

Research Article

## Pressurized seawater as an antifouling treatment against the colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri* in mussel aquaculture

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### Editor's note:

This paper is a contribution to the proceedings of the 3rd International Invasive Sea Squirt Conference held in Woods Hole, Massachusetts, USA, on 26–28 April 2010. The conference provided a venue for the exchange of information on the biogeography, ecology, genetics, impacts, risk assessment and management of invasive tunicates worldwide.

### Abstract

The development of effective mitigation techniques against *Botryllus schlosseri* and *Botrylloides violaceus* colonizing blue mussel aquaculture operations has not been well studied. The objectives of our research were to determine the efficacy of using pressurized seawater in the mitigation of colonial tunicate fouling and to identify optimal treatment timing and frequencies in reducing tunicate biomass. Treatment trials using high- (~700 psi) and low-pressure (~40 psi) seawater spraying were conducted in St. Peters Bay and Savage Harbour, PEI, from May to November 2009. The use of high-pressure seawater was an effective anti-fouling measure for these species, causing significant reductions in tunicate biomass. In contrast, low-pressure seawater had no discernable effect. The timing of treatment was found to be the most important factor affecting efficacy, with reductions in tunicate biomass increasing in magnitude the closer the treatment occurred to harvest. Treatment frequency did not affect tunicate biomass. In addition, fewer treatments also resulted in less nuisance mussel spat fouling the mussel socks. Colonial tunicate fouling did not affect adult mussel growth and productivity, and no evidence of smothering or crop loss was observed.

**Key words:** mussel aquaculture, aquatic invasive species, *Botryllus schlosseri*, *Botrylloides violaceus*, farm management, mitigation

### Introduction

The spread of non-indigenous tunicates has had ecological and economic impacts world-wide as these species become dominant in marine biofouling communities (Lambert and Lambert 1998; Carver et al. 2003; Coutts and Forrest 2005; Gittenberger 2009). Invasive tunicates typically proliferate on artificial substrates such as rock walls, wharf pilings, navigational buoys, floating docks, vessel hulls, and aquaculture infrastructure (Lambert and Lambert 2003; Forrest et al. 2007; Tyrrell and Byers 2007; Locke et al. 2009a). As a consequence, increased management costs are often associated with the

introduction and establishment of non-indigenous tunicates, resulting from changes in handling and defouling procedures, as well as the implementation of regulatory restrictions to reduce the risk of spread to unaffected areas (Forrest et al. 2007; Locke et al. 2009b).

Shellfish aquaculture industries worldwide have been strongly affected by the spread of non-indigenous tunicates. A combination of factors including increased maintenance and processing costs, production losses resulting from inter-specific competition for space and resources (Lesser et al. 1992; Carver et al. 2003), and crop loss associated with smothering and detachment threaten the viability of the industry in some

areas (Boothroyd et al. 2002; Ramsay et al. 2008a; Locke et al. 2009a). This has prompted research to develop mitigation strategies that reduce tunicate fouling. For anti-fouling measures to be practical, they must be cost-effective, environmentally benign, and minimize product loss and negative impacts on shellfish production, without compromising food safety (Braithwaite and McEvoy 2005; Locke et al. 2009a).

In Prince Edward Island (PEI), Canada, two solitary tunicates [*Styela clava* (Herdman, 1881) and *Ciona intestinalis* (Linnaeus, 1758)] and two colonial tunicates [*Botryllus schlosseri* (Pallas, 1766) and *Botrylloides violaceus* Oka, 1927] have been established since the late 1990s (Ramsay et al. 2008b; Locke et al. 2009a, b). These species have caused considerable challenges for local mussel (*Mytilus edulis* Linnaeus, 1758) and oyster [*Crassostrea virginica* (Gmelin, 1791)] culture industries, particularly with respect to the solitary tunicates *S. clava* and *C. intestinalis* (Carver et al. 2003; Howes et al. 2007; Locke et al. 2007; LeGresley et al. 2008; Locke et al. 2009a). Compared to the two solitary tunicates, little research has focused on developing effective mitigation techniques for *Botryllus schlosseri* and *Botrylloides violaceus*, and the impacts of these species on mussel productivity have not been well described (Carver et al. 2006). PEI mussel farmers currently use pressurized seawater to remove colonial tunicates, a method adapted from mitigation strategies used against *C. intestinalis* in the region. Alternative treatments that have been explored for *Botryllus schlosseri* and *Botrylloides violaceus* include the use of fresh water, brine, lime, and acetic acid immersion, with exposure to ~5% acetic acid for >15 s proving the most effective (Carver et al. 2006; Forrest et al. 2007). In New Zealand, several methods were used in an attempt to eradicate the colonial tunicate *Didemnum vexillum* Kott, 2002 from the region, including water blasting, air drying, chlorine dosing, and wrapping wharf pilings with plastic (Coutts and Forrest 2007). Although several of these methods were effective in mitigating colonial tunicate fouling, they are not necessarily practical or feasible in an aquaculture setting. As a consequence, high-pressure spraying is widely used in PEI, as it has proven to be cost-effective, capable of being applied with minimal training, and without any observable effect on mussel quality. However, the efficacy of this treatment has never been

formally evaluated for colonial tunicates, and mussel growers currently lack information on optimal treatment timing and frequencies required to improve farm management practices.

The objectives of this study were to a) determine the efficacy of pressurized seawater to mitigate colonial tunicate fouling, b) identify optimal treatment timing and frequencies for reducing tunicate biomass, and c) evaluate the impact of treatment on mussel productivity. We also document the impact of *Botryllus schlosseri* and *Botrylloides violaceus* on mussel growth and productivity, the growth of the tunicate species, and the effect of treatment on settlement of nuisance mussel spat.

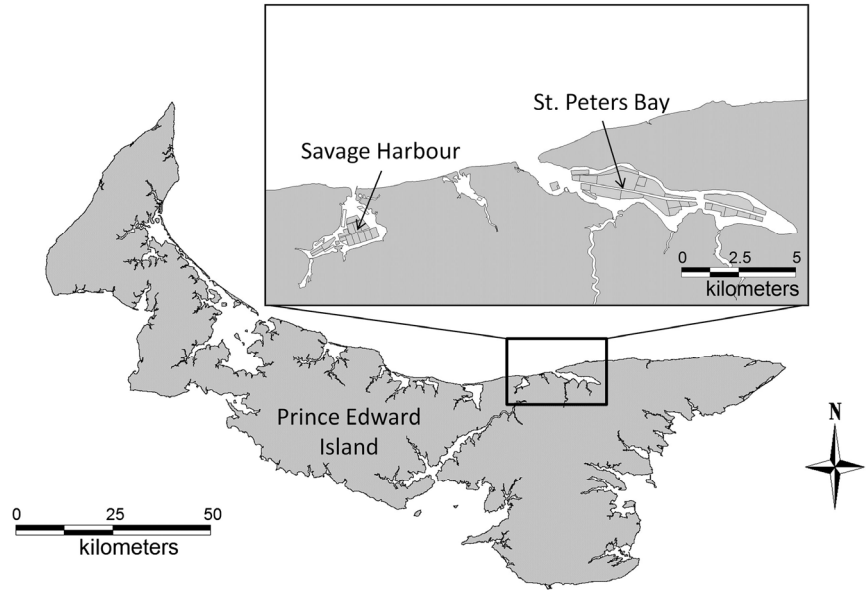
## Methods

### *Treatment application and sample collection*

Pressurized seawater trials were conducted in two semi-enclosed bays, St. Peters Bay (46°25.005'N, 62°41.247'W) and Savage Harbour (46°24.764'N, 62°50.458'W), located along the north shore of PEI (Figure 1). Sites were selected based on the presence of active mussel culture operations, existing populations of the colonial tunicates *Botryllus schlosseri* and *Botrylloides violaceus*, and the absence of *C. intestinalis* and *S. clava*.

In May 2009, 270 mussel socks were seeded and deployed on long-lines at existing leases in both St. Peters Bay and Savage Harbour. At the time of deployment, two to three mussel socks were sampled per bay to determine mussel density, biomass and length (Table 1). Two above-water, pressurized seawater treatments were tested for their efficacy in reducing tunicate biomass on mussel socks: high-pressure seawater (~700 psi applied with a commercial pressure washer using a single rotary nozzle) in St. Peters Bay and low-pressure seawater (~40 psi applied with a hose) in Savage Harbour. Treatment pressures were selected based on existing mitigation methods used by mussel growers in each bay. Both treatments were applied manually as opposed to using the sprayer-boxes used in *C. intestinalis*-infested bays. High- and low-pressure treatment efficacy was evaluated using 18 treatment groups based on combinations of treatment timing (date of treatment) and frequency (number of treatments) (Table 2). Treatment groups were distributed along longlines in St. Peters Bay and Savage Harbour using a randomized block design with each line divided

**Figure 1.** Map of Prince Edward Island, Canada, with an inset indicating the two study sites, St. Peters Bay and Savage Harbour. Shaded regions within each bay indicate areas occupied by mussel leases.



**Table 1.** Stocking characteristics for mussel socks deployed in St. Peters Bay and Savage Harbour in May 2009. For comparison with this study’s results, mussel density and weight are presented per 15 cm.

Stocking characteristics	St. Peters Bay	Savage Harbour
Deployment date	29 May	22 May
Mean mussel density per 15 cm (SD)	98.3 (1.1)	47.8 (1.5)
Mean mussel length (SD)	27.1 mm (5.4)	38.9 mm (4.8)
Mean mussel weight per 15 cm (SD)	92.7 g (1.6)	Not recorded
Sock length (m)	1.8	1.4
Socking material	Go Deep 6XL	Go Deep 6XL

**Table 2.** Treatment schedule for each treatment group in St. Peters Bay (high-pressure) and Savage Harbour (low-pressure). ‘x’ indicates the dates when pressure-washing was applied to mussel socks. Control socks were sampled on each treatment date (i.e. every 3 wk).

	Treatment date		Treatment group															Control		
	St. Peters Bay	Savage Harbour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		16	17
T1	17 Jul	15 Jul	x	x	x	x	x	x												
T2	6 Aug	5 Aug		x	x	x	x		x	x	x	x								
T3	2 Sep	31 Aug			x	x	x	x		x	x	x		x	x	x				
T4	22 Sep	18 Sep				x	x			x	x	x		x	x	x	x			
T5	15 Oct	15 Oct					x	x			x				x		x	x		

into three blocks and five socks from each treatment group replicated within each block.

Between 15 July and 15 October 2009, five treatment applications (T1 to T5) were carried out in three week intervals in both bays (Table 2). During each application, 15 designated mussel socks per treatment group (five socks per

block) were thoroughly sprayed with pressurized seawater (~10 s per sock) according to the treatment schedule. Nine 30 cm sock sections were sampled from the base of randomly selected untreated mussel socks (three per block) on each treatment date to evaluate natural changes in tunicate biomass and mussel

productivity throughout the trial. In November, nine mussel sock sections (30 cm long, three sections per block) were harvested from each treatment group in each bay, resulting in a total of 162 samples per bay.

Environmental variables including temperature ( $^{\circ}\text{C}$ ), salinity (PSU; practical salinity units), dissolved oxygen ( $\text{mg L}^{-1}$ ), chlorophyll ( $\mu\text{g L}^{-1}$ ) and turbidity (NTU; nephelometer turbidity units) were monitored hourly throughout the trial using two water quality monitors (Wet Labs, OR). The monitors were deployed adjacent to the treatment socks in both bays.

#### Laboratory analysis

The ends of each 30 cm sock section were discarded, leaving a 15 cm section for analysis. Mussels and tunicates were separated, and all other epifauna discarded. Colonial tunicates were grouped by species and weighed (wet weight,  $\pm 0.01$  g). Adult mussels ( $>40$  mm) were counted and weighed while mussel spat ( $<40$  mm; seed mussels) was weighed but not counted.

A random subsample of 20 mussels from each 15 cm sock section was analyzed for shell length and condition index. Shell length was determined by measuring the greatest distance from the umbo to the anterior margin ( $\pm 0.01$  mm). Mussels were steamed and their meats removed before pooling meats and shells, which were dried in an oven at  $70^{\circ}\text{C}$  for 48 h. The condition index was calculated as  $100 \times \text{Wdm} \div \text{Wds}$ , where  $\text{Wdm}$  = pooled dried meat weight and  $\text{Wds}$  = pooled dried shell weight. When large quantities of samples were collected, mussel sock sections were maintained out of water in cold storage ( $\sim 4^{\circ}\text{C}$ ) for up to four days prior to processing.

#### Data analysis

The effect of high- and low-pressure treatments on mussel productivity and tunicate biomass were assessed using two-factor ANOVA with treatment group and block as fixed factors and mussel and tunicate variables as dependents. When significant treatment effects were identified, *post hoc* analyses were conducted using Fischer's least significant difference test to determine whether treatments differed significantly from controls. Results were considered statistically significant at  $p < 0.05$ . Weight and count data were square-root transformed prior to analysis to ensure homoscedasticity. The

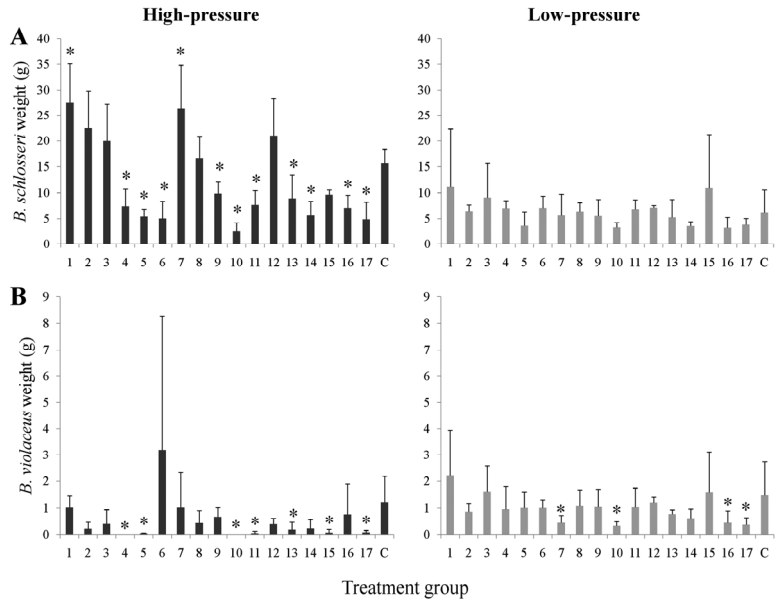
relationships between tunicate and spat biomass relative to treatment timing and frequency were explored using linear regression and the coefficient of determination ( $R^2$ ). Hourly environmental data were averaged per day, and correlations between sites and tunicate biomass were examined using the Pearson correlation coefficient ( $r$ ). All analyses were conducted using SYSTAT 11 for Windows (SYSTAT 2004).

## Results

#### *Treatment efficacy and optimal treatment time*

In St. Peters Bay, high-pressure washing significantly reduced *Botryllus schlosseri* biomass in most treatment groups compared to the control, with the greatest reduction (84%) occurring in treatment group 10 (treated every three weeks starting in early August) (Table 3; Figure 2A). In general, larger reductions in tunicate biomass were observed among groups which received treatment closer to the November harvest date (e.g. in October (T5), see Table 2), regardless of treatment frequency. For example, *Botryllus schlosseri* biomass was similar between treatment groups 5 and 17, both of which were treated in October, though group 5 was treated five times and 17 only once. Less substantial reductions in tunicate biomass were observed among samples which received their last treatment earlier in the trial (i.e., before October). Mussel socks which were only treated prior to and during the initial colonial settlement (T1 and T2) had the largest tunicate biomass at harvest. In fact, a significant increase in tunicate biomass relative to control socks was observed in treatment groups 1 and 7, which were treated once in mid-July and early August, respectively (Figure 2). A linear regression of *Botryllus schlosseri* wet weight versus the duration of time post-treatment supported these observations with greater tunicate biomass occurring among socks treated earlier in the season (Figure 3). This pattern was also observed for both species in Savage Harbour. In contrast, there was no relationship between *Botrylloides violaceus* biomass in St. Peters Bay and treatment date. While significant reductions of *Botrylloides violaceus* biomass were observed in seven of the 17 high-pressure treatment groups compared to the control (Table 3; Figure 2B), no clear factors such as timing of treatment characterized these groups.

**Figure 2.** Mean wet weight plus standard deviation of (A) *Botryllus schlosseri* and (B) *Botrylloides violaceus* collected from 15 cm mussel sock sections of each treatment group for high-pressure (St. Peters Bay, left panels) and low-pressure (Savage Harbour, right panels) treatments (n=9). C = control; \* indicates a treatment group significantly different from the control.



**Table 3.** Results of two-factor ANOVA testing for the effect of treatment on *Botryllus schlosseri* and *Botrylloides violaceus* weight, and on various mussel parameters. Significant results ( $p < 0.05$ ) are indicated in bold. For all tests:  $df = 2, 17$ .

Site	Variable	Mean-square	F-ratio	p
St. Peters Bay	<i>B. schlosseri</i> weight	11.542	11.376	<b>&lt; 0.001</b>
	<i>B. violaceus</i> weight	0.734	1.915	<b>0.024</b>
	Mussel weight	6.195	1.320	0.194
	Mussel count	0.764	1.577	0.083
	Mussel length	2.456	1.995	<b>0.017</b>
	Mussel condition index	1.510	1.942	<b>0.021</b>
	Mussel spat weight	31.762	6.887	<b>&lt; 0.001</b>
Savage Harbour	<i>B. schlosseri</i> weight	1.169	1.569	0.086
	<i>B. violaceus</i> weight	0.489	2.395	<b>0.004</b>
	Mussel weight	8.595	1.315	0.197
	Mussel count	0.516	0.968	0.499
	Mussel length	4.070	1.291	0.213
	Mussel condition index	0.961	1.645	0.066
	Mussel spat weight	7.618	0.947	0.522

The low-pressure treatments applied to mussel socks in Savage Harbour were ineffective at reducing colonial tunicate biomass. No differences in biomass were observed for *Botryllus schlosseri* (Table 3; Figure 2A), and only four treatment groups showed significant reductions for *Botrylloides violaceus* compared to control socks, though without any apparent pattern (Table 3; Figure 2B).

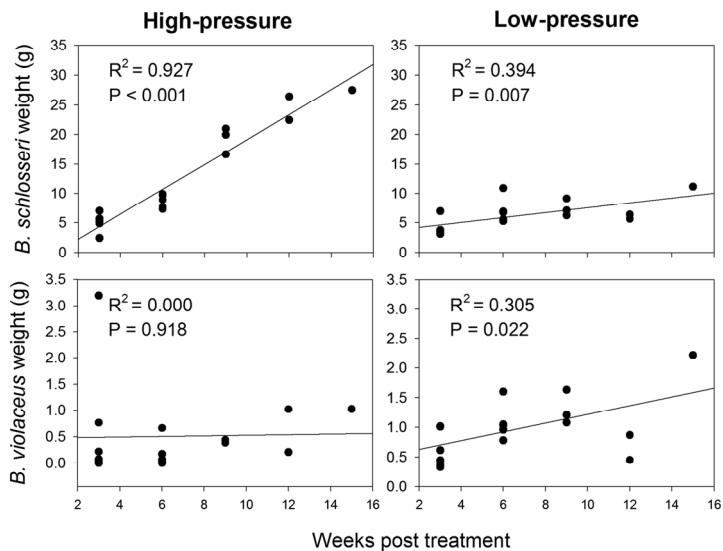
In general the mean biomass of *Botryllus schlosseri* was greater in St. Peters Bay ( $12.42 \text{ g} \pm 0.67 \text{ SD}$ ) than Savage Harbour ( $6.10 \text{ g} \pm 0.67 \text{ SD}$ ,  $F_1 = 43.908$ ,  $P < 0.001$ ). *Botrylloides*

*violaceus* was less prevalent at both sites, with a greater biomass in Savage Harbour ( $1.01 \text{ g} \pm 0.14 \text{ SD}$ ) compared to St. Peters Bay ( $0.55 \text{ g} \pm 0.14 \text{ SD}$ ,  $F_1 = 5.124$ ,  $P = 0.243$ ).

#### Mussel productivity

No effects of either treatment or tunicate fouling were detected on mussel productivity in either bay. Except for two treatment groups (6 and 11; see Table 2), there were no statistically significant differences between mussel weight, count, length, and condition index among the

**Figure 3.** Linear regressions plotting wet weight of *Botryllus schlosseri* and *Botrylloides violaceus* versus duration of time post high- and low-pressure treatment.



treatment groups relative to control samples, and these two differences were not biologically significant (Table 3; Figure 4).

Relative to control socks, mussel spat biomass in St. Peters Bay was significantly higher in almost all treatment groups that were pressure-washed more than once, regardless of when the socks received the initial treatment (see Table 2; Table 3; Figure 5A). Spat biomass was related to treatment frequency ( $R^2 = 0.61$ ,  $P < 0.001$ ), with greater biomass occurring on socks treated multiple times. This pattern was not observed among the low-pressure treatments in Savage Harbour ( $R^2 = 0.03$ ,  $P = 0.531$ ), where no significant differences in spat biomass were observed between treatment groups and control socks (Table 3; Figure 5B).

#### Tunicate and mussel growth

Based on data collected from untreated socks, temporal patterns in tunicate biomass varied between bays (Figure 6A, B). In Savage Harbour, colonies of *Botryllus schlosseri* and *Botrylloides violaceus* were first observed during the second sampling date (5 August). Mean tunicate biomass then peaked on the third sampling date (31 August; *Botryllus schlosseri*:  $14.63 \text{ g} \pm 6.43 \text{ SD}$ ; *Botrylloides violaceus*:  $6.80 \text{ g} \pm 3.33 \text{ SD}$ ) and steadily declined until harvest in November. In St. Peters Bay, colonies of *Botryllus schlosseri* were first observed on the

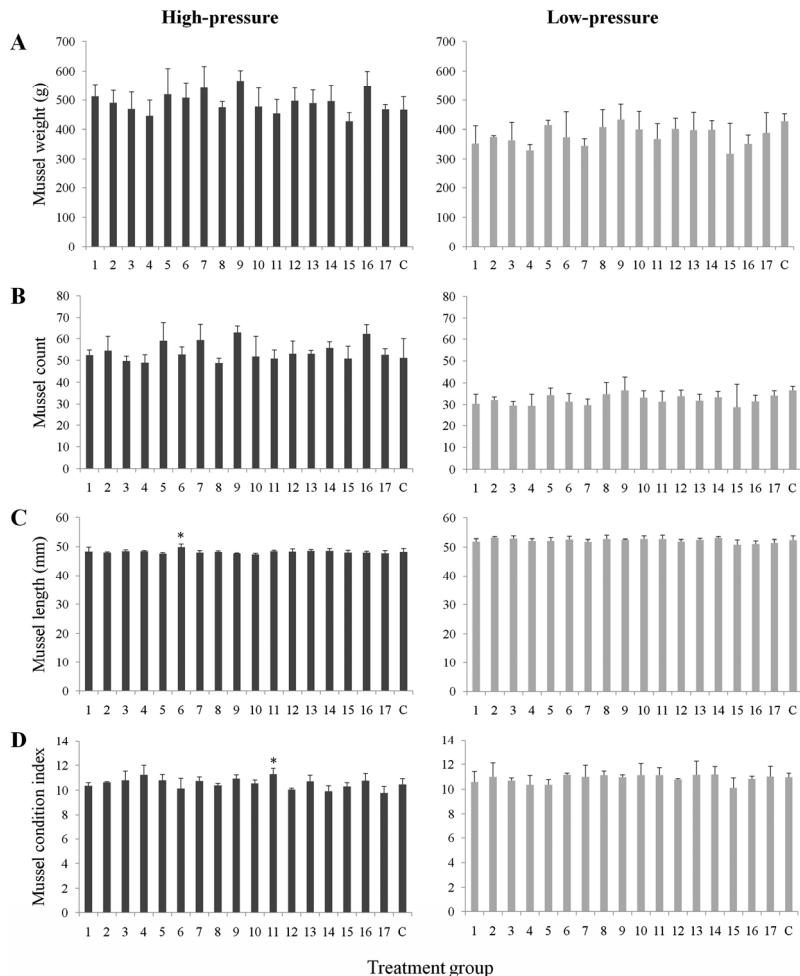
second sampling date on 6 August, while *Botrylloides violaceus* was not observed until the third sampling date on 2 September. Mean tunicate biomass in St. Peters Bay steadily increased throughout the summer and autumn months, peaking for both species on the fifth sampling date on 15 October (*Botryllus schlosseri*:  $29.25 \text{ g} \pm 15.20 \text{ SD}$ ; *Botrylloides violaceus*:  $1.44 \text{ g} \pm 2.12 \text{ SD}$ ) and then declined until harvest. The greatest biomass of combined colonial tunicates observed on a 15 cm long mussel sock section was 53.78 g, which was observed in St. Peters Bay on 15 October.

With respect to mussel productivity, a general increase was observed in mussel weight and length for St. Peters Bay and Savage Harbour (Figure 6C, D), while a decrease was observed in mussel density and condition index for both bays (Figure 6E, F).

#### Environmental parameters

Environmental parameters were similar between St. Peters Bay and Savage Harbour throughout the trial (Figure 7), but did not correlate significantly with tunicate biomass. Temperature varied from approximately  $15^\circ\text{C}$  in early July to a high of  $23^\circ\text{C}$  in late August, before decreasing to a low of  $6^\circ\text{C}$  in mid-November (Figure 7A). Salinity was more variable in St. Peters Bay than Savage Harbour ranging from 22.8 to 28.6 PSU and 26.0 to 28.5 PSU, respectively (Figure 7B).

**Figure 4.** Effect of treatment on mussel productivity. Means plus standard deviation of (A) mussel weight, (B) count, (C) length, and (D) condition index for 15 cm mussel sock sections from each treatment group for high-pressure (St. Peters Bay, left panels) and low-pressure (Savage Harbour, right panels) treatments (n = 9). C = control; \* indicates a treatment group significantly different from the control.

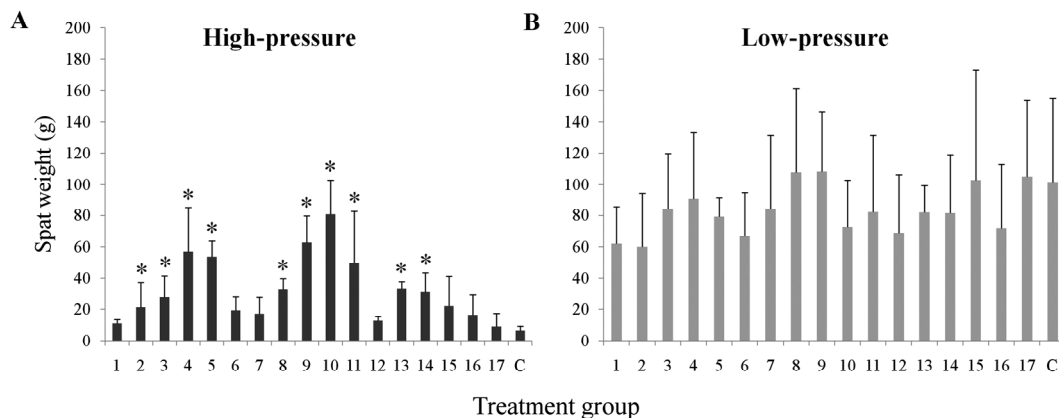


Dissolved oxygen concentrations were similar between bays but varied throughout the trial, initially occurring around 8 mg L<sup>-1</sup> and then declining sharply in late July to 2 mg L<sup>-1</sup> before gradually returning to original levels throughout the remainder of the trial (Figure 7C). Chlorophyll generally ranged between 1 and 4 µg L<sup>-1</sup>, although spikes exceeding 5 µg L<sup>-1</sup> occurred in St. Peters Bay in July and October, and in Savage Harbour in August (Figure 7D). Turbidity was low throughout June, July, and early August, ranging from 0 to 3 NTU in both bays (Figure 7E). Between August and October, turbidity increased but was also more variable, ranging from 0 to 4 NTU. In November, turbidity decreased in St. Peters Bay while it peaked at 10 NTU in Savage Harbour.

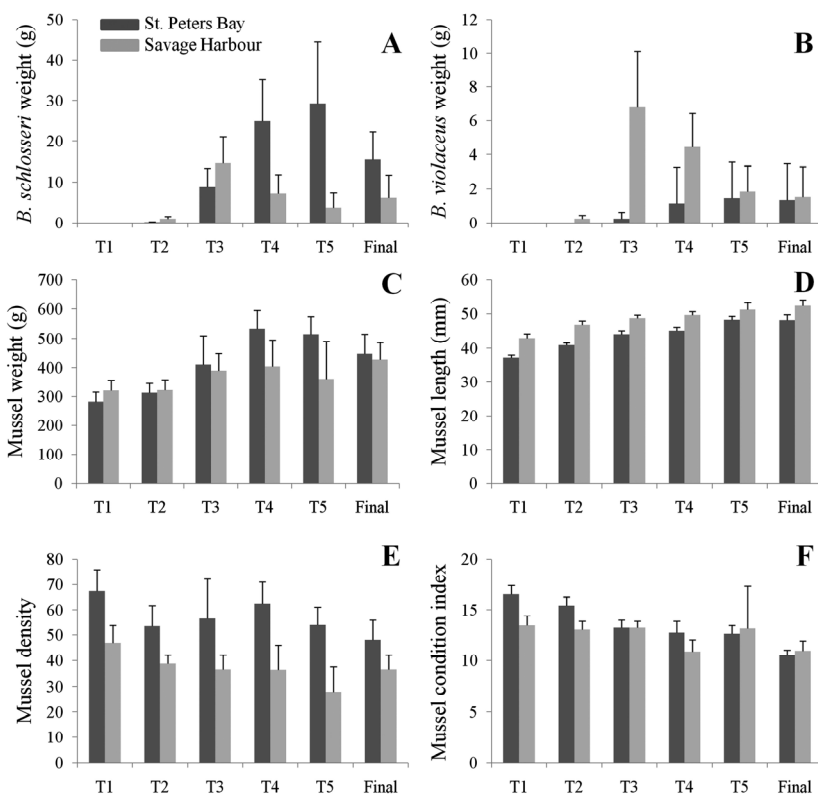
## Discussion

### *Effect of timing and frequency on treatment efficacy*

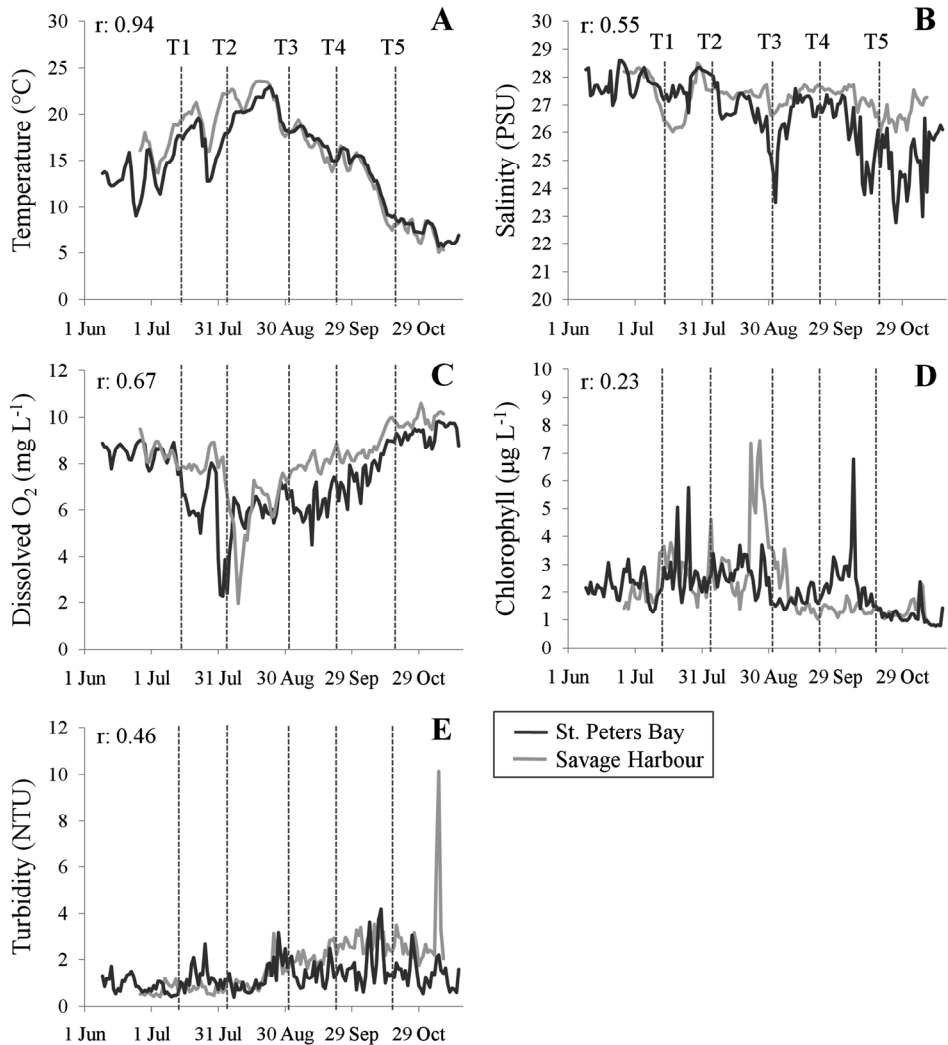
High-pressure spraying was effective at reducing the biomass of the colonial tunicates *Botryllus schlosseri* and *Botrylloides violaceus* on mussel socks in St. Peters Bay, while low-pressure spraying was relatively ineffective in Savage Harbour. Direct comparisons between high- and low- pressure treatment types were confounded however as treatments could not be replicated between bays for logistical reasons. As a consequence it cannot be ruled out that relative treatment efficacy may have been affected by differences in local conditions, such as mussel



**Figure 5.** Mean wet weight plus standard deviation for mussel spat collected from 15 cm mussel sock sections of each treatment group (see Table 2) for (A) high-pressure treatments in St. Peters Bay and (B) low-pressure treatments in Savage Harbour ( $n=9$ ). C = control; \* indicates a treatment group significantly different from the control.



**Figure 6.** Wet weight of (A) *Botryllus schlosseri* and (B) *Botrylloides violaceus*, as well as (C) weight, (D) length, (E) density and (F) condition index of mussels collected from untreated mussel socks from 15 July to 16 November 2009 in Savage Harbour and St. Peters Bay. Data represent mean values ( $n = 9$ ) obtained from 15 cm sock sections with error bars representing the standard deviation. T1-T5 indicates treatment application date (see Table 2), and 'Final' indicates data collected from control socks at harvest in November.



**Figure 7.** Environmental data collected from Savage Harbour and St. Peters Bay between June and November 2009 for (A) temperature, (B) salinity, (C) dissolved oxygen, (D) chlorophyll, and (E) turbidity. For each variable, similarity between bays is indicated by the Pearson correlation coefficient  $r$ . T1 to T5 represent treatment application dates (see Table 2). NTU = nephelometer turbidity units.

stocking characteristics, the magnitude of tunicate infestation, or environmental factors. In St. Peters Bay, treatment timing rather than frequency was the most important factor affecting efficacy, with reductions increasing in magnitude the closer the treatment application occurred to harvest. This was likely the result of tunicates re-colonizing treated mussel socks over time, either through budding of surviving zooids (Manni et al. 2007) or colonization through settlement of free-swimming larvae (Yamaguchi 1975; Carver et al. 2006). High-pressure

spraying also appeared to have a “priming” effect on mussel socks, in the sense of preparing the surface for settlement. Socks which were treated only prior to the initial colonial settlement (i.e., treated before early August) exhibited the greatest biomass of colonial tunicates. Most likely, the removal of competing epifauna provided a more suitable substrate for early growth and development of the tunicates. As a consequence, high-pressure spraying limited to the start of the growing season may have actually facilitated the settlement of

colonial tunicate larvae. Treatment effects were more pronounced for *Botryllus schlosseri* than *Botrylloides violaceus*, likely due to the relatively low and highly variable biomass of *Botrylloides violaceus* observed on mussel socks throughout the trial.

Tunicate biomass varied seasonally, increasing during the summer months and declining in the autumn, presumably in response to falling water temperatures. However, the timing of the biomass decline was inconsistent between bays, commencing in mid-September in Savage Harbour and in early November in St. Peters Bay. The reason for this inconsistency could not be explained using the environmental data collected. Factors known to influence tunicate growth and survival such as temperature, salinity, dissolved oxygen, turbidity, and chlorophyll (Carver et al. 2006), were relatively consistent between bays, but did not correlate significantly with tunicate biomass. This likely reflected the low statistical power of the correlation due to the small sample size available for analysis (n=6), rather than a lack of influence of environmental variables.

#### *Effect of treatment and tunicate fouling on mussel productivity*

Colonial tunicates were found to have little impact on mussel productivity throughout the study period. Although competitive interactions between *Botryllus schlosseri*, *Botrylloides violaceus*, and *M. edulis* have not been well described, our findings are consistent with those of Lesser et al. (1992) who found no significant source of food competition between *M. edulis* and *Botryllus schlosseri*. There is likely to be overlap between particle sizes preferred by *M. edulis* (2 to 16  $\mu\text{m}$ , but capable of extracting particles up to 100  $\mu\text{m}$ ) (Vahl 1972; Bayne et al. 1976; Lesser et al. 1992) and those deduced for *Botryllus schlosseri* and *Botrylloides violaceus* based on the capture efficiency of other ascidians (greatest capture efficiency expected at 2 to 3  $\mu\text{m}$ , but capable of extracting particles from 0.5  $\mu\text{m}$  up to the diameter of the esophagus) (Randløv and Riisgård 1979; Bone et al. 2003; Carver et al. 2006). However, *M. edulis* employs a substantially higher clearance rate relative to *Botryllus schlosseri*; in individual comparisons, single mussels filtered food (3 to 16  $\mu\text{m}$  particle range) 20 to 30 times faster than individual colonies 1  $\text{cm}^2$  in size (Lesser et al. 1992). Thus, mussels should be able to sustain high feeding

rates and maintain normal growth and development in the presence of the colonial tunicates *Botryllus schlosseri* and *Botrylloides violaceus*. One limiting factor for mussel feeding rates would be obstruction of the valves by tunicate colonies, which has been reported by Lesser et al. (1992) for *Botryllus schlosseri* and Bullard et al. (2007a) for *Didemnum* sp. We observed some mussels entirely covered by colonial tunicates, but valve openings were maintained, allowing continued siphoning by the mussel. It should be noted that these results are for adult mussels and do not necessarily apply to mussel seed on spat collectors, which were not assessed in our study.

High- and low-pressure treatments had no detectable impact on mussel productivity regardless of treatment timing or frequency. Differences in mussel length and density observed between bays resulted from differences in initial stocking size and density and not treatment type.

#### *Management implications*

Although pressurized seawater treatment was effective at removing colonial tunicate fouling from mussel socks, our results indicated that regular treatment may not be necessary, given that biomass of *Botryllus schlosseri* and *Botrylloides violaceus* did not influence mussel productivity. Interestingly, the frequency of high-pressure spraying was strongly and positively correlated with the weight of mussel spat on the socks. While spat collection on specially deployed collector ropes is desirable and used as seed source by PEI mussel growers, new spat settlement on established mussel socks is considered a nuisance (PEI DAFA 2003). Regular removal of colonial tunicates and other epifauna from the mussel socks may have decreased space and resource competition to the benefit of mussel spat, leading to improved spat growth rates and encouraging further settlement of mussel spat throughout the summer months.

In addition, a major concern with high-pressure washing is colony fragmentation. Colony fragments have the potential to reattach and form new colonies, potentially aiding in the dispersal of the species on an aquaculture lease or bay level by increasing the reservoir of propagules (Coultts and Sinner 2004; Bullard et al. 2007b; Forrest et al. 2007; Paetzold and Davidson 2010).

## Conclusion

The use of high-pressure seawater was an effective anti-fouling measure for *Botryllus schlosseri* and *Botrylloides violaceus*. However, results were effective only in the short term, with colonial tunicates re-establishing over time. In contrast, low-pressure seawater was not effective at reducing tunicate fouling. One or few treatments with high-pressure seawater, close to the harvest date, proved most effective at reducing both tunicate and nuisance spat fouling. Colonial tunicate fouling had no discernable impact on mussel productivity in Savage Harbour and St. Peters Bay. Based on this information, an optimal treatment strategy for colonial tunicate fouling would be to limit the application of high-pressure seawater until just before harvest to obtain cleaner mussels for processing. Reducing the frequency of high-pressure spraying would not only drastically reduce management costs for mussel growers, but also limit the amount of associated colony fragmentation, thus decreasing the potential for subsequent spikes in local levels of infestation and dispersal.

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