

## Research Article

## A field trial to determine the optimal treatment regime for *Ciona intestinalis* on mussel socks

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### Abstract

The invasive tunicate species *Ciona intestinalis* (Linnaeus, 1767) has had an economic impact on the aquaculture of the blue mussel *Mytilus edulis* (Linnaeus, 1758) in Prince Edward Island (PEI). This tunicate fouls mussel socks suspended on long lines in the water and reduces both weight and numbers of mussels, decreasing overall productivity. This study determined the relative effects of high pressure water treatment schedules over a four month period on two sites in the Murray and Brudenell Rivers on PEI. Results indicated that initiating treatment early (July) in the season and treating another two or three times during the season had the greatest effect on reducing tunicate numbers and size and enabling greater mussel productivity. While the most effective treatment may ultimately be site-specific, the two sites in this study support the notion that beginning treatment when tunicates are small is one of the most significant parts of the treatment plan. Two or more treatments (approximately monthly) are then needed to effectively control *C. intestinalis*, and allow mussels to reach their full growth potential.

**Key words:** aquaculture, aquatic invasive species, farm management, high-pressure water, mitigation strategies, *Mytilus edulis*

### Introduction

An intrinsic issue with off-bottom shellfish culture methods, such as long lines systems, is the potential for biofouling (Lutz Collins et al. 2009a; Adams et al. 2011). Indeed, controlling and mitigating biofouling can result in significant costs for commercial shellfish culture operations (Fitridge et al. 2012). Fouling organisms grow in unwanted locations, such as on aquaculture gear, as well as on the cultured animals themselves, and thereby impede the efficient growth of the organism and the maintenance of the entire culture system (Adams et al. 2011). The impact of biofouling on shellfish aquaculture can be severe and sometimes devastating for the industry (Watson et al. 2009). Ascidians or sea squirts are

among the most common biofoulers to shellfish aquaculture operations (Eno et al. 1997; Campbell 2002; Ross et al. 2002, 2004). Not surprisingly, the introduction, establishment and proliferation of invasive ascidians are becoming frequent phenomena in Atlantic Canada, in particular, those mediated by human actions (Carlton and Geller 1993; Ruiz et al. 1997; Cohen and Carlton 1998; Ruiz et al. 2000; Paetzold et al. 2012). The impact of invasive tunicates on the mussel aquaculture industry in the Atlantic region has proved indeed to be quite detrimental (e.g. Carver et al. 2003; Lutz-Collins et al. 2009b). The PEI mussel culture industry uses the longline system of production. Mussels are held in suspension in the water column in socks (meshed sleeves) hung from longlines, which are suspended along or below the water surface (PEI Aquaculture Alliance 2015).

Culturing of the blue mussel, *Mytilus edulis* (Linnaeus, 1758) on Prince Edward Island (PEI), is currently being challenged by invasive tunicates, its greatest threat since the establishment of the industry in the early 1980's. Four exotic tunicate species have recently been identified in PEI waters: *Ciona intestinalis* (Linnaeus, 1767) (Vase tunicate), *Styela clava* (Herdman, 1881) (Clubbed tunicate), *Botryllus schlosseri* (Pallas, 1766) (Golden Star tunicate) and *Botrylloides violaceus* (Oka, 1927) (Violet tunicate) (Locke et al. 2007). These invasive tunicates have all established populations in numerous bays and estuaries and are now impacting the mussel aquaculture industry (Locke et al. 2007; Ramsay et al. 2008a). They rapidly colonize the artificial substrates created by the suspension of mussel long-lines within the water column (Lambert and Lambert 2003; Forrest et al. 2007; Paetzold et al. 2012) and have become the main fouling organisms on mussel socks and gear. In addition to causing reductions in mussel productivity by competing for food and space with cultured mussels (Daigle et al. 2009), these tunicates have also added substantial additional weight to mussel aquaculture gear which results in additional labour and crop loss (Ramsay et al. 2008b). This rapid and heavy fouling of mussel socks has become a major problem for mussel producers in PEI, and has led to increased production and processing costs, limiting the profitability of the industry (Locke et al. 2009). The solitary *C. intestinalis* has become particularly difficult to control and eradicate from aquaculture gear and is now considered the single main threat to the industry on PEI (Carver et al. 2003, 2006; Daigle and Herbing 2009).

Considerable time and many resources have been invested on studying and mitigating the effects of *C. intestinalis* and other tunicate species over the last several years. These studies have focused on tunicate ecology (Carver et al. 2003; Howes et al. 2007; Bourque et al. 2007; Arsenault et al. 2009; Ramsay et al. 2009), new husbandry practices to decrease tunicate fouling (Thompson and MacNair 2004; Ramsay et al. 2008b; Daigle et al. 2009), new treatments to limit the fouling success (Carver et al. 2003; Bakker et al. 2011; Paetzold and Davidson 2011; Parent et al. 2011; Paetzold et al. 2012), feasibility studies examining eradication strategies (Edwards and Leung 2009), and dynamic models developed to better understand the growth and treatment implications of *C. intestinalis* and *S. clava* populations (Patanasatienkul et al. 2014). As a result, a variety of treatments to kill or remove tunicates from mussel socks

and gear have been tested in PEI, including immersion and spraying with fresh water, application of 4% sodium hydroxide, brine solution and 5% acetic acid (Carver et al. 2003; MacNair et al. 2006; Forrest et al. 2007). High pressure water is currently the most common treatment against *C. intestinalis* on PEI (Paetzold and Davidson 2010, 2011) and is the treatment of choice in this study.

Only a few studies have focused on the impacts of invasive tunicates and their mitigation measures on mussel productivity (see LeBlanc et al. 2007; Locke et al. 2009; Arens et al. 2011). Arens et al. (2011), for example, showed that the use of high pressure water was effective at decreasing the weight of both *B. schlosseri* and *B. violaceus* on mussel socks, but the reductions observed were only short-term, as both species quickly recolonized the socks. From anecdotal evidence, mussel growers perceived a decrease in mussel productivity as a result of heavy fouling by *B. schlosseri* and *B. violaceus* on mussel socks, though no such decrease was observed in Arens et al.'s (2011) study.

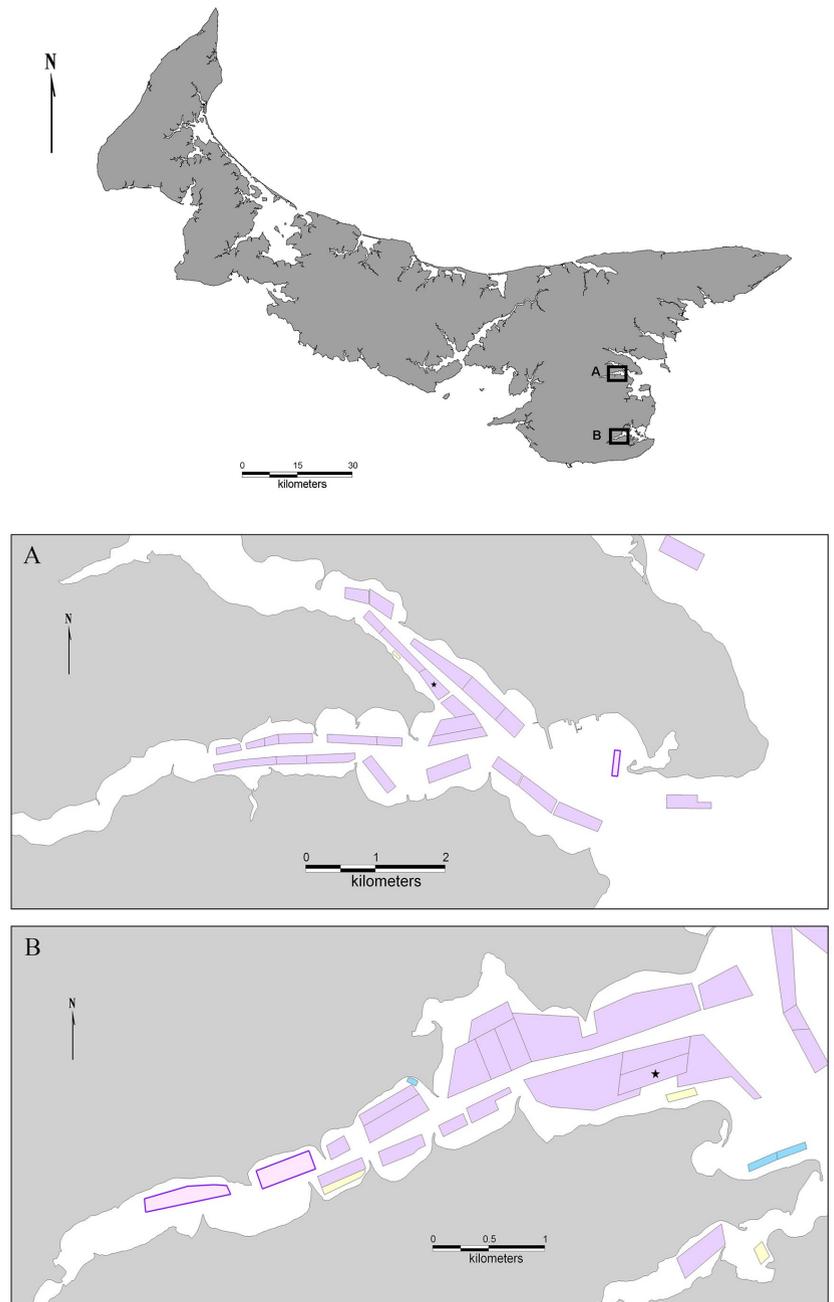
The objective of this study was to assess the effect of frequency and timing of high pressure water spray treatments on the mitigation of *C. intestinalis* on mussel socks and on the subsequent mussel productivity of two mussel aquaculture operations.

## Materials and methods

### *Site selection and sock characteristics*

Two river systems in eastern PEI, the Brudenell River (BR) and the Murray River (MR), were selected for the trials conducted in this study, based on their large number of mussel leases, high levels of *C. intestinalis* infestation and high winter mortalities (approximately 99%) of *C. intestinalis* during the three previous years (John Davidson and J Hill, personal observations). Aquaculture leases within each system were selected based on the reputation and willingness of the mussel growers to participate in this study. Representative experimental sites within each lease were selected following the lease holder advice (Figure 1). The depth of both systems was fairly similar: 8 m and 6 m in BR and MR respectively.

For each lease, 105 mussel socks of 2.5 m in length and an average stocking density of approximately 500 mussels per meter were obtained from each mussel grower and deployed on June 2<sup>nd</sup> and June 3<sup>rd</sup>, 2010 at the experimental sites in BR and MR, respectively.



**Figure 1.** Map of Prince Edward Island, indicating the location of Brudenell River (Insert A) and Murray River (Insert B), shaded areas within each system represent mussel leases; the \* identifies the approximate location of experimental sites.

Prior to deployment, three mussel socks were sampled from each lease to determine initial mussel and socking characteristics (Table 1).

#### *Experimental design*

Five distinct treatments were established based on current practices regarding time (date) and

frequency of pressure water treatment (Table 2). Some socks were treated in October only (hereafter labeled 1O), others in July and September (2JS), August and September (2AS), in July, August, and September (3JAS), and in July, August, September, and October (4JASO) (Table 2). Two additional treatments consisted of controls, either lifted (socks were mechanically lifted from the

**Table 1.** Deployment and socking characteristics in Brudenell River and Murray River. Mean values are followed by standard deviations.

Stocking characteristics	Brudenell River	Murray River
Deployment date	June 2, 2010	June 3, 2010
Mean mussel density per 0.3 m	161 ± 30.5	172 ± 34.0
Mean mussel length (mm)	40.3 ± 2.3	39.0 ± 2.0
Mean mussel weight per 0.3 m (g)	947.9 ± 195.9	964.8 ± 166.6

**Table 2.** A summary of the treatment schedule applied in Brudenell River (BR) and Murray River (MR).

Location and treatment date		Treatment codes and timing of application						
BR	MR	1O	2JS	2AS	3JAS	4JASO	Control Lifted	Control Unlifted
Jul-22	Jul-23		■		■	■		
Aug-24	Aug-26			■	■	■		
Sep-23	Sep-24		■	■	■	■		
Oct-25	Oct-26	■				■		

water the same way treated socks were, but no water pressure treatment was applied), or unlifted (socks remained untouched underwater). Treated socks were exposed to high pressure water which varied, on each farm, from 200 psi to 600 psi of pressure depending on the perceived attachment strength of the mussels, as determined by the operator. The spray apparatus had a nozzle size of 1.4 mm with a spray spread of 24°. The high pressure water caused both removal of the tunicate from the mussel gear (~90%) and mortal injury to *C. intestinalis* (~10%) (John Davidson, personal observations). These treatments reflect the variability in current practice in aquaculture operations.

The five treatment and two control groups were distributed along a long line at each site using a randomized block design, with each long line divided into three blocks and five socks from each treatment and control group replicated within each block. Distance between socks was approximately 40 cm. Socks were inspected monthly to ensure they remained buoyant and separated from the bottom.

### Sampling of socks

After all pressure water treatments were applied according to the schedule summarized in Table 2, sampling of socks took place on November 24<sup>th</sup> and 29<sup>th</sup>, 2010 and subsequently on May 4<sup>th</sup> and 5<sup>th</sup>, 2011 in BR and MR, respectively. During each sampling event, the middle three socks of the five replicate socks from each treatment and control group within each of the 3 blocks were

collected. This resulted in a total of nine socks from each treatment and control being sampled. At each sock being sampled, the bottom 30 cm was discarded to minimize potential interactions between socks and the substrate. The next 30 cm section from the bottom was then collected and carefully transferred to the laboratory. Due to feasibility, samples from November and May had to be collected from the same individual socks and, therefore, were from slightly different positions (depth) within the sock. Samples could not be collected from one of the treatments (1O) due to the complete loss of mussels and tunicates by November. Therefore, this treatment was no longer considered in the comparisons conducted (see below).

Initial mean *C. intestinalis* lengths were determined on separate study socks from MR and BR on July 22<sup>nd</sup> and 23<sup>rd</sup>, 2010 respectively, before any treatment was applied in the experimental sites. The mean length in MR and BR was 33 mm (SD=3) and 21 mm (SD=3) respectively. A continuous temperature probe (Vemco) was deployed on the study sites in BR and MR from June 25<sup>th</sup> to September 5<sup>th</sup>, 2010.

### Laboratory analysis

Within two hours of collection, samples were transported in a cooler to the laboratory and processed to separate and label the contents of the 30 cm sock sections into (1) mussels (living) and (2) *C. intestinalis*. Dead mussels, silt, other organisms on the sock, and the socking material were all discarded.

For each sample, the total weight and abundance of mussels was determined immediately after sampling. Thirty mussels were then randomly selected to determine shell length and frozen for later analysis of the condition index, the ratio of meat weight to shell weight (Abbe and Albright, 2003). Similarly for each sample, the total wet weight and abundance of *C. intestinalis* was determined and 50 specimens were randomly selected by blind division and measured to determine length. Enough time was allowed to ensure that the tunicates were relaxed before the measurements were conducted.

### Statistical analysis

Separate comparisons were carried out for each of the locations and times (November 2010 and May 2011) to determine the effects of treatment regimes on mussel productivity and infestation of *C. intestinalis*. Mussel productivity was estimated as mussel fresh weight, count, condition index, and length. Level of infestation was measured by *C. intestinalis* weight, length, and abundance. One-way ANOVAs were used to assess differences among treatment regimes at a significance level of 0.05. Analyses could not include location due to numerous interaction effects for the major variables. ANOVA assumptions of normality and equal variance were assessed in each analysis.

## Results

### *Mussel productivity: Assessed in November 2010*

In MR, socks treated three (3JAS) or four times (4JAS0) had a significantly higher mussel weight per 0.3 m sock (1,793 g  $\pm$  342 SD and 1,613 g  $\pm$  588 SD respectively), compared to socks treated two times beginning in August (2AS) (750 g  $\pm$  397 SD) and the lifted control (373 g  $\pm$  402 SD) (Figure 2A). Treating twice beginning in July (2JS) yielded significantly more weight per sock than the lifted control ( $p < 0.001$ ). Likewise, in BR, socks treated three or four times had greater mussel weight (1261 g  $\pm$  246 SD and 1,436 g  $\pm$  206 SD respectively) than socks treated twice beginning in August and the lifted control (636 g  $\pm$  150 SD and 797 g  $\pm$  279 SD respectively). Socks treated twice beginning in July had significantly greater mussel weight (1,153 g  $\pm$  361 SD) than socks treated twice beginning in August ( $p < 0.001$ ) (Figure 3A).

In MR, socks treated three (3JAS) or four (4JAS) times had significantly more mussels per

0.3 m sock (135 $\pm$ 27 SD and 127 $\pm$ 44 SD respectively) than socks treated two times beginning in August (2AS) and the lifted control (58  $\pm$  30 SD and 43 $\pm$ 42 SD, respectively) ( $p < 0.001$ ). Likewise, in BR, socks treated three or four times had significantly more mussels (109  $\pm$  30 SD and 117  $\pm$  20 SD respectively) than those treated twice beginning in August and the lifted control (62  $\pm$  15 SD and 75  $\pm$  25 SD respectively). Treating twice beginning in July (2JS) had significantly more mussels (98  $\pm$  25 SD) than treating twice beginning in August (62  $\pm$  15 SD) ( $p < 0.001$ ) (Figure 3B).

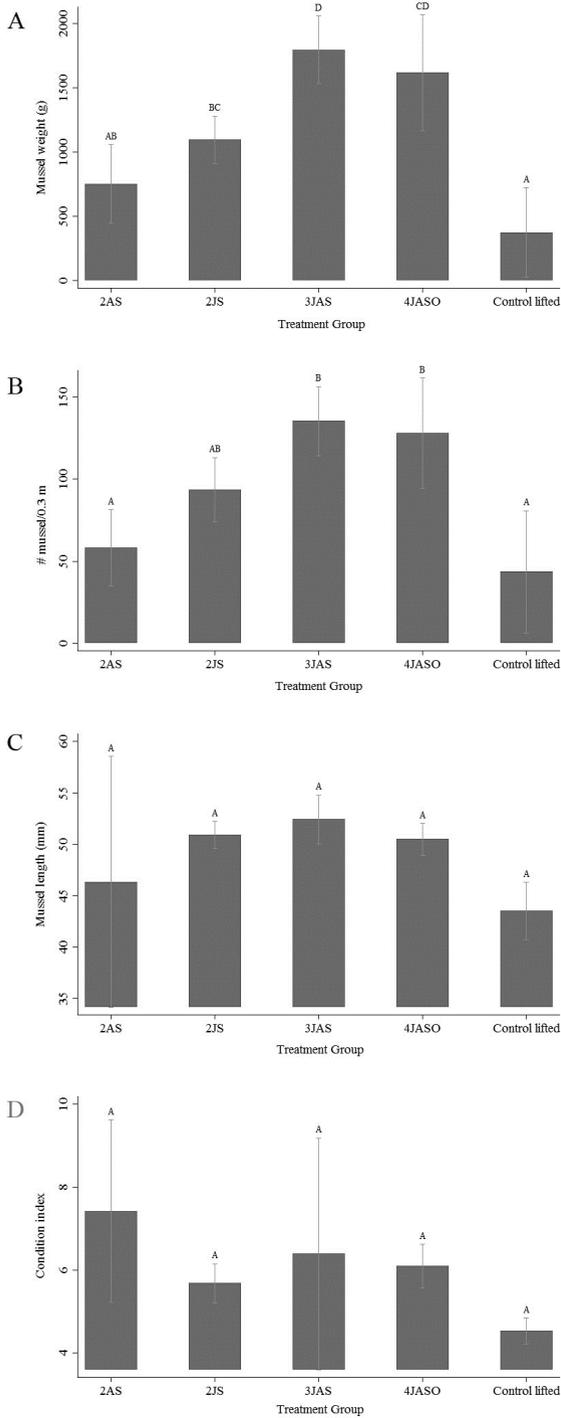
There were no statistically significant differences in mussel length between the treatment groups or the lifted control in both BR and MR ( $p = 0.06$  and  $0.09$  respectively). However, a consistent trend was observed in both locations. Treating three (3JAS), four (4JAS0), or two times beginning in July (2JS) yielded slightly larger mussels than did socks in the lifted control and those treated twice beginning in August (2AS group) (Figure 2C, 3C).

The condition index was generally higher in BR (mean 7.47, range 6.9–8.5) than in MR (6.03, 4.5–7.4). With regards to treatments, there was a significant difference in condition index in BR ( $p = 0.027$ ) where treating three times (3JAS) had a significantly higher CI than the lifted controls (Figure 3D). No significant differences were detected in MR ( $p = 0.106$ ) (Figure 2D).

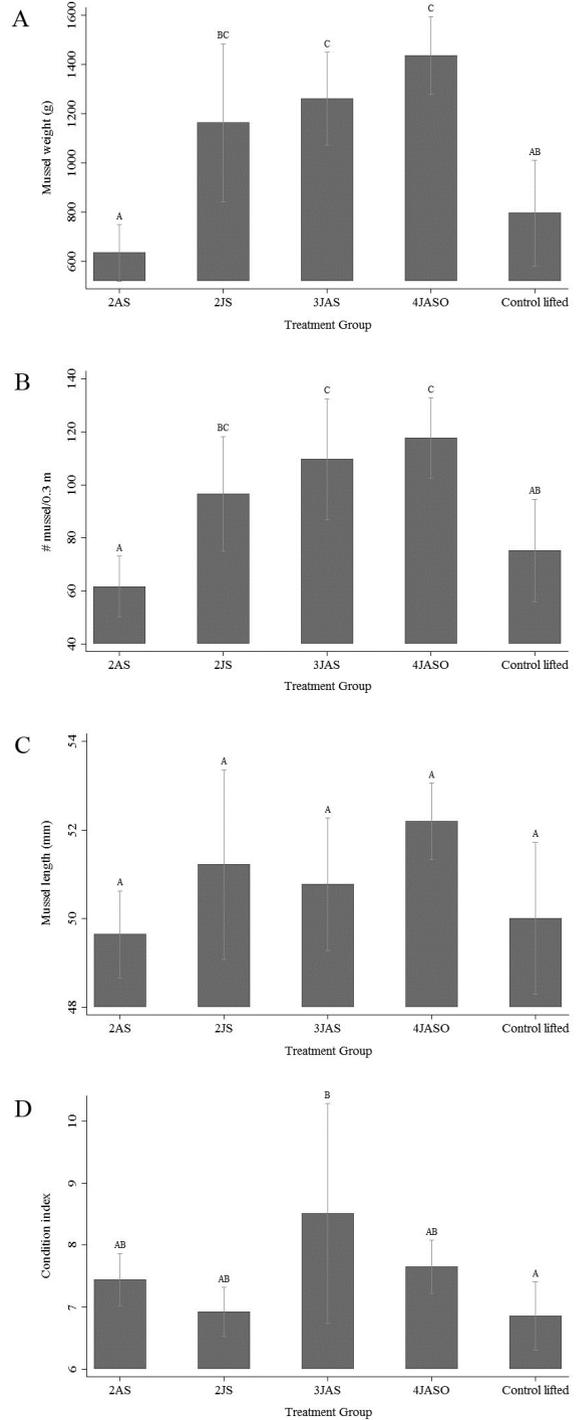
### *Mussel productivity: Assessed in May 2011*

In MR, socks treated three times (3JAS) and four times (4JAS0) had significantly higher mussel weight per 0.3 m sock (means of 2,443 g  $\pm$  332 SD and 1,955 g  $\pm$  345 SD respectively) compared to socks treated two times beginning in July (2JS) and August (2AS) (1,090 g  $\pm$  434 SD and 1,115 g  $\pm$  421 SD respectively). The latter also had significantly higher weight than the lifted or unlifted controls (283 g  $\pm$  137 SD and 161 g  $\pm$  52 SD respectively) ( $p < 0.001$ ) (Figure 4A). In BR, socks treated three and four times had significantly greater weight (1,391 g  $\pm$  210 SD and 1,696 g  $\pm$  261 SD respectively) than socks treated twice beginning in August (2AS) and the lifted controls (860 g  $\pm$  428 SD and 750 g  $\pm$  234 SD respectively). Socks treated two times beginning in July (2JS) had significantly greater weight (1,288 g  $\pm$  526 SD) than the lifted control ( $p < 0.001$ ) (Figure 5A).

Similar to the mussel biomass, in MR, socks treated three or four times had significantly higher mussel numbers per 0.3 m sock (means of

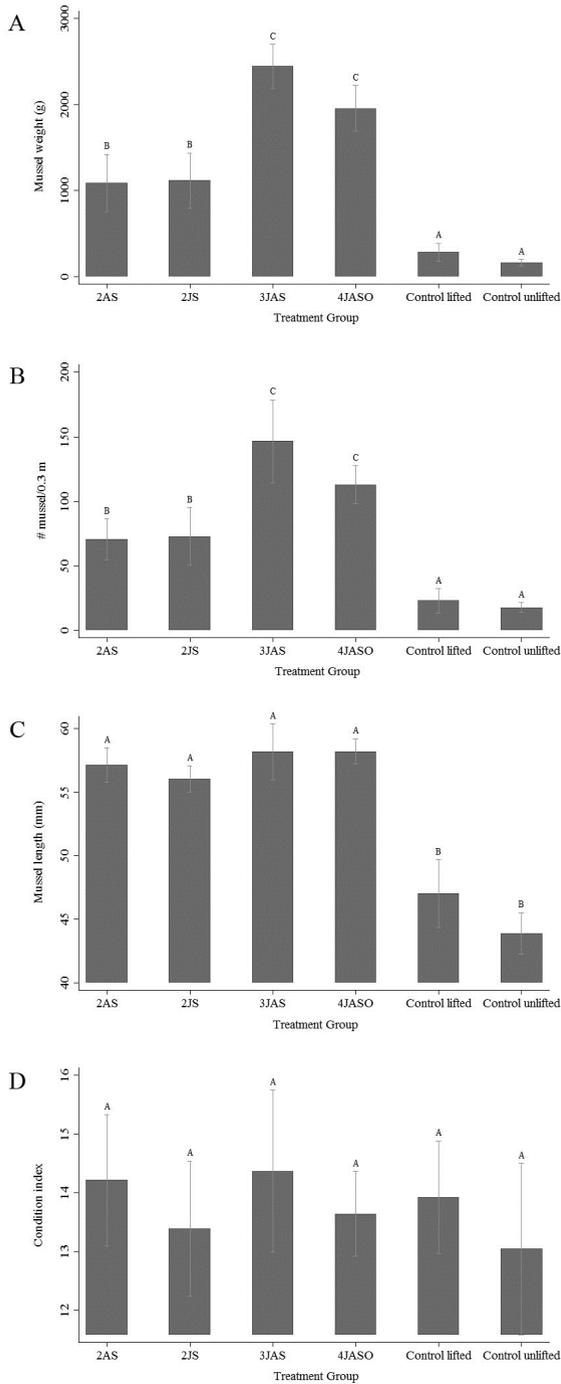


**Figure 2.** Mussel productivity in Murray River assessed during November 2010. (A) mean mussel weight, (B) # mussels / 0.3 M, (C) mean mussel length & (D) mean mussel condition index. All the values correspond to means (95% CI).

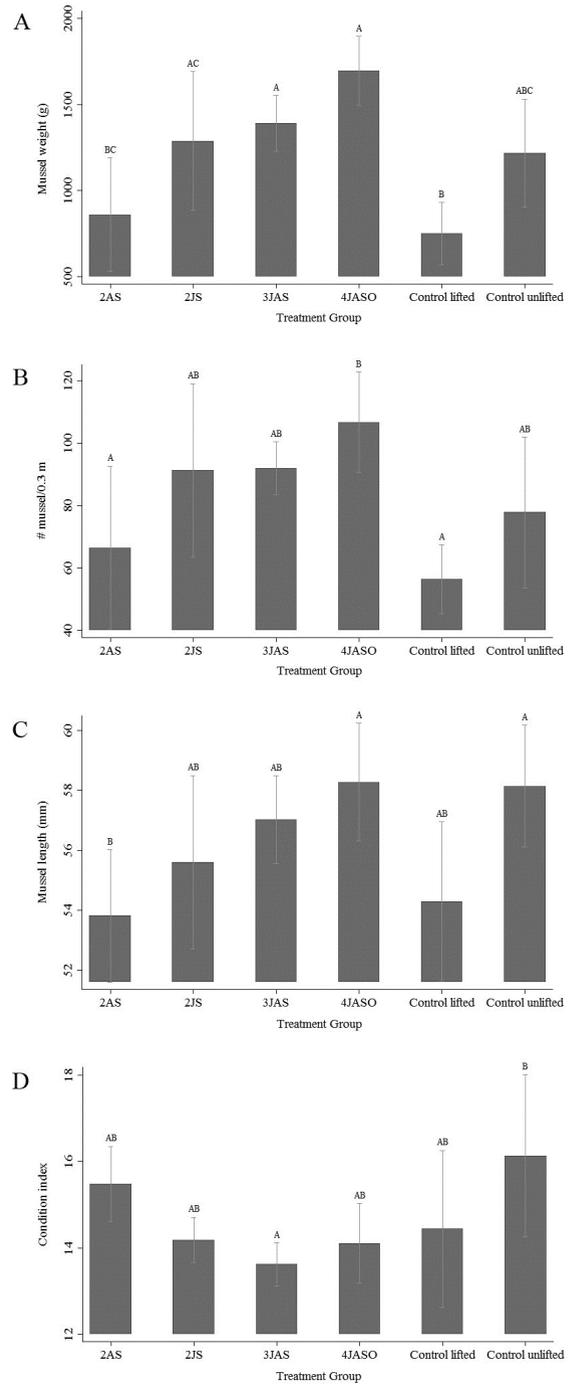


**Figure 3.** Mussel productivity in Brudenell River assessed during November 2010. (A) mean mussel weight, (B) # mussels / 0.3 M, (C) mean mussel length & (D) mean mussel condition index. All the values correspond to means (95% CI).

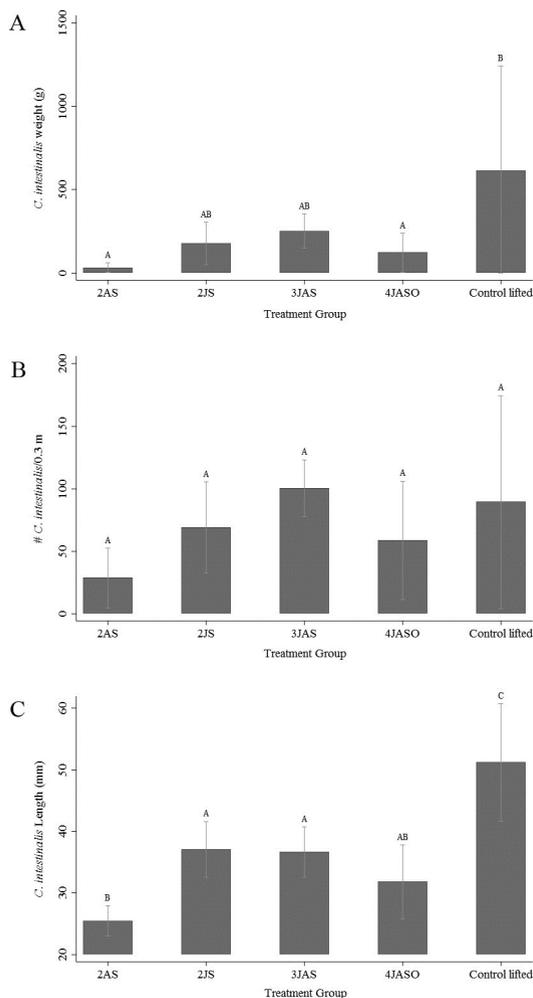
Treatment regime for *Ciona intestinalis* on mussel socks



**Figure 4.** Mussel productivity in Murray River assessed during May 2011. (A) mean mussel weight, (B) # mussels / 0.3 M, (C) mean mussel length & (D) mean mussel condition index. All the values correspond to means (95% CI).



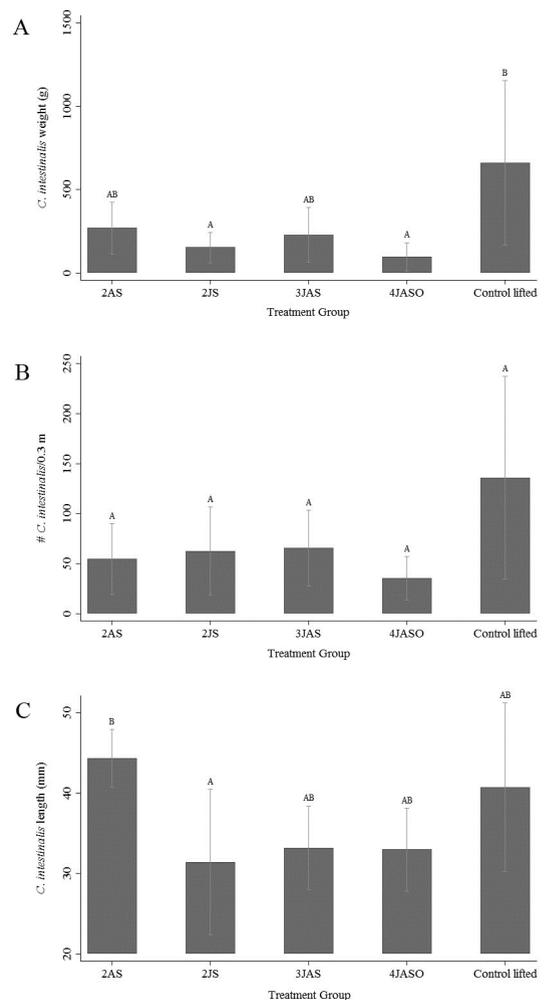
**Figure 5.** Mussel productivity in Brudenell River assessed during May 2011. (A) mean mussel weight, (B) # mussels / 0.3 M, (C) mean mussel length & (D) mean mussel condition index. All the values correspond to means (95% CI).



**Figure 6.** *Ciona intestinalis* infestation in Murray River assessed during November 2010. (A) mean *C. intestinalis* weight, (B) # *C. intestinalis* / 0.3 M & (C) mean *C. intestinalis* length. All the values correspond to means (95% CI).

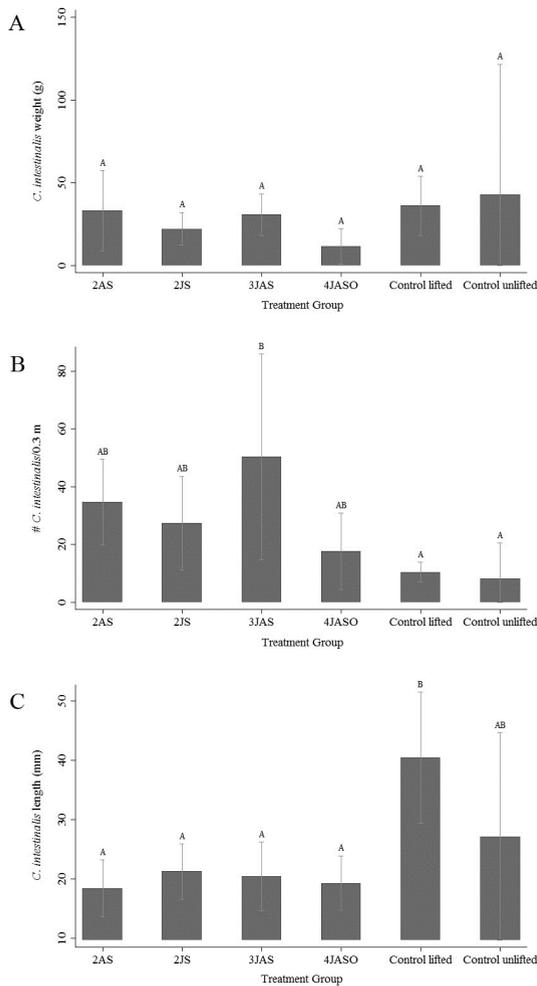
147 ±42 SD and 112 ±19 SD mussels respectively) compared to socks treated two times (70 ± 21 SD and 73 ±29 SD mussels) which were significantly heavier than the controls (18 ±5 SD and 23 ±12 SD respectively) ( $p < 0.001$ ) (Figure 4B). In BR, socks treated four times (107 mussels ±21 SD) had significantly more mussels than socks treated two times beginning in August and the lifted control (66 ±34 SD and 56 mussels ±14 SD respectively) ( $p = 0.002$ ) (Figure 5B).

In MR, all the treatments had significantly larger mussels than the 2 control groups ( $p < 0.001$ )



**Figure 7.** *Ciona intestinalis* infestation in Brudenell River assessed during November 2010. (A) mean *C. intestinalis* weight, (B) # *C. intestinalis* / 0.3 M & (C) mean *C. intestinalis* length. All the values correspond to means (95% CI).

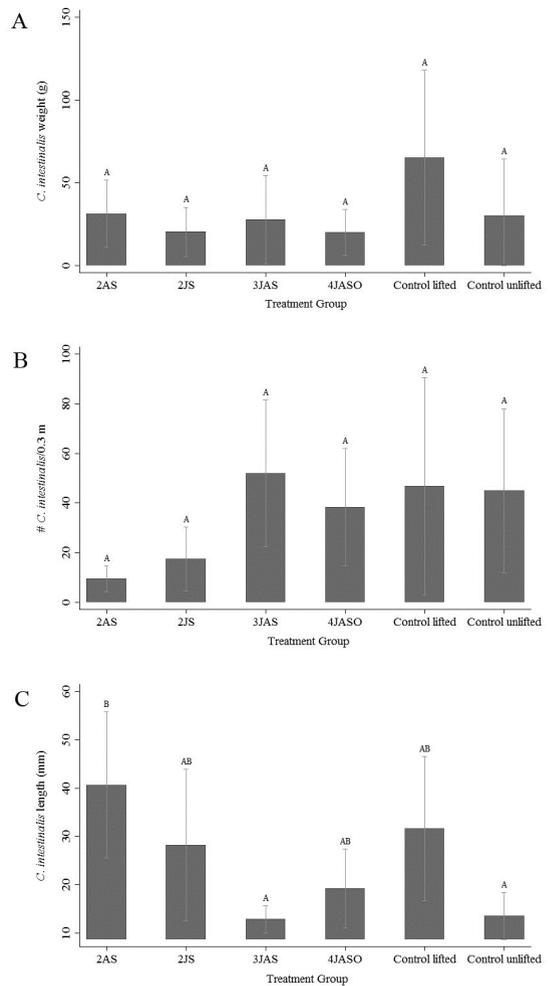
(Figure 4C). In BR, mussels were significantly larger in the group treated 4 times (4JASO) and the unlifted control (58.3 mm ±2.6 SD and 58.1 mm ±2.6 SD respectively) than socks treated twice beginning in August (2AS) (53.8 mm ±2.9 SD) ( $p = 0.005$ ) (Figure 5C). Similar to the sampling conducted in November, there was a significant difference in condition index in BR ( $p = 0.014$ ) but not in MR ( $p = 0.424$ ) (Figure 4D, 5D). In BR, the mean condition index was 14.22, with a range of 13.6–15.5, while in MR the mean was lower (13.75) with a range of 4.5–7.4.



**Figure 8.** *Ciona intestinalis* infestation assessed during Murray River May 2011. (A) mean *C. intestinalis* weight, (B) # *C. intestinalis* / 0.3 M & (C) mean *C. intestinalis* length. All the values correspond to means (95%CI).

*Ciona intestinalis* infestation: November 2010

In MR, the lifted control (mean of 614.6 g ± 730.8 SD) had significantly greater weight of *C. intestinalis* than the group treated twice beginning in August (2AS) and the group treated 4 times (4JASO) (means of 31.6 g ± 37.7 SD and 121.3 g ± 152.5 SD respectively) (p = 0.013) (Figure 6A). In BR, the lifted control 659.1 g ± 641.6 SD was significantly heavier (than the groups treated two times beginning in July (2JS) and the group treated four times (4JASO) (means of 152.5 g ± 115.8 SD and 107.3 g ± 114.8 SD respectively) (p = 0.010) (Figure 7A).

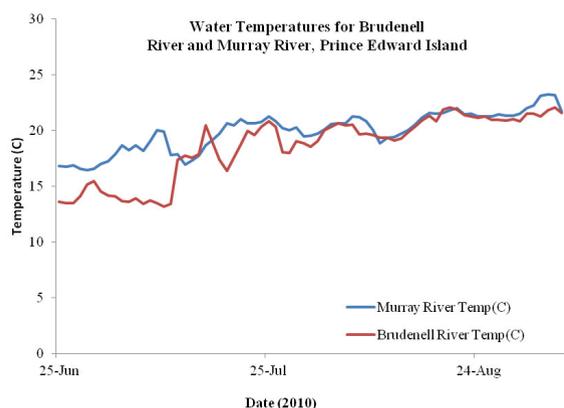


**Figure 9.** *Ciona intestinalis* infestation in Brudenell River assessed during May 2011. (A) mean *C. intestinalis* weight, (B) # *C. intestinalis* / 0.3 M & (C) mean *C. intestinalis* length. All the values correspond to means (95%CI).

There were no significant differences in number of *C. intestinalis* on the socks between treatment groups and controls in both MR (p = 0.1571) and BR (p = 0.0707) (Figure 6B, 7B). With regards to length, *C. intestinalis* were significantly longer in the lifted control group than in the treated groups (p < 0.001) in MR. Meanwhile in BR there was no such effect (p = 0.406) (Figure 6C, 7C).

*Ciona intestinalis* infestation: May 2011

In MR and BR, there was no significant difference in *C. intestinalis* weight between the treatments or controls (p=0.760 and 0.172 respectively) (Figure



**Figure 10.** Water temperatures in the experimental sites in Brudenell and Murray Rivers, Prince Edward Island.

8A, 9A). In MR, the number of *C. intestinalis* in the controls was lower than in the treatments (Figure 8B) ( $p=0.004$ ), but the length was greater in the controls ( $p=0.003$ ) (Figure 8C). In BR, there was no significant difference in number of *C. intestinalis* ( $p=0.087$ ) (Figure 9B), but the lengths were significantly different ( $p=0.001$ ) (Figure 9C).

#### Temperature profile

Water temperature was generally lower in BR than in MR from June 25<sup>th</sup> to approximately July 25<sup>th</sup>, 2010, and then similar up to the end of August (Figure 10). Initial water temperatures for BR and MR, on June 25<sup>th</sup>, were 13.6°C and 16.8°C, respectively. Water temperature was not recorded after September 5<sup>th</sup>.

#### Discussion

Overall, the results of this study suggest that the most advantageous high pressure water mitigation strategies are multiple (3–4) treatment regimes starting relatively early in the growing season (July). This conclusion is supported by the patterns observed in mussel productivity, particularly high weight values, and reductions in *C. intestinalis* abundance with respect to the other treatment regimes. These results do not necessarily apply to every mussel operation, in particular if they are subject to different growing seasons and varying water quality parameters such as temperature and food availability. However, they were gathered

from two geographically separated and independently managed locations which are representative of mussel operations in Atlantic Canada, and are also consistent with some aspects of the early life history of *C. intestinalis*.

In this study, mussel productivity was assessed by four measurable variables: mussel biomass, count, shell length and condition index. Among them, mussel biomass is the most important variable for the financial success of a mussel farm (as price is paid on a per weight basis) but is obviously related and dependent on the other three variables (CBC News 2012; PEIDAF 2015). The main cause behind the changes in weight among treatment groups was the number of mussels per unit sock. This suggests that the main effect of the tunicates is not necessarily a reduction in mussel growth and health, as expressed by shell length and condition index, but rather the fall off of mussels from the socks. Intuitively, one would think that the more treatments are applied to a sock, the more mussels would be knocked off by the frequent high pressure water treatment. However, in reality the opposite seems to be happening. The removal of the tunicate weight from the longline by high pressure water treatment allows for a more secure attachment of the mussels to the sock (Babarro et al. 2008) and, therefore, promotes a decrease in the overall mussel fall off.

A review of results per location indicates that in Brudenell River and Murray River, treating three or four times resulted in socks with the greatest mussel weight at the sampling in November, as compared to controls or treatments applied only two times. This increased weight was the result of significantly higher numbers of mussels on the longline, rather than an increase in the size of the mussels or their condition index. Shell length and condition index in fact only contributed a minor component to the mussel weight. One might speculate that increasing treatments on the longline would increase mussel thread production and attachment enhancement (Young 1985; Lachance et al. 2008). However recent unpublished research (Davidson and Hill, pers. comm., 1 May 2015), suggests that increased treatment does not increase mussel byssus production and attachment strength. An explanation is not available for this observation.

Interestingly, in Brudenell River there was no significant difference in mussel productivity between treating three, four or two times beginning in July while in Murray River there was a difference between the multiple treatments and the two treatments beginning in July. An explanation

for this difference likely relates to the second factor involved in a successful mitigation strategy: how early to treat the socks (Arens et al. 2011). The size of the tunicates at the beginning of the treatment seems to explain the observed pattern. Once pressure water treatment was first applied, Brudenell River had tunicates with a mean length of 21 mm while those in Murray River had a length of 33 mm. The increased strain on the byssal attachment of mussels with larger tunicates, as was the case in Murray River, could have caused increased mussel fall off during the initial treatment of the socks. Brudenell River, which for unknown reasons was infested with smaller tunicates at that time, may not have experienced the same level of fall off.

If the initial pressure water treatment is applied to mussel socks with smaller tunicates (i.e. relatively early in the season), this may allow growers to restrict the number of treatments to two, for example. If this doesn't have a substantial effect on the loss of mussel productivity, as these results suggest, this may account for a considerable saving in the cost of treatment. The rationale for initiating early treatment is further supported by the reduced mussel productivity observed in both sites, when the treatment was initiated in August (i.e. when tunicates were already larger in size). It might be also assumed that an increased water pressure would be required during treatments when larger tunicates were present. This entails the risk of increased mussel fall off, and as a consequence, reduced overall productivity (Babarro et al. 2008). Although this may occur occasionally in these and other mussel operations, the water pressure was held constant for all treatment groups on the individual farms. Further evidence of the importance of timing of the initiation of the treatment comes from the treatment that had to be discontinued. Delaying the first treatment to October resulted in complete sock failure even before the treatment was completed. As the socks were lifted from the water, the combined weight of the mussels and tunicates caused both to detach from the sock and fall into the water.

The effects of different treatment regimes on mussel productivity continued into the following spring as witnessed by the sampling in May after the winter ice. The increase in mussel weight over the winter can be largely explained by the dramatic increase in the mussel condition index from November to May in both Murray and Brudenell Rivers. This is consistent with the past experiences of mussel growers in the region (Smith and Ramsay 2014). Specifically, this increase

could be most likely related to the spring bloom between April and May, when a high level of phytoplankton is present in the water and facilitates feeding and rapid growth in the mussels.

Unlike the mussels, there were not clear patterns distinguishing the effects of the different treatment regimes on the *C. intestinalis* populations. During the fall sampling, and as expected, the only difference observed was an increase in weight in the control groups. During the winter sampling, this study found evidence of reduced tunicate weight in both river systems, but this was most likely related to the usual winter mortality affecting these populations, and was unrelated to treatment (Ramsay et al. 2009). There were no discernable "lasting effects" associated to individual treatment regimes. Winter mortality of *C. intestinalis* is a common but not completely understood phenomenon in the waters of PEI (Ramsay et al. 2009; Fisheries and Oceans Canada 2010), and its interaction with mitigation strategies requires further research.

In summary, the goal of selecting a treatment regime is to maximize mussel productivity, which for the mussel industry is the result of a balance between mussel growth and tunicate treatment strategies. Although treatment strategies may be site-specific, the results of this study indicate that *C. intestinalis* populations can be effectively controlled with multiple consecutive monthly (3–4) treatments, and that is the most effective treatment regime. These results also suggest that initiating treatment early in the growing season, when tunicates are smaller, is more effective than starting later in the growing season due to increased risk of fall offs and loss of mussels. A first treatment in July appears to be critical for facilitating strong mussel attachment and, subsequently, mussel abundance and weight. The anticipated harvest time of the crop does not appear to be an important factor in determining the treatment regime employed to mitigate *C. intestinalis*.

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