

Fouling of Mussel (*Mytilus edulis*) Collectors by Algal Mats, Dynamics, Impacts and Symptomatic Treatment in P.E.I. Canada

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ABSTRACT: Biofouling of spat collectors by green algal species on the *Mytilus edulis* (blue mussel) farms of Prince Edward Island in eastern Canada has been a problem for over 6 years. This problem is a symptom of increasing eutrophication in the bays and estuaries of the Maritimes. The fouling consists of primarily *Cladophora* sp. and *Enteromorpha* sp. that develop rapidly during June and August. Biomass doubles 2 to 4 times per week forming extensive algal mats that accumulate on all parts of the aquaculture equipment. Mussel spat settling at the same time either do not attach to the collectors or become attached to algal filaments. To deal with this problem several environmentally safe chemicals were tested in the laboratory for their toxicity to *Cladophora* sp. and to mussel spat. A concentrated brine solution had little or no effect on survivorship of mussel spat while causing death of *Cladophora* sp. cells by osmotic stress. This treatment was effective reducing fouling in field trials on commercial farms when used 2 to 3 times over two months. This information was quickly applied by the industry and the result was spat volume yields of over 6000 ml per treated collector versus less than 1000 per untreated collector.

KEYWORDS: *Cladophora*, mussel aquaculture, biofouling, algicide.

INTRODUCTION

Aquaculturists place structures in the water for extended periods of time resulting in biofouling with several associated impacts. Without preventative or remedial measures biofouling can quickly affect the quality of rearing environments. Biofouling communities on sea cages for fish culture can impact the water quality inside the cage by restricting water exchange¹. Algal biofouling of oyster culture bags can limit growth and survivorship of the organism². Industrial users of water for cooling such as power plants have used a range of biocides for controlling biofouling³ However, aquaculturists are very limited in the type of chemical or measures used against biofouling since they must provide a high quality food stuff⁴.

Over the past 20 years there has been a steady increase in nitrification of rivers, brooks and streams entering Prince Edward Island's (PEI) coastal waters and this has been reflected in algal macrophyte blooms.^{5,6} The source of the problem is eutrophication of estuaries that provides ideal conditions for growth of macro algal blooms. Maximum levels of inorganic nitrogen required to prevent macro algal blooms are less than 1.0 mg l⁻¹. In general most estuaries and lagoons of P.E.I. exceed this nitrogen level and summer blooms occur regularly.⁷ During our study the nitrate levels in Rustico Bay averaged 1.6 mg l⁻¹ and at times were over

4.0 mg l⁻¹. While nitrogen is a driving factor, precipitation, soil erosion, and hydrodynamics of the water body are secondary factors.

Since 1998 mussel (*Mytilus edulis* L.) aquaculturists in PEI have been impacted by bio-fouling of their mussel spat collectors by mat forming filamentous green macro algae. Mussel growers set out rope collectors in early summer to capture the settlement stages (spat) of naturally occurring mussel larvae. The collectors are stripped in the fall of the mussels (known as seed) for stocking and placement on grow-out longlines. The algae settle and grow on the collectors and the mussel spat that has already set on the collectors prior to algal settlement will migrate off the collector rope into the algal filaments. New mussel larvae will set directly onto the algal filaments. The algal mat becomes very dense and heavy and often the mat and attached mussels will fall from the collector when the longline is agitated (either by wind and wave action or by the growers as they attempt to handle the lines).

In the past growers in Rustico Bay have attempted to salvage seed by stripping the algal mat from the collectors with very poor results. Growers have also tried hydrated lime (calcium carbonate) dipping and removal of collectors after the harvest to kill the algae. The problem has continued to plague an industry that is worth \$60,000,000 CDN per year and has cost individual growers in excess of \$100,000 per year.

This study investigated the dynamics of algal biofouling on mussel collector ropes including settlement, growth and mortality of the algae and mussel spat.

Laboratory experiments tested the effectiveness of algaecides with *Cladophora sp.* and their incidental toxicity to *Mytilus edulis*. Field trials were used to compare to laboratory results, then successful treatments were applied in full scale commercial trials by mussel aquaculturists.

MATERIALS AND METHODS

Spore Settlement

Algal reproduction and recruitment were monitored using 25 unglazed ceramic plates, 15 by 15 cm square, placed at one station in Rustico Bay on May 28th. Plates were drilled in the centre and screwed to a 1 meter by 5 cm by 2 cm piece of wood. Plates were cultured in Provasoli enriched seawater at 15°C and 100 mmol photons m⁻²s⁻¹ in 14:10 light dark regime. Five new ceramic plates were added each week and were removed after 2 weeks for culture. The growth in enriched media allowed identification and estimation of densities of the plant taxa settling on the collectors. Three 1 cm² samples were scraped from each plate weekly for microscopic observation. A qualitative estimate was made of plate coverage of the plates by the plants when they became macroscopic.

Settlement on Collectors

Commercial mussel spat collectors were also utilized to monitor the timing of algal recruitment. Standard collectors were 1.9 m pieces of 10 mm polyethylene rope weighted with lead. Ten new collectors were attached to longlines weekly and were replaced in 2 week cycles. A 5 cm section of the rope was scraped from the top, middle, and bottom of each collector. Each 5 cm sample was spun for 20 seconds in a salad spinner (to remove excess water), weighed to 0.1 g, and the predominant species of algae were identified.

Although the ratio of algae to mussel seed in the biomass on mussel spat collectors in the above experiment was not determined, the composition of biomass on untreated (control) collectors placed in the water in association with the chemical treatment trials was determined (field treatment experiments). The mussel spat was separated from algal filaments and dewatered as above and weighed to 0.1 g. These collectors were included in experimental treatment trials but were not disturbed except to sample 5 cm sections of three collectors at every monitoring period of each experiment in each area.

GROWTH

Growth chambers were used to monitor the growth

of *Cladophora sp.* Kutzing. on a weekly basis. '*Enteromorpha*' sp. Link. was not used in this experiment as it was not an important component of the algal mat during the peak spat collection period. The authors were aware of recent changes (2003) in taxonomy placing *Enteromorpha* spp. in *Ulva*. For this paper we have retained '*Enteromorpha*' to keep the references prior to 2003 in this paper consistent and familiar.

Twelve 0.5 mm mesh containers were stocked with 0.5g-2.5g (dewatered) *Cladophora sp.* and left at a depth of 30 cm for 1 week. The chambers were spun for 20 seconds to remove excess water weight and the algae were weighed to 0.1g. The chambers were then restocked and set in the same location. Specific growth was calculated with the following formula:

$$SG = \frac{[(\ln(W_f) - \ln(W_i)) \times 100]}{T}$$

$\ln W_f$ = natural logarithm of the final weight

$\ln W_i$ = natural logarithm of the initial weight

T = time in days.

Selection of an Algaecide

The chemical agents chosen for testing were; saturated brine, 300 ppt. NaCl; 5% acetic acid C₂H₄O₂, vinegar; 4% hydrated lime, Ca(OH)₂; 30% and 60% sucrose, C₁₂H₂₂O₁₁; 5% citric acid C₆H₈O₇·H₂O, and elevated water temperature. Brine and sucrose were considered for their potential to cause high osmotic stress. Vinegar had been a treatment for tunicates on mussel farms. Hydrated lime was used regularly to kill settlement stages of starfish. Citric acid was a food grade chemical that could have similar effects to vinegar.

Experiments were conducted in 2 litre glass dosing trays. Each agent was applied to the plant material for 15 and 30 second immersion times. Two grams of fresh plant material were exposed to the treatment agent by placing plant material in a hand sieve and moving the sieve back and forth in the dosing tray for the selected duration. Following the exposure the plant material was rinsed of the agent by agitating it in clean seawater for 15 sec and then placing it in 500 ml of clean seawater for 15 minutes. Microscopic observations of 3 sub samples of plant material were conducted following each treatment. The treated plants were then placed in individual recovery containers and moved to a flow through seawater holding system. Twenty-four hours later 3 sub samples of each treatment unit were collected for a second set of microscopic observations. Each treatment trial was conducted in triplicate for each chemical and exposure time.

Evaluations of the effects of the treatments on the plant cells were made with a compound microscope at 10X magnification. Observations were made of the

number of cells that had swollen cell contents, shrunken cells and cells that were empty of contents in three subsamples of the treated plants - both at 15 minute and at 24 hours post treatment. The index utilized to quantify the cells affected was as follows:

1: ≤ 2 cells, 2: $> 2 < 10$, 3: $\geq 10 < 50$, 4: $\geq 50 < 100$, 5: ≥ 100

Algaecide Toxicity to Mussel Spat.

Once it had been established that vinegar (in the initial field trials) and brine (in the lab trials) were toxic to green algae the sensitivity of mussel seed to these chemicals was tested in laboratory trials. Four 2 litre Pyrex trays were filled with: vinegar 5%, brine 300 ppt, and ambient seawater (controls). Thirty mussel spat were selected between 4.0 and 5.0 mm shell height. Only seed that were actively producing byssal threads and observed attaching to the surfaces of the holding containers were chosen for the trial. The seed were placed in a dousing sieve and exposed, with agitation, to 5% vinegar for 20 seconds, 300 ppt brine solution for 20 seconds, 300 ppt. brine for 30 seconds, and control (seawater) for 30 seconds. The seed were then removed and rinsed in ambient seawater. Following the rinse they were moved to recovery tanks and held in mesh trays in a flow through seawater system for 24 hours. After 24 hours a count was made of the number of mussel seed attached to any surface in the container by byssal threads, the number unattached, and the number gaping and unattached. The same observations were made at 48 hours. The experiment was repeated three times.

Field Treatment Experiments

To treat collectors attached to longlines a trough 3 m by 0.5 m by 0.3m deep was filled with the test solution was attached to a mussel work boat. Sections of longlines with a minimum of 10 commercial mussel collectors attached were immersed in treatment agents for 15 seconds, 300 ppt. brine and 5% vinegar were applied to collectors in 3 separate sections within the treatment area. Three sections were left untreated, and 3 sections were immersed in a trough containing only seawater, to account for any effects handling the longlines and collectors may have had. The handled only sections were considered as controls for statistical comparisons while the untreated sections were monitored to track the undisturbed development of biofouling on collectors in each area

This portion of the study was designed to determine the minimum number of treatments required to sufficiently manage green algae (if multiple treatments proved to be necessary). Single treatments were made on July 14th, July 26th, August 3rd, August 10th, and August 17th. Second, third and fourth treatments were

applied at two week intervals from the first single treatment dates. Samples were collected 2 weeks after treating to monitor the effects of the treatments and the growth of the algae. Final treatments were applied during the week of August 27th-September 2nd.

Samples were collected 2 weeks after each treatment application to monitor the effects of the treatments and the growth of the algae. To monitor the algae/spat on collectors without physically removing collectors, 5 cm sections of the collector scraped from the top, middle, and bottom of 3 collectors per section. All biomass was removed and mussels were separated from the algae. Excess water was removed and the weights of algae and spat/seed mussels were recorded to 1g.

The one way ANOVA model was used for multiple comparisons of means, the test of significance was a Tukey comparison within a 95 % confidence interval of significant differences.

Commercial Treatments on Mussel Farms

Throughout the summer a number of treatments to clear algal mats were applied to mussel seed collectors by commercial growers. Treatment schedules varied from multiple treatment events by some growers to no treatment. Some growers added other agents than the ones recommended by researchers. Lime was added to deal with starfish along with brine for the algae. When vinegar was added it was usually of concentrations below 5%.

Treatment histories including treatment agents and treatment date(s) applied were recorded by interviewing growers for all leases surveyed in the bay (Table 1). In

Table 1. Schedule of commercial treatments applied to mussel seed collectors in Rustico Bay, July-September, 2004.as described by mussel growers.

Station number	Treatment	Dates (approximately)
1,2,3,10,11	Untreated	n/a
4	Brine&Lime	September 1 st Mid-September
5,6	Brine, Brine & Lime	Late July, Late August
7	Brine & Vinegar X2 Brine & Vinegar,	Early August Mid-August
8	Brine & Lime X1 Brine X2, Brine & Lime	Early September Late July, EarlyAugust Late August/ Early September
9	Brine, Brine & Vinegar	Date unknown, Mid-August
12,13	Brine & Lime Brine Brine Brine & Lime	Early September Late July/Early August Mid-August Late August

mid-October, 13 stations in Rustico Bay were selected for sampling. At each station 5 collectors chosen at random from each of 3 lines were stripped of all organisms (N=15). Algae were hand-separated from mussel seed and wrung or spun for 20 seconds to remove excess water. Weights (to 0.1 g) and volumes (to 25 ml) were recorded for algae and for seed. The length of each collector was measured and weights were corrected to eliminate any variation in sample weights/volumes due to different collector lengths.

RESULTS

Spore Settlement

Peak recruitment of *Cladophora sp.* occurred during the first week of August (Figure 1). All plates collected in August developed 100% cover of *Cladophora sp.* germlings. Prior to this time and from September onward '*Enteromorpha*' *sp.* was the most abundant germling. In August recruited algae on mussel spat collectors was 100% *Cladophora sp.*, the dominant species in the entire area at this time. This also corresponded to the period of maximum total *Cladophora sp.* biofouling on the mussel spat collectors that were placed in the field every two weeks (Figure 1).

During the monitoring period (August 9th to November 7th) algal fouling was continuous on control collectors with no clear trends in abundance (Figure 2). The lack of a clear trend and the 'high-low' pattern of weights for both the algae and the mussel seed may be explained by the constant build up and subsequent naturally caused stripping of algae and seed from the collectors. Mussel seed weight was highly variable but went through several highs and lows. Algae ranged from a high of 45% of total bio-fouling wet weight on collectors in early August to a low of 12% in late October. The biomass on collectors in October was predominantly mussel seed (Figure 2).

Growth

Weekly specific growth decreased during July; exponential growth began in early August, peaked in mid August and then declined to early September (Figure 3). There was a short recovery in growth rate until mid-September followed by a final decline in the fall.

There was not enough *Cladophora sp.* available to stock the growth chamber until early-mid July, and it was difficult to find at that time. It is suspected that since recruitment was limited prior to August 5th the algae used to stock the growth chamber prior to August

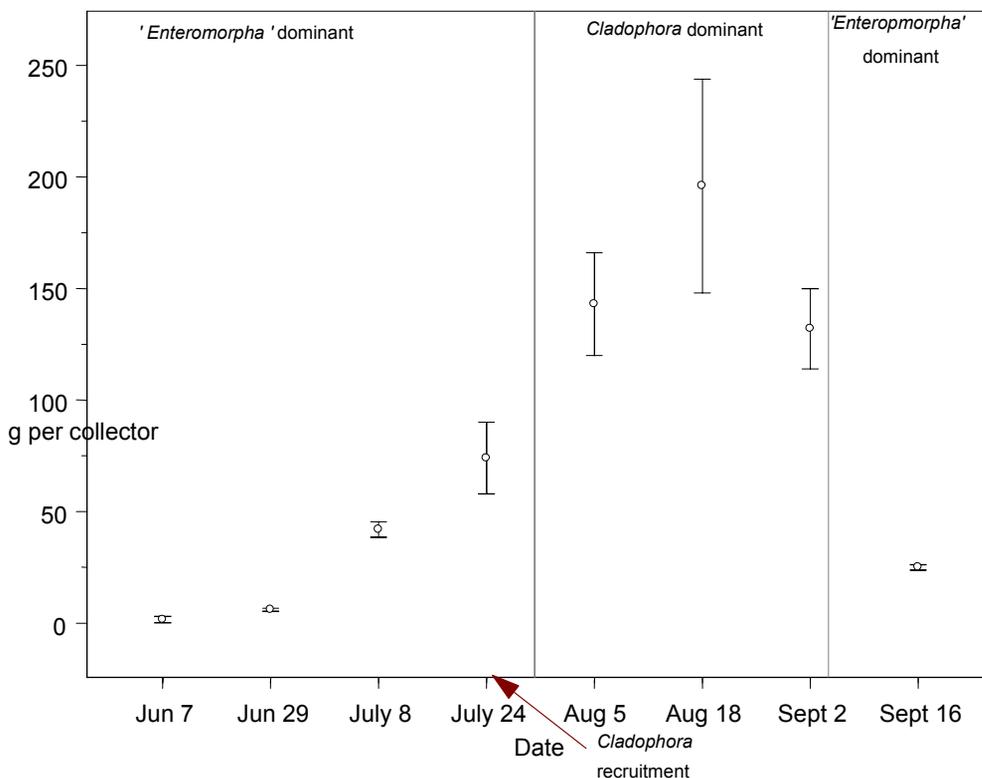


Fig 1. Mean and standard deviation of total biofouling wet weight on rope collectors placed in the field every two weeks summer to fall 2004 Rustico Bay. Vertical lines describe the dominant germlings as observed on ceramic settling plates.

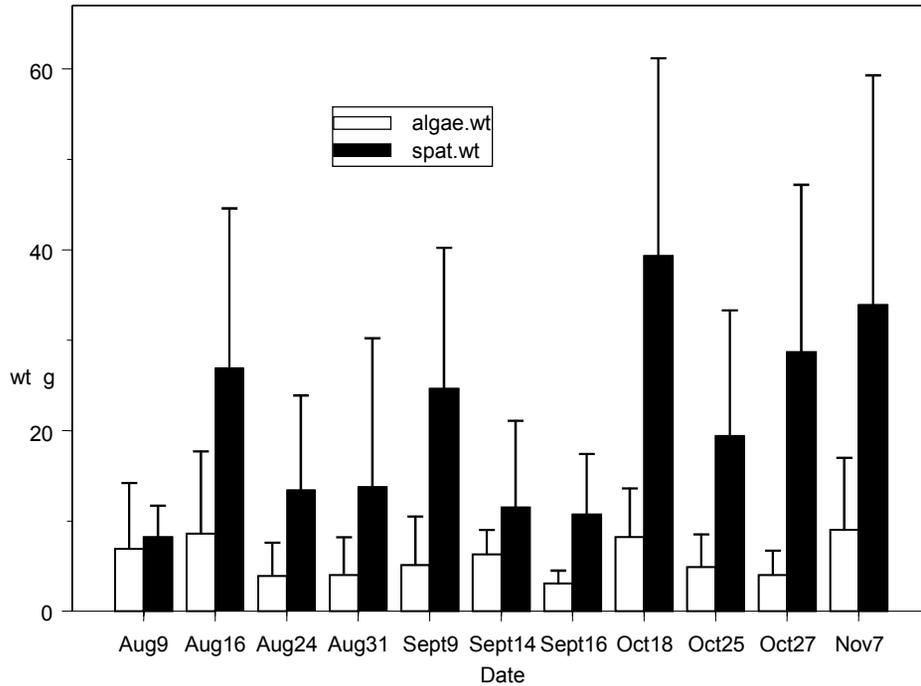


Fig 2. Mean and standard deviation of mussel seed and algae wet weight from 5cm scraped samples of collectors placed as controls in treatment experiments on seed collecting sites late summer to fall 2004, Rustico Bay.

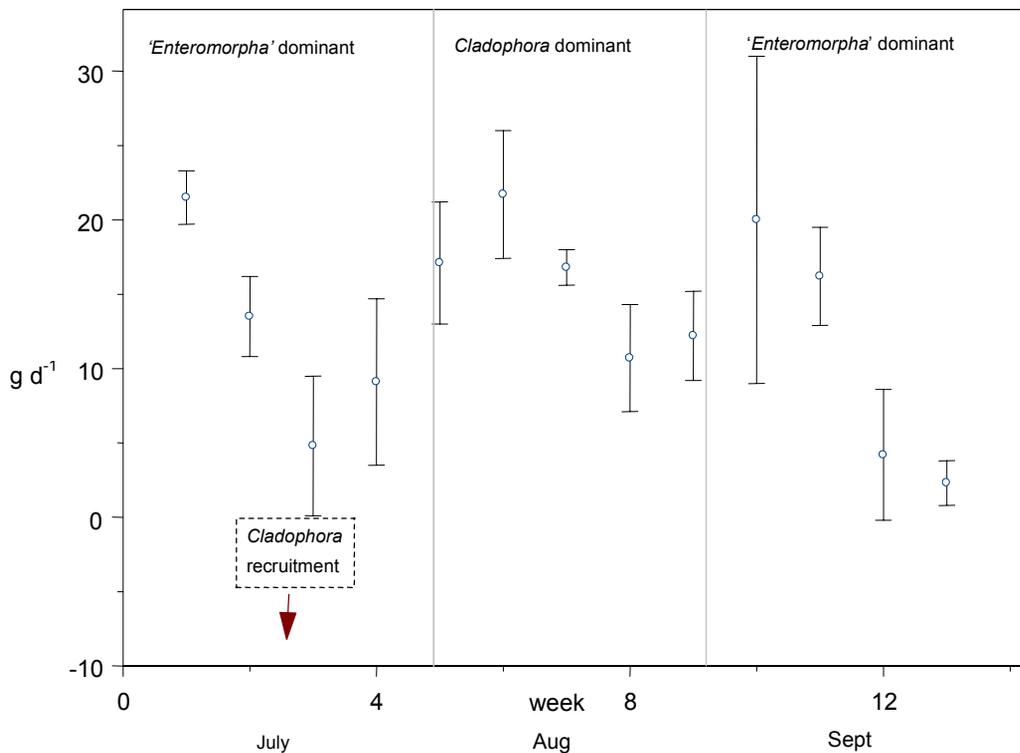


Fig 3. Mean and standard deviation of growth rate for *Cladophora* g d⁻¹ held in growth chambers over approximately 2 week intervals, from July until October 2004. Vertical lines indicate dominance of algal germlings as observed in culture of ceramic settling plates.

5th had been in the bay since the previous summer. This might explain the decline in growth rate from July 14th-29th (Figure 3). Reproductive structures were observed in June, August, and September. No massive mortality of green algae was observed throughout the season.

Algaecide Selection

Shrunken cells was the only consistent cell damage observed in the microscopic observations of cell condition. The chloroplasts pulled away from the cell walls and pinched into the middle of the cell. Cell damage was most noticeable with the 300 ppt salt treatment at both the 15 and 30 second immersion times. Cell damage was present at both the 15 minute and 24 hour recovery periods (Figure 4). Longer exposure time or higher temperature did not result in more damage within the limits of the index for the salt treatment. Sucrose 30% treatment effects appeared to be enhanced by higher (30 °C) ambient temperature (Figure 4).

Algaecide Toxicity to Mussel Spat

Twenty four hours following algaecide treatment the numbers of mussel spat exhibiting negative response (unattached) in brine treatments were not significantly different from the controls. (Figure 5). In contrast over 60% of mussel spat were unattached and/or gaping

open when exposed to 5% vinegar. The results were similar at the 48 hour observation.

Field treatment Experiments

Repeated brine treatments that began in July resulted in total mussel spat weights 4 to 5 times greater than controls and vinegar treatments by late August (Figure 6). This result was consistent and significant through the summer and fall. Repeated vinegar treatments either killed spat or prevented spat from growing in every experiment. The spat fall could not compensate for regular treatments of vinegar, since the residual population was impacted as well as any newly settled spat. Vinegar appears in field trials despite its toxicity to mussel spat because field and laboratory experiments were run concurrently.

Single treatments of vinegar did not have a clear and consistent negative impact on spat when compared to single brine treatments (Figure 6). Spat was constantly settling on the collectors and any mortality that resulted from treatment could have been offset by additional settlement on a surface clear of algae.

Field treatment trials provided very definitive results when comparing the effects of repeated vinegar treatments with repeated brine treatments on algal biofouling. Repeated treatments of brine were the most

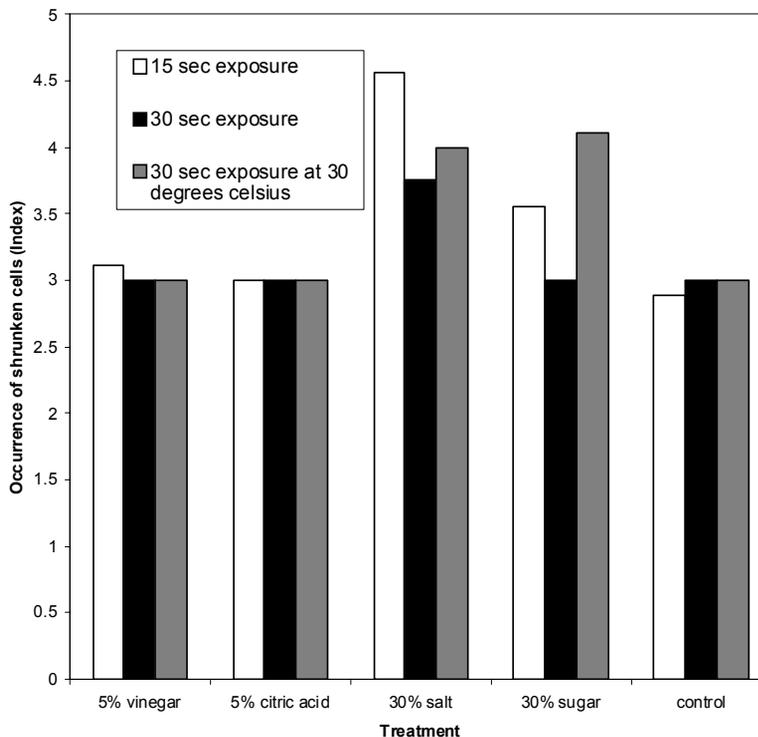


Fig 4. Cellular damage in *Cladophora* plants exposed to vinegar, citric acid, salt and sugar for 15 and 30 seconds compared with controls, observed at 15 minutes post treatment. Cell damage index 1-5.

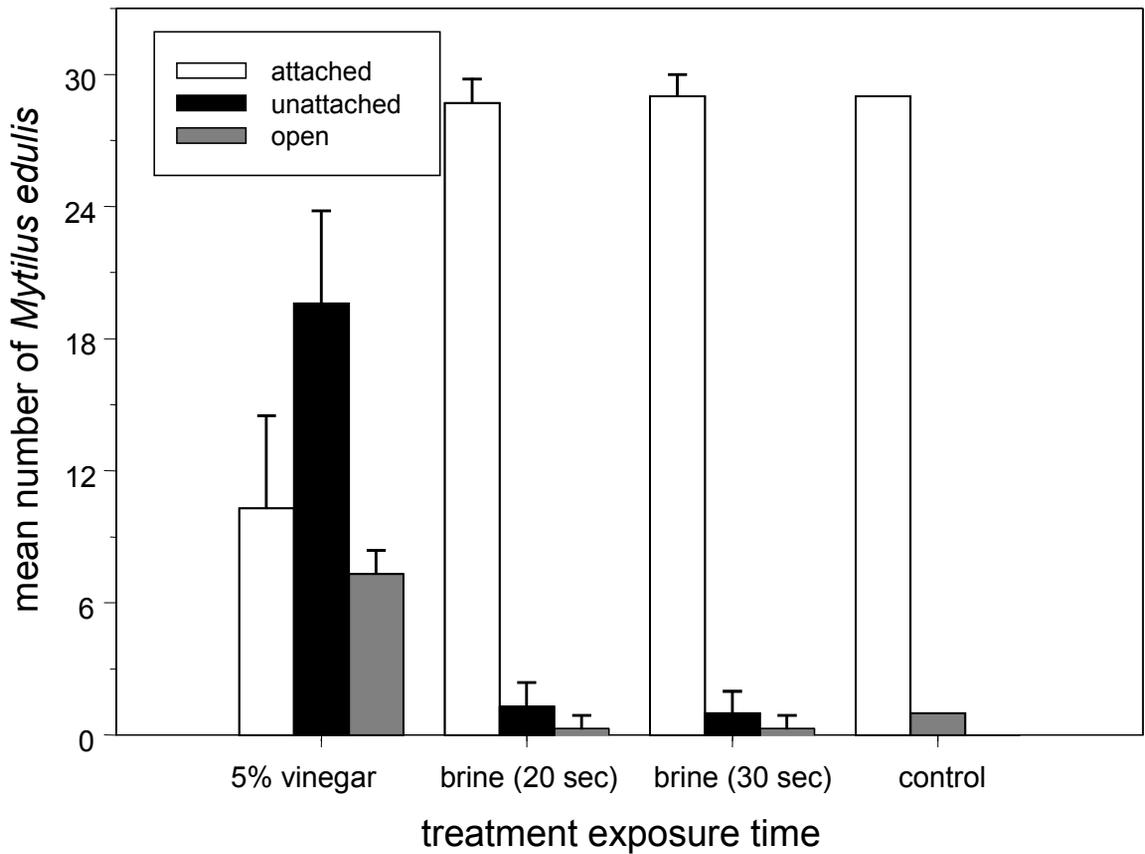


Fig 5. Response to treatment; mean number and standard of mussels in each of three states, 24 hours after treatment with 5% vinegar and 300 ppt. brine of 30 total exposed individuals.

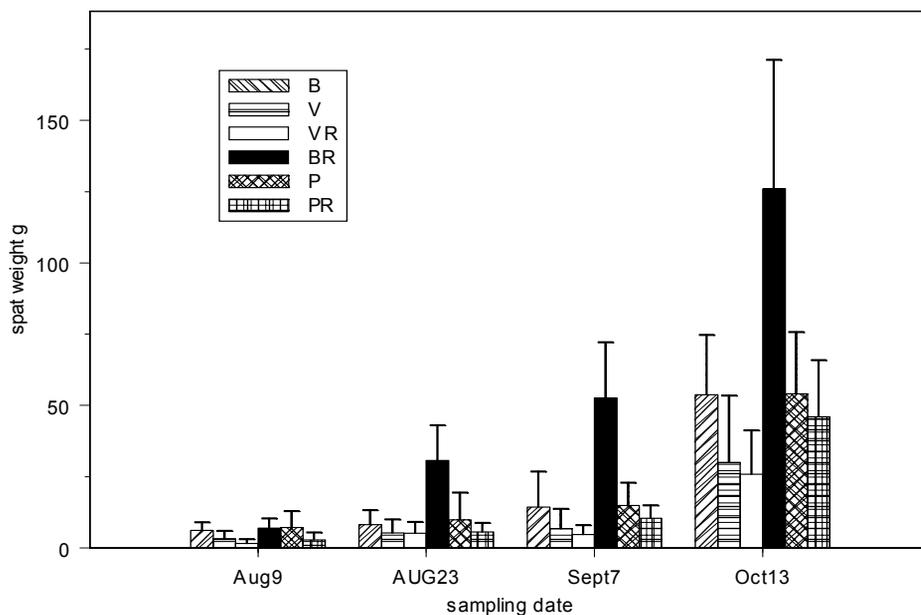


Fig 6. Mean and standard deviation of mussel spat wet weight per 5 cm scraped section of collectors on sampling dates following treatment by brine 300 ppt (B), brine 300 ppt repeated (BR), vinegar 5% (V), vinegar 5% repeated (VR), controls handled once (P), and controls handled multiple times (PR), Rustico P.E.I. 2004.

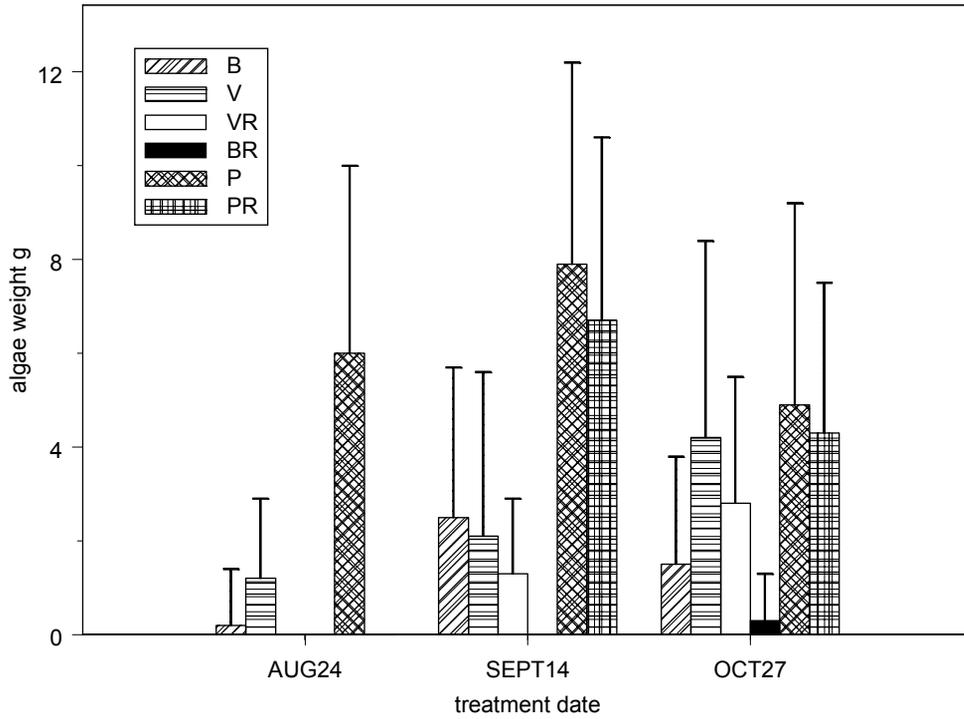


Fig 7. Mean and standard deviation of algae weight per 5 cm scraped section of collectors on sampling dates following treatment by brine 300 ppt. (B), brine 300 ppt.,repeated (BR), vinegar 5% (V), vinegar repeated 5% (VR), treatments and controls handled once (P), and controls handled multiple times (PR), Rustico P.E.I. 2004.

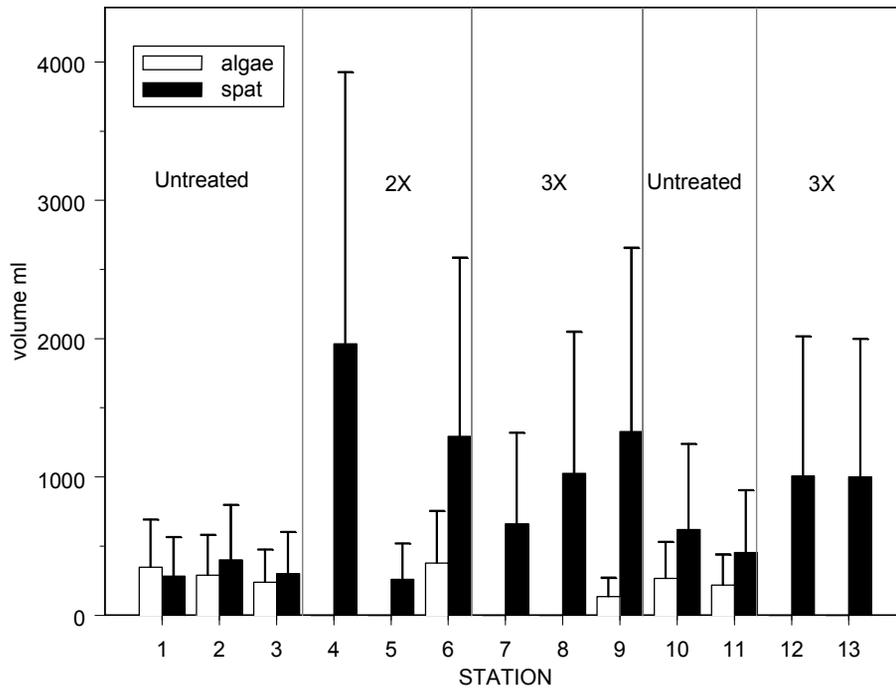


Fig 8. Mean and standard deviation of algae and seed volume harvested from lines treated commercially for algal biofouling (data corrected for a standard collector length) on mussel seed farms in Rustico Bay PEI Oct 6-7, 2004.

effective in reducing the algal fouling on collectors to zero or near zero levels (Figure 7). Repeated treatments will impact any newly settled algal biofouling at a stage when it is most vulnerable to treatment.

When the collectors were treated only once, the new spat settling in the short term encountered only the collector as a settlement surface. If there were no subsequent treatments new growth of algae was quickly re-established from the spore bank. During the course of the treatment trials some collectors (both untreated and once-treated) became stripped of *Cladophora* when lines were agitated by wind and waves. Along with algal biomass, spat that had settled in the algal biomass was lost. Repeated treatments kept the collector clean of algae and allowed for continuous spat settlement directly on the collector or on previously settled seed (Figure 7).

Commercial Field Trials

Farmers who did not treat spat collectors had very low final mussel seed yield and an equal amount of algae among collectors (Figure 8). Farmers who made at least two series of treatments had significantly higher seed yields and lower algae fouling than untreated stations. The areas that were treated 3 times had the best overall yield of seed and least algal fouling and were significantly better than all other leases in Rustico Bay (Figure. 8).

The robustness of brine as a treatment is reflected in the success of the commercial treatments in Rustico Bay. Commercial applications of treatments were made over several weeks by several crews operating on kilometres of seed lines. It is unlikely 3 separate operators were consistent in each area with their treatment applications. Variations in the immersion times, the concentration of brine in the trough, and the degree of handling of the lines were to be expected. There was, despite all the variables, a significant difference in the volume of seed harvested from areas treated three times versus areas treated twice with brine.

DISCUSSION

Identification of a single *Cladophora* species that is the "cause" of the build up of algal mats in P.E.I. estuaries is problematic due to the need for revision of the classification of Cladophorales in general. In the Baltic *Cladophora glomerata*, a fresh and brackish water species, is named as the main component in large blooms and mats.⁸ In the Mediterranean *Cladophora albida* is a component of mats forming in the Venice lagoon nitrified environment. Locally green algal mats have been described in the Bay of Fundy.⁹ However, these authors only identify the algae to genus '*Enteromorpha*'

spp. and *Cladophora spp.* The earliest key for algae in this region Taylor,¹⁰ recorded one *Cladophora* species as mat forming, (*Cladophora magdelana*) *Cladophora albida*.¹¹

'*Enteromorpha flexuosa*' is the most common '*Enteromorpha*' species in our estuarine waters forming drifting mats.¹¹ '*Enteromorpha intestinalis*' is common as an attached plant and can appear on mussel backlines in abundance that may resemble mats. The thicker thallus and lack of fine branching does not form intermeshed mats.

Green algae become a problem with spat collection when they foul mussel collectors acting as physical barriers to settlement. When given a choice of natural settlement substrata, mussel spat will settle on filamentous algae such as *Cladophora spp.* in preference to other algal species and second only to previously settled mussels.¹² Artificial habitat (monofilament 0.4mm) is the preferred substrate over *Cladophora* in a ratio of 3.5 to 1 (opp. cit.). However, when a collector has a population of *Cladophora* the first substratum encountered by the spat will be the algal filaments. In general, mussels become the competitive successional dominant species in the natural environment. Effective mussel collection requires the mussel seed to become dominant on collectors immediately.

Several factors must be considered when choosing a treatment agent to remove the algae on the collectors. There are common chemical solutions designed as algaecides for everyday use. Algaecides with copper as a common active ingredient. are used in potable water supplies and some hatchery operations. Others are halogen based chemicals and simazine herbicide compounds. While all these chemicals are registered for use in some jurisdictions for holding ponds and swimming pools, they are not compatible for use in association with shellfish aquaculture.¹³ Natural substances have been used to control algae, such as mustard seed extract and barley straw.¹⁴ Recently algal extracts have been tested as settlement inhibitors for macroalgae.¹⁵ For this project, only chemicals that would be delivered in a pulse directly to the collectors and were of low overall environmental risk and low cost were considered. There was no risk of increasing the baseline salinities or acidity of Rustico Bay by applying brine or vinegar to collectors with dipping troughs.

The effectiveness of a brine solution's toxicity to green algae is based on osmotic stress. The differential in concentration between cell contents and the bath of brine is large. The water in the cell moves across the cell membrane into the salt solution and thus the remaining contents of the cell shrink away from the cell wall. The osmotic stress created by the brine (300 ppt.) is well beyond that resulting from the 0 to 35 ppt of the normal

estuarine environment commonly encountered by *Cladophora* spp. Salinity in Rustico Bay ranged from 20 to 26 ppt. during the 2004 monitoring period.

Mussel larvae and spat are in general sensitive to toxins. Some species have been used as sub-lethal indicators of heavy metal toxicity.¹⁶ *Mytilus edulis* pumping rates respond to stress by pauses for up to 3 minutes. Filtration rates drop with an increase in toxicant concentration. Mussels however, can recruit and survive in moderately toxic environments and accumulate toxins.¹⁷ The lack of toxicity of brine to mussel seed may reside in the ability of the mussel to quickly sense the high salt levels and their tolerance for salinity in general. It appears they have sufficient time to shut their valves and seal themselves from the solution for the short time (15 seconds) they were exposed. Since mussels thrive in estuarine environments, they must naturally react to slight changes in salinity. The toxicity of vinegar to mussels may be related to the inability of the mussel to react quickly enough to prevent the solution from entering the shell. Then the acidity of the vinegar may act on critical organs to either stress or kill the animal.

Timing is an important factor in the application of treatments. It appears that in Rustico Bay, 2004, early August was the best time to treat collectors, if treating once-only. In early August the fastest growth was observed in captive *Cladophora* (growth chamber), and *Cladophora* became the dominant species recruited onto settlement plates. The most rapid increase in weights of biofouling on short-term and long-term collectors was also observed in early August. Control collectors had a biomass composition of about 45% algae (by weight) in the samples collected on August 9th. This was the highest percentage of algal fouling in all sample sets analyzed. Algae made up a smaller portion of the biomass through late August/September/October but visual observations revealed that algae had begun to fall off collectors by late August, taking mussel seed with it. If initial treatments are applied too late, there will be less available seed on collectors, and spat settling post-treatment might not have sufficient time to reach a reasonable size for socking by October/November.

Brine treatments are providing only symptomatic treatment for the problem of green algae biofouling. The farmers had a treatment cost of \$40.00 per longline and a return from that line of seed worth \$450. However, the ultimate threat to the Rustico Bay ecosystem is the overall build-up of algal biomass and subsequent biodegradation that can produce hypoxic or anoxic conditions killing organisms. The decrease in oxygen levels in mid summer on the bottom and at the head of the Rustico estuary is a warning of the potential for anoxic events. In general the symptoms of eutrophication must be dealt with in the short to

medium term to "fix" the problem. This involves a complex interaction of environmental stewardship and socio-economics.

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