The effect of mitigation strategies for the clubbed tunicate *Styela clava* on mussel collectors

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ABSTRACT

The culture of the blue mussel *Mytilus edulis* in Prince Edward Island (PEI) began in the 1970's. Production has since increased, such that PEI produces 80% of Canada's cultured mussels and in 2004 accounted for over $36 million CND. Recently, an increase in fouling organisms has lead to a change in the culture methods used by mussel growers across PEI. The focus of this study is on two fouling organisms that have become pests in several mussel growing areas; the invasive solitary clubbed tunicate *Styela clava* and several algae species collectively referred to as macroalgae (identified as *Cladophora*, and *Enteromorpha*). These are specifically devastating for the seed collection stage, and a primary objective of the mussel industry is to eradicate both with the application of one treatment.

The goal of our first study was to examine the effects of two chemical treatments on *S. clava*, macroalgae, and mussel seed at the seed collection stage. The treatments used were 5% acetic acid and 4% hydrated lime. The former is used to mitigate *S. clava* on mussel socks, while the latter is used to eradicate starfish from collectors and socks. Results strongly support 4% hydrated lime as the treatment of choice for controlling tunicate and macroalgae fouling at the seed collection stage. Collectors receiving this treatment had the highest mussel abundance and the lowest tunicate abundance, and had lower macroalgae weights than untreated control collectors. In addition, we examined when the treatments should be applied to the collectors. Results showed that treating twice with 4% hydrated lime, first in early August and again in late August, was successful at mitigating fouling by *S. clava* and promoting a set of mussel seed. These treatment times coincide with the main settling period of both starfish and *S. clava*, which occur in conjunction with water

temperatures that promote spawning by these species. The use of acetic acid as a mitigating treatment was effective at controlling macroalgae and *S. clava* fouling, however it would not be recommended on mussel collectors as it led to high seed mortality. A laboratory trial to determine the effect of 5% acetic acid on juvenile mussels (<1 yr) was conducted following the completion of the field trial. Through allelic frequencies examined by electrophoresis, it was determined that the genetic changes observed after acetic acid treatments could have a potential impact on salinity and temperature tolerance of mussel populations in suspension culture. A possible decrease of phenotypic plasticity of mussel culture by these genetic changes could eventually decrease the productivity of cultured mussels. Nevertheless, more results need to be obtained to confirm this hypothesis.

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List of Abbreviations

AFRI	Aquaculture and Fisheries Research Initiative
ANOVA	Analysis of Variance
AVC	Atlantic Veterinary College
°C	degree Celsius
CI	condition index
cm	centimetre
CND	Canadian
DAFAF	Department of Agriculture, Fisheries, Aquaculture and Forestry
DF	degrees of freedom
DFO	Department of Fisheries and Oceans
EC	Enzyme Commission
Gpi	glucose phosphate isomerase
kg	kilogram
km	kilometer
L.	Linnaeus
Lap	leucine aminopeptidase
m	metre
MLH	multilocus heterozygosity
mm	millimetre
MMP	Mussel Monitoring Program
Mpi	mannosephosphate isomerase
MT	metric tonnes
n	sample size
N	Nitrogen
O	Oxygen
Odh	octopine dehydrogenase
P	P-value
PEI	Prince Edward Island
pers. comm.	personal communication

pH	parts Hydrogen
Pgm	phosphoglucomutase
SD	standard deviation
SE	standard error
SMN	Shellfish Monitoring Network
spp.	species
Tx	treatment
μm	micrometre
yr	year

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Chapter 1 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

Prince Edward Island (PEI) is the leading producer of cultured mussels in Canada, accounting for over 80% of the national total produced. Since the culture of the blue mussel *Mytilus edulis* began in the 1970s, production has increased such that in 2004, nearly 17,500 MT of mussels were produced in PEI, with a landed value of $23.5 million CND (Smith 2005). The mussel industry is made up of 123 growers operating 291 leases and 8 processing companies, provides employment for 2500 workers and is a significant contributor to the PEI economy (Department of Fisheries and Oceans 2006). In recent years, annual production has reached a plateau, particularly due to the lack of lease availability.

Regardless of the location or method used in Atlantic Canada, the production of mussels is done by collecting mussel seed, maintaining the farm through husbandry practices, and harvesting the finished product (Newell 2001). In PEI, mussels are cultivated using the longline system. This system is composed of subsurface buoyed backlines that are anchored at each end, with mussels growing on collectors or in socks that are suspended vertically along the backlines (LeBlanc et al. 2003). The use of this system is suitable to PEI's water systems as the bays, rivers and estuaries that are used for mussel culture are typically shallow and protected. The longline system is applied in all mussel growing areas in the province. The entire process from seed collection to harvest is approximately 18 to 24 months, when mussels reach a size of approximately 50-60 mm.

In the past ten years, an increasing amount of biofouling has led mussel growers to adapt their husbandry practices in order to continue producing a quality product. The arrival of several invasive species and an increase in macroalgae growth has occurred in several of PEI's mussel growing areas. This biofouling has resulted in increased production costs for the removal of competing organisms. Biofouling is also causing problems with the seed collection stage of the production cycle. Considering that this situation will unlikely disappear and fouling species may further spread to non-infested areas, mitigation strategies are being examined in an attempt to control targeted species. However, little is known about the effects treatment methods may have on the mussel population.

This study was developed to target two organisms that have been fouling mussel aquaculture sites in PEI: the clubbed tunicate *Styela clava* and several species of filamentous macroalgae (*Cladophora sp.* and *Enteromorpha sp.*). A field trial was designed to examine the impacts of mitigation strategies at the seed collection stage. Two common treatments were assessed for their impact on 1) the clubbed tunicate, 2) macroalgae, and 3) the mussel population. A further laboratory trial looked into the effects of common anti-tunicate treatment on the genetic variability of juvenile mussels, <1 yr old.

1.2. Mussel production in PEI

1.2.1. The mussel Mytilus

1.2.1.1. Genetics

Three mussel species are cultivated in Canada; *Mytilus edulis*, *M. trossulus*, and *M. galloprovincialis*. The three *Mytilus* species can be separated by their

genetic and morphological differences (McDonald et al. 1991), as well as their environmental preferences (Gardner 1996). *Mytilus galloprovincialis* is a temperate warm-water species that can tolerate tidally exposed locations, although it is highly sensitive to salinity variations. Meanwhile, *M. edulis* is a temperate cold-water species that tolerates brackish waters, and *M. trossulus* is a cold-water species that is able to endure very low (6-7‰) and high (35‰) salinities (Gosling 1992a, Gardner 1996). In Atlantic Canada and southern Maine USA, *M. edulis* and *M. trossulus* exist in specific and defined areas that overlap in several places. Hybridization between the species can occur where they overlap, although in Atlantic Canada this hybridization is limited (Bates and Innes 1995, Mallet and Carver 1995, Saavedra et al. 1996, Comesaña et al. 1999). Morphological differences are detectable between allopatric populations, while in sympatric populations, the morphology is increasingly similar between species (Gardner 1996). Similarities in the sympatric populations could be an effect of the common environmental conditions (Gosling 1992b, Gardner 1996, Innes and Bates 1999) although hybridization could play a role as well.

Unpublished data by Landry et al. show that for PEI mussel populations < 1 yr old, 7% were identified as *M. trossulus* in 1997 and ~1% in 1999. The hybrid zone for *M. edulis* and *M. trossulus* occurs in the cold, high salinity waters off Nova Scotia and PEI and the cold, low salinity waters off Newfoundland and the Gulf of St. Lawrence (Varvio et al. 1988, McDonald et al. 1991, Gardner and Thompson 2001, Penney et al. 2002, Moreau et al. 2004). *Mytilus trossulus* is also the dominant mussel species in the Bras D'Or Lakes Cape Breton, which have low salinities (Mallet and Carver 1999). In some areas, the two species can be

differentiated by shell morphology; *M. edulis* has a more rounded shape while *M. trossulus* has an elongated shape (Mallet and Carver 1995, Innes and Bates 1999). Of the two, *M. edulis* is the preferred species for aquaculture purposes. *Mytilus edulis* has shown to have greater growth rates, both in terms of somatic tissues and shell, compared to *M. trossulus* which tends to have a lower survival rate in commercial development (Mallet and Carver 1995, Penney et al. 2002).

1.2.1.2. Environment

In the Maritime region, mussels can withstand temperatures below freezing and as high as 25°C (Mallet and Myrand 1995). Temperatures from 10-20°C are optimal for growth (Seed 1976). *Mytilus edulis* tolerates salinities from 0-31‰ (Mallet 1989). The ideal salinity is ~26‰ (Department of Fisheries and Oceans 2003).

1.2.1.3. Food

Mussels are filter-feeders that selectively feed on particles suspended in the water column. This includes phytoplankton, bacteria, detritus and organic matter (Bayne 1976, Jørgensen 1990). Food particles 2-100 μm in diameter can be consumed, although particles 3-7 μm are retained with 100% efficiency (Bayne 1976). Mussels can also select high quality food that enhances soft tissue growth (LeBlanc et al. 2003).

1.2.1.4. Reproduction

Mussels are a dioecious species. In the Atlantic region, spawning occurs from mid-May to late September (Mallet 1989). In PEI, the main spawning event generally begins in late May or early June. Spawning is a response to temperature (10 - 12°C), an abundance of food, or a physical disturbance (Mallet 1989). When

spawning occurs, the eggs and sperm are discharged and fertilization takes place in the water column (Mallet and Myrand 1995). Following fertilization, the embryo goes through several transformations. The first stage is the 'trocophore stage'. At this stage, cilia appear and the embryo begins to swim (Bayne 1976). This is followed by the secretion of the shell, at which point the trocophore becomes a veliconcha for a period of several weeks. During this 'veliger stage' the larvae have a swimming organ (the velum), a functional gut, and a shell (Bayne 1976). The larvae consume food from the water column during this stage. The 'pediveliger stage' is marked by the development of a pedal organ. It is at this stage that the larvae will find and settle on a substrate and then undergo metamorphosis. Metamorphosis of the mussel larva includes several morphological changes; the secretion of byssal threads, the collapse and disappearance of the velum, the formation of the labial palps, and the re-orientation of the organs in the mantle cavity (Bayne 1976). The secretion of the adult shell follows metamorphosis, and the resulting post-larva is termed a 'plantigrade' (Bayne 1976). Once metamorphosis occurs, the mussels secrete byssal threads and attach to solid substrates such as rocks, filamentous algae, or collectors (Mallet 1989). At this stage, the mussels are capable of changing locations to find a suitable substrate. This is done by using their foot to crawl or by floating in the water column (Mallet 1989). When conditions are ideal (food, temperature, salinity), the complete transformation is 20 days (Bayne 1976).

1.2.2. Seed collection

The collection of mussel seed is a seasonal occurrence in PEI. Successful recruitment of mussel seed at a collection site is dependant on the duration and

timing of the settlement period, the number of viable larvae produced, larval-retention within the system, and predation (Department of Fisheries and Oceans 2006). The PEI Department of Agriculture, Fisheries and Aquaculture (DAFA) provide growers with the Mussel Monitoring Program (MMP), which notifies growers of mussel seedfall. The timing and abundance of seedfall is predicted by examining the numbers and sizes of mussel larvae in the water. In PEI, collectors are generally put out in late May or early June, depending on the information communicated by the MMP. To obtain seed, mussel collectors are used. These are ~1.5 m long pieces of frayed polypropylene rope that are attached to the longline ~40 cm apart. These collectors provide an attractive substrate for larval settlement. When mussels reach a size of 10-25 mm, which could be in the fall or the spring following settlement, the seed is harvested and socked. Mussels are stripped from the collector ropes, put through a declumper and graded before being socked at densities ranging from 500 to 1000 mussels per m (McDonald et al. 2002). When seed from collectors is in short supply, growers may also choose to harvest seed that has settled on mussel beds, most of which can be accessed during low tide events.

The potential for hatchery-reared seed has been discussed as an option for mussel growers in PEI. At present, the economics of the wild seed collection heavily outweigh those of hatchery-produced seed in PEI (Brake et al. 2001). Seed hatcheries do exist in North America, with the advantage of implementing selective breeding programs, having more control over seed quality, and having the option of producing polyploids for superior growth and sterility (Brake et al. 2001). Hatcheries also ensure that there are no seed shortages. In addition, it is beneficial in areas where fouling by species other than mussels occurs. Given these

advantages, the industry in PEI could benefit from hatchery-produced seed. Initial set-up is costly, and there is still a successful wild collection occurring, so the hatchery concept may not be a practical or economically feasible option in PEI for some time.

1.2.3. Genetic variability

The degree of heterozygosity measured on multiple polymorphic allozyme loci can reflect the performance of a mussel population (Tremblay et al. 1998a, Tremblay et al. 1998b). Numerous studies have shown a relationship between multiple locus heterozygosity (MLH) and physiological fitness in bivalves (Koehn and Gaffney 1984, Rodhouse et al. 1986, Hawkins and Bayne 1991, Mitton 1993, Bayne and Hawkins 1997, Tremblay et al. 1998a.). Although this relationship has been thoroughly studied, there has yet to be an explanation for the correlation between heterozogosity and fitness (Hansson and Westerberg 2002). However, two theories have been suggested to explain this relationship; direct overdominance and associative overdominance (Gosling 1992b, David 1998, Hansson and Westerberg 2002). Direct overdominance stems from the belief that the correlation is caused by some inherent advantage of being heterozygous at the scored loci and possibly the linked loci. Associative overdominance assumes that being homozygous (at linked loci) is unfavourable. Hansson and Westerberg (2002) cite three more recent hypotheses that are based on the previous assumptions. These hypotheses are the direct, local, and general effects hypotheses. The direct effect hypothesis is when there is a heterozygote advantage, resulting from functional overdominance at the scored loci. The local effect hypothesis is when the heterozygosity advantage is a result of the effects of homozygosity at closely linked loci. And finally, the general

effect hypothesis is when the heterozygote advantage is a result of the effects of homozygosity at genome-wide distributed loci. The local and general effect hypotheses together reflect the associative overdominance theory (Hansson and Westerberg 2002).

This relationship between heterozygosity and fitness parameters may be relevant to the aquaculture industry. Genetic differentiation can occur within populations located in the same area. Mussel stocks less than 10 km from one another have proven to be as genetically diverse as populations that are many kilometres away (Penney and Hart 1999). The discovery of a positive correlation between individual growth rate and survival to the degree of heterozygosity measured at several polymorphic enzyme loci is of particular importance (Koehn and Gaffney 1984, Gentili and Beaumont 1988, Zouros et al. 1988, Borsa et al. 1992, Alfonsi et al. 1995, Tremblay et al. 2001, Myrand et al. 2002, Hilder and Soulé 2004, Lesbarrères et al. 2005). Tremblay et al. (2001) and Myrand et al. (2002) have attributed survival in suspension-cultured mussels under stressful conditions (i.e. high water temperatures, air exposure) to the relationship between MLH and fitness. The increase in heterozygosity of the mussels was due to the stressful conditions that created selective mortality of the homozygotes. Mussels with the highest MLH demonstrate a higher level of fitness, which increases the scope for growth due to lower maintenance metabolic rates (Tremblay et al., 2001). Any factor that reduces the overall genetic variability may compromise the ability of a species to adapt to environmental change. Loss of variation within the population will result in a convergence towards one type and a narrower range of options.

1.3. The clubbed tunicate

1.3.1. Distribution

The clubbed tunicate is a solitary ascidian (Order: Pleurogona; Suborder: Stolidobranchia; Family: Styelidae). It is native to Asian waters, ranging from the Kurile Strait, Sea Okhotsk, to southern Siberia, Japan, Korea, and the coast of China, at least as far south as Shanghai (Abbott and Johnson 1972). Presently, *S. clava* can be found on the coasts of California, the eastern United States, southern Australia, northwest Europe (Lützen 1999), Washington State, Vancouver Island, and PEI (Lambert and Lambert 2003). The worldwide spread of this species is the result of anthropogenic transport. Lambert (2005) suggests that vectors of transport are hull fouling and introductions of shellfish and seaweeds that had tunicate species settled on them.

In PEI, the clubbed tunicate was first identified on mussels harvested in the Brudenell River in 1998 (Thompson and MacNair 2004). It has since spread to several other mussel growing areas, including Montague River (1999), Murray River (2000), Vernon/Orwell Rivers (2001), St. Mary's Bay (2001), Cardigan River (2002), the March Water area of Malpeque Bay (2002), and the Darnley Basin on the North Shore (2005) (Figure 1-1). The spread of the clubbed tunicate within the bays and rivers is both temporal and seedial. In March Water, the tunicates were first identified as a few individuals in a small space on a mussel lease. Within a few years, the population had established itself and is now spreading among the leases with increased biomass (Davidson et al. 2005).

It is thought that the clubbed tunicate arrived to PEI via foreign ships coming from infested areas. The introduction could have been through ballast water or hull

attachment. As for its movement around the Island, vectors for transport include boat traffic, movement of shellfish, and effluent from processing plants (Davidson et al. 2005). Regulations have since been implemented by the Department of Fisheries and Oceans (DFO) to restrict the movement of shellfish out of, between, or within tunicate infested areas.

1.3.2. Biology

The tunicate body is shaped like a medieval club, and consists of an elongated oval body and a long tapering peduncle that ends in the holdfast (Lützen 1999). The external surface, known as the tunic, is wart-covered and brown in adults, while wartless and a yellowish-brown colour in younger tunicates. The tunic provides a substrate for a variety of organisms to settle on and they are often observed covered with smaller tunicates as well as other sessile species. The tunic also protects against desiccation, and *S. clava* can survive out of water for several days (Thompson and MacNair 2004). The tunicates are stalked, that is, they are slimmer and twisted at the base (pedal end) and wider and not twisted at the top. Younger individuals are not stalked. Tunicates as large as 180 mm have been found in Murray River and Brudenell River (Thompson and MacNair 2004).

The environmental conditions for *S. clava* are typical of PEI's mussel growing areas, which are generally sheltered and do not experience environmental fluctuations. They tend to prefer sheltered locations that do not have strong wave action (Lützen 1999). *Styela clava* is not an estuarine species, although they can survive in conditions where salinity gets as low as 8‰ for short periods of time (Sims 1984). They tolerate a wide range of temperatures, from -2 °C to at least 23°C (Holmes 1976). *Styela clava* is known to attach to a variety of substrates, but has

shown to prefer artificial substrates to natural substrates. In PEI, *S. clava* has a strong attraction for gear used in longline aquaculture systems where it fouls buoys, ropes, concrete blocks, scope lines, back lines, and floating oyster bags (Thompson and MacNair 2004). It is widely known that these surfaces can create assemblages of species that would not be found on natural surfaces (Lambert and Lambert 1998, Connell 2000, Holloway and Connell 2002, Lambert and Lambert 2003). Solitary tunicate species thrive on artificial substrates where there is limited space available and they can reach lengths of 15 cm and out-compete other species that cannot reach out as far into the water (Lützen 1999, Lambert and Lambert 1998, Lambert and Lambert 2003). Settlement generally occurs on structures that are already fouled, making it a secondary settler. Densities on such structures can be very high, with up to 6,000 tunicates per metre being reported on mussel socks in Murray River (Thompson and MacNair 2004).

Food in the form of phytoplankton and other organic particles is filtered from the water through the anterior incurrent siphon. Water and waste is expelled via the excurrent siphon, which is located laterally to the incurrent siphon. Size ranges of 2 – 40 μm have been consumed in trials, although it is possible that larger sizes could be ingested as 40 μm was the largest particle size used (D. Bourque, DFO Moncton, pers. comm.). 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* can also ingest the larvae of other aquatic species. Osman et al. (1989) found that *S. clava* was consuming the larvae of the American oyster *Crassostrea virginica*. This could affect the settlement rates and may reduce population sizes near and around *S. clava*.

The clubbed tunicate is hermaphroditic, a feature that is common in ascidians. The life-history of *S. clava* has been documented for populations inhabiting PEI waters (Thompson and MacNair 2004, Davidson et al. 2005, Bourque et al. in press). Gonads are mature from June to October. *Styela clava* has a relatively long spawning period that begins when the water temperature is above 15°C (Thompson and MacNair 2004). More specifically, larvae are found in the water column and recruitment typically occurs from the third week of June to late October (Figure 1-2; Figure 1-3). Larval concentration was highest in mid-August and was followed by a rapid drop. In 2003, peak recruitment occurred September 23.

The clubbed tunicate is a broadcast spawner that utilizes external fertilization and small larvae, as opposed to the colonial species that fertilize internally and have large larvae (Tarjuelo and Turon 2004). The larval stage of the clubbed tunicate has been reported to last 24 to 28 hours at 20°C (Holmes 1976). Tunicate larvae are nonfeeding, so dispersal, settlement and metamorphosis must take place before the energy supply is depleted (McHenry 2005). Once settlement occurs, growth is rapid. Thompson and MacNair (2004) reported a 30-40 mm increase in body length from late July to early November. Growth decreases in November and is followed by limited or no growth from December to April when water temperatures hover around 0°C. High mortality occurs over the winter months. Mortality levels have been reported as high as 60% for juveniles and 80% for adults (N. MacNair, pers. comm.). Although mortality is high, surviving individuals recover and spawn again the following summer. These surviving individuals can be up to 100 mm in length

(Thompson and MacNair 2004). The larger individuals have most likely survived two winters, but it is not known if any survive a third.

1.4. Macroalgae

There are numerous types of macroalgae in PEI. Dominant algae species are red (Rhodophyta), brown (Phaeophyta), green (Chlorophyta), and blue-green (Cyanophyta). It is generally the filamentous red and green species that foul aquaculture equipment. Red macroalgae include *Polysiphonia*, *Ceramia*, while the green macroalgae include *Enteromorpha*, *Ectocarpus*, *Chaetomorpha*, and *Cladophora*. Taylor (1962) reported the association between shells and the groups *Enteromorpha*, *Ceramia*, and *Polysiphonia*. In 2004, there were several blooms of green algae in mussel growing areas that had a negative impact on the mussel seed industry (Smith 2005). When macroalgae fouling occurs on mussel seed collectors, the seed migrates outwards onto the macroalgae. In conditions where there is wave action, the macroalgae will fall from the collectors, taking the seed with it. This has resulted in seed shortages in PEI (Smith 2005).

Red algae can have three distinct forms, which some authors refer to as plants; male, female, and tetrasporophyte (Hanic 1974). The male plants produce non-motile sperm that rely on water currents to reach the female plants. Once the female plant is fertilized, it produces carpospores, which are released into the water column. These carpospores settle onto a hard substrate and become tetrasporophytes, which often resemble either the male or female plant (Hanic 1974). It is the tetrasporophytes that produce the male and female plants. This is done by the release of spores, half that become female plants and half that become male

plants (Hanic 1974).

The green species also have three plants involved in their lifecycle, although it is markedly different from that of the red algae. In this group, both the male and female plant produces male and female gametes that both resemble sperm (Hanic 1974). Fusion of the two gametes occurs when they meet in the water column, which results in a diploid plant. Once this plant is mature, it produces spores. Half of these spores develop into female plants, while the other half develop into male plants (Hanic 1974).

Macroalgae settlement occurs in mid-July, with large mats being present on collectors and the longline by mid-August.

1.5. Biofouling of mussel aquaculture

There are a variety of problems that can be encountered during the production of mussels in PEI. Although they are usually rectified through improved husbandry practices, they continue to occur and cause substantial monetary losses through the loss of product, increased labour, and purchase of new equipment. Predation is a primary concern for the industry. Predation of mussel culture is predominantly by starfish, crabs, and diving ducks like the Greater Scaup *Aythya marila* and the Long-Tailed duck *Clangula hyemalis*. Environmental variation can also lead to loss of product, for example high water temperatures and high wind events like those that occurred during Hurricane Juan in 2003. Fouling by aquatic species presents a major challenge for mussel growers, although studies have shown that fouling does not reduce the quality of mussels in PEI (LeBlanc et al. 2003, Davidson et al. 2005, Department of Fisheries and Oceans 2006). Production costs

increase considerably when fouling occurs. Invasive species can increase fouling problems associated with aquaculture gear, as well as competition with mussels for habitat and food. These invaders generally do not have any natural predators, which puts them at an advantage over the native species that can have one or several predators.

Fouling of mussel gear occurs at all stages of production. There are two major fouling groups of mussel aquaculture in PEI, the invasive tunicates and species of filamentous macroalgae. Both of these groups have become problems in the past decade. This has been initiated by a variety of factors; an increase in vectors for invasive species, altering environmental parameters, and changes in land-use patterns.

A succession of introduced tunicates has occurred in mussel growing areas; *S. clava* in the Brudenell River (1998), the vase tunicate *Ciona intestinalis* in the Montague River (2004), and the violet tunicate *Botrylloides violaceus* and golden star tunicate *Botryllus schlosseri* in Savage Harbour (2004). Since they were first reported, the tunicates have spread to numerous mussel growing areas and some areas are now experiencing the presence of multiple tunicates species. All four tunicate species are considered to be a threat to the sustainability of the PEI mussel industry although it is the solitary tunicates that compete with mussels for habitat. Competition between tunicates and mussels is primarily for space and potentially for food. There is also increased labour that must be applied to the production and mitigation process.

The solitary *S. clava* has affected mussel production in several areas. One of these areas is Murray River, which has very high abundances of *S. clava*. This

species has been considered a serious pest to growers in this area since 2001 when infestation rates increased significantly. The clubbed tunicate effectively fouls mussel lines, buoys, boats, and mussel collectors and socks. This results in additional costs in labour to clean fouled gear, for mussel processing, and to dispose of tunicate waste (Thompson and MacNair 2004). A conservative estimate of the additional cost of producing mussels in infested bays suggests there is an increase of approximately $0.25 CND/kg added to the final production costs (Thompson and MacNair 2004).

Fouling by filamentous macroalgae species, particularly the green macroalgae *Cladophora sp.* and *Enteromorpha sp.*, is another biofouling issue for the mussel industry. This is especially harmful at the seed collection stage. Macroalgae settle directly on the mussel collectors, coating the rope surface and growing into large, rope-like algal "mats". The mussel seed will then settle directly on the algae or migrate off the collectors and onto the algal mats. As the algae grows, the mats become heavier which can result in both the macroalgae and mussel seed stripping away from the collector if they are lifted from the water or agitated by wind or wave action (Smith 2005). In these circumstances, seed loss can be substantial, jeopardizing the source of seed for socking.

The presence of starfish (*Asterias vulgaris*) on mussel growing equipment occurs seasonally in PEI. Starfish spawning occurs in May or early June and starfish are first found on socks and collectors by the end of July (MacKinnon et al. 1993). Of all the commercially cultured bivalves in PEI, mussels are the preferred prey species for starfish (Miron et al. 2005). Starfish are especially detrimental to seed collectors. Starfish on first-year mussels can benefit growers as they remove the

second set of seed, but on collectors, they grow rapidly and consume such large amounts of mussel seed that the crop used for stocking leases is jeopardized (MacKinnon et al. 1993).

1.6. Mitigation strategies

Various mitigation strategies are used in the mussel industry. These strategies are used to remove predators and/or fouling organisms. For this reason treatments are region specific in the growing areas of PEI, and variations in treatment type and application occur between growers as well as between years. The stage of production is another determining factor. A treatment used in the seed collection stage may not necessarily be used in the socking stage. For the most part, these treatment regimes are chemical treatments, although some physical and biological treatments are also used. An example of biological control is when the growers lower the longline so that the socks touch the bottom. This allows crabs (*Cancer irroratus*) to climb on the socks to damage any tunicates or starfish, and clean off any smaller mussels or those that are moribund (Ellis 2002).

Chemical treatments are used to mitigate the clubbed tunicate. Investigations into an adequate treatment have been ongoing since the arrival of the clubbed tunicate (Davidson et al. 2005). An assortment of treatments has been tested with varying results. A spray of 5% acetic acid solution has been most effective for controlling the clubbed tunicate on PEI mussel socks (Davidson et al. 2005) and a brine bath dip was successful for *S. clava* fouling of oyster baskets in Ireland (Minchin and Duggan 1988). Treatments of lime, brine, and acetic acid have been successful in eradicating tunicate spp. from oyster gear in PEI and Nova Scotia

(MacNair and Smith 1998, Carver et al. 2003). The mode of action for these treatments has not been documented, although preliminary results are being gathered at the AVC (G. Johnson, pers. comm.).

Experimental trials have been conducted in order to find a suitable treatment for fouling macroalgae. Solutions of brine have shown success in mitigating macroalgae from mussel collectors (Sharp et al. 2005). However, with the presence of filamentous macroalgae becoming more prolific in mussel growing areas, treatment regimes must be further tested and developed. It would also be ideal to mitigate for tunicates and macroalgae with the same treatment.

Chemical treatments are also used to control the effect of starfish on mussel collectors and socks. Starfish are a common predator to cultured mussels. The consumption of mussels by starfish is impressive; immediate action is required if mussel loss is to be avoided. Treatment is a dip of 4% hydrated lime solution that is generally applied when five or more starfish are observed on a collector or sock (N. MacNair, DAFA, pers. comm.). Lime baths are efficient in that mussels are usually unaffected while the lime affects the tube feet of the starfish and causes mortality. These tube feet are part of the vascular system; the chemical affects the transport of food and respiratory gases in the starfish, which leads to death (MacKinnon et al. 1993). This treatment regime occurs in late July, and sometimes growers must treat again if there is another set of starfish that occurs in late August or early September.

1.7. Objectives

The objective of the first study was to develop a mitigation strategy for the control of the clubbed tunicate and macroalgae on mussel collectors, while still

promoting a healthy mussel population. It is hoped that this strategy could be implemented into the current husbandry practices used during the seed collection stage of mussel production in PEI. The objective of the second study was to determine if treatments of 5% acetic acid had an effect on the physiological fitness of < 1 yr mussels. This treatment is commonly applied to mussel socks to mitigate the clubbed tunicate.

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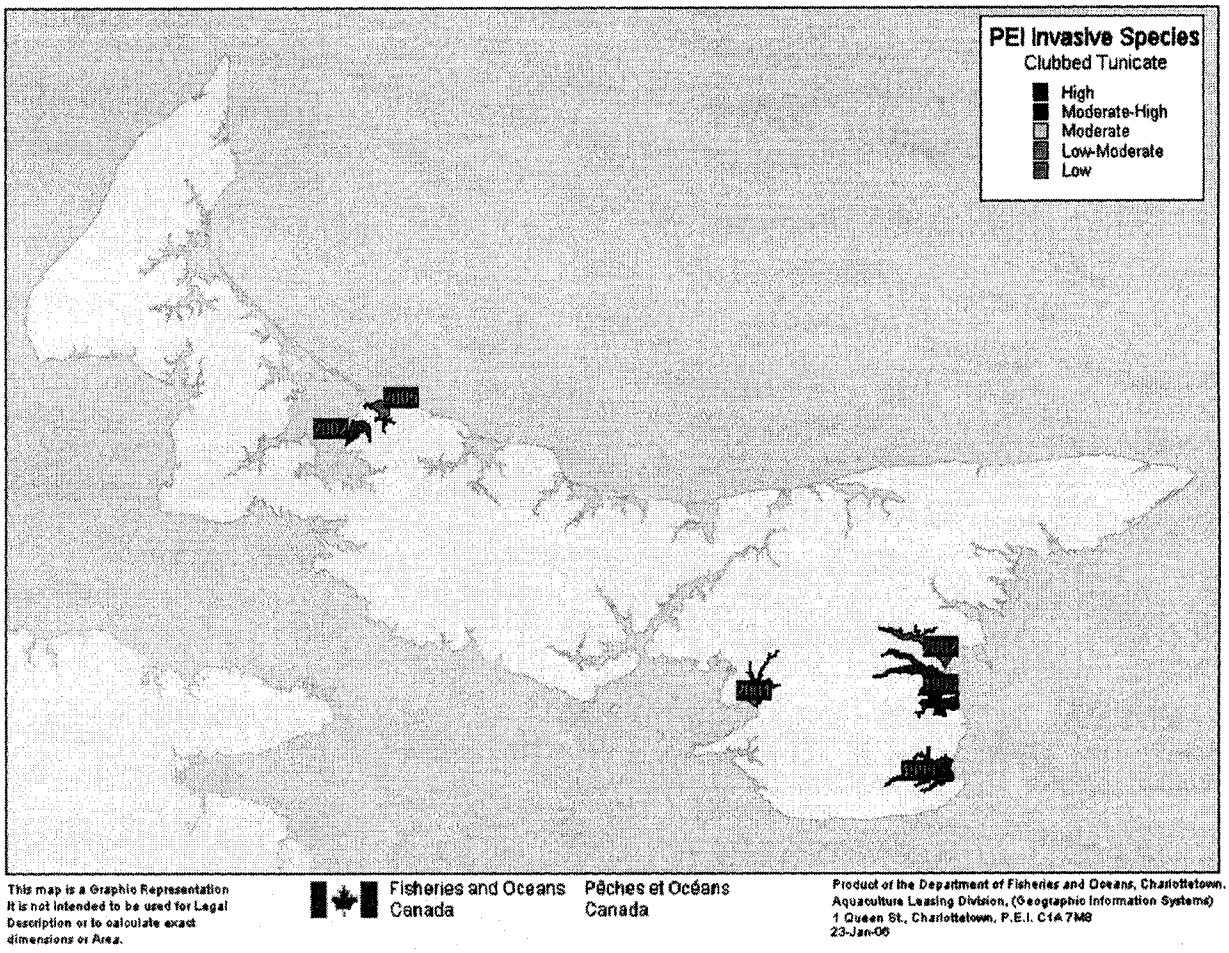

Figure 1-1 Current distribution of the clubbed tunicate *Styela clava* in Prince Edward Island, Canada. Numbers represent the year *Styela clava* was first identified (1998 – Brudenell, 1999 - Murray River, 2001 – Vernon/Orwell Rivers, 2002 – Marchwater and Cardigan, 2005 – Darnley Basin).

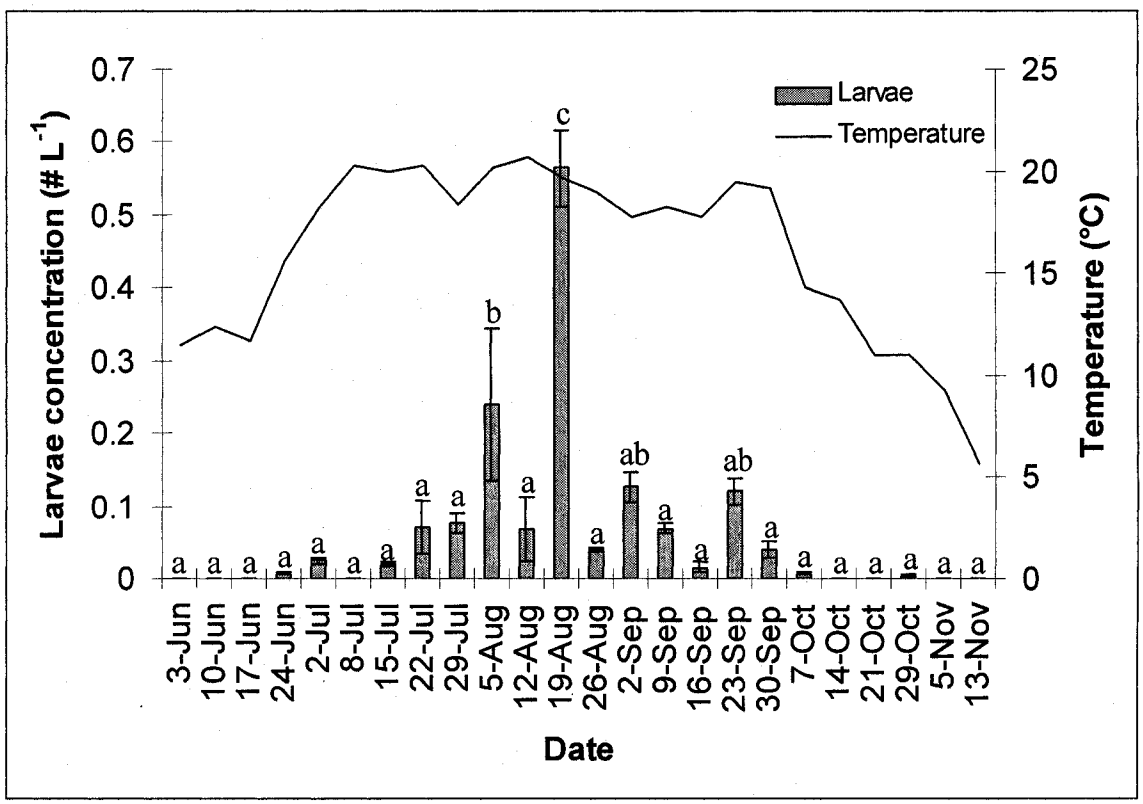

Figure 1-2 *Styela clava* larval concentrations vs. water temperature in Murray River, PEI, in 2003 (Davidson et al. 2005).

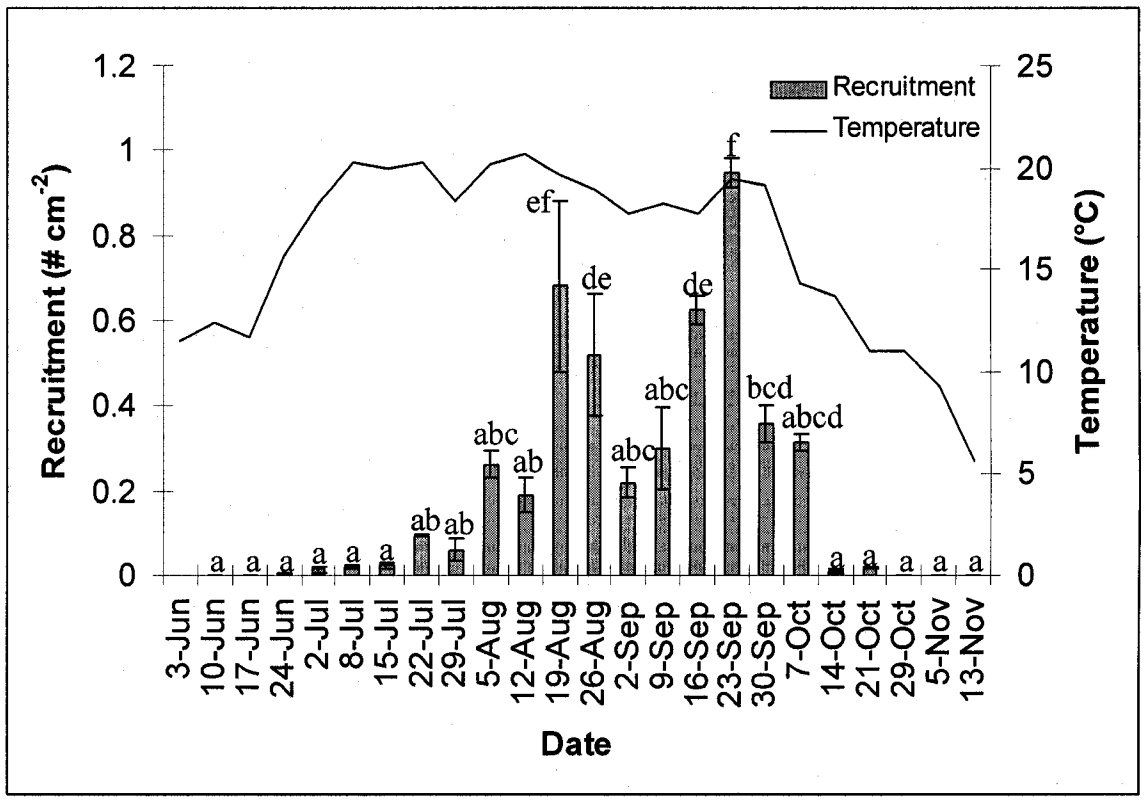

Figure 1-3 *Styela clava* larval recruitment vs. water temperature in Murray River, PEI, in 2003 (Davidson et al. 2005).

Chapter 2 THE EFFECT OF PEST CONTROL ON MUSSEL SEED PRODUCTION IN PRINCE EDWARD ISLAND, CANADA

2.1. Abstract

The blue mussel (*Mytilus edulis*) aquaculture industry in Prince Edward Island, Canada, depends on the success of wild mussel seed collection in the early summer months. The presence of the invasive clubbed tunicate (*Styela clava*) and several species of filamentous macroalgae have been negatively affecting seed collection as they are efficient biofouling organisms. Treatments of 5% acetic acid and 4% hydrated lime were applied to mussel collectors to determine which treatment regime was most effective at reducing tunicate and macroalgae biomass, while still promoting mussel seed abundance. Treatments had a significant effect on mussel seed collection; untreated control collectors had 100% more tunicates and almost 50% more macroalgae present at the end of the trial compared to treated collectors. Treatment with hydrated lime was successful at controlling the clubbed tunicates on mussel collectors while still achieving high mussel seed collection. Collectors treated with hydrated lime had a mean (±SE) mussel abundance of 104.6 ± 26.9 per 30 cm as compared to 38.7 ± 13.1 for acetic acid and 0 for the controls. The abundances of tunicates showed a reverse trend on collectors treated with hydrated lime (99.3 ± 22.7 per 30 cm) in relation to acetic acid (148.6 ± 26.3) and the controls (489.8 ± 21.1). In terms of timing, treatment with hydrated lime in early August and again in late August resulted in significantly higher mussel abundances (253.4 ± 56.8) and mussel lengths (19.51 ± 1.25 mm), while remaining free of tunicates. The timing of this treatment targeted the major spawning period of the

clubbed tunicate as well as the settlement period of starfish, which prey on mussels and have the ability to deplete mussel seed quickly and efficiently. For controlling macroalgae on mussel collectors, a treatment of acetic acid successfully reduced the biomass by 42%,however, there was considerable mussel mortality.

2.2. Keywords

Mytilus edulis, *Styela clava*, macroalgae, fouling, mitigation strategies, seed collection

2.3. Introduction

The clubbed tunicate *Styela clava* Herdman is a solitary ascidian that has recently arrived to the estuaries of the Atlantic Canadian province of Prince Edward Island (PEI) (Figure 2-1). *Styela clava* is native to the Sea of Okhotsh, Sea of Japan, and the coasts of Japan, Korea and northern China (Abbott & Johnson 1972). Through various methods of introduction, the distribution of *S. clava* has become worldwide. The species has spread to the east and west coasts of the United States, southern Australia, and Northwest Europe (Lützen 1999). It can also be found on both coasts of Canada, and has been identified in New Zealand waters in the past year (Biosecurity New Zealand 2005). It was first identified in PEI in 1998, and has since spread to several areas around the island. *Styela clava* has the ability to heavily foul blue mussel *Mytilus edulis* L. growing equipment as well as compete with cultured mussels for food and space, and has caused concern regarding the sustainability of the mussel industry (Thompson & MacNair 2004). The cost of this infestation is substantial to the mussel industry, mainly through mitigation efforts,

extra labour costs, and processing expenses.

In addition to *S. clava*, mussel growers are also concerned with biofouling by macroalgae and starfish. Large masses (mats) of filamentous macroalgae grow on mussel seed collectors and can cause significant seed loss (Smith 2005). Starfish are a common predator of the cultured mussels (MacKinnon et al. 1993). These starfish grow rapidly and consume large quantities of the small mussels. Both of these fouling organisms can diminish the mussel seed supply necessary for the fall socking period.

Biofouling of mussel aquaculture sites in PEI is in part due to the longline system that is used for cultivation. This method consists of ropes and individual socks that hang vertically in the water column from a subsurface buoyed backline (LeBlanc et al. 2003). Mussel culture begins with the collection of seed in early summer. Collection is done by providing pelagic mussel larvae an opportunity to settle on frayed ropes that are hung vertically in the water column. Settlement occurs in early to late June following the major spawning event in late May or early June. The juvenile mussels are then left to grow on the collector ropes until the fall when they are removed, declumped, graded, and placed in socks for the grow-out stage. The collector ropes also provide habitat for the fouling macroalgae, starfish, and *S. clava*, which recruits better on newly deployed artificial substrates compared to natural surfaces (Lambert & Lambert 2003, Lambert 2005). The location of seed collection also plays a role in the severity of biofouling. In most cases, mussel culture in PEI is done in the bays and estuaries that are not exposed to harsh elements such as wave action and strong currents. This type of habitat is ideal for tunicate recruitment (Lambert and Lambert 1998).

Various treatment regimes are used to control biofouling on mussel culture sites, depending on the grower and lease location. Presently, in PEI, infestations of *S. clava* on mussel socks are treated by spraying a 5% acetic acid ($C_2H_4O_2$) solution, which can be purchased as white table vinegar (pH of ~3.0). Macroalgae fouling on mussel collectors in PEI are controlled with a dip in a brine solution (a saturated salt solution) (Smith 2005). Dips in 5% acetic acid have also shown some success with macroalgae fouling, although mussel mortality is reportedly high (Sharp et al. 2005). In addition to these treatments, solutions of 4% hydrated lime (calcium hydroxide, $Ca(OH)_2$) have been used to eradicate starfish from mussel collectors (pH of ~12.0) (MacKinnon et al. 1993). These treatments are generally applied when the fouling organism is first observed on the culture gear.

There are limited publications dealing with the control of *S. clava* on aquaculture equipment (Minchin & Duggan 1988, Thompson & MacNair 2004, Davidson et al. 2005). Treatment trials have yielded inconsistent results, although chemical treatments seem to yield the most promising results. There is no recommended treatment for controlling *S. clava* infestations on mussel collectors. Results from field and laboratory experiments conducted by Davidson et al. (2005) indicate that treatments of 5% acetic acid have the most potential for controlling *S. clava* on mussel socks. Carver et al. (2003) have also observed mortality of the solitary tunicate, *Ciona intestinalis*, using acetic acid in Nova Scotia. While this treatment effectively causes tunicate mortalities, it has also caused high mussel seed mortality (Davidson et al. 2005, Sharp et al. 2005).

In order to find a suitable treatment regime for controlling the settlement and growth of *S. clava* and macroalgae on mussel collectors, treatments of 5% acetic

acid and 4% hydrated lime were evaluated in a field trial. The goal of this study was to develop a mitigation strategy for the control of *S. clava* and macroalgae on mussel collectors, while minimizing the impact on mussel health. The objectives of the project are to determine 1) the effect of the treatments on the targeted species, 2) the effect of the timing of the treatment application on the targeted species, and 3) the effect that the combination of treatment and time of application has on the targeted species.

2.4. Materials and Methods

2.4.1. Study Area

The field experiment was conducted between June and November 2004 in Murray River (Latitude: 62.5694° Longitude: 46.0255°), located on the southeastern side of PEI. Murray River is a major mussel aquaculture area that consistently provides a reliable mussel seed set, has an established population of *S. clava* and an abundance of macroalgae, and has the infrastructure and resources available to conduct field trials. Water temperature ranged from 9.4 to 22.57°C throughout the study period, with an average of 17.61°C.

2.4.2. Experimental Design

In the spring of 2004, prior to the primary mussel spawning event, 100 mussel collectors were deployed on a single longline in Murray River, PEI. The collectors consisted of ~12-14 mm diameter frayed polypropylene rope pieces of an average length of 1.5 m. Collectors were separated by ~40 cm along the longline. The collectors were subjected to a series of treatments in July and August of 2004. The experimental design was a randomized block design, with the longline divided

into five sections of 20 collectors in order to control for environmental variation along the length of the longline (Figure 2-2).

Within each block, six collectors were assigned to each of three treatment groups: i) 5% acetic acid, ii) 4% hydrated lime, or iii) salt water, which acted as a manipulation control. In addition, two stationary control collectors were untreated and not removed from the longline for any period of time; measurements on the stationary controls were not included in the data analysis. For each treatment group, treatments were either applied once (on July 22, August 2, 12, or 24) or twice (on July 22 and August 12, or on August 2 and 24).

2.4.3. Treatment Procedure

The treatments consisted of solutions of 5% acetic acid and 4% hydrated lime. The control solution was water taken from Murray River, at a salinity of ~27‰. This application controlled for air exposure, agitation and stress on the collectors during treatment. The acetic acid was not diluted with water, while the hydrated lime powder was mixed with salt water taken from Murray River to obtain a concentration of 4%. Treatments were applied by dipping the collectors in one of the three solutions for 15 seconds and then rinsing them in seawater for 15 seconds. The seawater was obtained from Murray River and changed after each collector. The rinse was used to decrease the effect of residual treatment solutions from neighbouring collectors.

2.4.4. Sampling Protocol

All collectors were removed from the water on November 3, 2004 and placed into individual plastic bags to prevent falloff of mussels, macroalgae, or

tunicates during handling/transport. The collectors were stored in their individual bags in a –20°C freezer until they were processed in the lab.

2.4.5. Laboratory Analyses

In the laboratory, each collector was analyzed individually. After blinding the operator to the treatment group and week of treatment application, measurements taken from each collector were: dry macroalgae weight; mussel condition index, mussel length and abundance; and *S. clava* length and abundance. For all mussel measurements, only mussels >10 mm were included in the study. This excluded mussels on the collectors from a second set of seed that would have settled after the treatments concluded.

Macroalgae and all biotic material except mussels and tunicates were manually removed from each collector. The macroalgae was separated from the other biotic material, placed in an aluminium pan and dried for 24h in a drying oven at 80°C. The dry weight of the macroalgae was determined with a precision balance (Denver Instruments, 0.01 g accuracy).

Sections 10 cm long were removed from the top, middle, and bottom of the collector to obtain a 30 cm representative sample for the entire collector. The top sample was taken 30 cm down from the top-end of the rope, the middle was taken another 30 cm down, and the bottom was taken another 30 cm down from the middle section. For each of the three 10 cm sections, shell length of all mussels and body lengths of all *S. clava* were measured using a digital calliper (Mitutoyo, 0.01 mm accuracy). The abundance of both mussels and tunicates was determined by counting all individuals in the three sections.

Mussels were stripped off the remaining collector pieces (other than the 3 x 10 cm samples) and 30 were randomly selected for condition index (CI) based on the methods described in Abbe and Albright (2003). Mussel meat was removed from the shell and dried in a drying oven at 80°C for 24h. The dry meat was then removed from the oven and weighed using an analytical balance (Denver Instruments, 0.0001 g accuracy) to obtain the dry meat weight. The empty shells were also dried for 24h and then weighed to get the dry shell weight. The CI is calculated as the ratio of the dry meat weight over the dry shell weight and multiplying by 100 (Abbe & Albright 2003).

$$CI = \frac{Dry\ meat\ weight\ (g)}{Dry\ shell\ weight\ (g)} \quad x\ 100$$

2.4.6. Statistical Analyses

Data for macroalgae weight, CI, mussel and tunicate length, as well as mussel and tunicate abundance were analyzed separately. All analyses were carried out at the collector level; collector averages were used for CI, mussel length and tunicate length. Initial statistical models included effects of treatments (5% acetic acid, 4% hydrated lime, and control), application week(s) (6 groups), the treatment*week interaction, and blocks (1-5), but non-significant effects were removed in final models. Multiple comparisons for significant effects used a Bonferroni correction. The scale of the linear statistical model depended on the specific outcome, and its compliance with model assumptions, as evaluated from model residuals and diagnostics (Dohoo et al. 2003). Box-Cox transformations were investigated to determine an approximately optimal transformation. In detail,

macroalgae weights and tunicate abundance were square-root transformed, CI and mussel length were untransformed but the control treatment was excluded due to lack of mussels. Initially, a rank-based analysis for mussel abundance was carried out (to account for a large number of collectors with zero counts), but the comparison of the two chemical treatments could be done on square-root transformed counts. As a substantial portion of tunicates were below 5 mm in size (and therefore unmeasurable), tunicate length was assessed both by the proportion of tunicates <5 mm, and by the length of measurable tunicates. The former were analyzed by a logistic model adjusted for overdispersion, and the tunicate lengths were log-transformed before analysis. Whenever full compliance with the linear model could not be achieved, the P-values of statistical tests were confirmed by permutation tests (Anderson & Ter Braak 2003). All statistical tests were interpreted at a 5% significance level. The statistical analysis was carried out using Stata Version 9 (StataCorp LP, College Station, TX).

2.5. Results

The main mussel spawning event occurred during the first week of June (Smith 2005), while the peak spawning period for *S. clava* occurred during the third week of August (Bourque 2006, in press). Based on visual inspection of the collectors, mussel seed recruitment occurred by the end of July. However, shortly after deployment and treatments, species assemblage on the collectors was noticeably different and highly distinguishable between treatments by the end of the trial period. Of the 90 collectors receiving treatment of manipulation, only 30 collectors retained mussel seed at the end of the study period. Only the collectors

treated with 5% acetic acid and 4% hydrated lime had mussels present (10 and 19 collectors respectively), while the control collectors had none. Tunicate recruitment and retention occurred on 73 of the 90 collectors at the end of the experimental period, including all 30 manipulated control collectors.

Macroalgae weight

All collectors had values for macroalgae weight. No statistically significant effects were obtained for weeks or the treatment*week interaction, but there was a strong treatment effect ($P = 0.003$). The treatment effect was attributable to a higher macroalgae weight for controls (Table 2-1).

Condition Index

Only 23 collectors had sufficient mussels to be included in this analysis (8 collectors for acetic acid, and 15 collectors for hydrated lime). No significant effects were found.

Mussel length

Mussels survived on 29 collectors (10 collectors for acetic acid, and 19 collectors for hydrated lime). A significant effect was observed for the treatment*week interaction ($P < 0.001$) and subsequent comparisons using Bonferroni correction indicated significantly larger mussels on collectors that had been treated with hydrated lime at two dates; a single treatment August 2 and a double treatment August 2 and August 24 (Table 2-3).

Tunicate length

Larger tunicates (≥5 mm) were found on 62 collectors, while smaller tunicates (<5 mm) were found on 68 collectors. Proportions of the small tunicates showed significant effects of the treatment*week interaction ($P = 0.001$). When

performing pairwise comparisons among the treatment*week combinations, the collectors receiving a chemical treatment had significantly higher proportions of small tunicates than the control collectors across all treatment times, except for double treatments at August 2 and 24. Collectors that received a single treatment of acetic acid on August 24 and a double treatment on August 2 and August 24 had only (100%) small tunicates (Table 2-3). The length of measurable tunicates had a significant effect at the treatment*week interaction ($P = 0.003$). Among the treated collectors, the smallest tunicates were found for a double treatment of acetic acid on August 2 and August 24 (Table 2-3).

Mussel abundance

Only 29 collectors had mussels at the end of the field trial (10 for collectors treated with acetic acid, and 19 for collectors treated with hydrated lime); the abundance was significantly lower in the control group. For the two chemical treatments, there was a significant treatment*week interaction ($P = 0.02$). Mussel abundance was highest for collectors that received a single treatment of hydrated lime August 2 and a double treatment of hydrated lime August 2 and August 24 (Table 2-3).

Tunicate abundance

Tunicates (both small and large) were found on 73 collectors, with treatment being the only significant effect on the tunicate abundance ($P < 0.001$). The collectors receiving the manipulated control had the largest number of tunicates, but there was no difference between the two chemical treatments (Table 2-1).

2.6. Discussion

The impact of fouling organisms on mussel aquaculture is widespread in PEI. Because the seed collection stage is the beginning of mussel production, the development of a treatment that controls tunicates and macroalgae, while still allowing for a healthy set of seed, would be beneficial to the mussel industry. This study investigated the effect of treatments on two biofouling organisms, *S. clava* and macroalgae, and the impact on mussel seed collection. The most striking observation from this study shows the critical importance of using treatment regimes to ensure mussel seed collection, in view of the null results on non-treated collectors (controls). Only treated collectors were able to sustain a set of mussel seed. This is most likely due to starfish predation, which was an uncontrolled variable in this study on biofouling. MacKinnon et al. (1993) have indicated the necessity of applying a chemical treatment to mitigate starfish recruitment at the seed collection stage in order to avoid loss of seed. Treatments of 5% acetic acid and 4% hydrated lime were effective at differing degrees for controlling the two fouling groups. The most successful treatment regime, in terms of reducing the infestation of *S. clava,* while still obtaining a high recruitment level of mussel seed, was the application of hydrated lime in early (2^{nd}) and late (24^{th}) August (Figure 2-3). This treatment was effective in reducing *S. clava* infestations, and resulted in successful mussel seed collection. For macroalgae control, it is evident that chemical control is necessary as acetic acid and hydrated lime treatments produced the best results as compared to the control solution. Unfortunately, the observed seed mortality from the acetic acid may limit the use of this treatment as a means of mitigating macroalgae fouling.

Based on the results of our study, the incorporation of hydrated lime treatments into the care and management of mussel seed collectors is suggested, especially where there are infestations of *S. clava*. A double treatment of hydrated lime applied in early August and again in late August was successful in mitigating *S. clava* and promoted an abundant mussel seed population, while maintaining low amounts of fouling macroalgae. When this strategy was used in Murray River, which has one of PEI's heaviest *Styela* infestations, collectors had a set of mussels and limited tunicate fouling (only 8 tunicates, all <5 mm). The timing of this treatment coincides with the primary settlement periods of both *S. clava* and starfish. MacKinnon et al. (1993) stated that starfish appear on collectors at the end of July. Therefore, our first hydrated lime treatment would have eradicated the starfish. Although *S. clava* are continuous spawners, Bourque et al. (2006, in press) identified August 19 as the major spawning period. *Styela clava* have a short pelagic larval stage of 24-48 hours, so the second treatment would have occurred shortly after the major settlement period. The mode of action of the chemical treatments is not known for *S. clava*, however, the juveniles could be more susceptible to treatments than the older, larger individuals. This could be because their tunic has not developed its characteristic leathery toughness.

There are many benefits to using a hydrated lime treatment regime. Hydrated lime can be easily obtained, is relatively inexpensive, and to date no negative impacts on the environment have been observed. The solution can also be prepared while in the field by mixing the powder with salt water (40g powder/l of water). Also, this chemical has already been included into the husbandry practices of mussel culture in PEI. Dips of hydrated lime are used to eradicate starfish from

mussel collectors when they appear in late July and sometimes when there is another set in late August. The collectors are treated immediately when starfish are observed because they can devastate the seed collection process (MacKinnon et al. 1993). The use of one treatment strategy to effectively control for two fouling species is advantageous to mussel growers. With the success of this treatment strategy, growers can reduce the labour necessary to produce collectors with a viable set of mussel seed.

The use of acetic acid on mussel collectors is not recommended. Acetic acid caused sufficient *S. clava* mortality and decreased macroalgae fouling, but significant mussel mortality was observed. Further trials should be performed using different application methods or a diluted solution of acetic acid that may perform better in terms of maintaining mussel seed. Acetic acid could potentially be used on aquaculture gear that has been fouled with *S. clava* and macroalgae, but not where mussel seed is involved. The recommended brine treatment should be considered when macroalgae fouling occurs (Sharp et al. 2005).

2.7. Conclusion

Treatment must be applied to seed collectors in areas where *S. clava* and macroalgae fouling occur in order to obtain adequate seed collection. A double treatment of 4 % hydrated lime in early August and again in late August was efficient for mitigating *S. clava* from mussel collectors. The incorporation of this treatment regime into the husbandry practices at mussel culture sites with similar *S. clava* infestations is advised if they are not already in use. Results fit well with the present husbandry practices for seed collection and maintenance in PEI, since

starfish treatments are generally conducted twice. For controlling macroalgae on mussel collectors, a single treatment of 5% acetic acid was effective at reducing macroalgae abundance, although there was considerable mussel mortality, therefore it is not advised. Further work is necessary to determine how hydrated lime and acetic acid affect the physiology of *S. clava*, as well as < 1 yr mussels.

2.8. Acknowledgement

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Table 2-1 Mean ± SE (SE = SD/√n) for macroalgae dry weight (g), mussel condition index, mussel shell length (mm), length of larger tunicates (≥5 mm), mussel abundance and tunicate abundance and percentage of small tunicates (<5 mm). Sample size (*n*) indicates the number of collectors that were used in the analysis.

Treatment	Macroalgae Weight	Condition Index	Mussel Length	Tunicate Length (≥5mm)	Mussel Abundance	Tunicate Abundance	% of Small Tunicates
5% Acetic Acid	2.41 ± 0.33 *(n = 30)*	17.10 ± 0.49 *(n = 8)*	13.93 ± 0.85 *(n = 10)*	10.26 ± 0.64 *(n = 13)*	38.7 ± 13.1 *(n = 30)*	148.6 ± 26.3 *(n = 30)*	91% *(n = 13)*
4% Hydrated Lime	2.69 ± 0.56 *(n = 30)*	17.01 ± 0.66 *(n = 15)*	16.08 ± 0.82 *(n = 19)*	13.02 ± 1.4 *(n = 19)*	104.6 ± 26.9 *(n = 30)*	99.3 ± 22.7 *(n = 30)*	92% *(n = 21)*
Control	4.14 ± 0.43 *(n = 30)*	N/A[#]	N/A[#]	24.89 ± 0.87 *(n = 30)*	0 *(n = 30)*	489.8 ± 21.1 *(n = 30)*	96% *(n = 30)*

Values given as mean ± SE (SE = SD/√n).
[#] No mussels present on the collectors

Table 2-2 The mean ± SE (SD/√n) macroalgae weight (g), mussel condition index, mussel shell length (mm), length of large tunicates (≥5 mm), mussel abundance for treated collectors, separated by the week they were treated (n = 15). Percentage of small tunicates represents the proportion of small tunicates (<5 mm) in comparison to the total tunicate abundance.

Date	Macroalgae Weight	Condition Index	Tunicate Length (≥5mm)	Mussel Length	Mussel Abundance	Tunicate Abundance	% of Small Tunicates
July 22	2.61 ± 0.60	15.81 ± 1.09	16.98 ± 1.78	14.21 ± 0.69	49.5 ± 21.5	266.9 ± 60.0	28%
August 2	2.28 ± 0.45	16.79 ± 1.12	18.7 ± 2.68	15.65 ± 1.49	82.4 ± 42.6	263.9 ± 64.2	25%
August 12	3.7 ± 0.83	18.17 ± 0.15	14.92 ± 2.27	15.36 ± 3.25	15.3 ± 10.5	267.3 ± 50.7	54%
August 24	3.58 ± 0.73	18.87 ± 0.09	22.72 ± 2.03	11.68 ± 0.26	6.3 ± 3.5	222.3 ± 46.8	39%
July 22 and August 12	3.4 ± 0.74	15.51 ± 0.63	15.54 ± 2.74	15.87 ± 1.38	41.3 ± 20.1	274.3 ± 55.8	41%
August 2 and August 24	2.91 ± 0.62	18.11 ± 0.97	25.42 ± 2.72	17.02 ± 1.48	91.8 ± 35.9	180.6 ± 60.7	28%

Values given as mean ± SE (SE = SD/√n).

Table 2-3 Table showing the variables that were significantly influenced by treatment*week. Estimated levels (with 95% confidence intervals) is represented for the mussel shell length (mm) and mussel abundance, while the length of the large tunicates (≥5 mm) is shown by the mean with confidence intervals, and the percentage of small tunicates is the proportion of small tunicates (<5 mm) to the total tunicates present, with confidence intervals.

Treatment	Week	Mussel Length	% of Small Tunicates	Length of Large Tunicates	Mussel Abundance
5% Acetic Acid	*July 22*	15.36^{a} [10.63, 20.08]	$56\%^{a}$ [41%, 71%]	12.57^{ab} [9.58, 16.50]	96.0^{ab} [3.0, 267.7]
	August 2	12.65^{a} [6.77, 18.53]	$49\%^{a}$ [34%, 64%]	11.44^{ab} [8.04, 16.28]	46.4^{ab} [4.3, 133.1]
	August 12	12.11^{a}	$94\%^{b}$ [85%, 98%]	9.16^{a} [6.96, 12.07]	24.6^{a} [15.5, 70.2]
	August 24	N/A[#]	$100\%^{b}$ [62%, 100%]	14.04^{ab} [8.83, 22.32]	0^{a} [0, 0]
	July 22 and August 12	18.51^{ab}	$90\%^{b}$ [78%, 96%]	7.86^{a} [6.13, 10.07]	43.6^{ab} [27.5, 124.4]
	August 2 and August 24	12.31^{a} [0, 33.59]	$100\%^{ab}$ [4%, 100%]	N/A[#]	21.8^{a} [10.6, 63.2]
4% Hydrated Lime	*July 22*	13.35^{a} [11.12, 15.58]	$37\%^{a}$ [22%, 56%]	12.66^{ab} [10.01, 16.01]	52.6^{ab} [0.6, 143.8]
	August 2	18.66^{b} [16.99, 20.32]	$70\%^{a}$ [49%, 85%]	10.51^{ab} [8.03, 13.76]	200.8^{ab} [6.9, 561.4]
	August 12	18.61^{ab}	$90\%^{b}$ [77%, 96%]	8.66^{a} [6.75, 11.10]	21.4^{a} [13.5, 61.0]
	August 24	11.68^{a} [10.55, 12.82]	$58\%^{a}$ [37%, 77%]	20.00^{b} [15.19, 26.34]	19.0^{a} [0.3, 52.1]
	July 22 and August 12	14.99^{a} [8.56, 21.43]	$86\%^{b}$ [69%, 94%]	8.58^{a} [6.53, 11.27]	80.4^{ab} [2.0, 223.1]
	August 2 and August 24	19.51^{b} [16.03, 23.00]	$100\%^{ab}$	N/A[#]	253.4^{b} [84.8, 457.7]

Values given as mean ± SE (SE = SD/√n).
[a-b] Within a column, means without a common superscript were significantly different ($P < 0.05$).
[#] Too few samples to include in analysis.

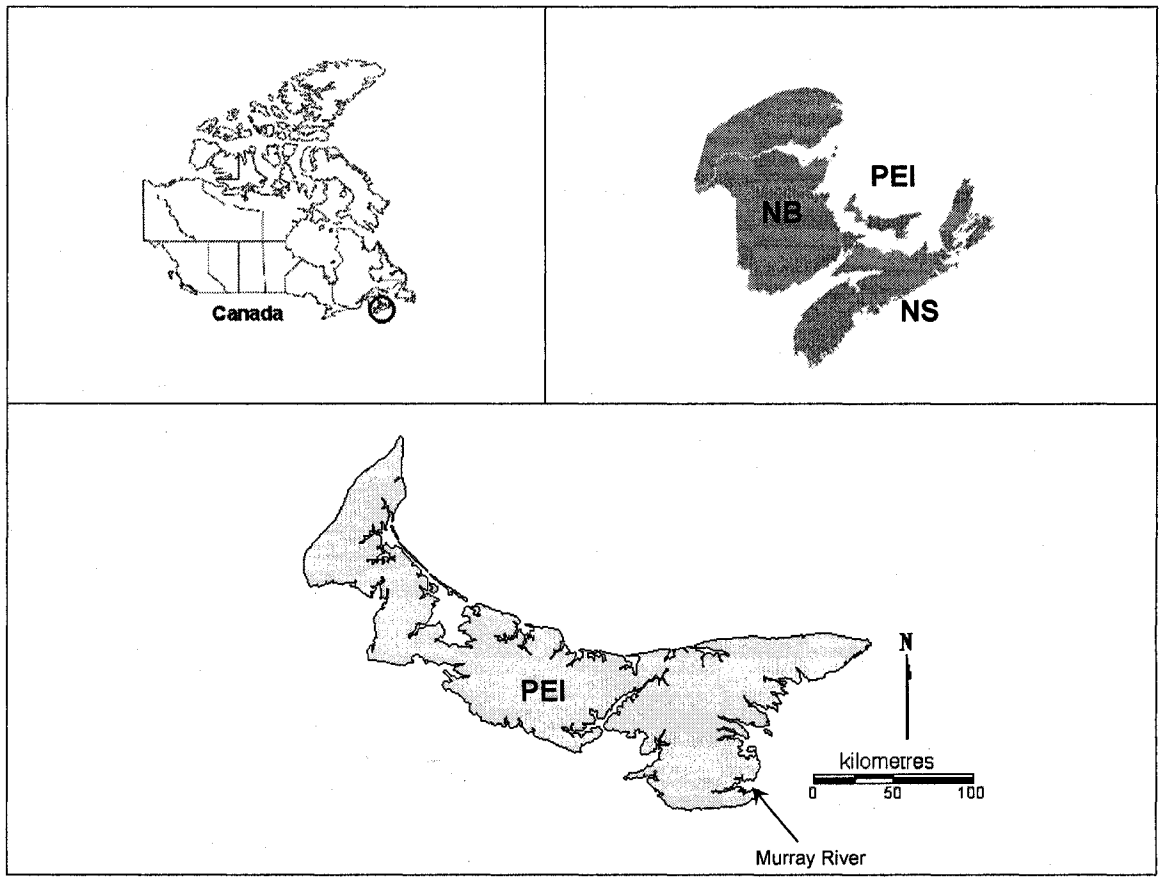

Figure 2-1 Map of Canada, the Atlantic provinces of Prince Edward Island (PEI), New Brunswick (NB), and Nova Scotia (NS), and PEI showing the field trial site, Murray River.

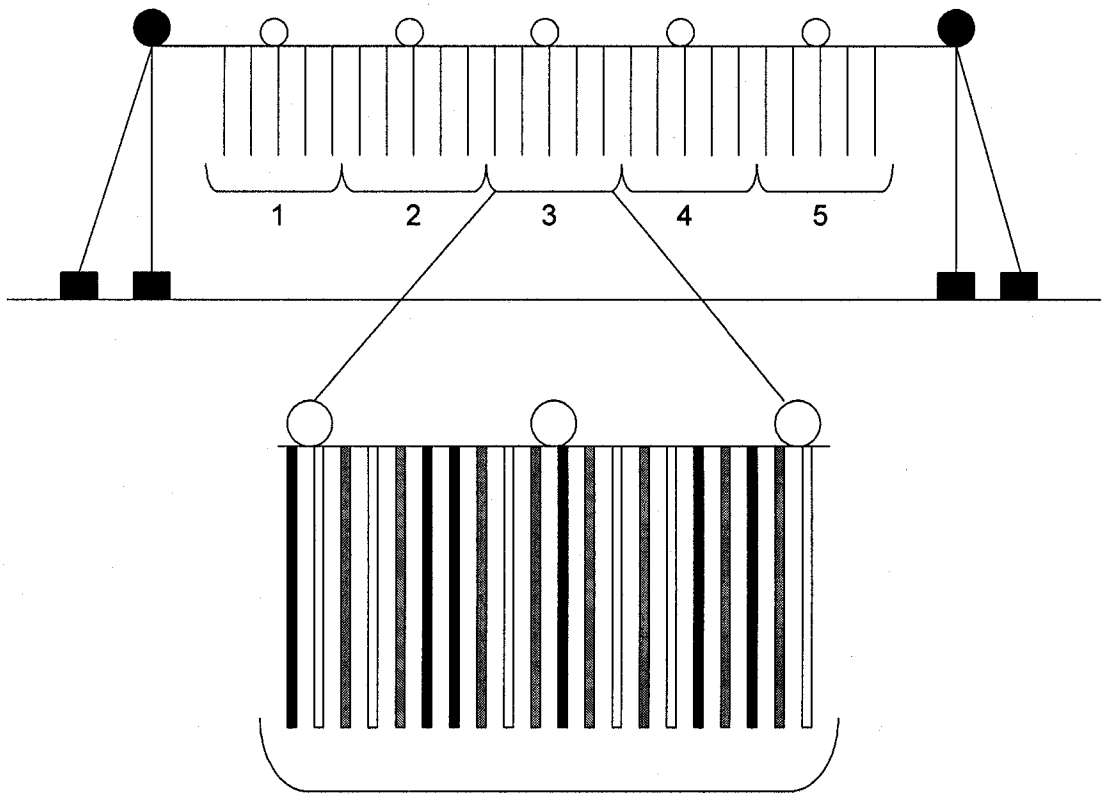

Figure 2-2 Schematic representation of a typical longline containing blocks of collectors; the actual longline used in the trial had five blocks. Each block contained two control collectors (represented as the striped collectors) and six replicates for each treatment of 4% hydrated lime, 5% acetic acid, and manipulated controls (represented as white, gray, and black collectors, respectively). For each of the three treatments, there were six treatment periods (four single treatments and two double treatments).

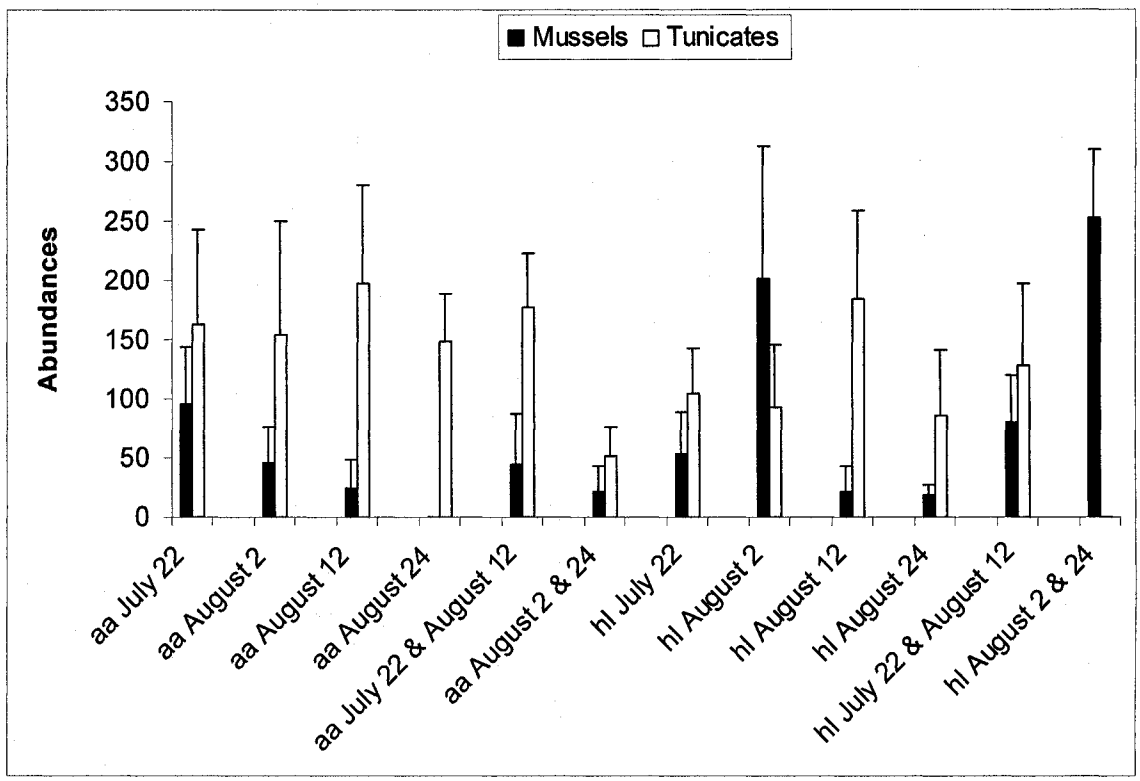

Figure 2-3 The mussel and tunicate abundances separated by the treatment (aa = 5% acetic acid, hl = 4% hydrated lime) and the week that the treatment was applied to the collectors (n = 5).

Chapter 3 THE EFFECT OF A CLUBBED TUNICATE (*STYELA CLAVA*) TREATMENT ON THE PHYSIOLOGICAL FITNESS OF JUVENILE CULTURED MUSSELS (*MYTILUS EDULIS*)

3.1. Abstract

The cultured mussel industry in PEI has been greatly affected by the recent invasion of several tunicate species. One in particular, the clubbed tunicate *Styela clava*, has a negative effect on the production of mussels because of its ability to foul the growing equipment. Mitigation strategies are being developed and implemented into the current husbandry practices in order to control the effects of this fouling species. The use of chemical treatments is prolific, with sprays of 5% acetic acid (table vinegar) being used frequently to eradicate the tunicates from mussel socks. Unfortunately, this chemical has been known to cause significant mortality in juvenile (< 1 yr old) mussels, and could affect the overall fitness of the population. In order to determine the effect of this treatment on mussel populations, we treated mussels (< 1 yr old) with 5% acetic acid to produce ~50% mortality. The surviving mussels were then subjected to electrophoresis trials to determine heterozygosity through allozyme analysis. Although there was no significant decrease in heterozygosity post-treatment, the allelic frequency of the allozymes measured was affected by the treatment. Comparisons of the allelic frequencies for the three groups found differences for *Pgm*, *Lap*, and *Mpi*. These genetic differences could attribute potential inability of a mussel population to withstand salinity and temperature fluctuations.

3.2. Keywords

Mytilus edulis, *Styela clava*, heterozygosity, allelic frequency

3.3. Introduction

Prince Edward Island (PEI) is the leading producer of the cultured blue mussel *Mytilus edulis* in Canada. The culture of this species contributes significantly to the province's aquaculture industry. Recently, the arrival of several invasive tunicate species has been causing problems with the cultured mussel industry. The main issue is the increased production costs that are involved in growing mussels in infected areas. In PEI, mussels are grown using the longline system, which provides habitat for tunicates as it consists of socks and collectors hanging vertically in the water column. At the seed collection stage, where collector ropes are hung in the water column, competition arises between the juvenile mussels and tunicates, specifically the solitary clubbed tunicate *Styela clava*. Mitigation strategies have been ongoing in an effort to eradicate the tunicates from aquaculture gear, mainly through the application of chemical treatments. Treatments of 5% acetic acid have proven to be successful in mitigating the clubbed tunicate from mussel socks in infested areas (Davidson et al. 2005).

While the main interest is the control of tunicate fouling, the impact of the chemicals on the mussel population is also of importance to the industry. The increased use of chemical treatments in mussel culture operations has lead to the question of how these treatments affect the mussel population. In subjecting mussels to treatments currently in use, we can see how the population responds to such stress. This is an important factor in determining a potential treatment regime

for controlling tunicates on mussel growing gear. There are several tools that can be used collectively to see the effects of treatments, including gross observations (growth, abundance), condition indices, and allozyme analysis.

Both *M. edulis* and *M. trossulus* populations have been found in the Atlantic region (Varvio et al. 1988, McDonald et al. 1991, Gardner and Thompson 2001, Penney et al. 2002). The distribution of *M. edulis* in Atlantic Canada overlaps with that of *M. trossulus*, and hybrids of these two species have occurred (Bates and Innes 1995, Mallet and Carver 1995, Saavedra et al. 1996, Comesaña et al. 1999). The waters surrounding PEI and the Magdalen Islands, including the Northumberland Strait, have been found to be primarily populations of *M. edulis* (Tremblay et al. 1998c).

For this study, mussel genetics was looked at through allozyme analysis by using gel electrophoresis. There have been cases of positive correlations between multiple locus heterozygosity (MLH) and physiological fitness indicators (growth and fecundity) in several species of molluscs (Koehn and Gaffney 1984, Rodhouse et al. 1986, Beaumont and Zouros 1991, Hawkins and Bayne 1991, Mitton 1993, Bayne and Hawkins 1997, Tremblay et al. 1998b). In addition, Tremblay et al. (1998a, 1998b) and Myrand et al. (2002) found that heterozygosity can indicate a mussel population's ability to withstand stressful events.

The objective of this experiment was to determine if treated first-year mussels had a higher MLH score than the control mussels, indicating that more heterozygous individuals could withstand the stress of being subjected to treatments. It was also necessary to identify the species represented in our study as either *M. edulis* or *M. trossulus*. The study of the MLH-fitness correlation is based on the

comparison of the mean heterozygosity of control mussels and the surviving mussels that had been subjected to a chemical treatment.

3.4. Materials and Methods

3.4.1. Chemical treatment

Cultured first year mussels were obtained from Georgetown, PEI and kept in a saltwater recirculation tank. Water temperature in the tanks was a constant 14.5°C during the trial. Groups of 300 mussels were randomly separated into 3 mesh bags. For the experiment, one bag was kept as the initial population to be used as the control group, and two of the bags (total = 200) were treated with 5% acetic acid (commonly known as white table vinegar). Treatments were applied to each bag by first shaking the bag, and then dipping it into the solution. After dipping, the mussels were rinsed for 15 seconds in water taken from the circulating tanks. The bags were then put back into the tanks to observe mortality each day for up to one week after the treatment. Mortality was determined by mussel appearance and response; gaping mussels that would not respond to tactile stimulation of the mantle were regarded as dead. After the 1-week period, live mussels from each treatment were frozen in a - 80°C freezer for electrophoretic study of enzymes as described in Tremblay et al. (1998b).

3.4.2. Gel electrophoresis

Surviving mussels from the treated and control group were thawed and the shell length was measured (mm). A small piece of the digestive gland was homogenized in an approximately equal volume of homogenization buffer (0.2 M Tris-HCl, pH 8.0, with 30% sucrose, 1% polyvinyl-polypyrrolidone, 0.1% NAD, 5

mM dithiolthreitol, 1 mM phenylmethylsulphonyl fluoride), centrifuged at 15,000 g for 30 minutes at 4 °C, and the supernatant was applied to a horizontal cellulose acetate gel (as per Hebert and Beaton 1989). Five polymorphic allozymes were analyzed: glucose phosphate isomerase (*Gpi*, EC 5.3.1.9), phosphoglucomutase (*Pgm*, EC 5.4.2.2.), leucine aminopeptidase (*Lap*, EC 3.4.11.1), mannosephosphate isomerase (*Mpi*, EC 5.3.1.8), and octopine dehydrogenase (*Odh*, EC 1.5.1.11). Alleles were named A, B, etc. in order of electrophoretic mobility, such that the slowest allele was called A. For each treatment, 48 mussels were individually analyzed.

3.4.3. Species determination

A section of the foot was removed from the 48 mussels in the initial population and species identification were done by PCR (*Polymerase Chain Reaction*) using the diagnostic loci marker *glu*-5. This method allows distinguishing between the two species, *M. edulis* or *M. trossulus*, and hybrid individuals (Rawson et al. 1996). For mussel DNA isolation, we used QIAGEN® DNeasy ™ Tissue Kit and 50 ng of DNA served as PCR template.

3.4.4. Statistical analysis

Allelic and genotypic frequencies for the polymorphic loci were obtained using GENTIX 4.05 (Belkhir et al. 1998). The fixation index (*F*is), which represents the M0endelian equilibrium and deviation from Hardy-Weinberg equilibrium, was calculated using GENEPOP 3.3 (Raymond and Rousset 1995). The *F*is value was not reported when a dominant allele had a frequency >0.8 because of the predominant influence of rare alleles. Significant differences between allelic frequencies were tested using the Fisher exact test implemented in

GENEPOP 3.3.

3.5. Results

3.5.1. Chemical treatment

Preliminary trials indicated that in order to achieve 50% mortality of the mussels, a 90 second dip in the 5% acetic acid was necessary. Final mortality was 38% for mussels in the first group and 28% for those in the second group. The mussel length (mean ± standard error) was 26.02 ± 0.28 mm (Table 3-1).

3.5.2. Species determination

The results from the mussels sampled in this study found that the population was 100% *Mytilus edulis*.

3.5.3. Allelic frequencies

The results for the allelic frequencies for the two treatments and the initial population are detailed in Table 3-1. All groups showed polymorphism at all loci except *Odh* and *Lap* for the initial group, and *Odh* for the treated mussels. No large deficit in heterozygosity was observed for each treatment and loci, except for *Gpi* and *Mpi* for the initial mussels and those in the treatment 1 group, and only for *Mpi* for mussels in the treatment 2 group. The three tested groups of mussels showed significant differences in their allelic frequencies for *Pgm*, *Lap*, and *Mpi* (Fisher exact test; DF = 10; $P < 0.001$).

3.6. Discussion

The population used in the study was pure *Mytilus edulis*, despite reports indicating the presence of *M. trossulus* in low amounts (7% in 1997 and <1% in

1999) (Landry et al. unpublished). In PEI, water temperatures can exceed 20°C, which would favour the more temperate *M. edulis* species. Prevalence of *M. trossulus* has also been more prolific in more northerly areas of the Atlantic region, such as Newfoundland (Varvio et al. 1988, McDonald et al. 1991, Gardner and Thompson 2001, Penney et al. 2002).

No important changes in heterozygosity were detected in the treated populations; however, the treatment seemed to induce a change in the allelic frequency of the allozymes measured. The impact of this potential change in the mussel population prior to receiving treatment is not known, however, the implication of specific loci adapting to environmental conditions is a possibility. Comparisons of the allelic frequencies for the three groups found differences for *Pgm*, *Lap*, and *Mpi*. These genetic drifts could have several impacts in mussels population. Allozyme variability of *Lap* locus is related to salinity tolerance. Salinity variability in estuaries causes differences in the allelic frequencies of this locus (Koehn and Hilbish 1987). Similar to salinity variation, heat fluctuation has an effect on the *Pgm* and *Lap* loci (Gardner and Skibinski 1990). In several species of beetle, butterfly, sea anemone and barnacle, *Pgi* and *Mpi* genotypes were associated to thermal tolerance and to availability of dietary sugars (Hoffmann 1983, Schmidt & Rand 2001, Neargarder et al. 2003, Watt et al. 2003). *Pgi* and *Mpi* are important parallel enzymes regulating glucose through glycolysis and thus play a major role in cellular energetics. Our results suggest that the change in allelic frequencies observed after acetic acid treatments could change the salinity and temperature tolerance of mussel populations in suspension culture.

It is suggested that heterozygous individuals have an advantage over

homozygous individuals in mussel populations. Heterozygous mussels under stress survive longer than homozygotes under the same stress (Myrand et al. 2002, Beaumont and Toro 1996). It has been documented that heterozygous mussels have a lower protein turnover that results in energy conservation (Bayne and Hawkins 1997). The survival of mussels in stressful conditions has been credited to the higher fitness of heterozygotes due to lower metabolic needs resulting in better stamina under stress (Myrand et al. 2002). With no apparent effect of treatment on the heterozygosity in this experiment, it is suggested that 5% acetic acid does not cause a stressful event that would select for the more heterozygous individuals in a population. Further studies should be conducted with larger populations from different regions to ensure the validity of this statement.

3.7. Conclusion

The use of acetic acid as a means of mitigating *S. clava* infestations should not be recommended for mussel seed collectors. The results from this trial indicate the effect the treatment has on the genetic makeup of the mussel population. Although there was no change in heterozygosity, the alteration of the allelic frequencies of the treated mussels shows that treatments of acetic acid could have negative effects on the success of the population at later stages of development. The changes in allelic frequencies exhibit the potential for future effects due to environmental fluctuations (i.e., salinity and temperature).

3.8. Acknowledgement

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Table 3-1 Allelic frequencies for the two 5% acetic acid treatment populations and the initial population. Significant probabilities ($P < 0.05$) for divergence of Hardy-Weinberg equilibrium are in bold.

	Gpi	*Pgm*	*Mpi*	*Odh*	*Lap*	All loci
Mytilus edulis (initial population)						
A	0.13	0.33	0.07	0.03	0.08	
B	0.58	0.37	0.78	0.88	0.83	
C	0.29	0.30	0.16	0.08	0.08	
D	/	/	/	0.01	/	
Ho	0.50	0.62	0.22	0.17	0.21	0.34
He	0.56	0.66	0.37	0.23	0.29	0.42
Fis	**0.12**	0.08	**0.40**	/	/	
n	48	47	45	48	48	48
Mytilus edulis (treatment group 1)						
A	0.11	0.17	0.06	0.02	0.18	
B	0.63	0.70	0.76	0.93	0.74	
C	0.26	0.14	0.18	0.05	0.08	
D	/	/	/	0.00	/	
Ho	0.35	0.42	0.23	0.15	0.44	0.32
He	0.53	0.47	0.39	0.14	0.41	0.39
Fis	**0.34**	-0.14	**0.42**	/	-0.04	
n	48	48	48	48	48	48
Mytilus edulis (treatment group 2)						
A	0.15	0.24	0.16	0.06	0.17	
B	0.56	0.42	0.57	0.80	0.41	
C	0.29	0.34	0.27	0.14	0.42	
D	/	/	/	0.00	/	
Ho	0.47	0.75	0.44	0.23	0.55	0.49
He	0.58	0.65	0.57	0.34	0.63	0.55
Fis	0.20	0.12	**0.25**	/	0.14	
n	47	48	48	47	44	48

Chapter 4 SUMMARY AND CONCLUSIONS

The development of the cultured mussel industry is a success story for aquaculture in PEI. However, the introduction of several aquatic invasive species and the increase in fouling organisms causes concern for the future of the industry. The research included in this thesis investigates the results of using chemical treatments to mitigate biofouling by both an introduced tunicate and macroalgae at the seed collection stage. The purpose of the project was to examine the effects of the treatments on the fouling species, as well as the < 1 yr mussel population. Methods used to determine the effects were growth measurements, abundances, and condition indices, while the use of heterozygosity was used to evaluate mussel seed performance following treatment.

The first experiment conducted was a field trial designed to develop a treatment regime for the mitigation of the clubbed tunicate, *Styela clava*, and macroalgae on mussel collectors in Murray River, PEI. Treatment solutions of 5% acetic acid and 4% hydrated lime were chosen for the trial, and a saltwater control was used to control manipulation of the collectors. Acetic acid has a history of causing *S. clava* and macroalgae mortality on mussel socks, however, when used in trials where mussel seed (< 1 yr) were present, seed loss was high (Davidson et al. 2005, Sharp et al. 2005). Hydrated lime is a common chemical used in the production of cultured mussels to eradicate starfish infestations, which can consume large amounts of seed and lead to substantial economic loss (MacKinnon et al. 1993). This chemical has shown to have insignificant effects on the mussel seed such that mortality does not occur. Following a treatment period of four weeks,

during which treatments were applied on a weekly basis, treatments were applied once to some collectors, while others received a double treatment using the same chemical. The results obtained from this trial are positive and should be acknowledged as such. Overall, hydrated lime was shown to be the most effective treatment for mitigating infestations of *S. clava* and macroalgae. Treatment of hydrated lime resulted in the successful recruitment and retention of a viable mussel population while decreasing *S. clava* populations. Acetic acid caused *S. clava* mortality, however seed retention and abundance was decreased. A double treatment of hydrated lime applied early in August and again late in August was found to be the best treatment. Mussel abundance was greater than any other treatment group, while having no tunicate infestation. Furthermore, there was a noticeable decrease in macroalgae abundance as compared to the control collectors.

The hydrated lime treatment regime that is recommended was also effective at dealing with starfish predation, which was an uncontrolled variable. Starfish predation occurred throughout the study. Collectors that did not receive a chemical treatment were exposed to starfish predation such that the mussel seed population was depleted, leaving an area for *S. clava* recruitment and settlement.

In view of this study, treatment must be applied to collectors to ensure successful mussel seed recruitment. This cannot be stressed enough, especially with the increase in fouling organisms present in PEI waters. The strategy recommended here is realistic and shows much potential within the mussel industry as this treatment is already commonly used for the eradication of starfish. Further benefits include the low cost, and the ease with which the treatment solution can be mixed on board mussel boats. A disadvantage of this chemical is that it is unpleasant to work

with, and if not handled correctly, can be caustic and irritating to the skin.

The laboratory trial designed to monitor the genetic effects of an experimental treatment (5% acetic acid), which has been shown to successfully cause *S. clava* mortality, indicated that mussels (<1 yr) may be negatively affected. Research by Myrand et al. (2002) indicated that more heterozygous individuals are better able to survive under stressful conditions. Changes in heterozygosity at the mussel seed stage after treatment could indicate if the population is being influenced by the chemical. The results showed no differences in heterozygosity, although there was a change in the allelic frequencies of the treated mussels. This change in allelic frequencies observed after acetic acid treatments could change the salinity and temperature tolerance of mussels populations in suspension culture. Should there be fluctuations in these environmental conditions, the population may be at risk. Therefore, the effect of treatment with acetic acid on mussel collectors should be further investigated, as it could lead to a decrease in the survival of the mussel seed population.

The results from these experiments show that husbandry practices for mitigating fouling organisms can affect seed collection, as well as population genetics. The results from the field trial show that there is a successful treatment regime that can be used to mitigate infestation by *S. clava* and macroalgae. The laboratory trial was useful in that it identified an otherwise undetectable effect that acetic acid has on the mussel seed population.

The mussel industry is dependent on future research and investigations into methods of controlling tunicates on growing gear. With the recent arrival of three more tunicate species, the vase tunicate *Ciona intestinalis*, the violet tunicate

Botrylloides violaceus, and the golden star tunicate *Botryllus schlosseri*, the cultured mussel industry will have to find a method of controlling multiple tunicate species in mussel growing areas. This will include documenting the life cycles of the tunicates in PEI, and continue investigating treatments that will mitigate invasive tunicates from mussel gear. It is apparent that the species are not going to be eradicated, and further spreading of the species around the Island seems inevitable.

Recently (July of 2006), the solitary tunicate *C. intestinalis* was found on mussel gear in Murray River. Now that a treatment has been established to mitigate *S. clava*, further trials need to be conducted to find a treatment that can mitigate *C. intestinalis* as well. Ideally there should be a treatment regime that can be used to control both tunicate species without harming the mussel population. What will perhaps be most important in the discovery of such a treatment will be targeting the newly recruited individuals. Interestingly, the life histories of these two tunicates is quite different; where *S. clava* spawns at 15°C, *C. intestinalis* spawns much earlier when water temperature reaches only 8°C. With both species having short larval periods, settling times differ substantially, with *C. intestinalis* settling much earlier than *S. clava*. This may mean that treatments will have to start earlier, and perhaps continue throughout the summer months. Through cooperative efforts by the industry, academia, and governmental groups, the continuation of a sustainable mussel culture industry will help PEI remain as the dominant producer of high-quality mussels in Canada.

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