroxide, short and long wavelength ultraviolet light, ultrasound, infrared light, electricity, pressure washing, and sugar (Davidson *et al.*, 2005). While some of these treatments show promising results, optimal mitigation strategies will require new husbandry and lease management practices to be an integral component of the solution.

In Italy, mussel growers periodically remove the socks from the water and place them on drying racks to allow for the tunicates to desiccate and die, or mussels are re-socked to remove fouling tunicates (G. Arsenault, pers. comm.). In South Africa, growers re-sleeve their mussels immediately following the recruitment of *C. intestinalis* (Hecht and Heasman, 1999). Results from N. MacNair (unpubl.) have indicated that with increasing stocking density of mussels at the time of socking, there was a decrease in the abundance of *S. clava*. In New Zealand, they have used several mechanical techniques to remove tunicates, including a submersible vacuum (Sinner and Coutts, 2003).

1.3.4. Interspecific competition between tunicates

Successful recruitment, defined as cumulative larval settlement over some longer period of time minus any post settlement mortality during that same period (Osman and Whitlatch, 1995a), is the critical criteria for establishing a population in a new ecosystem. How successful recruitment is depends on several factors including: post-settlement predation (Osman and Whitlatch, 1998); availability and type of substrate for settlement (Osman and Whitlatch, 1995b; Osman and Whitlatch, 1995a; Wieczorek and Todd, 1997; Osman and Whitlatch, 1998); larval size and density (Hurlbut, 1993; Marshall and Keough, 2003); food availability (Myers, 1990); risk of mortality from desiccation and/or siltation (Osman and Whitlatch, 1995a); and any other changes to the physical environment, such as extreme seasonal temperature fluctuations.

Osman and Whitlatch (1998) found predation of newly settled and juvenile life stages has the strongest influence on recruitment, with the snails *Mitrella* and *Anachis* consuming new recruits, and with fish and larger invertebrates preying on juvenile solitary ascidians, mussel recruits, and some bryozoan individuals. Different species appear to escape predation at different life stages with most bryozoans and the ascidian *Botrylloides* escaping before they are one week old, the colonial ascidian *Botryllus* escaping after 2-3 weeks, solitary ascidians only escaping as adults, and mussels perhaps never completely escaping (Osman and Whitlatch, 1998). Most species of invertebrates favour one substrate over another, as is the case with *C. intestinalis*, which, in a study by Weiczorek and Todd (1997), settles more readily on substrate bearing natural species biofilm. The numbers of both attached and trapped larvae increased with biofilm age.

Previous studies on tunicate competition have focused on control of new recruitment by resident adults, observing a variety of mechanisms. 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 preying on or overgrowing newly attached individuals (Osman and Whitlatch, 1995ab). Osman and Whitlatch (1995b) found that few settling individuals of any species were able to successfully attach to the surface of an established resident adult population. They concluded that "the principle effect of the resident species (which they studied) seems to be the removal of habitable space and the apparent aggregation of settlers in the remaining unoccupied areas" (Osman and Whitlatch, 1995b). According to Durante (1991) the main reason for settlers to aggregate include: increased likelihood of gamete encounter, and enhanced probability of survival on substrate where conspecifics have successfully settled and metamorphosed. Larvae have the ability to detect the presence of species whose competitive ability exceeds their own and will attempt to settle elsewhere (Grosberg, 1981; Young and Chia, 1981).

Osman and Whitlatch (1995b) conducted a study on the effect of resident adults on larval settlement using four species of tunicates: *B. Schlosseri*, *B. diegensis*, *Mogula manhattensis* (Dekay), and *S. clava*. They found that as the abundance of the resident adult population increased, the successful settlement of larvae decreased. Settlement onto the surfaces of tunicate species was rarely observed in this study. "All the settling species that were investigated were able to either differentiate between panel surfaces and those biological surfaces present, or were inhibited by the resident adults from attaching

to their exposed surfaces" (Osman and Whitlatch, 1995b). The probability of settlement onto adults of any of the four species of tunicates tested was extremely small in their experiments (Osman and Whitlatch, 1995b) and they conclude that the principle way in which resident sessile species affect recruitment is by changing the quantity of available space (Osman and Whitlatch, 1998).

Prior background has focused on how settlement and recruitment levels of invertebrates, particularly tunicates, can be manipulated by environmental factors. It is also of interest to observe how established individuals and colonies interact with tunicates post-settlement. Osman and Whitlatch (1995a) identify three factors affecting survivorship including (1) predation or overgrowth by residents, (2) added physical structure for firmer attachment and (3) camouflage from motile predators. In their study to determine the effect of resident adults on post-settlement mortality, Osman and Whitlatch (1995a) concluded "resident species influenced settlement, but had little effect on post-settlement mortality." It has been observed that some species of adult organisms such as the colonial tunicates *Diplosoma* and *Botryllus* (Osman and Whitlatch, 1995a) have the ability to overgrow or crush weaker spatial competitors (Connell, 1961; Hunt and Scheibling, 1997). Overgrowth by colonial organisms does not, however, pose a lethal problem to *Mogula citrina* because this solitary ascidian has the capability to keep its siphons exposed through holes in the colony (Sebens, 1986). Presumably, this would be true of any thick skinned solitary tunicate, such as *S. clava.*

Sutherland and Karlson (1977) demonstrated that some dominant species could persist on individual substrates by inhibiting subsequent recruitment of other species. Substrates at the same site immersed (or disturbed) at different times produced a mosaic

of dominance among the substrates within a site (Sutherland, 1974; Osman, 1977). Roughgarden (1998) says that, according to Gaussian precepts, no two congeneric species with similar requirements should be able to occupy the same niche for long.

1.4. Mussel Stocking Density

1.4.1. Effect on tunicate recruitment

Anecdotal reports from mussel growers indicate that increasing mussel stocking density has the effect of decreasing tunicate recruitment on mussel socks. There is no clear explanation for these observations but the following three hypotheses can be proposed. The first hypothesis is that there will be a decrease in exposed surface area per mussel as stocking density increases. Only mussels on the outside of the sock will be exposed to tunicate recruitment, leaving the mussels in the core of the sock free from tunicate fouling. The second hypothesis is that self-thinning will be more intense with increased stocking density (Fréchette and Lefaivre, 1990; Fréchette *et al.*, 1992; Fréchette *et al.*, 1996). When tunicates attach to mussels on a sock, they add additional weight to the mussels, thereby stressing the byssal attachment. In doing this, the tunicates may act as a catalyst in the self-thinning hypothesis, stressing the byssal threads of the mussel to the point of failure. Both the mussel and the tunicates attached to it would be removed from the mussel sock. The end result would be a clean, unfouled inner core of mussels. The third hypothesis is that an increase in stocking density decreases tunicate recruitment because there would be more filtering action with more mussels. Filtration by mussels may be setting up micro-currents around the sock which keeps the tunicate larvae from settling, once again making high density most effective (Green *et al.*, 2003). Also, as

mussels are filtering for food, they may take in surrounding larvae before attachment occurs and expel them as pseudofaeces, thereby decreasing the viability of the organism (Mileikovsky, 1974; André *et al.*, 1993; Davenport *et al.*, 2000; Lehane and Davenport, 2004).

1.4.2. Effect on mussel production

Several studies have focused on the effect of initial stocking density on mussel production over the last 20 years (Frechétte *et al.*, 1996; Lauzon-Guay *et al.*, 2005ab). Some are attempting to model mussel growth using a variety of parameters, including zooplankton and phytoplankton biomass (Dowd, 1997), seed size and density (Fréchette *et al.*, 1996; Lauzon-Guay *et al.*, 2005a), stock and site (Dickie *et al.*, 1983; Mallet *et al.*, 1987; Waite *et al.*, 2005), and food and space (Fréchette and Lefaivre, 1990). Drapeau *et al.* (2006) conducted an epidemiological field trial to determine best farm management practices using sock spacing, seed size, seed density, sock length, and longline spacing. Optimal stocking density has been defined by Carver and Mallet (1990) as the stocking density at which yield is maximized without negatively affecting growth rate. The aim of this particular thesis was not to determine which mussel stocking density maximizes yield, but rather which stocking density shows the least amount of fouling by tunicates without significantly affecting growth rates or biomass of mussels.

In order to determine the optimal density for reducing tunicate recruitment on mussel socks, it is necessary to evaluate the effect of stocking density on mussel growth rates and condition. Density dependence in cultured populations is not infrequent and has the potential to act at different scales within whole systems (Fréchette and Bacher, 1998). Several studies have been published (Lauzon-Gauy *et al.*, 2005a; Drapeau *et al.*, 2006)

which indicate that as stocking density increases in a production system, the growth rate and condition of the mussels decreases. As stocking density increases, growing conditions become crowded and there is a reduction in the per-individual ration of food and space, which in turn reduces the average body size, resulting in a negative correlation between body size and stocking density (Petraitis, 1995). Density-dependent regulation of growth rate and reproductive effort has been documented in many populations of infaunal as well as epifaunal bivalves (Fréchette and Lefaivre, 1990).

As mussels are grown in socks there exists a limited amount of space for their growth. As such, a process known as self-thinning often takes place, whereby populations undergo competition-driven mortality or spacing out (Grant and Kramer, 1990). It is an intraspecific process (Petraitis, 1995) caused by physical interference or crowding and exploitive competition for food (Fréchette *et al.*, 1992). The most obvious form of physical interference is the impairment of the shell opening (Fréchette *et al.*, 1992) which is a critical factor in controlling mussel pumping rate (Jørgensen *et al.*, 1988). Fréchette *et al.* (1992) suggested that when crowding interferes with normal feeding, condition index will decrease inversely to mussel size.

All of the above information indicates that as stocking density increases, mussel growth and condition decreases; however it is not clear whether there is a lower stocking density at which growth and condition begin to decrease. If stocking density is too low, mussels may not be forced out of the socking material and therefore into a better position for food acquisition (personal observation). Carling (1992) showed that the presence of neighbours slows the water current flow, thereby increasing the residence time of

suspended particles in the area. Prins *et al.* (1995) also found that the optimum population density for mussels are not the lowest ones.

Fréchette *et al.* (2005) described stocking density as the most central issue in aquaculture management. Stocking density has an effect on both the amount of food and space available to each individual mussel in a sock. Growth and survival have been shown to be density dependent in many populations of benthic suspension feeders, but it is generally difficult to determine whether food or space is the limiting factor (Fréchette and Lefaivre, 1990). Alunno-Bruscia *et al.* (2000) found that survival was negatively correlated with density, but did not differ significantly between food regimes, which would indicate space is the limiting factor. They also concluded that the survival time of mussels was negatively related to density indicating that mussels at high densities died earlier than mussels at low densities. Food, space or light are in short supply at high densities which results in intra-specific competition (Brichette *et al.* 2001).

When conducting experiments on mussel density and size on morphology, Lauzon-Guay *et al.* (2005b) found that in some instances mussels became narrower and had a lower tissue-to-shell ratio at high densities. They started with initial densities ranging from 100 to 800 mussels per 30 cm. It was expected that there would be more substantial differences between densities regarding shell dimensions, but the inconsistent effect of density on mussel morphology was thought to be due to density dependent mortality and fall-off. Another study by Lauzon-Guay *et al.* (2005a) found that initial stocking density had little effect on mussel length throughout the experiment, but did affect tissue-to-shell-ratio and survival. Survival of small seed generally decreased with increasing density while survival of large seed was not affected. In both experiments,

mussel seed grown at low or high initial density reached commercial size in the same period of time.

Fréchette *et al.* (1996) conducted some modeling experiments in which they used field trials to determine Optimal Stocking Density (OSD). Using a classical approach, they found the OSD to be 400 mussels per 30 cm, but with the biomass-density relationship (BN) approach it was suggested the OSD be between 120 and 200 mussels per 30 cm, using a medium sized mussel. The recommended stocking density for suspension grown mussels is 180 mussels per 30 cm according to Mallet (1989). The precise OSD will depend on the growing conditions of the area, but in general most mussel producers stock between 150-200 mussels per 30 cm (N. MacNair, pers. comm.).

1.5. Objectives

Prince Edward Island has seen the arrival of four exotic tunicate species in the last decade which have established and become invasive in various locations around the Island. It is of critical importance to determine best management practices to cope with this incursion to ensure the viability of the mussel industry which is currently left vulnerable. The study area chosen was Brudenell estuary because all four tunicate species have been documented to exist.

In order to identify best management practices it is important to understand where the greatest challenge exists. In 2004 and 2005, three of the four tunicates arrived with the other having been detected in 1997 and currently well established. The first part of the study focused on the competitive interactions among these four species to determine if there was one species which emerged as the dominant competitor. That is, we

investigated which of the four species would establish itself as the dominant fouling organism in a system where all four species are known to exist.

Secondly, as a prerequisite in any management plan to control an unwanted pest, the basic biology must be understood. Reproduction, early life history, and the development of the tunicates were investigated. By conducting this study, we determined if tunicate species and abundance and their recruitment changed over time. In addition, this study investigated the possibility that inter-specific competition was affected by the order of tunicate species recruitment. That is, does having *C. intestinalis* settle first influence the settlement patterns of other tunicates, which settle after *C. intestinalis*?

The effect of mussel stocking density on the recruitment of tunicates needs to be explored. Mussel growers usually use a moderate density of 150-200 mussels per 30 cm, although, in some cases, densities can be found outside this range. Tunicate recruitment was monitored on mussel socks at various densities and thereafter for growth. Growth was quantified by length, weight, and abundance. Mussel productivity was assessed by length, Condition Index (CI), and mortality.

On PEI mussel socking is typically done during the fall of the year, but can also be done in the spring. Therefore, it was appropriate for this project to follow the timeline of the industry. The project determined the effect of fall socking vs. spring socking on mussel productivity and tunicate establishment. It was thought that spring socking did not give the mussels enough time to "come out" of the socking material and adequately attach their byssal threads. Industry believed that at high densities, significant mortality and/or fall-off of mussels occurred due to intraspecific competition, weight of the additional biomass and weakening of byssal threads during the summer months.

Therefore, socking was done in both fall and spring to determine if mortality and/or fall-off were significant.

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Chapter 2 PROCESS OF INVASIVENESS AMONG EXOTIC TUNICATES IN PRINCE EDWARD ISLAND, CANADA

Ramsay A, Davidson J, Landry T and Arsenault G. The process of invasiveness among exotic tunicates in Prince Edward Island, Canada. Biological Invasions (submitted).

2.1. 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 the second exotic tunicate reported in that system 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 an exotic species being detected, its establishment, invasiveness, and eventual nuisance is presented.

2.2. Keywords

aquatic invasive species; *Botrylloides violaceus*; *Botryllus schlosseri*; *Ciona intestinalis*; exotic species; nuisance species; *Styela clava*

2.3. Introduction

The introduction and establishment of exotic species, defined as a species deliberately or accidentally released into an area in which it has not occurred, has become an increasing problem in recent years (Binggeli, 1994). Non-indigenous species have greater opportunity of "hitching a ride" to exotic places through several mechanisms. The main vectors identified in the marine and freshwater environments include: attachment to ship hulls, transfer through ballast waters, 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 nuisance in its host area (Stachowicz *et al.*, 1999; Locke *et al.* 2007). An exotic species is considered invasive when its population becomes self-generating, typically expanding and competing with indigenous species, and exists in a free-living state in the wild (Binggeli, 1994). Both indigenous and exotic species can become nuisance or pest species, by interfering with the objectives or requirements of producers (Binggeli, 1994).

Prince Edward Island (PEI) has 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 tunicate species have been identified in the waters surrounding PEI (MacNair, 2005; Locke *et al.*,

2007). The introduced species are: *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 of 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 by 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 preying on or overgrowing newly attached individuals (Osman and Whitlatch, 1995ab). Established individuals and colonies interact with other tunicates post-settlement. Osman and Whitlatch (1995b) identified three factors affecting survivorship including (1) predation or 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 have the ability to overgrow weaker spatial competitors (Connell, 1961; Hunt and Scheibling, 1997), such as the colonial tunicates *Diplosoma* and *Botryllus* (Osman and Whitlatch, 1995b).

The four 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 have also shown to be a significant fouling organism to the cultured mussel, *Mytilus edulis* (L.), thereby classifying them as nuisance species. Production and processing costs are increasing and the tunicates are competing for food and space with the mussel (Thompson and MacNair, 2004). In addition to affecting the economically important industries associated the infested waters, these species have the potential to alter the local fauna and flora, displacing native species (Berman *et al.*, 1992).

The goal of this study was to document the invasion process of an exotic species from its successful establishment to its eventual categorization as a nuisance species in an environment already successfully invaded by another exotic tunicate. A more thorough in-depth understanding of the tunicate population dynamics and the process of becoming a nuisance species can be achieved through this study. Information on the successful establishment and spread of an exotic species is essential for policy makers when determining initial responses and management scenarios for new introductions to uninfested areas.

2.4. Materials and Methods

2.4.1. Study Area

The Brudenell estuary, located at the eastern end of PEI, was chosen for this study due to its particular site characteristics (Figure 2.1). Georgetown Harbour is a deep water port, located on the north side of the estuary and is considered a likely site for species to

be introduced through shipping traffic. This estuary was the site for the first detection of both *S. clava* and *C. intestinalis* in PEI. This estuary is also highly productive and economically important for the cultured mussel *M. edulis*, generating high growth rates (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).

2.4.2. Experimental Design

A series of collector plates, 10 x 10 cm pieces of PVC, were used as the primary substrate for colonization by tunicates. 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 metres 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 the same 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 in weight).

2.4.3. Laboratory Analysis

Tunicates were removed from the collector plates and sorted by species. The total tunicate weight, by species, was measured for the entire collector plate; hydriods, caprella, and other species were removed prior to weighing. In 2003 and 2005, the samples were frozen and then later thawed to determine mean tunicate length and abundance. All individual tunicates larger than 5 mm were measured and counted. In

2006, mean length and abundance was determined by measuring and counting all individuals from 25 % of the collector plate within 36 hrs of removal from the water. There was no apparent difference in the length of the tunicates within this length of time. Tunicates were measured from their holdfast to the distal edge of the incurrent siphon. *Ciona intestinalis* exhibited a tactile response during handling and would contract, thereby reducing its length. As a solution to this problem, tunicates were spread out on a tray and left for 15 minutes to relax. Measurements were then taken, before the tunicates could contract. Care was taken to accurately measure the tunicate without touching the tunicate with the calipers.

2.4.4. Statistical Methods

Tunicate abundance (total # of tunicates on bottom surface of collector plate), mean weight and length, within species, were compared between sample years using two-sample t-tests. 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. Comparisons among the two tunicates by weight between sampling years were analyzed using the non-parametric Kruskal-Wallis test, as a result of unequal variances between years. Pairwise comparisons among the two tunicates between sample years were done using two-sample t-tests with unequal variances and Bonferroni correction. All data were analyzed using Minitab 15 (© 2006 Minitab Inc.). All estimates are reported as mean ± standard error.

2.5. 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* on collector plates, in the fall of 2003, was estimated at 351.8 ± 44.5 with a mean length of 7.2 ± 0.7 mm (Table 2.1 and Figure 2.2). The abundance and distribution of *S. clava* in that system was not evaluated in 2004. However, that year, *C. intestinalis* was identified in the upper reaches of the Montague River in limited abundance through the use of dive surveys (Figure 2.1). Reports from MacNair (2005) indicated that *C. intestinalis* remained in a small area of Montague River, with *S. clava* greatly exceeding (> 100X) 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 (Figure 2.2). 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, with an average of 267.8 ± 28.4 individuals per collector plate, but this decrease was not statistically significant ($P = 0.131$). *Styela clava* weight on the collectors increased marginally between 2003 and 2005 ($P = 0.057$), but there was a significant difference ($P < 0.005$) in the length of *S. clava*, increasing to 23.5 ± 1.7 mm from 7.2 ± 0.7 mm in 2003.

In 2006, two years after *C. intestinalis* was first identified, it had spread throughout the estuary. Its abundance had risen to alarming proportions, averaging 444.4 ± 42.3 individuals per collector plate, significantly greater ($P < 0.005$) than 2005

abundance. The weight and mean length of *C. intestinalis* had increased from 2005; weight increasing from 22.5 ± 8.4 to 2007 ± 218 g ($P < 0.005$) and length increasing from 28.8 ± 3.7 to 55.5 ± 1.8 ($P < 0.005$). The number of *S. clava* had declined to near eradication with sporadic observations of older animal (> 1 year old) on mussel culture gear, and zero abundance on the collectors (Figure 2.2). By the end of this study, *C. intestinalis* abundance had risen above *S. clava* abundance at its height in 2003. The weight of *C. intestinalis* on the collector plates was far greater (and significantly different, $P < 0.005$) than *S. clava* had ever been, averaging 2007.0 ± 218.0 g in 2006 compared to a maximum of 117.0 ± 17.0 g for *S. clava* in 2005 (Figure 2.3).

2.6. 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 greatest threat, especially to the well established mussel aquaculture industry. Within two years of being identified, *C. intestinalis* had established as the dominant fouling species for mussel culture 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?

In 2006, it became evident that *C. intestinalis* was a strong competitor for spatial resources. Recruitment of this species of tunicate was observed to start one month earlier (June) compared to *S. clava* (July). The reproductive cycle of *C. intestinalis* starts when

water temperatures reach 8 °C (Carver *et al.*, 2003; Howes *et al.*, 2007; refer to Chapter 3), while the reproductive cycle of *S. clava* only starts when water temperature exceeds 12 °C (Bourque *et al.*, 2006). This 4 °C gap (corresponding to approximately one month) provides *C. intestinalis* a significant recruitment advantage over *S. clava* (Stachowicz *et al.*, 2002). 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). Filtration may also play a role in this inter-specific competition.

Unpublished 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.* (refer to Chapter 3) only one observation was made of *S. clava* settling on *C. intestinalis*. This was late in the field season and it appeared that at this time, the tunic walls of *C. intestinalis* were thick enough for *S. clava* settlement. This late in the season, however, the recruitment level of *S. clava* was 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 one year of age. However, in PEI, *C. intestinalis* exhibits a long and continuous breeding season (refer to Chapter 3). New recruits can reproduce in their first year and there is even a possibility of three generations within a single season. Two important factors contributing to *C. intestinalis* success in these rivers are (1) a long continuous reproductive season and (2) the presence of a mucoidal tunic to hinder settlement of other species.

Previously established criteria to define exotic, invasive and nuisance species can be used here to illustrate the dynamics between the two foremost fouling organisms, *S.*

clava and *C. intestinalis*. As clearly illustrated in Figure 2.2, the abundance of *S. clava* peaked between 2003 and 2005, while *C. intestinalis* was still in its establishment phase in 2005. *C. intestinalis* only entered its invasive phase after 2005, when its population shows signs of self-regeneration and expansion. At this point, it is possible that the invasion of *C. intestinalis* was starting to dominate habitat niches for other species, including *S. clava*. In 2006, *C. intestinalis* abundance has increased well above *S. clava*, replacing it as the main nuisance species for mussel aquaculture in the Brudenell estuary. *Styela clava* is no longer considered to occur in sufficient numbers to warrant the 'nuisance' label in this area, and could eventually be re-classified as exotic to this area. Other *S. clava* infested areas in PEI such as Malpeque Bay and Murray River, where *C. intestinalis* has not established, have stable or increasing populations, 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 interesting to observe if the process of invasiveness for *C. intestinalis* will be similar.

2.7. Acknowledgements

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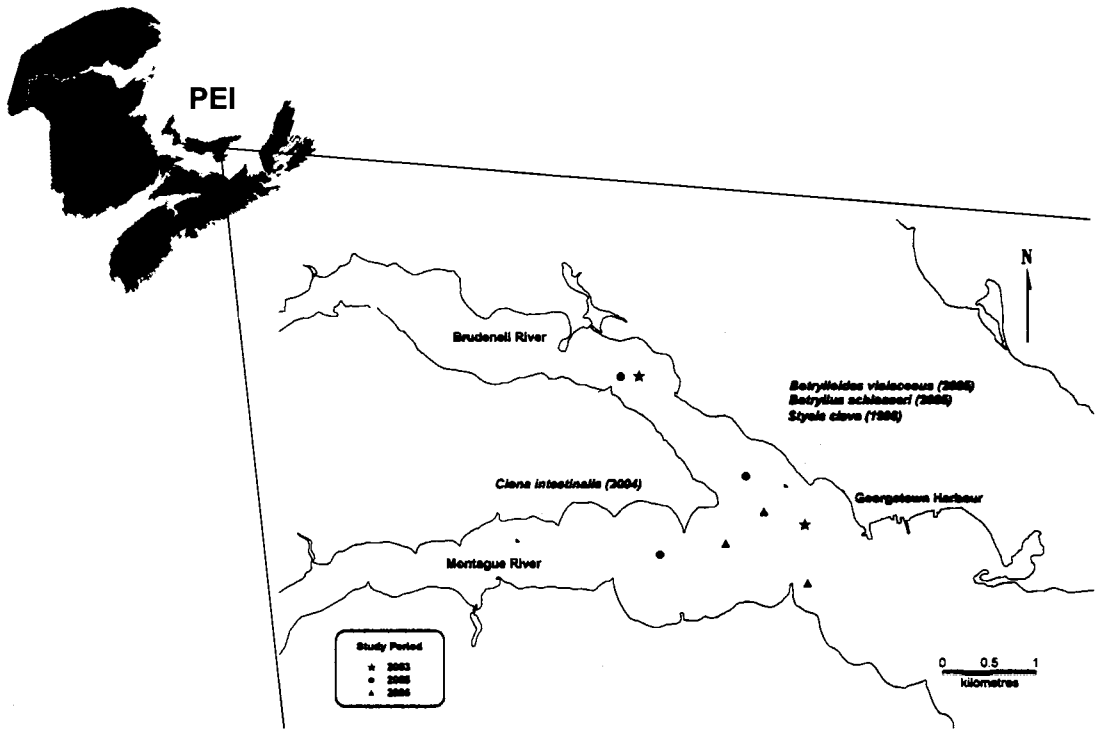


Figure 2.1 Map of the Brudenell estuary with sample sites and the areas of initial tunicate species detection identified, in PEI (Map created using MapInfo Professional 7.0).

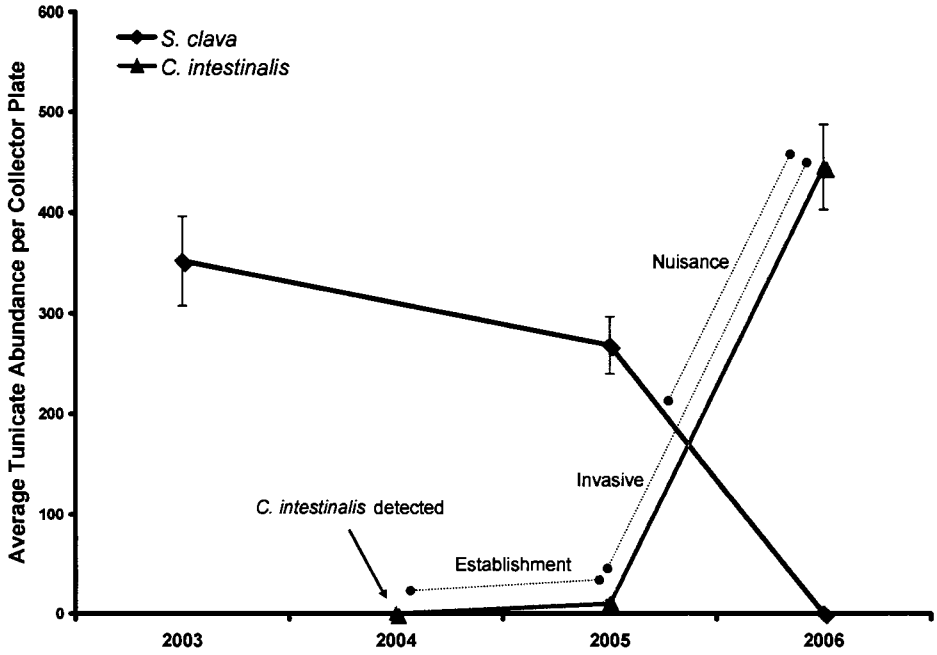
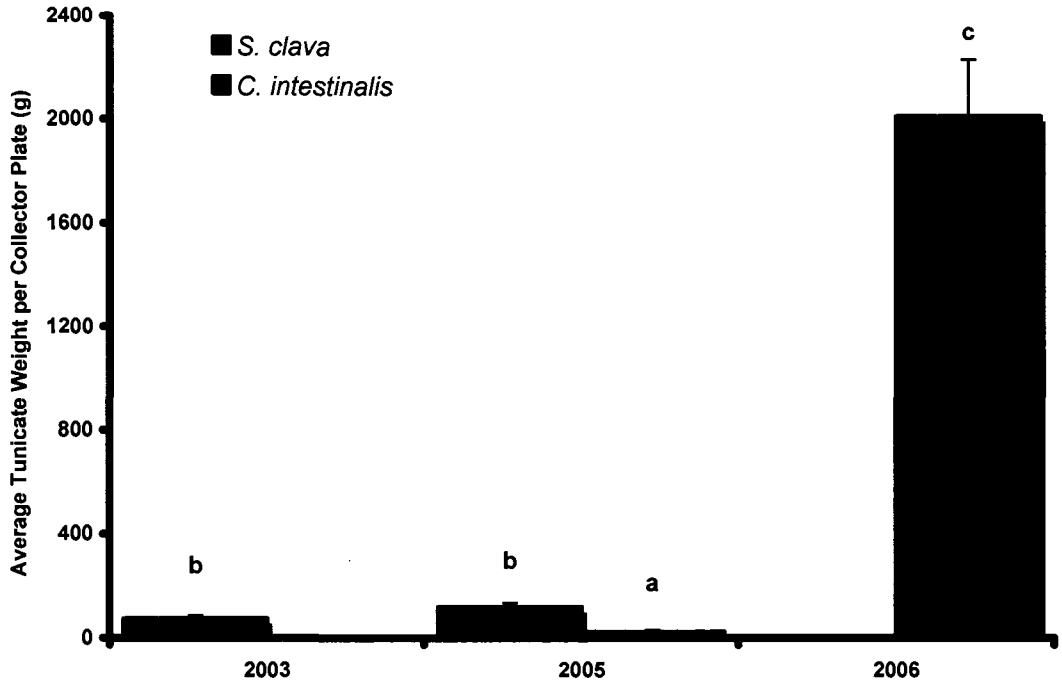


Figure 2.2 Mean abundance of *S. clava* and *C. intestinalis* by sampling year in 2003, 2005 and 2006. Stages of establishment, invasiveness, and nuisance are indicated for *C. intestinalis*.



[a-b] means without a common subscript were significantly different ($P < 0.05$)

Figure 2.3 Mean weight (± SE) of *S. clava* and *C. intestinalis* by sampling year in Brudenell estuary.

Table 2.1 Tunicate abundance, weight, and mean length (± SE), by species, in the three years of the study.

Year	*Styela clava*			*Ciona intestinalis*		
	Abundance	Weight (g)	Length (mm)	Abundance	Weight (g)	Length (mm)
2003	351.8 ± 44.5 [a]	73.1 ± 13.8 [a]	7.2 ± 0.7 [a]	N/A	N/A	N/A
2005	267.8 ± 28.4 [a]	117.0 ± 17.0 [a]	23.5 ± 1.7 [b]	9.9 ± 3.5 [a]	22.5 ± 8.5 [a]	28.8 ± 3.7 [a]
2006	0 ± 0 [*]	N/A	N/A	444.4 ± 42.3 [b]	2007.0 ± 218.0 [b]	55.5 ± 1.8 [b]

[*] excluded from statistical analysis

[a-b] within a column, means without a common subscript were significantly different ($P < 0.05$)

Chapter 3 REPRODUCTION, EARLY LIFE HISTORY AND SEASONAL DEVELOPMENT OF THE INVASIVE ASCIDIAN *Ciona intestinalis* IN PRINCE EDWARD ISLAND, CANADA

Ramsay A, Davidson J, Bourque D and Stryhn H. Reproduction, early life history and seasonal development of the invasive ascidian *Ciona intestinalis* in Prince Edward Island, Canada. ICES Journal of Marine Science (submitted).

3.1. Abstract

In 2004, the exotic tunicate, *Ciona intestinalis*, was first detected in Montague River, Prince Edward Island. Since that time, this previously exotic species has become a nuisance for local aquaculture industries with varying degrees of severity. The reproductive ability and rapid biomass accumulation of *Ciona intestinalis* creates a challenge for management. Reproductive biology and development of *C. intestinalis* was investigated to assist aquaculturalists in developing control strategies and to understand the risk of spread to other aquaculture sites. The study included *C. intestinalis* gonad development, larval abundance and settlement, and subsequent development post-recruitment. Histology indicates gonads are developed and are capable of spawning from May to December. Larvae were observed in water samples from the first sampling in early August through November, with a distinct peak in early October. Recruitment on experimental collectors, however, occurred from mid June until early December, with a peak in late-August. A rapid increase in biomass was documented in late July, six weeks after the initial recruitment. No substantial increase in *C. intestinalis* biomass was observed after mid August. A detailed understanding of the life cycle of *C. intestinalis* is considered a prerequisite in any management effort to mitigate its impact.

3.2. Keywords

Ascidian; *Ciona intestinalis*; Gonad development; Larval concentration; Recruitment; Reproductive period, Seasonal dynamics

3.3. Introduction

Culturing the blue mussel, *Mytilus edulis*, on Prince Edward Island (PEI) is facing its greatest challenge since its beginning over 20 years ago. Four exotic species have been introduced into Prince Edward Island (PEI) waters, and have become invasive, nuisance species (refer to Chapter 2). These four ascidians; *Styela clava* (clubbed tunicate), *Ciona intestinalis* (vase tunicate), *Botryllus schlosseri* (golden star tunicate), and *Botrylloides violaceus* (violet tunicate) have proved to be significant fouling organisms to the cultured mussel industry, especially in terms of increased costs of production and processing (Thompson and MacNair, 2004) and potential competition for space and food. As such, they have the potential to negatively affect growth rates and meat yields of the cultured mussel. Mitigation strategies for tunicates on mussels and aquaculture gear have proven costly with inconsistent results (Carver *et al.*, 2003; Thompson and MacNair, 2004; Davidson *et al.*, 2005). A long-term strategy for the mitigation of tunicate impacts should include farm management practices that result in decreased biomass of tunicates on mussel gear and mussels. An understanding of the life cycle and recruitment success of these organisms is an essential prerequisite of formulating such management strategies.

The most recently identified tunicate, *C. intestinalis*, has proven to be highly successful in its new environment. Since its first detection in the estuarine section of

Montague River, PEI, in 2004, it rapidly spread and established itself in that system, as well as the adjacent Brudenell estuary (refer to Chapter 2). Prior to the introduction of *C. intestinalis*, these rivers were dominated by *S. clava* but now its population has declined to near non-existent levels with the increasing infestation of *C. intestinalis*.

The successful establishment of *C. intestinalis* is largely a result of its reproductive capacity. *Ciona intestinalis* is capable of producing gametes continually as long as temperatures are suitable (Carver *et al.*, 2006). The lower limit for spawning activity was observed at 8 °C in a Scandinavian population (Dybern, 1965; Gulliksen, 1972) and in Nova Scotia, Canada (Carver *et al.*, 2003). Carver *et al.* (2003) documented the presence of ripe eggs in the ovary from November (7 °C) through January (1 °C) although the animals were not spawning during this period. Signs of egg resorption were evident in February and March (-1 °C), with gamete production resuming in April, when water temperature increased to 4 °C. The total number of eggs produced by an individual in a lifetime has been estimated to be between 10 000 (Petersen and Svane, 1995) and 12 000 (Carver *et al.*, 2003). In contrast, Yamaguchi (1975) found that an individual was capable of producing 100 000 eggs in a lifetime in the Pacific Ocean. This geographical discrepancy may reflect the effect of environmental condition (such as temperature).

After the egg has been fertilized in the water column the tadpole-like larvae hatches 18 - 22 hrs later (Cirino *et al.*, 2002; Bullard and Whitlatch, 2004). Embryogenesis varies with temperature and generally the time required increases with decreasing temperature and the same is true of the larval stage (Carver *et al.*, 2006). In two studies of British populations, Millar (1952) reported the time to settlement to be 6-

36 hrs, whereas results from Jackson (2005) showed the settlement time to be between 2-10 days.

Observations on the life history of the *C. intestinalis* population in Lunenburg Bay, Nova Scotia, by Carver *et al.* (2003) were consistent with a pattern of two generations or recruitment peaks per year (Dybern, 1965). One recruitment event was observed in May-June and a second in August-September 2000 (Carver *et al.*, 2003). Howes (2007) conducted a monitoring program in the nearby Mahone Bay from 2002 to 2005 and also observed two recruitment peaks, generally lasting an additional month compared to those from Carver *et al.* (2003): May-July and August-October. In the study by Carver *et al.* (2003) average growth rate was estimated at 20 mm mo^{-1} at 10-20 °C growing to a maximum body length of 140 mm.

This study focused on the reproductive ability and seasonal development of *C. intestinalis* on PEI, investigating seasonal changes in gonad development, larval concentrations, recruitment patterns and biomass development. This information should be used in conjunction with newly developed husbandry and mitigation techniques to lessen the impact of *C. intestinalis* on mussel aquaculture.

3.4. Materials and Methods

3.4.1. Study Site

The confluence of the Brudenell and Montague Rivers, located at the eastern end of PEI, was chosen for this study because of its abundance of *C. intestinalis* (Figure 3.1). The associated rivers are very productive; in 2004, the growth rates for *M. edulis* were as high as 2.25 to 2.32 mm mo^{-1} (Department of Fisheries and Oceans, 2005). Montague

and Brudenell Rivers accounted in 2004 for 10.4 % of the total mussel production on PEI (Department of Fisheries and Oceans Canada, 2006). The temperature ranged from -1.5 °C during ice cover (February, 2006) to 21 °C in the summer (August, 2006), while the salinity ranged from 15 ppt in the fall to 30.1 ppt in the spring and the amount of dissolved oxygen ranged from 4.9 to 10.1 mg L^{-1}, with the higher levels being observed in the spring.

3.4.2. Histological Analysis

Six *C. intestinalis*, of at least 50 mm in length, were randomly sampled on a weekly basis from 24 February 2006 to 8 January 2007 to evaluate gonad development. Sections of ovary and testis were excised from fresh live specimens and fixed in a 10% formalin and seawater solution. Histological process and slide preparation followed the procedures of Howard and Smith (1983). Ovary and testis staging criteria established for *S. clava* by Parker *et al.* (1999) were modified for *C. intestinalis* staging in this study and were limited to three stages: developing, ripe, and spawned, based on ovary and testis size, presence of ripe spermatozoa and ova, and signs of regression of the gonad (Table 3.1). When multiple developmental stages were observed in a single sample only the most fertile stage was reported.

3.4.3. Larval Sampling

Weekly larval samples were collected near Site 2 (see Figure 3.1) with a bilge pump from 2 August to 4 December 2006 to evaluate *C. intestinalis* larval concentrations. A weight was attached to the pump so it could be lowered vertically into the water column. For each sample, the pump was raised and lowered approximately every 10 s through the first 2 m of the water column as to target the entirety of this

section of water. Water was pumped (50 L min^{-1}) through a 64 μm sieve for 3 min to collect larvae. Three samples were taken on each date and were always collected early in the afternoon. Sieve contents were fixed with ~10 % formalin. In the laboratory, samples were concentrated to 10 ml and placed into a Ward Counting Wheel. Larvae were identified and counted under a stereo microscope.

3.4.4. Recruitment

The magnitude of *C. intestinalis* recruitment (the accumulation of settled individuals minus post-settlement mortality over a pre-determined time period) during its reproductive season was estimated using 10 cm x 10 cm PVC plates suspended in a horizontal position from three mussel longlines at Sites 1-3 (see Figure 3.1), located 2 to 3 m below the water surface (see Bourque *et al.*, 2007). Three plates, separated by 0.5 m, were attached to a rope and tied to each of three mussel longlines for a 2 week period, at which time they were retrieved and preserved in 4 % formalin. This was repeated every 2 weeks from 1 May 2006 to 11 December 2006. In the laboratory, the number of ascidian recruits was estimated under a dissecting microscope. A transparent grid was designed for accurately counting juvenile tunicates on the collector plates. Species identification used the guidelines of Bullard and Whitlatch (2004). In this study, collector plates were directly deployed, as opposed to preconditioning by exposing collectors to sea water to allow biofilm to develop (Osman and Whitlatch, 1995; Bourque *et al.*, 2007). Preliminary assessment of the effect of preconditioning did not show significant difference with direct deployment.

3.4.5. Temporal Development

Biomass accumulation of *C. intestinalis* during its reproductive season was investigated by deploying 15 collectors in early May on each of the three mussel longlines. One collector was retrieved by SCUBA diving every two weeks for the entire field season until the end of November 2006 to document when *C. intestinalis* weight began to increase. Collector plates were removed from the collector rope and sealed in plastic Ziplock© bags for transport. Samples were quantified within 36 hrs of removal from the water because it was difficult to preserve specimens and still have accurate measurements. Total tunicate mass was recorded for the entire collector plate, and mean length and abundance were determined from ¼ of the collector plate. Hydroids, amphipods of the genus *Caprella*, and other species found on the collector plates were removed prior to determining tunicate weight. For tunicate abundance and mean length only specimens larger than 5 mm, from their holdfast to the branchial siphon, were considered. *Ciona intestinalis* exhibited a tactile response during handling and would contract, thereby reducing its length. To avoid false measurements tunicates were spread out on a tray and left for 15 min to relax. Measurements were then taken before the tunicates could contract.

From the beginning of May until mid-November 2006 new collector plates were deployed on each mussel longline every 2 weeks to determine the temporal development of *C. intestinalis*, with time of deployment manipulated. These collectors remained in the water column until the end of November 2006, at which time they were retrieved. The plates were quantified in the same manner as the collector plates deployed at the beginning of the field season.

3.4.6. Statistical Analysis

Larval concentrations were compared between sampling dates using a 1-way ANOVA. Recruitment and temporal development (abundance, weight and length) were analyzed using linear models (Christensen, 1996) to determine significant differences between sample sites (Figure 3.1), water column position (top, middle, and bottom) and sample/deployment dates. First order interactions were included but removed if non-significant. The model assumptions were assessed by residual analysis and Box-Cox analysis, and the dependent variables were subjected to transformation by the natural log, square root, or the cubic root transformations as appropriate to meet the model assumptions. In order to enable log transformations, values equal to zero were changed to half the value of the lowest, non-zero value included in the analysis prior to transformation. Sampling dates with zero or negligible occurrence of *C. intestinalis*, as well as extreme outliers, determined by the outlier detection test based on deletion residuals, were omitted from analysis. Pairwise comparisons between dates used the Bonferroni method. In presence of a significant interaction between dates and sites, the interaction was modeled as a random effect to allow for overall comparisons between dates and sites. Estimates are presented as least squares means with 95 % confidence intervals, backtransformed to original scale as appropriate. The significance level was set at 0.05. Data were analyzed using Minitab 14 (© Minitab Inc.) and SAS 9.1 (© SAS Institute Inc.).

3.5. Results

3.5.1. Histological Analysis

Results show both ovarian and testicular tissue in *C. intestinalis* to be regressing and/or spawned (stage 3) from 24 February to 7 April 2006. In the period from 24 April to 10 May 2006 ovaries were classified as developing (stage 1), and were ripe (stage 2) thereafter until the samples obtained 5 December 2006. Testes were determined to be developing from 24 April to 3 May 2006 and were ripe thereafter in samples taken from 10 May to 5 December 2006. Gonads were determined to be regressing and/or spawned again by 8 January 2007 (see Table 3.1).

3.5.2. Larval Concentrations

Larvae were present in the water column from the beginning of sample collection on 2 August until 22 November (Figure 3.2). Concentrations varied between sampling dates ($P < 0.001$), but showed an increasing trend from 2 August until 6 October, when a spike of 7.2 larvae L^{-1} was recorded. Apart from this one sample collection, larvae concentrations were between 0.03 and 1.88 larvae L^{-1}. Larval concentrations then decreased and the last record of *C. intestinalis* larvae in the samples was on 22 November.

3.5.3. Recruitment

The first recording of *C. intestinalis* was on 13 June at 0.3 individuals cm^{-2}, representing recruitment during the two preceeding weeks with a mean water temperature of 8.9 °C (Figure 3.3). Recruitment of *C. intestinalis* steadily increased throughout the summer until it peaked at 48.4 individuals cm^{-2} in the two weeks prior to 22 August, where mean water temperature reached a high of 17.7 °C. One plate from this collection

time had approximately 23 000 individuals recruited. The last observed recruitment was on 28 November when *C. intestinalis* abundance dropped to 0.1 individuals cm^{-2}. At this time, mean water temperature had dropped to 8.5 °C and continued to drop to a mean of 6.1 °C for the following two weeks. Recruitment levels were different between sample dates ($P < 0.001$), the three water column positions ($P = 0.015$, with the middle position having higher recruitment, although this trend was not consistent throughout the study period) and marginally different between the three sample sites ($P = 0.054$), with a significant sample date by site interaction ($P < 0.001$). Site 3 had significantly higher recruitment than the other two sites, when recruitment was high, from July to mid October.

3.5.4. Temporal Development

Ciona intestinalis was the dominant organism settling on the collector plates from the start of the study in May until late November. Tunicates were first observed on 13 June, with a density of 110 juveniles per collector detected with a dissecting scope (Figure 3.4). By 25 July, the tunicates could be quantified without a dissecting scope, with densities reaching a mean of 224 individuals per collector. Throughout the season, abundance remained similar, peaking in late October with a density of 429 individuals observed on a single collector plate. Total weight of tunicates per collector remained relatively low (< 10 g) until 11 July, thereafter, steadily increasing until the end of the field season, peaking at 2253 g per collector on 31 October (Figure 3.5). The decrease of approximately 10% in the mean weight on the collector plates at the last two sampling dates was presumably caused by tunicates falling off. Mean tunicate length was first quantified on 25 July at 22 mm, which represents a growth rate of approximately 1.5 mm

day^{-1} over the previous 2 weeks (Figure 3.6). Mean length continued to increase over the next month to 51.3 mm by 22 August (1.6 mm day^{-1}), reaching 69.4 mm by late November. Position in the water column had a significant effect on weight ($P = 0.009$, with the top position having higher weight, although this trend was not consistent throughout the study period) and abundance ($P = 0.026$, with the top position having higher abundance, although this trend was not consistent throughout the study period), but no effect on length ($P > 0.5$). Site 3 had greater tunicate abundance ($P < 0.001$) and weight ($P < 0.001$), but tunicate length was not different between the three sites ($p > 0.5$). Abundance, weight and length were different between retrieval dates in all analyses ($P < 0.001$) and there was a significant retrieval date by site interaction in abundance ($P = 0.036$), weight ($P < 0.001$), and length ($P < 0.001$).

Investigation into the effect of initial recruitment period on end-of-season presence of *C. intestinalis* showed abundance remaining high on collectors set from 1 May until 3 October, ranging between 55 and 483 individuals per collector plate (Figure 3.7). Thereafter, abundance declined to 0 individuals (> 5 mm). From 1 May to 25 July, the total weight on collector plates remained high, with weights between 878 g and 1970 g per collector plate (Figure 3.8). By 8 August, tunicate biomass had stopped accumulating at the rates seen previously and had declined to 130 g per plate. Collector plates deployed between 22 August and the end of the field season (14 November) acquired low biomass, with a mean weight below 75 g per plate. Abundance of *C. intestinalis* was greater at Site 3 ($P < 0.001$), while position in the water column had some effect ($P = 0.035$, with the top position having the lowest abundance, although this trend was not consistent throughout the study period). There was no difference in weight

between the three sites ($P > 0.5$), and a marginal difference between the water column positions was observed ($P = 0.068$). *Ciona intestinalis* mean lengths on collector plates set from 1 May to 25 July ranged from 57-73 mm (Figure 3.9). Mean tunicate length after 22 August remained below 20 mm and tunicates on collectors set out after 17 October were < 5 mm. Tunicate length was smallest at Site 3 ($P < 0.001$) and only a marginal difference was observed between water column positions ($P = 0.055$). Abundance, weight and length was different between retrieval dates in all analysis (P <0.001), and there was a significant deployment date by site interaction with weight and length data ($P < 0.001$).

3.6. Discussion

3.6.1. Reproductive cycle and early life history

The results show *C. intestinalis* to be a seasonal spawner in PEI because it has a temperate climate. Histology results are consistent with the findings by Carver *et al.* (2003). Collection of larvae began in August, after recruitment had already been observed, making it difficult to correlate larval concentrations with recruitment. Although no samples were taken prior to 2 August, recruitment data shows that larvae were present earlier on. This was confirmed by N. MacNair (pers. comm.) who observed larvae in the water column on 12 June 2007 at the same station used in 2006. Peak larval concentrations were observed in early October; recruitment peaked over a month earlier, suggesting that larval concentration is not indicative of recruitment success. Reasons for this apparent contradiction could be that larval samples were taken from only one point source, and that larvae were less viable later in the season. Multiple sampling locations

and larvae viability indicators could confirm this observation. In this study, the recruitment of *C. intestinalis* did not depend strongly on the position of the collector plates in the water column; however, position within the estuary did significantly affect recruitment, while water temperature was high. In the lack of environmental data to explain this finding, we hypothesize it to be due to water depth, availability of substrate in the vicinity of the collectors, and differences in water flow and currents between the three sites. Site 3 which had higher recruitment levels during the peak recruitment period from late July until mid October was near the shore, in shallower water, near a sandbar, which may have altered the water flow. It would also be of interest to deploy collectors which sample the entire water column to determine if there are differences in recruitment with depth. This study only sampled the 2 meters of the water column, where mussel product was being grown.

Recruitment of *C. intestinalis* closely corresponded to sea-water temperature. The first recruitment was observed when water temperatures rose above 8 °C and ceased when water temperatures fell below 8 °C. The recruitment levels increased as the temperature increased through the summer season. These results are consistent with the recruitment data reported by Carver *et al.* (2003) in Nova Scotia, although in PEI recruitment gradually increased to one major peak, while there were two peaks documented in Nova Scotia. Temperature has also been shown to be an important environmental cue for the spawning of *S. clava*, another invasive tunicate to PEI (Bourque *et al.*, 2007).

3.6.2. Effect on previously established tunicates

Prior to the introduction of *C. intestinalis*, *S. clava* was the primary invasive fouling tunicate of concern to the mussel industry. The reproductive period of *C. intestinalis* shown here is much greater than that of *S. clava* (Bourque *et al.*, 2007). Brudenell estuary has been shown to be converted from a *S. clava*-dominated system to a *C. intestinalis*-dominated system within 2 years of the introduction of *C. intestinalis* (refer to Chapter 2). This information should be of concern to mussel aquaculturalists in areas where *C. intestinalis* has not been introduced and highlights the need for monitoring, early detection and a rapid response.

3.6.3. Temporal Development

The present study enabled us to determine times when biomass of *C. intestinalis* was accumulating and when it stops accumulating during the reproductive season. These parameters are essential for determining when to apply mitigation techniques and how to appropriately alter husbandry practices. The two most important times to note are (1) late July when biomass begins to accumulate at an increasing rate, and (2) mid August when biomass on newly deployed gear does not accumulate to considerable weight. It appeared that although peak recruitment happened in early September, these new recruits did not continue to grow at the rates observed by previously settled individuals and were possibly less viable later in the field season as water temperature began to decline. While the first significant decline in biomass accumulation of *C. intestinalis* was observed in mid August we noted that the species was still recruiting until the end of November and biomass was still measurable until early October.

In 2006, mussel crop failures, due to the excessive fouling weight of *C. intestinalis*, were observed in the Brudenell estuary, PEI. Due to the cost and time requirements, the mussel aquaculture industry may consider one treatment (*i.e.* pressurized water, acetic acid or hydrated lime solution) for *C. intestinalis* in late July/early August, before new recruits had time to achieve considerable biomass. By early August, biomass was already 25 % of that observed during crop failures. This effort would stabilize the fouling weight on mussel crop through the reproductive season of *C. intestinalis*. Significant winter mortality of *C. intestinalis* was observed in the spring of 2007, which, if observed in subsequent years, would additionally reduce the biomass of *C. intestinalis* on mussel crop to a more manageable level. *Ciona intestinalis* recruitment levels may be variable temporally and geographically, and winter mortality has been inconsistent between years. Therefore aquaculturalists need to closely monitor their mussel crop for subsequent recruitment after treatment to determine the need for an additional treatment in late August/early September.

3.7. Acknowledgements

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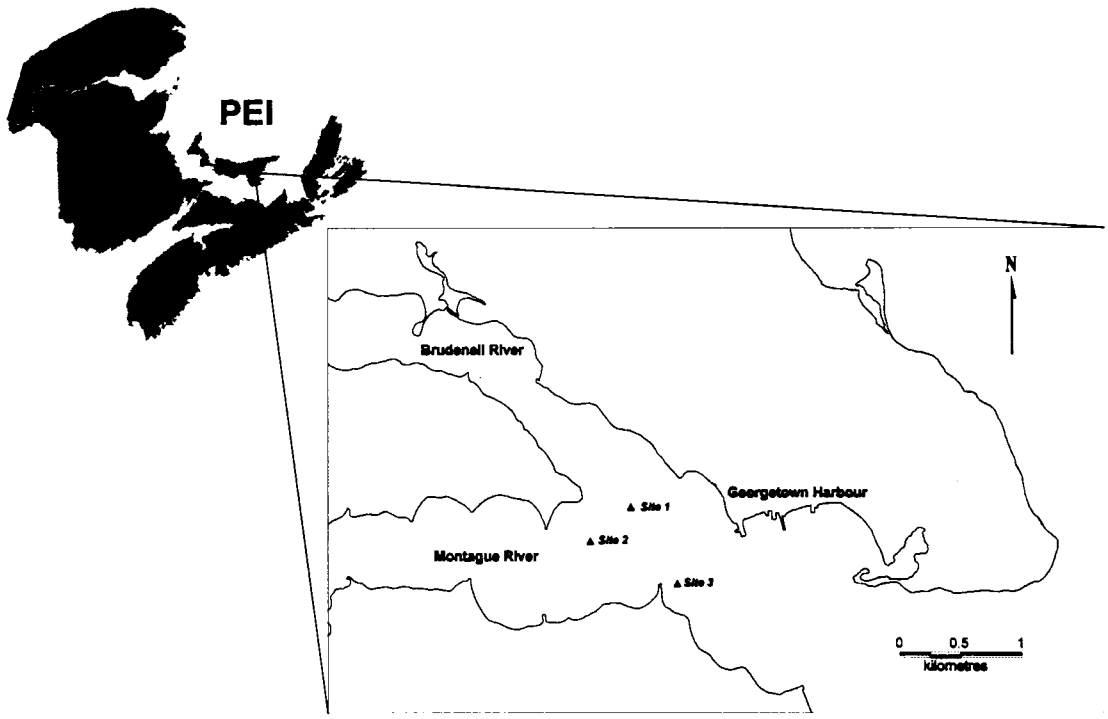
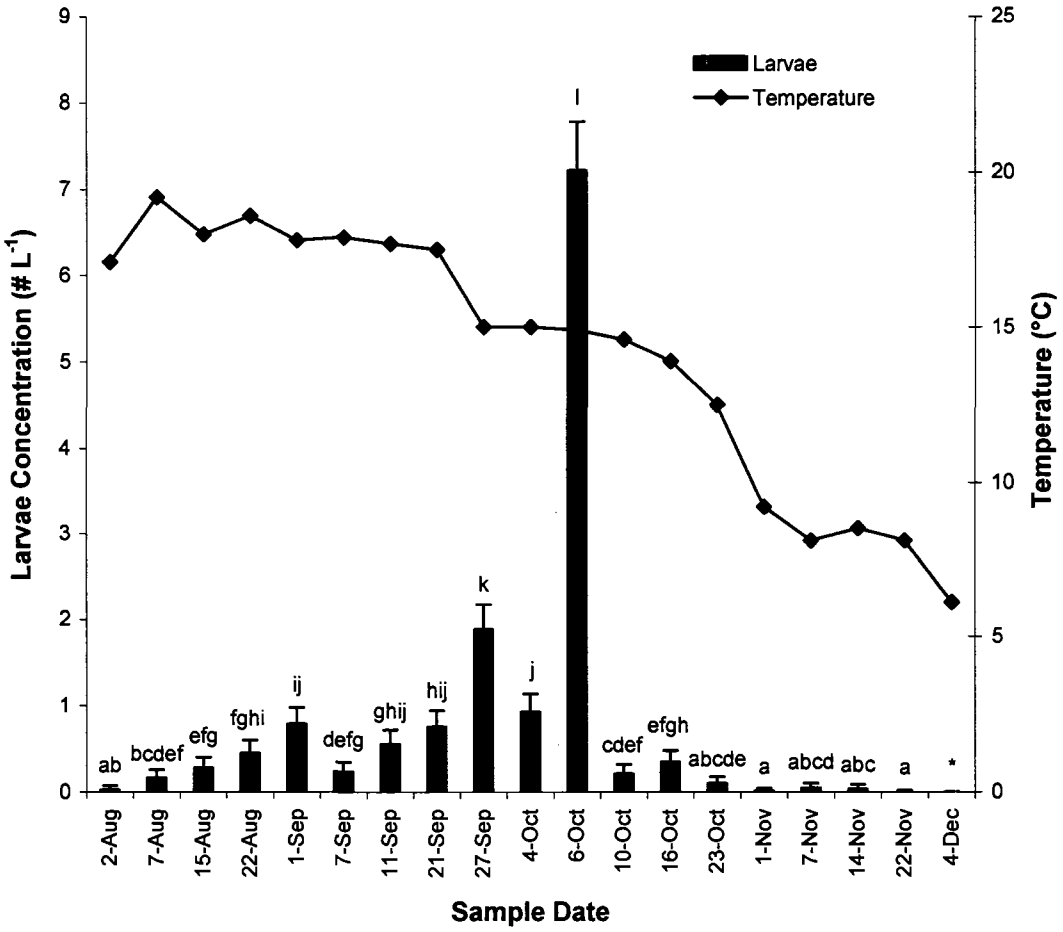
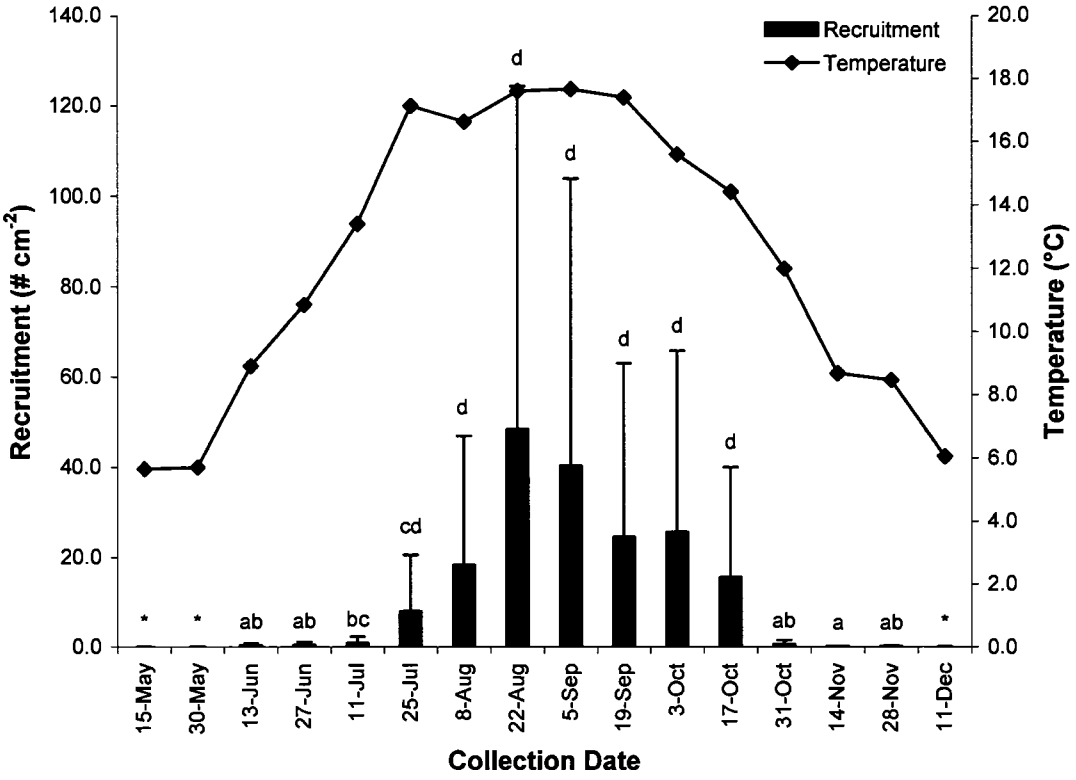


Figure 3.1 Map of Brudenell estuary, located in eastern Prince Edward Island on the east coast of Canada, indicating sample locations (Map created using MapInfo Professional 7.0).



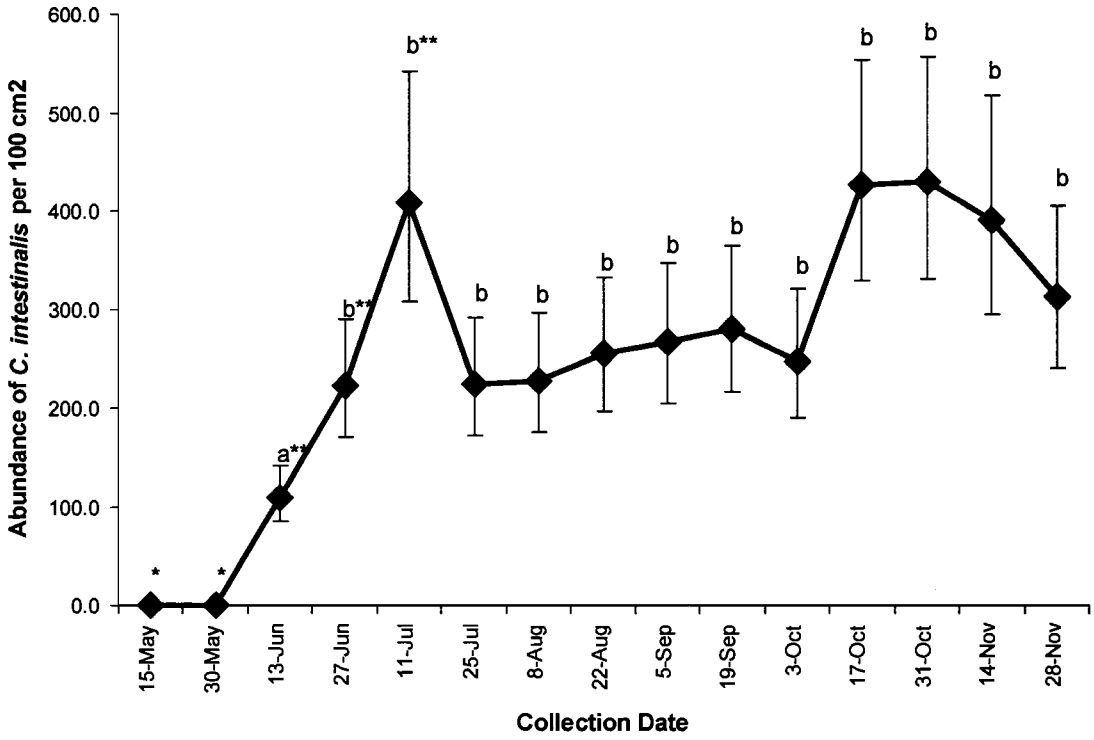
* Sample date was omitted from analysis.

Figure 3.2 Estimated *C. intestinalis* larval concentrations (with 95% CI) in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different (square-root scale analysis). Mean temperature (°C) of each sampling date is represented by the solid line.



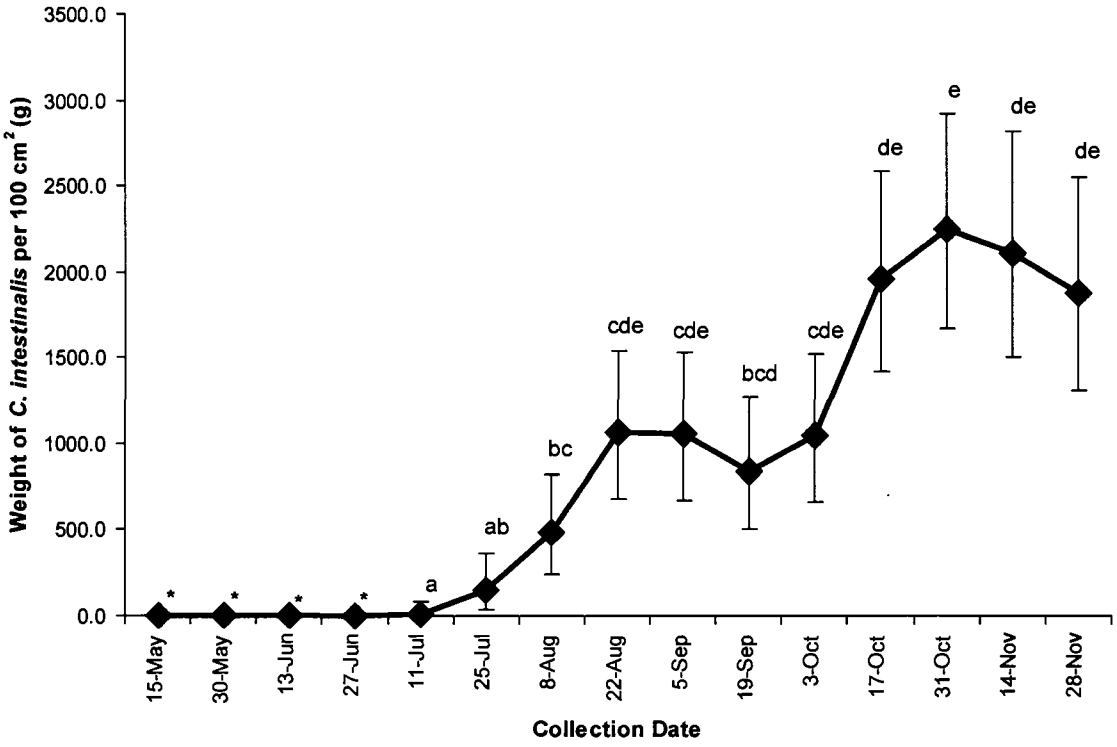
* Collection date was omitted from analysis.

Figure 3.3 Estimated *C. intestinalis* recruitment (with 95% CI) in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different (natural log scale analysis). Mean 2-week temperature (°C) of each sample period is represented by the solid line.



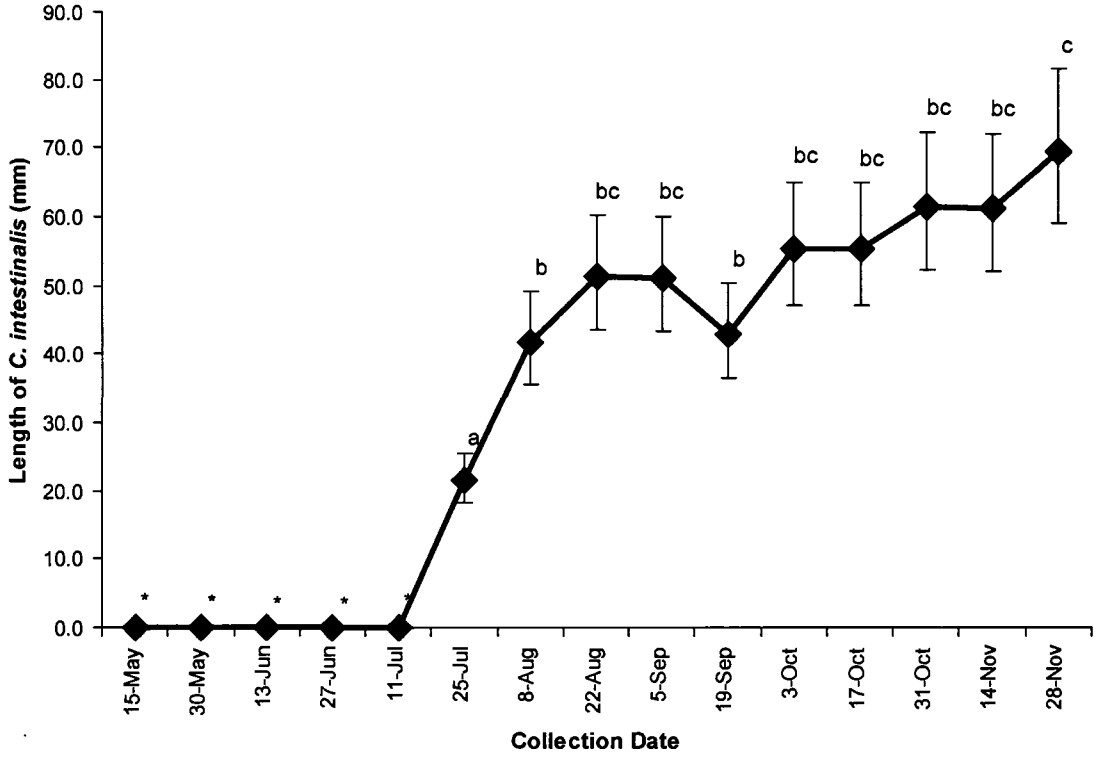
* Collection date was omitted from analysis.
** Individuals were counted under a dissecting scope and are < 5 mm.

Figure 3.4 Estimated abundance (with 95% CI) of *C. intestinalis* accumulated over time in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different (natural log scale analysis).



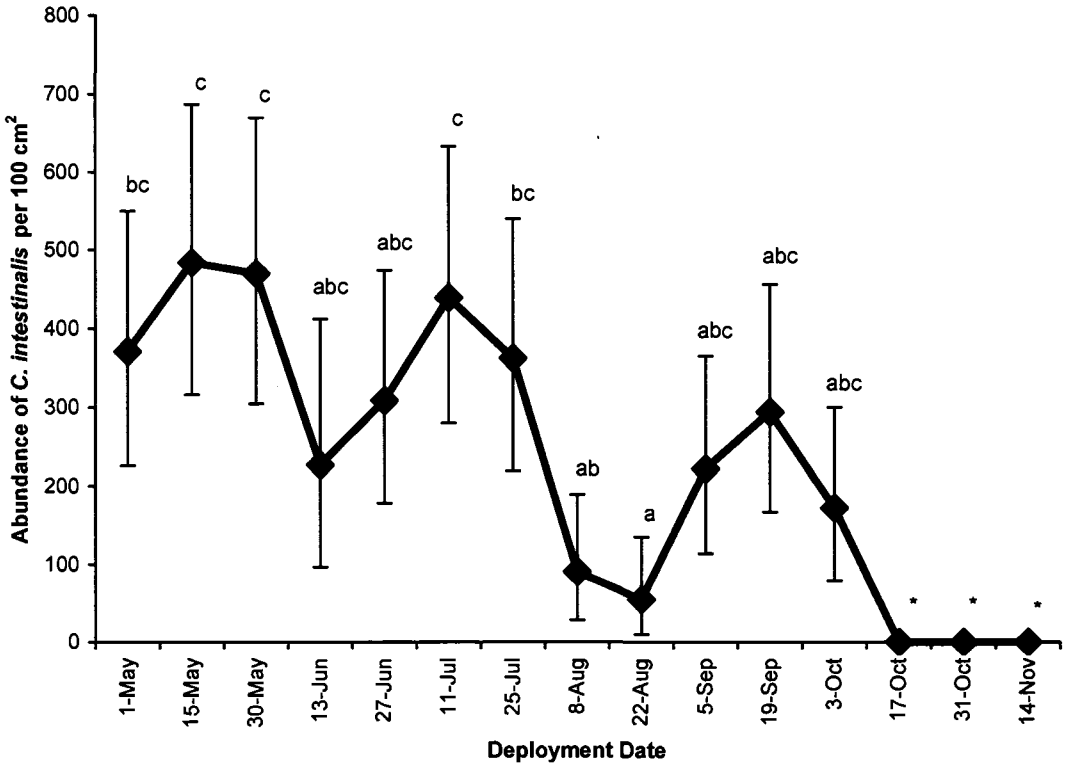
* Collection date was omitted from analysis.

Figure 3.5 Estimated weights (with 95% CI) of *C. intestinalis* accumulated over time in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different (square-root scale analysis).



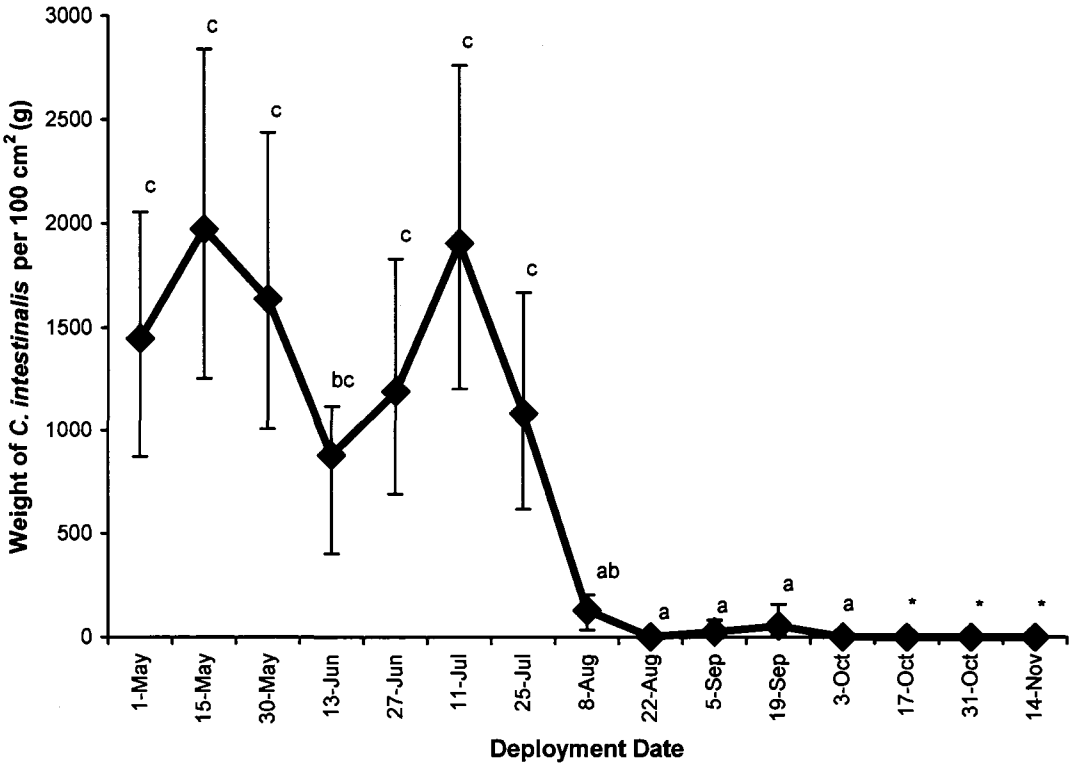
* Collection date was omitted from analysis.

Figure 3.6 Estimated length (with 95% CI) of *C. intestinalis* accumulated over time in 2006 in the Brudenell estuary; sample dates with the same letter were not statistically different (natural log scale analysis).



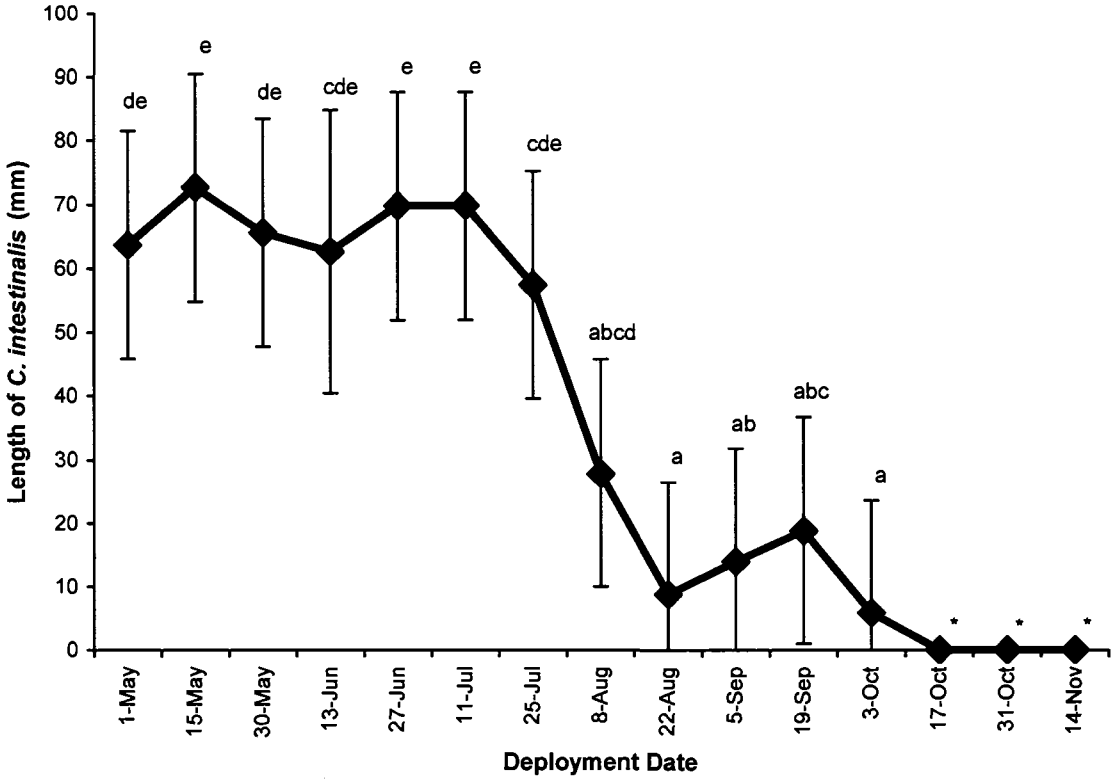
* Deployment date was omitted from analysis.

Figure 3.7 Estimated abundance (with 95% CI) of *C. intestinalis* accumulated over time, using different deployment dates, in 2006 in the Brudenell estuary; deployment dates with the same letter were not statistically different (square-root scale analysis). All samples were collected 28 November.



* Deployment date was omitted from analysis.

Figure 3.8 Estimated weight (with 95% CI) of *C. intestinalis* accumulated over time, using different deployment dates, in 2006 in the Brudenell estuary; deployment dates with the same letter were not statistically different (cubic-root scale analysis). All samples were collected 28 November.



* Deployment date was omitted from analysis.

Figure 3.9 Estimated length (with 95% CI) of *C. intestinalis*, using different deployment dates, in 2006 in the Brudenell estuary; deployment dates with the same letter were not statistically different (square-root scale analysis). All samples were collected 28 November.

Table 3.1 Staging criteria for histological assessment of *C. intestinalis* reproductive status

Stage	Description	Ovary	Testis
1	Developing	Ovary small, containing eggs in various maturational stages. A few ripe ova (150μm) may be present.	Testis small, containing various maturational stages of sperm. Some small aggregates of ripe spermatozoa may be present.
2	Ripe	Ovary large, containing eggs in various maturational stages. Up to 50-75 % of eggs may be ripe.	Testis large, containing medium to large aggregates of ripe spermatozoa.
3	Spawned/Regressing	Ovary decreasing in size, containing eggs in various maturational stages with some ripe eggs remaining. Eggs in later stages showing signs of regression.	Testis decreasing in size with frequent patchy areas containing sparse amounts of gametes.

Chapter 4 THE EFFECT OF STOCKING DENSITY ON TUNICATE RECRUITMENT FOR THE CULTURED MUSSEL *Mytilus edulis*

Ramsay A, Davidson J, Landry T and Stryhn H. The effect of stocking density on tunicate recruitment for the cultured mussel, *Mytilus edulis.* Aquaculture (submitted).

4.1. Abstract

Over the past decade, two exotic tunicates (*Styela clava* and *Ciona intestinalis*) have established in the Brudenell estuary of Prince Edward Island. *Ciona intestinalis* is now the most significant fouling organism, contributing > 99 % of the total fouling biomass on mussel farms. Tunicates have the potential to compete with mussels for space and food, potentially decreasing the growth rates and meat yields of cultured mussels. Anecdotal reports from mussel growers suggest that increased stocking density decreases the recruitment of the fouling organisms. To evaluate this hypothesis, 15 mussel socks of low, medium and high densities were placed on three longlines in the Brudenell estuary in the fall 2005 and in the spring 2006. Sampling was conducted in June, August and October to determine the effect of different stocking densities and socking time on mussel productivity and *C. intestinalis* recruitment and growth. Mussel condition and shell length were not significantly different and there was no difference in the weight of *C. intestinalis* between the three stocking densities by the end of the field trial in October. Time of socking did not have a considtently significant effect on the results. Mussel loss was between 50 and 60 % for all treatments, with no clear pattern being evident. Although promising results were observed in the August sampling fouling by *C. intestinalis* overwhelmed all three stocking density treatments by the end of the trial.

4.2. Keywords

Ciona intestinalis; husbandry; invasive species; mussel productivity; *Mytilus edulis*; stocking density; *Styela clava*

4.3. Introduction

Mussel aquaculture is an important industry on Prince Edward Island (PEI) with an annual production of 17 000 Mt valued at $107 M to the economy (Department of Fisheries and Oceans, 2006). In 1997, an exotic tunicate, *Styela clava*, was detected in the Brudenell estuary (Davidson *et al.*, 2005; Locke *et al.*, 2007). This species rapidly established itself, became invasive, and eventually a nuisance to the mussel aquaculture industry in the area. As a fouling organism, *S. clava* competed for space and food with *Mytilus edulis*, potentially decreasing mussel productivity (Thompson and MacNair, 2004). In 2004, another exotic tunicate, *Ciona intestinalis*, was detected in the Montague River and has become invasive to this river and the adjacent Brudenell estuary (MacNair, 2005). Since first detection its abundance and biomass in the estuary has dramatically increased and the abundance of *S. clava* has declined proportionally, with > 99 % of the fouling biomass, being attributed to *C. intestinalis* (refer to Chapter 2). It is currently classified as an aquatic nuisance species as it is affecting mussel production in infested bays and rivers and is considered a greater threat to mussel aquaculture than *S. clava* had ever been.

A variety of treatments have been employed to remove *C. intestinalis* from the mussel socks and associated aquaculture gear, including systematic replacement of buoys on the mussel longlines and treatments of entire crops using acetic acid, brine solution,

hydrated lime and pressurized water, as well as biological control using rock crabs (N. MacNair, pers. comm.; Carver *et al.*, 2003). However, these treatments showed inconsistent results in the reduction of tunicates on the mussel socks. Other treatments tried on *S. clava* with little or no success include NaOH (caustic soda), hot water, steam, paraffin wax, lanolin, molasses, mild detergents, citric acid, formalin, hydrogen peroxide, short and long wavelength ultraviolet light, ultrasound, infrared light, electricity, pressure washing, and sugar (Davidson *et al.*, 2005). While some of these treatments show promising results, optimal mitigation strategies will require new husbandry and lease management practices. In Italy, mussel growers periodically remove the socks from the water and place them on drying racks to allow for the tunicates to desiccate and die or mussels are re-socked to remove fouling tunicates (G. Arsenault, pers. comm.). In South Africa growers re-sleeve their mussels immediately following the recruitment of *C. intestinalis* (Hecht and Heasman, 1999). Results from N. MacNair (unpubl.) have indicated that with increasing stocking density of mussels at the time of socking there was a decrease in the abundance of *S. clava.*

The aim of this project was to evaluate if the stocking density of mussels can reduce the recruitment and growth of *C. intestinalis*, without significantly affecting mussel growth, mussel loss on socks and mussel Condition Index (CI). The mechanism for this phenomenon may be explained by the following three hypotheses. The first hypothesis involves the use of surface area. As stocking density increases, the total surface area per mussel will decrease, because only the mussels on the outside of the sock will be exposed to tunicate recruitment, leaving the mussels in the core of the sock free from tunicate fouling. The second hypothesis involves the concept of self-thinning on

mussel socks. The more mussels that are placed in the socking material, the more intense the self-thinning will be (Fréchette and Lefaivre, 1990; Fréchette *et al.*, 1992; Fréchette *et al.*, 1996). When tunicates attach to the mussels on the sock, they add additional weight to the mussels, thereby stressing the byssal attachment. In doing this, the tunicates may act as a catalyst in the self-thinning hypothesis. These outer mussels, which are fouled with tunicates, fall off, leaving a clean, unfouled inner core of mussels. Lastly, there exists the possibility that mussels, as they are filtering for food, take in tunicate larvae before attachment occurs and expel them as pseudofaeces, thereby decreasing the viability of the organism (Mileikovsky, 1974; André *et al.*, 1993; Davenport *et al.*, 2000; Lehane and Davenport, 2004). This would imply that higher stocking densities would be more effective in decreasing recruitment due to increased filtering action with more mussels. Filtering action by mussels may create micro-currents around the sock which would keep tunicate larvae from settling and causing high density to again be the most effective (Green *et al.*, 2003).

4.4. Materials and Methods

4.4.1. Study Area

The Brudenell estuary, located on the eastern coast of PEI, was chosen for this study because of its high abundance of *C. intestinalis* (see Figure 4.1). *Styela clava* and two colonial tunicates, *Botryllus schlosseri* (golden star tunicate) and *Botrylloides violaceus* (violet tunicate) are also reported in this area. The associated rivers in this estuary are very productive, with growth rates for *M. edulis* among the top 50 % recorded in PEI at 2.25 to 2.32 mm mo^{-1} (Department of Fisheries and Oceans, 2005). In 2004, the

Brudenell estuary and associated rivers accounted for 12.5 % of the total mussel production on PEI (Department of Fisheries and Oceans Canada, 2006). The temperature ranged from -1.5 °C during ice cover (February, 2006) to 21 °C in the summer (August, 2006), while the salinity ranged from 15 ppt in the fall to 30.1 ppt in the spring.

4.4.2. Experimental Design

Mussel seed was secured from St. Peter's Bay and socked into the appropriate density treatment, in December 2005 and April 2006. A total of 270 socks were used for this study. Fifteen socks of the three mussel stocking density treatments (low = 90 mussels per 30 cm, medium = 250 mussels per 30 cm, and high = 500 mussels per 30 cm) were randomly deployed in three blocks of five socks on each of three mussel lines for both fall and spring socked mussels (Figure 4.2). Longline sites were selected on the basis of availability from three mussel leases in the area; sites 1 and 2 were located at the confluence of the Montague and Brudenell Rivers estuaries in approximately 7.5 m of water while site 3 was situated near the shore in ~4 m of water. Mussels socked in fall 2005 were moved to different sections of the longline to reflect a new design developed for spring socking (see Figure 4.2). The effect of handling these socks was assessed by leaving five socks in their original position and moving the other 10 socks in each density treatment on each longline into the remaining two blocks of five socks. Socks were periodically checked to ensure adequate buoyancy was keeping socks off the bottom substrate, and starfish were removed.

4.4.3. Sampling Protocol

At each sampling (in June, August, and October, 2006), five socks of each density treatment were selected from each of the three sites. This was completed for socks

deployed in December 2005 and April 2006. The bottom 30 cm of the sampled socks was discarded to minimize interaction between the treatment and the bottom substrate. The next 30 cm section from the bottom was assessed.

4.4.4. Laboratory Analysis

Samples were processed immediately after collection to separate the sock section into (1) mussels and (2) *C. intestinalis*. Dead mussels, silt, and socking material were discarded. Samples were preserved by freezing and later thawed for quantification. Abundance of mussels was determined in each sample. Subsamples of fifteen mussels were randomly collected from each sample to determine mean mussel length and CI. Mussel length was determined by measuring from the umbo to the most distal edge and Condition Index (CI) was calculated according to the formulae given in Abbe and Albright (2003):

$$Condition\ Index = \frac{Dry\ meat\ weight\ (g)}{Dry\ shell\ weight\ (g)} \times 100$$

Ciona intestinalis was measured as total biomass on a sock section. Subsamples were taken to determine abundance and mean length. Specimens smaller than 5 mm (holdfast to the distal edge of the incurrent siphon) were not considered for abundance and mean length evaluation.

4.4.5. Statistical Analysis

Separate analyses were carried out for each sampling time (June, August and October) to assess the effect of mussel stocking density (low, medium, and high) and socking time (fall vs. spring) on mussel productivity (abundance, CI and length) and *C. intestinalis* fouling (abundance, length and weight). Mussel productivity and *C. intestinalis* fouling were analyzed using linear models (Christensen, 1996) to determine

significant differences between the two treatment factors (stocking density and socking time) and mussel longline site which was considered a blocking variable. First and second order interactions between these variables were investigated, and the within-line blocking was included as well. In the presence of significant interaction between longline site and a treatment variable, longline site was modeled as a random effect to allow for overall comparisons between treatments. The handling pressure associated with moving mussels socked in December 2005 the following spring was assessed in a separate analysis for a linear model, including also the stocking density and blocking variables. The model assumptions were assessed by residual analysis, and the dependent variables *C. intestinalis* weight and abundance, as well as mussel abundance for the August sampling were subjected to transformation by the natural log transformation to meet the model assumptions. Pairwise comparisons between treatments were done using the Bonferroni method. Estimates are presented as least square means ± standard error for all treatment combinations, backtransformed to original scale as appropriate. The significance level was set at 0.05. Data were analyzed using Minitab 15 (© Minitab Inc.) and SAS 9.1 (© SAS Institute Inc.). Mussel loss was calculated as a ratio of mean number of lost mussels for each treatment in October compared to initial stocking densities in December 2005 and April 2006. Standard errors were computed using the delta method (Weisberg, 2005), as this was a non-linear function.

4.5. Results

4.5.1. Mussel Productivity

Mussel seed used for the late fall deployment (December 2005) had similar length between the three stocking density treatments, with a mean of 26.4 mm (Table 4.1), while mussel seed deployed in April 2006, were marginally larger in the medium and high density treatments (30.2 mm and 31.5 mm), with mussels in the low density treatment averaging 26.5 mm in length. During June 2006 sampling, *C. intestinalis* was not present on the mussel socks or associated aquaculture gear. At this time, a significant interaction between stocking density and socking time was detected ($P = 0.037$) for shell length, with mussels from spring socking being different between the low density (29.5 mm) and the high density (31.8 mm) treatments. This was expected as the low density group had an initial lower length. In August, *C. intestinalis* was present on mussel socks. Mussel length was similar between stocking density ($P = 0.28$) and time of socking ($P = 0.17$), but there was a marginal (2.0 mm) but significant difference between longline sites ($P < 0.001$). Mussels at site 2 were an average length of 37.0 mm whereas mussel length at sites 1 and 3 were 39.4 and 39.3 mm, respectively. In October, mussel length had increased to a mean of 46.0 mm. Again, no significant differences were observed between stocking density ($P = 0.31$) or stocking time ($P > 0.5$) but the difference between longline site ($P = 0.029$) remained with site 2 having the lowest mussel length. No apparent reason for this difference at site 2 was evident.

Mussel CI in June was lower in the high stocking density group at 25.5 compared to the low and medium density treatments at 27.9 and 27.6, respectively ($P = 0.028$) but not significantly different between socking times ($P = 0.078$) (Table 4.1). Longline site

was significant ($P < 0.001$), with site 3 showing an average CI of 23.2, compared to 27.8 and 30.1 at sites 1 and 2, respectively. The condition of mussels in the August sampling was similar between stocking density ($P > 0.5$) and socking time ($P = 0.39$), with the site effect still present, but to a lesser degree ($P = 0.026$); the average CI values were 12.7, 11.7, and 12.1 at sites 1, 2, and 3, respectively. In October, no difference in CI between treatments was detected ($P > 0.5$), but there was a site difference ($P < 0.001$). The site difference is not considered biologically significant as average CI was between 10.8 and 12.8 for the three sites.

Mussel loss from the time of socking until the end of the field trial in October was similar between density treatments and time of socking (Figure 4.3). There was approximately 50 % mussel loss in all treatments, with the highest loss (> 60 %) observed in the high density treatment from the fall socking (Table 4.1).

4.5.2. Tunicate Fouling

Ciona intestinalis was not present in the initial sampling in June, on the fall or spring socks. By August 2007, *C. intestinalis* had recruited on all experimental socks and its abundance was significantly different between socking time ($P < 0.001$); fall socks had ~30 % more *C. intestinalis* (Figure 4.4). There was also a significant ($P < 0.001$) geographical differences in *C. intestinalis* fouling, with socks on longline site 3, which had the lowest CI in June, having > 300 % more tunicates (475 *C. intestinalis* per 30 cm) compared to socks on the other two (157 and 116). In October 2006, *C. intestinalis* abundance was similar between density treatments ($P = 0.20$) and time of socking ($P = 0.29$); however, the longline site 3 still had the greatest *C. intestinalis* fouling by abundance ($P < 0.001$).

The average length of *C. intestinalis* (Figure 4.5) in August was different between stocking density ($P = 0.001$) and longlines ($P < 0.001$). *Ciona intestinalis* length on the longline with the highest *C. intestinalis* abundance and lowest CI (site 3) averaged 29.6 mm, compared to 21.6 and 20.0 mm on the other two. *Ciona intestinalis* length was higher on the low stocking density (25.4 mm) compared to the medium and high densities; 23.1 and 22.8 mm, respectively. In October 2006 the length of *C. intestinalis* was still different between longline sites ($P < 0.001$), with the same line consistently having the highest abundance and largest (59.1 mm) *C. intestinalis*. There was a significant trend of decreasing *C. intestinalis* length with increased stocking density in the treatments ($P = 0.020$); the average lengths were 54.9, 52.4, and 49.8 mm for the low, medium and high density socks, respectively.

In the August sampling, *C. intestinalis* biomass was not different between stocking densities ($P = 0.25$) or socking time ($P = 0.15$), but an interaction between the treatments was detected ($P = 0.013$). The low density socks from the fall socking had greater fouling of *C. intestinalis* per 30 cm of mussel sock (122.2 g) compared to an average 67.8 g on the other five treatments (Figure 4.6). *Ciona intestinalis* biomass at the same longline site with the highest abundance and length of the tunicates was > 400 % (233.5 g) than that on the socks at the other two longline sites (32.4 and 54.0 g). *Ciona intestinalis* biomass in October was different between longline site ($P < 0.001$), and a difference between time of socking was also detected ($P = 0.040$). Site 3 had an average of 1536.5 g compared to 863.1 and 693.2 g at sites 1 and 2, respectively. Spring socked mussels had lower *C. intestinalis* fouling (967.0 g) compared to fall-socked mussels (1094.9 g).

4.5.3. Effects of Handling

The experimental design was modified from the initial set-up, affecting 60 % of the socks from each density on each longline for the fall-socked mussels. Comparison of manipulated vs. non-manipulated socks only revealed two significant effects. Mussel CI was significantly higher on manipulated socks in June ($P = 0.010$) and lower in August ($P = 0.008$) while *C. intestinalis* weight remained significantly higher on manipulated socks ($P = 0.027$). All other factors analysed showed that manipulation had no apparent effect ($P > 0.1$).

4.6. Discussion

Tunicate colonization took place after the June sampling. It was observed that as stocking density increased, the average length of *C. intestinalis* decreased in both August and October samplings. This would suggest that higher stocking density is having an effect on tunicate recruitment or growth through a process involving competition for food or space. In the latter case, the probable mechanisms causing a decrease in *C. intestinalis* length through filtration are hypothesized to be by either filtering *C. intestinalis* larvae, resulting in an unviable organism or simply by creating a micro-environment not favourable to tunicate larvae settlement (Mileikovsky, 1974; Lehane and Davenport, 2004). As settlement and recrcuitment increased through the summer, the suggested initial competition effects of mussels on *C. intestinalis* seem to have less effect on its growth and recruitment. Although a trend was detected in *C. intestinalis* length, the abundance and weight results between various stocking densities and spring vs. fall socking showed no consistently significant results. In August, *C. intestinalis* abundance

was significantly greater on fall-socked mussels, but in October no difference was found. Biomass of *C. intestinalis* in August was significantly higher on the low density socks from the fall socking. The medium and high density treatments showed lower biomass, as well as the low density socks from the spring socking. In October, no stocking density effect was found, but there was lower *C. intestinalis* fouling on spring socked mussels. By the end of the field trial no benefit of increasing stocking density was observed.

In the development of new husbandry practices to mitigate and lessen the impact of fouling tunicates, it is necessary to ensure that these practices are not having detrimental effects on the cultured organism, *M. edulis*. This study suggests that in the presence of a significant fouling organism, *C. intestinalis*, increased stocking density is not associated with a decrease in mussel condition, mussel growth, or loss of mussels from the sock. In the absence of tunicates, increased stocking density at a bay-scale level has shown detrimental effects on mussel productivity (Waite *et al.*, 2005; Drapeau *et al.*, 2007). It is also important to note that the results of this study do not support the self-thinning (*i.e.* increasing loss of mussels with increasing stocking density) hypothesis (Lauzon-Guay *et al.*, 2005). It was hypothesized that with stocking mussels at a very high density, gradually the mussels would decrease to a level similar for lower stocking densities. It was also hypothesized that growth and condition index, after initial stocking, would be lower with higher stocking density, but steadily increase as density levels approach previous levels through self-thinning. There was no consistent evidence to support Lauzon-Guay's hypothesis in this study; however, no significant fouling organism was present in that study.

Based on the results of this study, it is possible to make some recommendations to the mussel industry on husbandry strategies to reduce the effects of *C. intestinalis*. At a medium stocking density of approximately 250 mussels per 30 cm, *C. intestinalis* length and biomass are less than that observed on the low stocking density in August. Mussel loss is marginally lower compared to the high density socks. However, by October 2006, all stocking density treatments are completely fouled by *C. intestinalis*, with a slightly lower biomass on spring socked mussels, which may not be economically or ecologically significant. Therefore, it is critical that any husbandry application to reduce the effect of tunicate infestation is combined with more direct treatment measures, applied at optimal times (refer to Chapter 3). Treatments using lime, acetic acid, or pressurized water are showing promising results (N. MacNair, pers. comm.). In Spain and New Zealand, mussel farmers are known to re-sock their mussels after fouling has affected their crop. Ideally, on PEI, farmers would initially stock their mussels at a high density in the fall or spring of the year, allow them to grow until tunicate biomass reaches a critical level and then re-sock at a lower density for the final growth phase (see Chapter 3). Although the main benefit of re-socking is the direct removal of *C. intestinalis* and other fouling organisms, mussels in lower densities, free of tunicate fouling, would likely have a higher productivity (Drapeau *et al.*, 2007). Additionaly, if an intermediate treatment is required during the initial nursery phase, the effort and cost to treat high density socks would be proportionally lower to the factor of mussel density increase.

Although not considered to be of primary interest in this research, a significant location effect was apparent. Location within the estuary did significantly affect mussel CI and *C. intestinalis* recruitment and growth. In June, mussel CI was much lower at site

3, although this difference was not present in the later samplings. *Ciona intestinalis* abundance, length, and biomass were consistently higher at site 3. With the lack of environmental data to explain this finding, we hypothesize lower water depth and differences in water flow and current at site 3 to be a possible cause. SCUBA divers reported increased current in the vicinity of this line. Site 3 was near the shore, in shallower water and near a sandbar, which may have altered the water flow, delivering more food. More investigation into this geographic difference and the role of local hydrodynamics is required.

4.7. Acknowledgements

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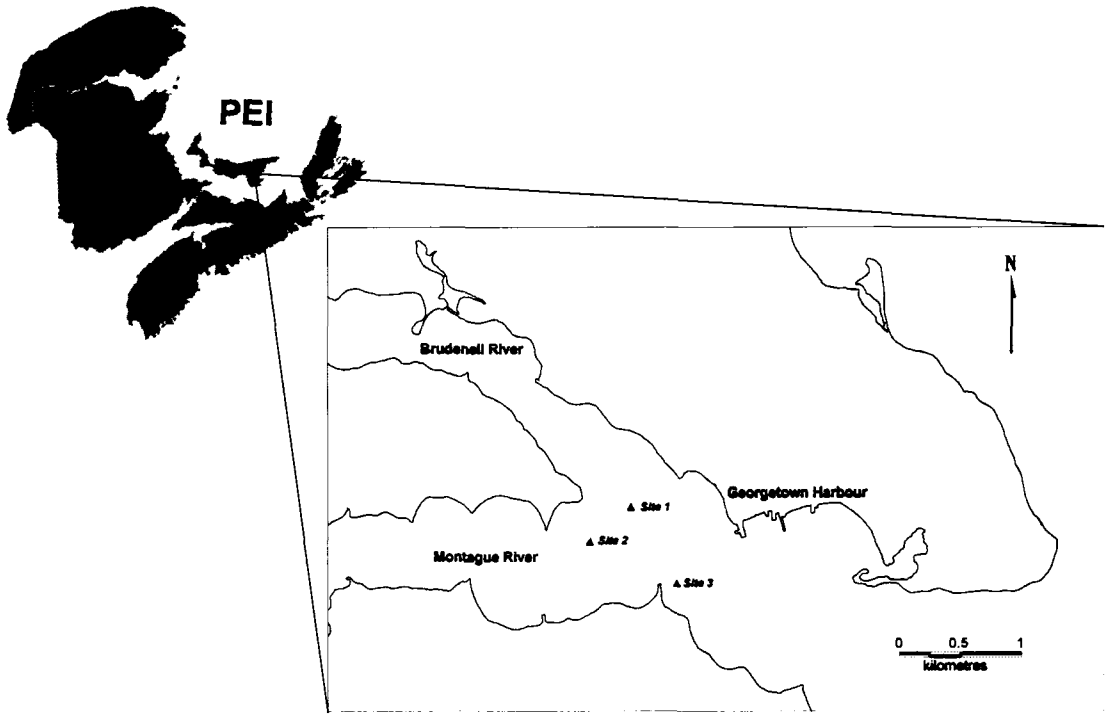


Figure 4.1 Map of Brudenell estuary, located in eastern Prince Edward Island on the east coast of Canada, indicating sample locations (Map created using MapInfo Professional 7.0).

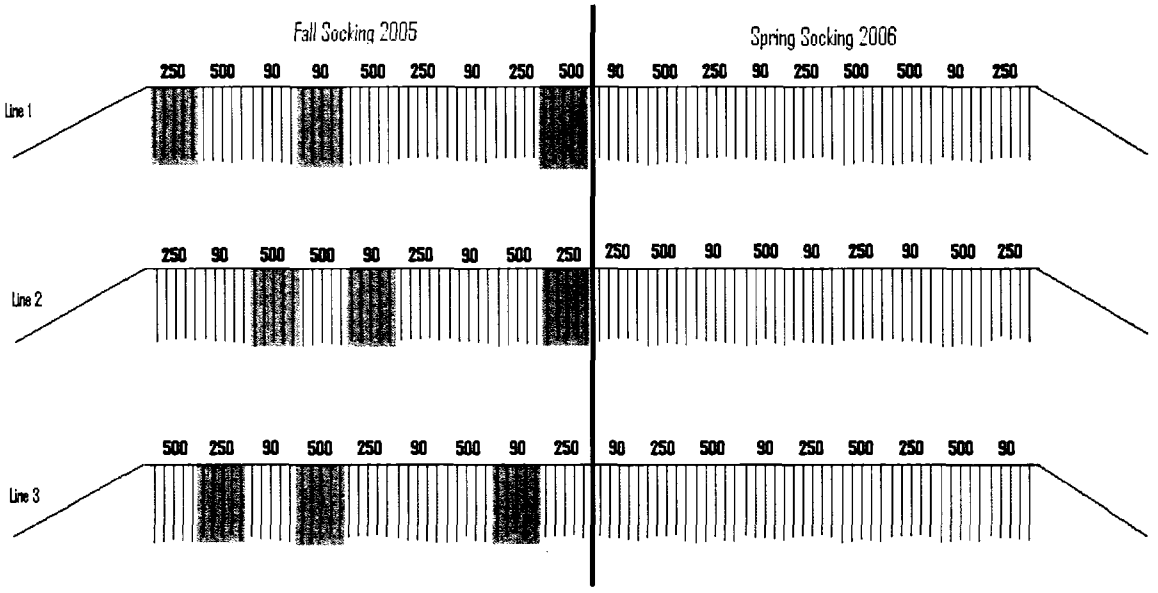


Figure 4.2 Experimental design showing the two treatments; socking time (fall and spring) and stocking density (three blocks of five socks on each longline within each socking time). Differing stocking densities are differentiated by the numeric values appearing above their group. Shaded groups are mussel socks which were not handled in the re-design of the experimental set-up.

Table 4.1 Descriptive mussel productivity statistics (LS mean ± SE) grouped within sampling time by time of socking and stocking density.

Time of Sampling	Time of Socking	Stocking Density	Condition Index	Length (mm)	Abundance
December	Fall *(Initial Deployment)*	Low	N/A	26.3 ± 0.1	103.6 ± 5.2 [a]
		Medium	N/A	26.4 ± 0.1	237.0 ± 5.2 [b]
		High	N/A	26.4 ± 0.1	527.8 ± 5.2 [c]
April	Spring *(Initial Deployment)*	Low	N/A	26.5 ± 0.3 [a]	116.4 ± 7.3 [a]
		Medium	N/A	30.2 ± 0.3 [b]	246.7 ± 7.3 [b]
		High	N/A	31.5 ± 0.3 [c]	329.5 ± 7.3 [c]
June	Fall	Low	27.5 ± 0.8 [b]	31.2 ± 0.6 [AB]	83.5 ± 7.4 [A]
		Medium	28.6 ± 0.8 [ab]	31.4 ± 0.6 [AB]	164.4 ± 7.4 [B]
		High	26.8 ± 0.8 [a]	31.4 ± 0.6 [AB]	304.0 ± 7.4 [D]
	Spring	Low	28.0 ± 0.9 [b]	29.5 ± 0.6 [A]	90.2 ± 8.5 [A]
		Medium	26.4 ± 0.8 [ab]	31.1 ± 0.6 [AB]	196.2 ± 7.4 [B]
		High	24.4 ± 1.1 [a]	31.8 ± 0.7 [B]	261.7 ± 10.4 [C]
August	Fall	Low	12.2 ± 0.3	39.3 ± 0.4	65.5 ± 2.9 [A]
		Medium	12.3 ± 0.3	38.7 ± 0.4	151.7 ± 6.7 [C]
		High	12.4 ± 0.3	38.3 ± 0.4	280.9 ± 12.4 [D]
	Spring	Low	12.0 ± 0.3	38.4 ± 0.5	71.7 ± 3.5 [A]
		Medium	12.2 ± 0.3	37.8 ± 0.4	122.1 ± 5.4 [B]
		High	12.1 ± 0.4	38.5 ± 0.6	185.3 ± 11.0 [C]
October	Fall	Low	11.7 ± 0.7	46.3 ± 0.5	51.4 ± 7.5 [A]
		Medium	11.1 ± 0.7	46.6 ± 0.5	114.7 ± 7.5 [B]
		High	12.0 ± 0.8	46.8 ± 0.5	203.8 ± 7.7 [D]
	Spring	Low	11.2 ± 0.8	45.8 ± 0.5	51.9 ± 8.2 [A]
		Medium	11.8 ± 0.7	46.9 ± 0.5	103.7 ± 7.5 [B]
		High	11.3 ± 0.8	46.7 ± 0.5	147.4 ± 9.2 [C]

[1, 2] indicates significant difference between socking times
[a-c] indicates significant difference between densities
[A-D] indicates significant difference between combined density and socking groups
Coding is absent if no significant treatment effect was detected

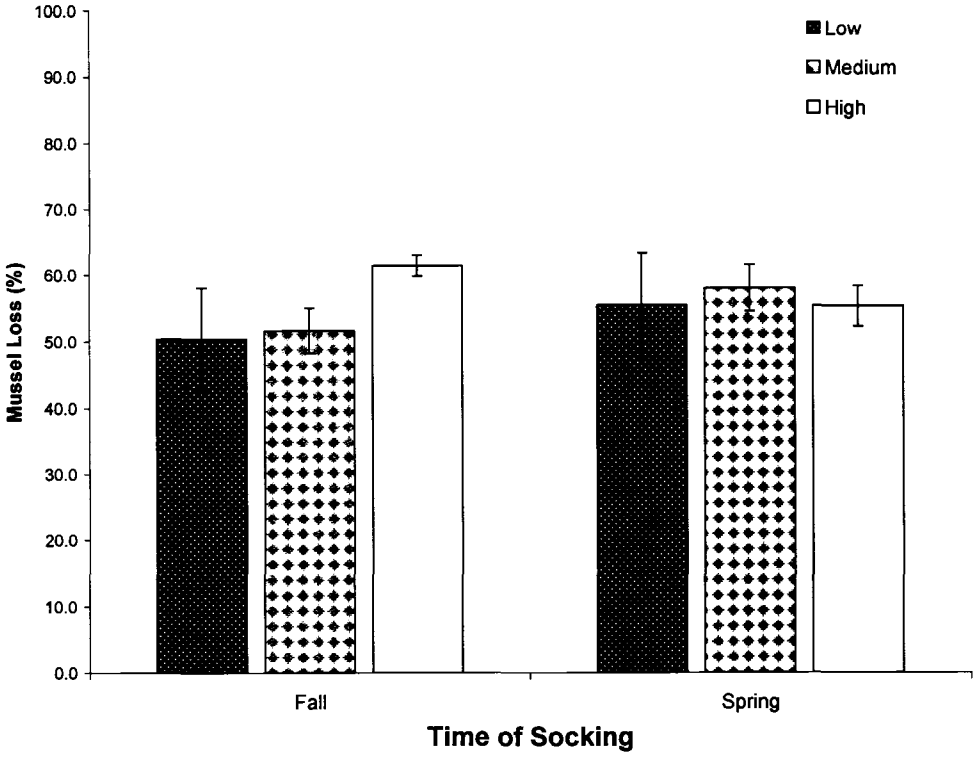
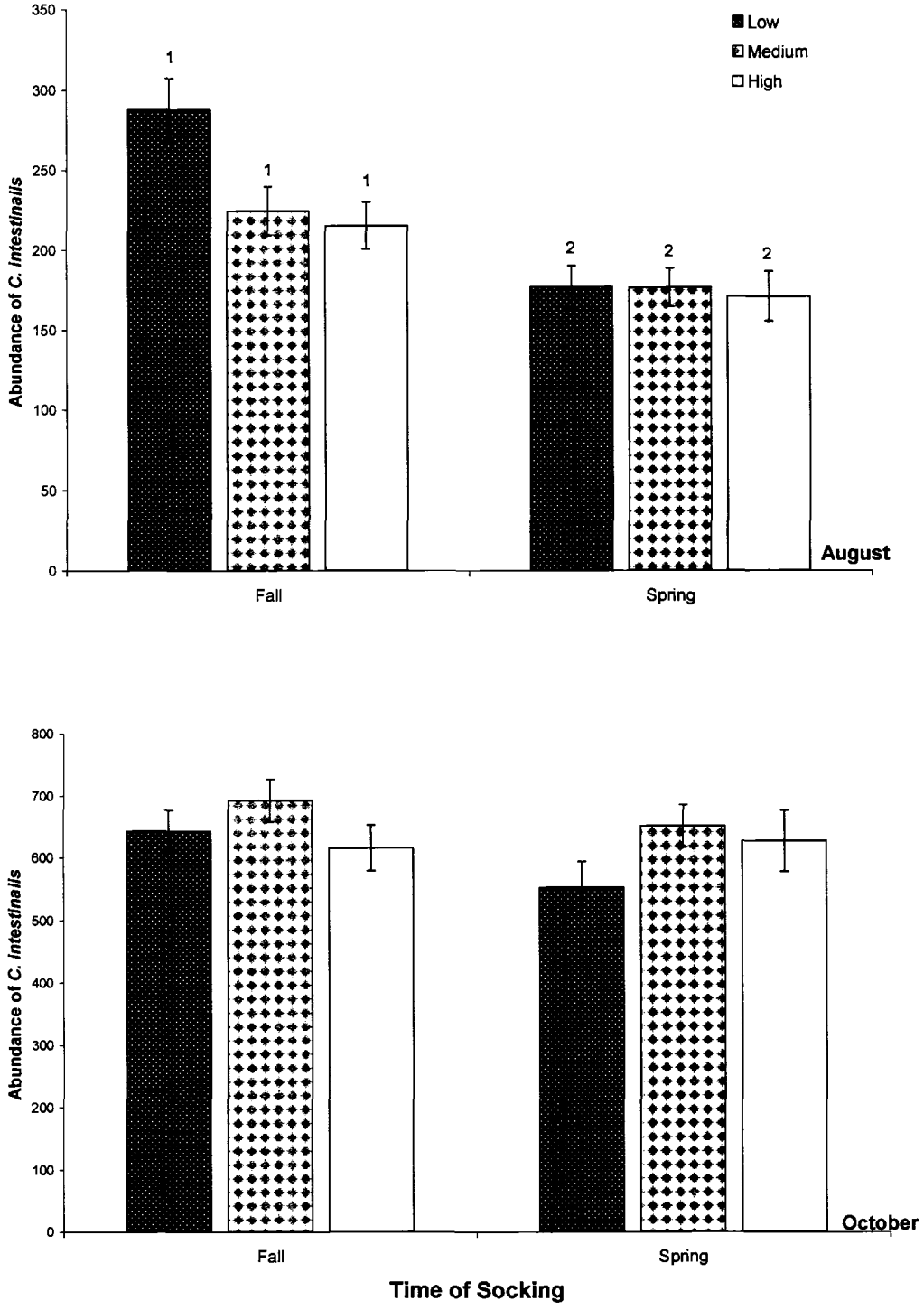
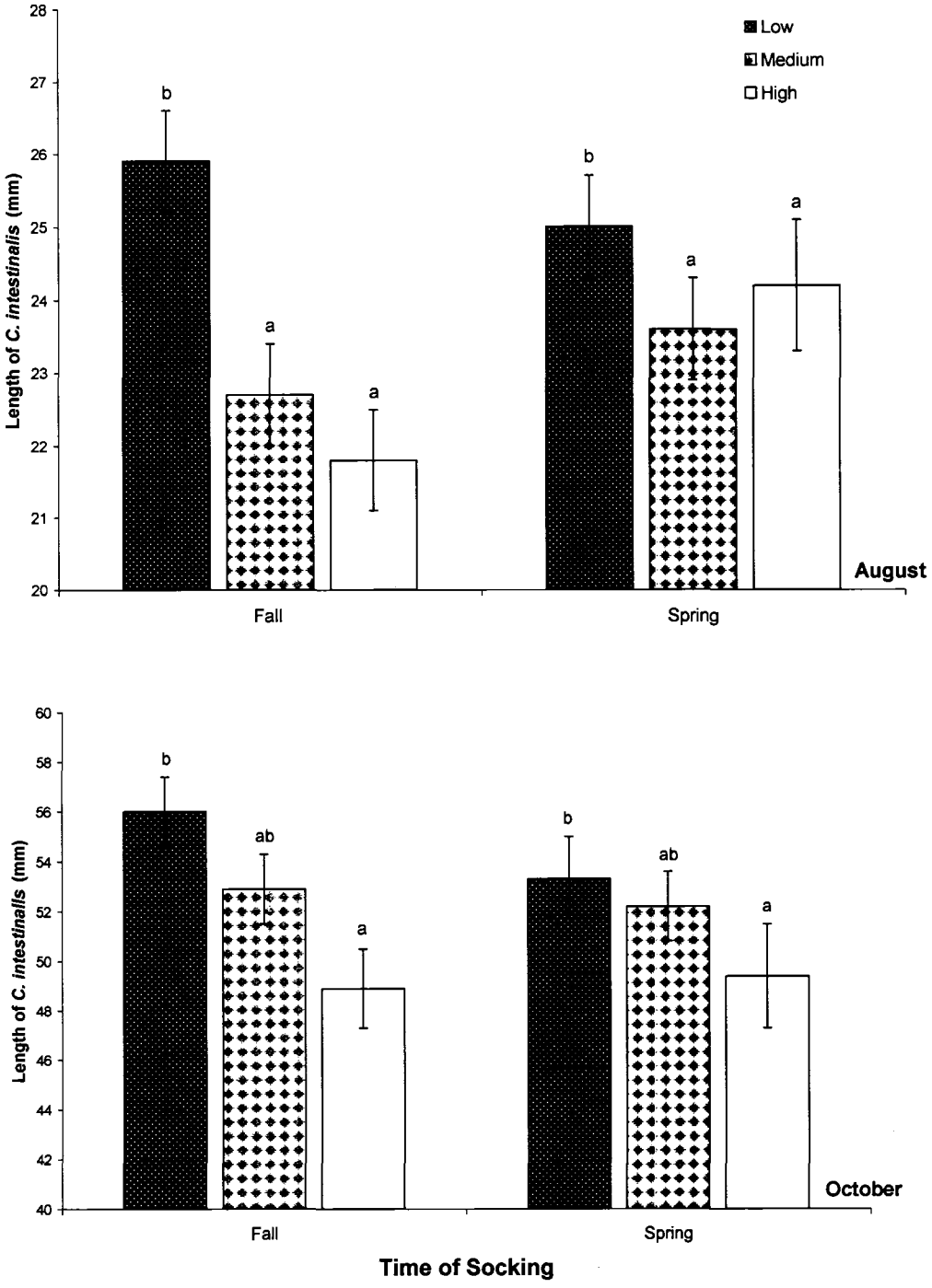


Figure 4.3 Mussel loss (%) by socking time (fall vs spring) and stocking density (low, medium, and high) at the time of last sampling in October.



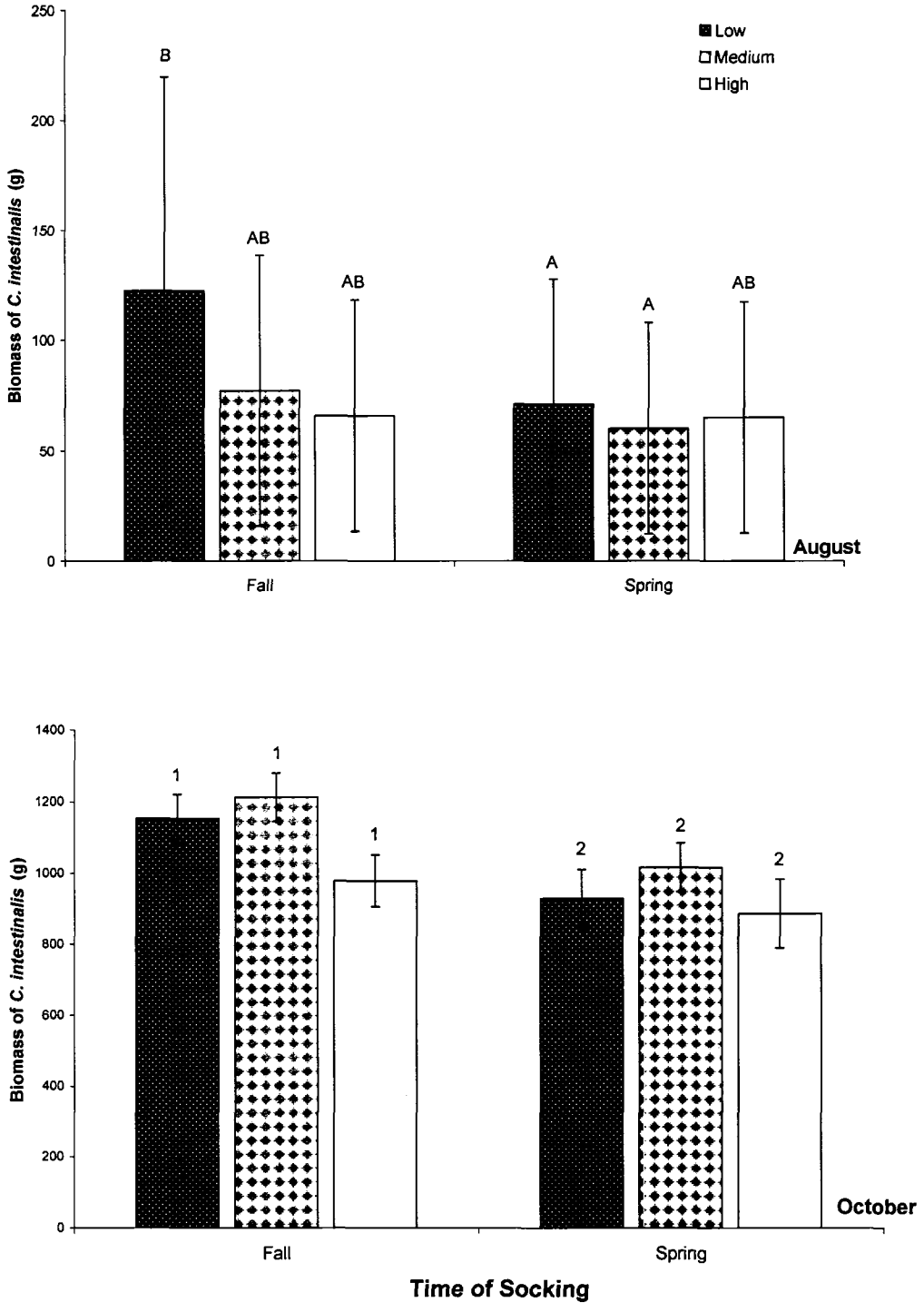
[1, 2] indicates significant difference between socking times
[a-c] indicates significant difference between densities
[A-D] indicates significant difference between combined density and socking groups
Coding is absent if no significant treatment effect was detected

Figure 4.4 Mean abundance (± SE) of *C. intestinalis* by socking time (fall vs spring) and stocking density (low, medium, and high) when measured in August and October.



[1, 2] indicates significant difference between socking times
[a-c] indicates significant difference between densities
[A-D] indicates significant difference between combined density and socking groups
Coding is absent if no significant treatment effect was detected

Figure 4.5 Mean length (± SE) of *C. intestinlais* by socking time (fall vs spring) and stocking density (low, medium, and high) as measured in August and October.



[1, 2] indicates significant difference between socking times
[a-c] indicates significant difference between densities
[A-D] indicates significant difference between combined density and socking groups
Coding is absent if no significant treatment effect was detected

Figure 4.6 Mean biomass (± SE) of *C. intestinalis* by socking time (fall vs spring) and stocking density (low, medium, and high) as measured in August and October.

Chapter 5 SUMMARY AND GENERAL CONCLUSIONS

Over the past two decades, the mussel (*M. edulis*) aquaculture industry has thrived on PEI as an economically viable and ecologically sustainable industry. Recently, however, this industry has been threatened by the invasion of exotic tunicates, which have become a nuisance in many of the primary mussel growing areas. Furthermore, with each subsequent nuisance species establishing, the effects and required mitigation efforts increase proportionally. In chronological order, the introduction of *S. clava*, *B. violaceus*, and *B. schlosseri* have all negatively affected the mussel industry, but were managed with some level of success. The most recent tunicate species to arrive, *C. intestinalis*, is proving to be a much more severe threat, with the potential to cripple production in infested areas. The research presented here serves primarily as a rapid assessment of reproductive and developmental potential of a newly invading species, considered prerequisites when determining control strategies, and as long-term management plans to control tunicate fouling.

The invasive success of a new exotic species was documented in the Brudenell estuary through the use of collector plates, dive surveys and observations from aquaculturalists. Prior to 2004, this estuary was dominated by the exotic tunicate species, *S. clava*, which had rapidly become a nuisance species. In 2004, *C. intestinalis* was identified in this system, along with the two colonial tunicate species, *B. violaceus* and *B. schlosseri*. Based on data gathered from collector plates in 2005 and 2006, a transition from a system dominated by *S. clava* to one dominated by *C. intestinalis* is documented. However, the colonial species were not observed on collector plates suggesting that they

did not successfully establish in this area, despite reaching nuisance level in other infested areas in PEI (*e.g.* Savage Harbour), where *C. intestinalis* and *S. clava* are not found. *Ciona intestinalis* became the foremost fouling organism within two years of identification as an exotic species with its ability to foul mussels and gear far exceeding that of *S. clava*. As a result, reproductive biology, early life history and development were investigated to assist in identification of control strategies.

Ciona intestinalis larvae were observed to be present in the water column from at least the beginning of August until the end of November, with a distinct peak observed in early October. Settlement and subsequent recruitment over a two-week period, however, showed *C. intestinalis* establishing from early June until mid-November. Recruitment clearly started when average water temperature rose to 8 °C and ceased at the end of the field season when water temperature dropped below 8 °C. Recruitment steadily increased through the field season, peaked in mid-August, and declined thereafter. It is curious that peak larval concentrations were observed after peak recruitment. One would expect the opposite scenario to be true where a large supply of larvae would indicate a proportionally large recruitment event. This observation could be due to *C. intestinalis* not being well adapted to the environmental conditions throughout the year on PEI, being successful only during the warmer months from June to August. Development of *C. intestinalis* was documented through the field season and showed that even though recruitment starts in early June no significant biomass is evident on the collector plates until late July. This information is critical to mussel growers who are investigating appropriate treatment strategies (*e.g.* high pressure water, vinegar spray, or lime immersion) for controlling the effects of tunicate infestation on their crop. Ideally,

mussel growers should begin treatment when *C. intestinalis* has reached a length between 10-20 mm. At this time, the mussel socks are still within handling limits of existing equipment and technology. Beyond this level of infestation, fouling on mussel socks can affect mussel productivity by decreasing growth and CI, as well as creating increased stress on the integrity of the sock structure. Complete crop failures have already been observed in heavily infested areas.

In determining optimal treatment times, new collector plates were deployed every two weeks and left in the water until the end of the field season to assess when *C. intestinalis* recruitment and subsequent growth was reduced. In this aspect of the study, there was a significant decline in *C. intestinalis* biomass after the first week of August. However, *C. intestinalis* continued to develop on collector plates deployed until the middle of October. This decrease in the development of *C. intestinalis* could be attributed to a reduction in water quality and water temperature. The rapid establishment of *C. intestinlalis* prior to August may have exceeded a sustainable level for the tunicate population in the estuary depleting food supply before it had time to replenish within a tidal cycle. The carrying capacity of the estuary may not be high enough to support the rapid establishment and growth of the tunicate population within a season.

Simultaneously with the study of the reproduction, early life history and development of tunicates, a large-scale field trial was initiated to determine the effect of mussel stocking density and socking time on tunicate recruitment and development. This study assessed primarily the level of *C. intestinalis* fouling on each of the three stocking density treatments (low, medium, and high). The mussel productivity was also assessed through growth, survival and condition index. The results of this study showed an initial

tendency towards lower *C. intestinalis* biomass on the higher stocking density groups, but this trend was not apparent by the end of the trial in October. The mussel stocking density had no significant effect on mussel productivity.

In consideration of all of the above results, the following mitigation strategies are proposed. Since no significant effect on mussel productivity was observed with increasing stocking density, a high density of mussels is not considered detrimental in areas with a significant infestation of *C. intestinalis*. It appears that increased stocking density does have potential to inhibit settlement of *C. intestinalis* by creating micro-currents around the sock perimeter. However, due to the high volume of larvae in the water column this effect maybe diluted and overwhelmed by repeated attempts at settlement by the species. Mussel producers, as mentioned above, should consider treating their high density socks once around late July, when *C. intestinalis* begins to show signs of significant growth. A subsequent treatment may be required in late August, as newly recruited individuals, post-treatment, begin to grow substantially. This could be in the form of another treatment or the same as the initial, but re-socking in New Zealand and Spain has shown to be highly beneficial (T. Landry, pers. comm.). This would involve harvesting the high density socks during mid-production cycle, removing the tunicate biomass, and re-socking the mussels at a much lower density. Three main benefits arise from this method: (1) removal of *C. intestinalis* biomass (2) any initial treatments applied would be more cost effective with a higher density sock (*i.e.* half the cost and half the time to treat one high-density mussel sock as opposed to two medium-density socks) and (3) increased mussel productivity with a lower stocking density, when

free of *C. intestinalis* fouling. The feasibility of this practice requires further investigation.

There is still much research to be conducted on mussel husbandry, treatment for invasive tunicates and the complete life history of the tunicates, especially *C. intestinalis*. Invasions of *C. intestinalis* documented in other areas of the world have shown a rapid success of the species in its new environment in the first few years post-introduction, followed by a marked decline (G. Lambert, pers. comm.). This was observed during the winter of 2007 in our study area, after the present study was completed. Dive surveys showed the species to be in greatly reduced quantity and tunicate debris to be floating in the water column and lying on the bottom sediments in a state of decomposition. Upon returning to view mussel socks previously infested by *C. intestinalis*, we found them mostly unfouled, except for barnacles. Reasons for this mass winter mortality are unclear, although divers in the fall 2006 observed unusual water clarity, which may suggest food depletion from the heavily infested area. *Ciona intestinalis* may have reproduced beyond a sustainable level in the estuary, depleted the food, and gone into winter in a malnourished state. Unable to sustain themselves, they may have died and decomposed. The decomposition of *C. intestinalis* may have compounded the problem by consuming higher levels of dissolved oxygen in the water column. Filtration potential of *C. intestinalis* and the effect of massive biomass of the tunicate decomposing require further investigation.

Another area requiring more investigation is the discrepancy between *C. intestinalis* larvae data and the recruitment data. Currently, it is only hypothesized that larvae become less viable later in the field season; thereby an increase in larvae numbers

in the water column may not correlate to increased recruitment. The current provincial tunicate larvae monitoring program collecting water samples to determine the number of tunicates in the water for mussel producers would be interpreted and used differently considering this late failure in recruitment. A similar study to document and correlate two-week recruitment with larval abundance is recommended.

Several other bays and estuaries in eastern PEI have been invaded by *C. intestinalis*, including Murray River. Early observations in the spring and summer 2007 indicate high levels of recruitment and development, as were documented by this study in 2006 in the Brudenell estuary. Research should focus on documenting the invasion, initiating a treatment plan similar to the one outlined above, and documenting winter mortality. Documenting the winter mortality would also require environmental parameters, such as chlorophyll, to determine food availability in the water column and the ability of the decomposition process (in the event of a mass mortality) to deplete dissolved oxygen.

Geographic variability needs to be addressed. This study utilized three longlines in the Brudenell estuary which were no more than 1 km apart from each other. One of these longlines consistently, during peak recruitment from July to mid October, had much higher recruitment. The reasons for this variability are unexplained. Water depth, currents, and the density of neighbouring mussel crop may be important variables in consideration of recruitment success. The collector plates used for this study stratified two metres of the water column and no recruitment difference was found with depth. It would be of interest to have collector plates stratify the entire water column to determine recruitment variability, if any.

Although this study does not address the theme of chemical treatments, other researchers are working in this area and two main components need to be addressed. Firstly, the environmental impact from the use of these chemicals in mass quantities within island bays and estuaries requires investigation. Secondly, the mechanisms by which these chemical treatments are affecting the tunicate life are in need of observation. Knowing the mechanism will enable researchers to direct their efforts and to develop more efficient application methods.

Exotic species have established in PEI waters over the past decade, including the four tunicate species (*S. clava*, *B. schlosseri*, *B. violaceus*, and *C. intestinalis*), the green crab (*Carcinus meanus*), and the oyster thief (*Codium fragilis*). The need for rapid response to new exotic species incursions requires attention. As mussel producers themselves observe the bays and estuaries they should be updated on current threats through meetings showing displays of the organisms. This monitoring should be accompanied by a follow-up confirmation by the appropriate governing organization (*e.g.* Department of Fisheries and Oceans). *Didemnum sp.*, a colonial tunicate species, is currently present on the east coast of the United States and field observations indicate that this is the dominant tunicate species. In New Zealand, this species has proven to be highly effective at establishing itself on mussel socks and gear, surpassing the level of fouling observed by *C. intestinalis*. Collaboration and communication between researchers around the world is increasingly important to ensure stakeholders are updated on potentially vital information for industry sustainability.