The use of food grade oil in the prevention of vase tunicate fouling on mussel aquaculture gear

Jiselle A. BAKKER, S. Christine PAETZOLD, Pedro A. QUIJÓN, Jeff DAVIDSON

Abstract

Current mitigation strategies against invasive tunicates on mussel aquaculture gear in Prince Edward Island concentrate on labour-intensive and costly fouling removal. Instead of removal, this study focused on preventing the settlement of the vase tunicate *Ciona intestinalis* and other fouling organisms by applying a layer of food grade oil to gear prior to recruitment. Laboratory tests established the adherence and persistence of shortening, a food grade oil with a melting point exceeding ambient water temperatures, to rope and mussels. In *situ tests* showed that shortening decreased *C. intestinalis* weight and abundance on buoys, spat collector ropes and collector plates but not on mussel socks. Fouling by algae and other tunicates was significantly reduced on most substrates. There were no detrimental effects of shortening treatment on mussel length and abundance on mussel socks, but total mussel weight was significantly lower on shortening-treated socks. Shortening treatment did not significantly affect mussel spat settlement on spat collector ropes, but further evaluation is required. Overall, shortening application has considerable potential for reducing tunicate and other fouling, particularly on buoys.

Keywords

Ciona intestinalis, mitigation, shortening, invasive marine species, prevention

Introduction, Hypotheses and Problems for Management

The blue mussel (*Mytilus edulis* L. 1758) aquaculture industry plays an important role in Prince Edward Island (PEI)'s economy, annually producing around 17.5 Mt of product and generating $48.7 million in revenue (DFO 2006). Over the past decade, the productivity of the industry has been challenged by the rising costs associated with biofouling of aquaculture gear. Central to the problem are four recently arrived species of invasive tunicates (class Ascidiacea): *Styela clava* Herdman 1881, *Botryllus schlosseri* Pallas 1766, *Botrylloides violaceus* Oka 1927 and *Ciona intestinalis* L. 1758. Their establishment in PEI estuaries was facilitated by the high nutrient load and the large amount of suspended artificial substrates in the form of aquaculture gear in these systems (Locke et al. 2007). The number of invasive tunicates species in PEI waters will likely increase in the future; at least two other species have recently been found in nearby waters: *Didemnum vexillum* Kott, 2005 in Eastport, Maine, US (Jennifer Dijkstra, Wells National Estuarine Research Reserve, Wells, ME, USA, personal communication) and *Diplosoma listerianum* (Milne-Edwards, 1841) in the Magdalen Islands, QC, Canada, in 2008 (Sarah Stewart-Clark, University of PEI, pers. comm.).

Excessive fouling on aquaculture gear requires increased manpower for the treatment and maintenance of mussel leases (personal observation). *Ciona intestinalis* (Fig. 1) is the most

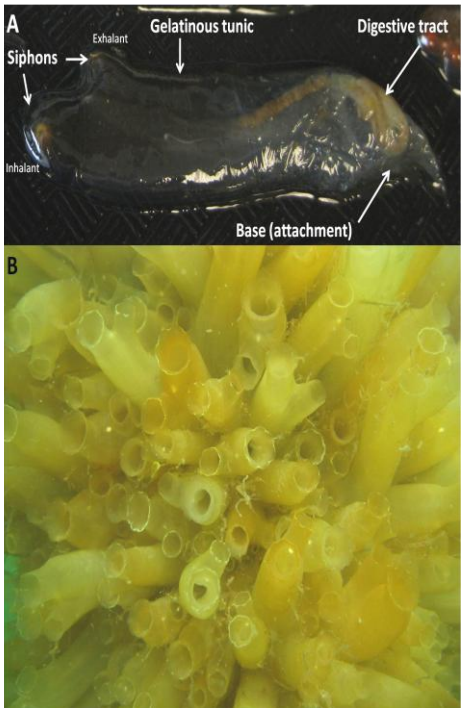

Figure 1. The solitary tunicate *Ciona intestinalis* with body parts indicated (A) and growing in large clusters in the field (B).

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detrimental fouler of aquaculture gear because of its rapid sexual maturation, short reproductive cycle, rapid growth (Ramsay et al. 2008), and the considerable weight it adds to aquaculture equipment (DFO 2006). Growers currently add mesh around their mussel socks (double-socking) to avoid crop loss and have implemented several methods of tunicate mitigation. These mitigation strategies focus on treating the mussel sock, causing mortality or removal of tunicates, primarily by application of high-pressure salt water (Carver et al. 2003).

An alternative approach to treatment is to reduce settlement and prevent the accumulation of fouling biomass. Anti-fouling paints or coatings are a traditional method of reducing fouling, but most contain compounds that have negative environmental effects such as copper or tri-butyl tin (Grinwis et al. 1998; Fisher et al. 1999; Armstrong et al. 2000; Katranitsas et al. 2003) and are not suitable for use in aquaculture (Braithwaite & McEvoy 2005). Non-toxic alternatives, such as paraffin wax, have been tested in PEI as anti-fouling coatings on buoys but did not persist on buoys over the winter months (Neil MacNair, PEI Department of Fisheries, Aquaculture and Rural Development, Charlottetown, PEI, pers. comm.). In this study, a food-grade oil with a melting temperature above the maximum water temperature in PEI (23 °C; DFARD 2008) was assessed as a potential anti-fouling on aquaculture gear. Solidified oil was expected to be more flexible and persistent in cold water than paraffin wax, and since it is a food product itself, it would likely not pose a food safety issue.

Reducing fouling on several types of mussel aquaculture gear such as buoys, socks and ropes used to collect mussel spat has multiple benefits for the industry: decreased labor and equipment costs by reducing the need for frequent fouling removal treatments; a decreased need for frequent replacement of heavily fouled buoys; preventing mussel fall-off on mussel socks; and avoiding space competition between mussels and fouling organisms on spat collectors. These substrates are widely used in mussel aquaculture operations and are comparable to substrates used in many other types of shellfish aquaculture. This information can also be complemented with data obtained from standardized surfaces, such as polyvinyl chloride (PVC) collector plates.

The objective of our study was to assess the efficacy of food-grade oil to reduce fouling on aquaculture gear utilizing a

Resumen

Las actuales estrategias de mitigación frente a los tunicados que actúan como invasores en las artes empleadas en viticultura - Isla del Príncipe Eduardo (Canada), - se restringen a la costosa eliminación manual e intensiva de las incrustaciones. En lugar de la eliminación, este estudio se centra en la prevención del establecimiento del tunicado *Ciona intestinalis* y de otros organismos incrustantes mediante la aplicación a maromas y exterior de mejillones, de una capa de grasa de calidad alimenticia con un punto de fusión superior a la temperatura ambiental habitual del agua. Mediante tests de laboratorio se estableció la adherencia y persistencia de la grasa y el establecimiento y evolución de *C. intestinalis* (peso y abundancia). La aplicación de grasa redujo el peso y abundancia de *C. intestinalis* en boyas, y dispositivos colectores (maromas y placas), pero no en las mallas de producción. Algas y tunicados incrustantes fueron reducidas significativamente en la mayoría de los sustratos. El tratamiento no provocó efectos negativos sobre la longitud de los mejillones ni sobre su abundancia, pero se redujo el peso total en las mallas de producción. La aplicación de grasa tiene un considerable potencial para reducir las incrustaciones de tunicados y otros organismos.

Palabras clave

Ciona intestinalis, mitigación, prevención, engrasado, especies marinas invasoras

combination of laboratory and field trials.

Methods

Oil adherence

Laboratory experiments were conducted to determine adherence of Golden All-Purpose Shortening (Dolphin Village, Canada) to different substrates over time. The shortening, a blend of partially hydrogenated soybean and cottonseed oil, was selected based on its relatively high melting point of 45-50 °C (ADM Packaged Oils 2010). Since temperatures of PEI estuarine waters have been recorded only as high as 23 °C, the shortening was expected to remain solid in the water throughout the summer season. All trials were conducted using artificial salt water at 26-27 ppt (Instant Ocean®).

To assess shortening adherence to rope, sections of dry or pre-soaked rope were dipped for five seconds in liquid shortening (heated to 87 °C) and then air-dried for 15 seconds (N = 3). In a separate experiment, similarly treated ropes (shortening heated to 48.7 °C) were placed in 1 L beakers filled with salt water (21-22 °C) and aerated to create water movement for visual observation of shortening adherence for a period of 5 days (N = 3).

Shortening adherence was also observed on 18 cm mussel sock sections and individual mussels, all of which were dipped into liquid shortening at 41.2 °C. For these trials, the water was aerated, changed every two days, and the mussels were fed daily with 2.3 mL of condensed algae paste (Innovative Aquaculture Ltd., Canada) per beaker. The presence of a shortening layer was monitored visually and by touch for 6 days until the experiment was terminated due to mussel mortality in both control and treated groups.

Treatment and deployment of experimental gear (field trial)

In order to test the efficacy of shortening treatments under field conditions, trials were conducted on a mussel lease in St. Mary's Bay (46° 07'32.22" N, 62°02'39.25" W) on the eastern shore of PEI in 2008 and 2009. In 2008, shortening was applied to styrofoam buoys (N = 5; 16 cm x 15.5 cm x 6.5 cm tapered; avg. 36 g shortening per unit), mussel sock sections (N = 6; 50 cm long; avg. 457 g shortening per metre), spat collector lines (N = 5; 180 cm long; avg. 22 g shortening per metre) and PVC collector plates (N = 15; 10 cm x 10 cm; avg. 13.6 g

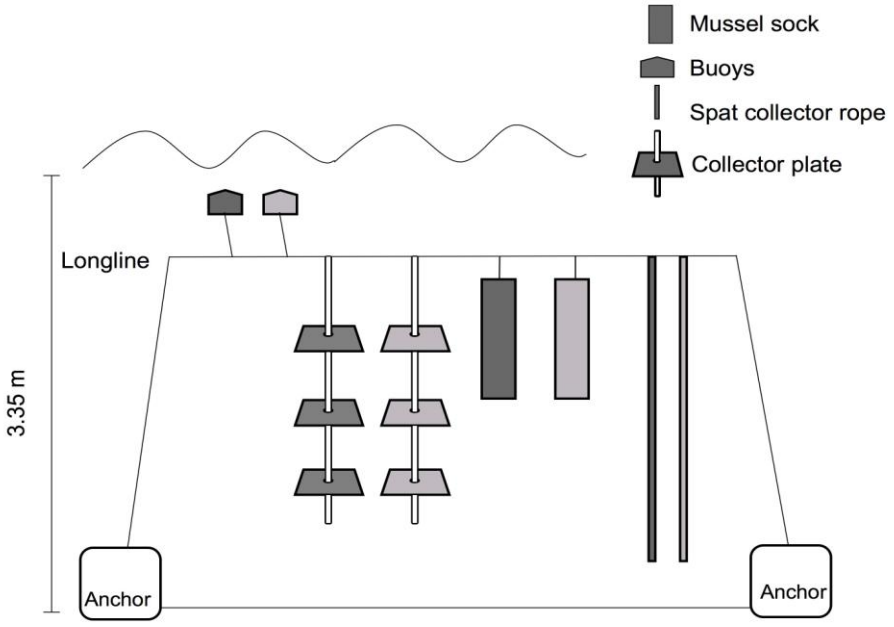

Figure 2. Experimental setup on a mussel longline in St. Mary's Bay, PEI. Mussel socks, buoys, spat collectors, and PVC collector plates were either dipped in shortening (treated) or left untreated (control). Replicates from each substrate were arranged alternating treated and untreated (indicated by different shading). Only one replicate per substrate and treatment is shown in this figure.

shortening per array of 3 plates), and matched with equal numbers of untreated substrates (controls). All substrates were deployed on an empty mussel long line between 8 and 11 July 2008. All substrates were weighed before and after treatment. Collector plates were deployed in arrays of three plates connected by a rope with 60 cm spacing between plates and a metal weight at the bottom (Fig. 2).

Collector plate arrays and buoys were treated in the laboratory by dipping them into liquid shortening (68 °C) followed by overnight storage at room temperature and deployment in the field the following day. Mussel socks were collected from St. Mary's Bay, pressure washed to remove existing fouling and shortened to 50 cm long sections. Mussel sock sections and spat collector ropes were dipped in liquid shortening (81.9 °C) in the field and attached to the experimental mussel long line. Treated and control substrates were alternated on the long line (Fig. 2) to ensure uniform experimental conditions such as water current direction and speed for all substrates. In 2009, five additional spat collectors (245 cm long) were treated with melted shortening, air-dried overnight and deployed on the same long line in St. Mary's Bay on 8 June. The treated spat collectors were alternated with five control spat collectors along the line with 50 cm spacing between collectors.

Field observations and sample processing

At the start of the 2008 field trial on 8 July, 90 untreated mussels were collected and measured to create a baseline for mussel length and allow growth comparisons between treated and control mussels post-deployment. All substrates were retrieved on 12 August 2008, weighed in the field and then brought to the laboratory for further analyses, taking care to avoid loss or mixing of biofouling from different substrates. In the laboratory, the fouling was removed from each substrate and sorted into three categories: "*C. intestinalis*", "Algae" and "Other Tunicates" (including the native *Molgula* sp. and the invasive *S. clava* and *B. schlosseri*). For the collector plates, only the fouling on the down-facing side was analyzed since this side is the preferred settling side for *C. intestinalis* and other invasive tunicates (personal observation). Measurements focused on tunicates greater than 5 mm in length only. The wet weight of organisms in each fouling category was recorded. All *C. intestinalis* were frozen and thawed before measuring length from base to siphon to avoid contraction of live animals leading to inaccurate length data. *C. intestinalis* were then counted. Where *C. intestinalis* abundance was very high, length and abundance of subsampled animals were recorded. The abundance of all mussels and the length of a subsample of 30 randomly chosen mussels per sock were recorded.

During the 2009 trial, spat collectors were retrieved on 29 September and analyzed as described above with the following minor modifications: Due to large amounts of mussel spat on the collectors, mussel abundance was determined using a linear equation obtained from plotting weight against abundance of subsamples of 20 to 100 randomly selected mussels. Since time did not permit complete processing of fresh samples, thawed weights instead of fresh weights of algae and other tunicates were taken after the material underwent one freeze-thaw cycle. In addition, no tunicate length measurements were taken during this trial.

Statistical analyses

Statistical analyses were performed using Minitab 15 and SPSS software. For the field trials, the Student's t test compared treated and control substrates when the assumptions (normality and homogeneity of variances within treatment groups) for parametric tests were satisfied. If necessary, data were square-root transformed (*C. intestinalis* density on mussel

socks, *C. intestinalis* fouling weight on buoys and algae fouling weight on buoys) or log transformed (*C. intestinalis* fouling weight on spat collectors). For data that could not be normalized by transformation (length of *C. intestinalis* on mussel socks), the non-parametric Mann-Whitney test was used. For collector plates, data from the three plates per array were averaged as they reflected the natural variation in fouling observed among the top, middle and bottom sections of mussel socks. *C. intestinalis* lengths from all 15 collector plates in each treatment group were averaged for two-way ANOVA (with treatment and plate location (top, middle, bottom of collector set) as factors) to compare the effect of shortening treatment on *C. intestinalis* growth. Weight and abundance data collected for mussel sock sections and spat collectors in the field experiment were standardized to 1 m to account for variations in length. To determine mussel growth, the average baseline mussel length was subtracted from each average replicate mussel length in the control and shortening-treated group post-treatment; significant differences in growth between the two groups were determined using the Student's t test. Power analyses were conducted in those variables where non-significant differences were observed between treated and control groups. To confirm t-test results in cases when normality assumptions could not be met we used a permutation test (which is independent of the distribution of the data) with 1000 randomizations. If the permutation test p-value was close to 0.05, the test was repeated with 10,000 randomizations. Since permutation tests confirmed all t-test p-values, only the latter are reported in this paper.

Results

Shortening adherence

Shortening formed a visible layer on rope sections which persisted for the five days observed in salt water in the laboratory. An average of 1.6 g of shortening adhered to 9.5 mm polypropylene rope sections. Although not quantified, there appeared to be a lower adherence to mussels in comparison to the other substrates.

During the 2008 field trial, a substantial amount of shortening adhered to the experimental substrates before their deployment, averaging 457 g m^{-1} on mussel sock sections, 13 g per collector plate array, 36 g per buoy and 22 g m^{-1} on spat collectors. Once deployed, the shortening was clearly visible on all substrates (Figs. 3 & 4, buoys and collector plates not shown). SCUBA diving observations in 2008 suggest that the layer of shortening remained adhered to treated spat collectors, buoys and collector

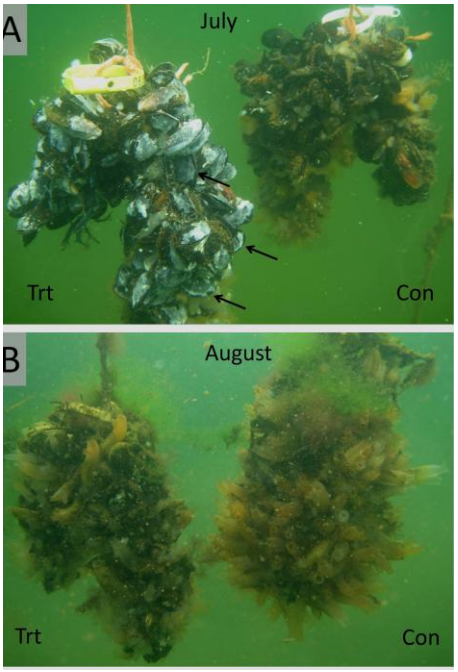

Figure 3 (left)**.** Representative experimental mussel sock sections (A) four days following deployment in July 2008 and (B) immediately prior to retrieval in August 2008. Mussel sock sections were either dipped in melted shortening (Trt) before deployment or left untreated (Con). Arrows indicate examples of shortening receding from the mussel valve edges.

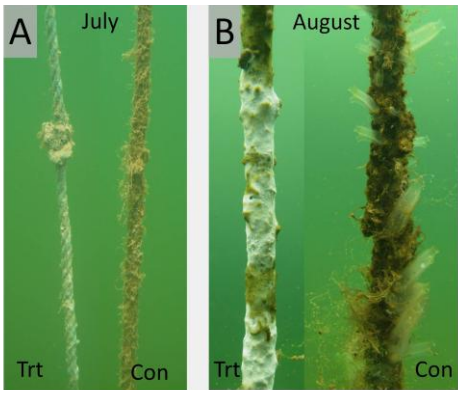

Figure 4 (right)**.** Representative experimental spat collector ropes (A) four days following deployment in July 2008 and (B) immediately prior to retrieval in August 2008. Spat collector ropes were either dipped in melted shortening (Trt) before deployment or left untreated (Con).

plates during the four-week study period (Figs. 4-6) but that the shortening was beginning to recede from the valve edges of mussels on mussel socks four days after deployment (Fig. 3A). The layer of shortening remained present on spat collectors for the duration of the 17 week field trial in 2009 and was still visible during the subsequent processing of samples.

Effect of shortening on settlement and growth of fouling organisms

In the 2008 field trial, *C. intestinalis* wet weight was significantly reduced relative to controls on all shortening-treated substrates except mussel sock sections (Fig. 7) and these

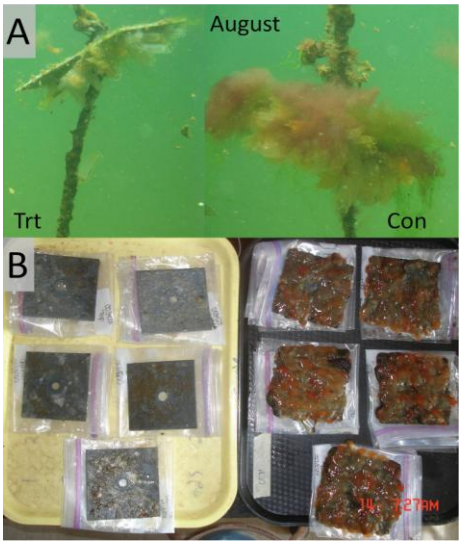

Figure 5. Experimental collector plates immediately (A) prior to and (B) after retrieval in August 2008, following a four-week deployment in St. Mary's Bay, PEI. Collector plates were either dipped in melted shortening (Trt) before deployment or left untreated (Con) .

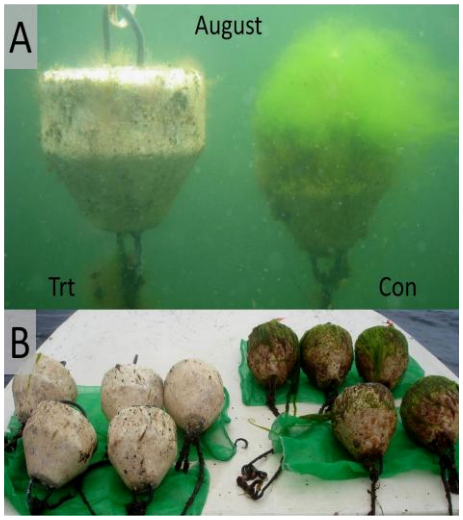

Figure 6. Experimental buoys immediately (A) prior to and (B) after retrieval in August 2008, following a four-week deployment in St. Mary's Bay, PEI. Buoys were either left untreated (control) or dipped in melted shortening (treatment) before deployment.

reductions were clearly visible on the substrates (Figs. 4-6). Reductions were 92 % on spat collectors, 99 % on collector plates, and 95 % on buoys ($p<0.001$ for all comparisons) (Fig. 7). The mean *C. intestinalis* weight per metre was 38 % lower on treated mussel socks than on control socks but this difference was not statistically significant ($p=0.298$).

The abundance of *C. intestinalis* on all treated substrates was significantly lower than on control substrates in 2008 (Fig. 8). Spat collectors, collector plates, buoys and mussel sock sections had reductions of 92 % ($p<0.001$), 86 % ($p<0.001$). 95 % ($p=0.001$) and 32 % ($p=0.028$) respectively (Fig. 8). *C. intestinalis* lengths were also 78% less on treated collector plates than on controls ($p<0.001$) (Fig. 9).

In 2009, similar reductions in *C. intestinalis* biomass and abundance were found on shortening-treated spat collectors. *C. intestinalis* wet weight and abundance were 96 % lower ($p=0.006$) (Fig. 7) and 95 % lower ($p<0.001$) (Fig. 8) respectively, on treated spat collectors compared to controls.

In 2008, algal wet weight was significantly reduced on treated buoys and spat collectors but not on treated mussel socks when compared to controls (Table 1). Compared to controls, an 87% reduction in wet weights of other tunicates was observed on treated buoys, but not on the other substrates (Table 1). In 2009, algal wet weight on shortening-treated spat collectors was not significantly different from controls. The biomass of other tunicates also did not differ between shortening-treated and control substrates in the 17-week field trial in 2009.

Four weeks after deployment in 2008, the fouling associated with control mussel socks was dominated by *C. intestinalis*, with 834.1 g m^{-1} in average wet weight in comparison to 76.5 g m^{-1} and 2.5 g m^{-1} average wet weight for algae and other tunicates, respectively. Algae made up the

heaviest fouling category on control buoys and control spat collectors (59.4 g and 33.0 g, respectively), compared to *C. intestinalis* (9.5 g and 12.5 g respectively) and other tunicates (4.7 g and 0.6 g, respectively). Compared to *C. intestinalis* and algae fouling, other tunicate weight was negligible, never exceeding an average of 5 g per substrate.

Effect of shortening on mussels

Mussel length, growth and density were not significantly different between controls and treated socks, but mussel biomass was 23 % lower on treated mussel socks than on control socks (Table 2). Spat settlement in 2008 was unusually low on both control and treated spat collectors (Table 2). In contrast, more spat was noted on untreated sections of the ropes supporting the collector plates than on rope segments that had been dipped into shortening along with the collector plates. In 2009, mussel spat settlement was observed on both control and treated spat collectors. There was no significant difference in the mussel biomass or abundance between treated and control spat collectors (Table 2).

Discussion and Conclusions

Efficacy of shortening at reducing C. intestinalis and other fouling

Shortening effectively reduced *C. intestinalis* biomass and abundance on spat collector ropes for a period of 17 weeks, and on buoys, mussel sock sections and collector plates for a period of four weeks. Shortening was also effective at reducing the amount of other

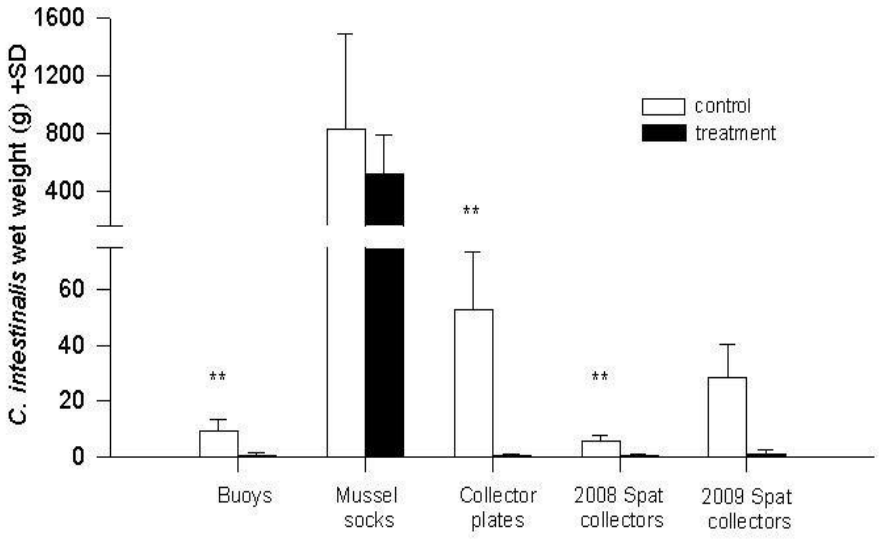

Figure 7. Effect of shortening treatment on *C. intestinalis* wet weight on four substrates. Mussel sock data and spat collector data are standardized to 1 m. Differences between shortening-treated and control groups for each substrate were detected using Student's t test. * indicates $p < 0.05$, ** indicates $p < 0.001$. SD = standard deviation.

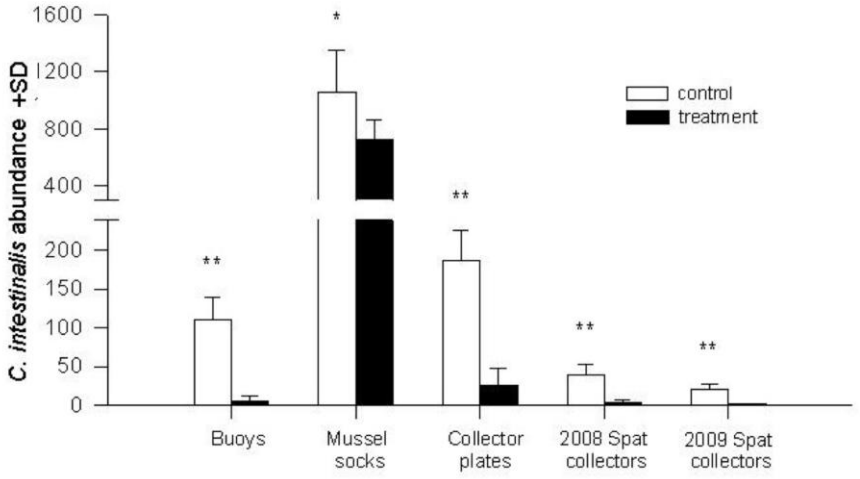

Figure 8. Effect of shortening treatment on *C. intestinalis* abundance on four substrates. Mussel sock data and spat collector data are standardized to 1 m. Differences between shortening-treated and control groups for each substrate were detected using Student's t test. * indicates $p < 0.05$, ** indicates $p < 0.001$. SD = standard deviation.

fouling organisms (algae and other tunicates). Fouling reduction was especially high on buoys and spat collector ropes, making shortening a potentially useful commercial treatment against biofouling on these substrates. Since mussel farmers continue to try various methods for treating algae fouling on spat collectors, identifying the capacity of shortening to reduce algae fouling as well as tunicate fouling is particularly important (Sharp et al. 2006).

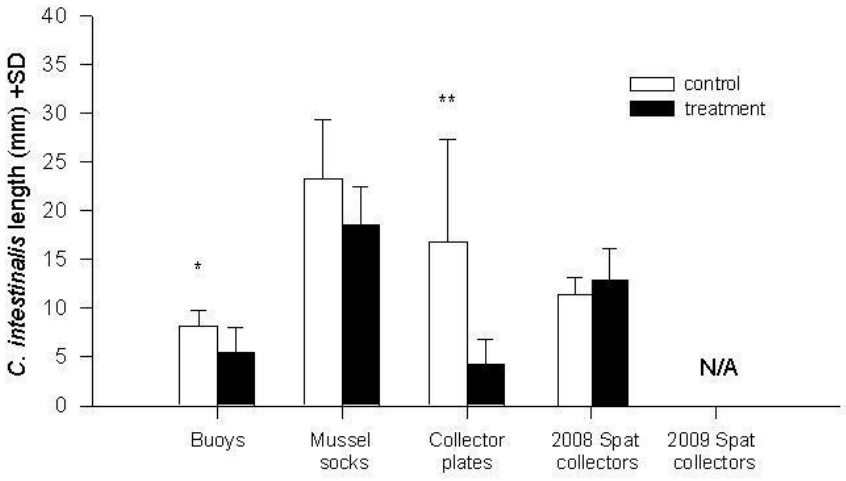

Figure 9. Effect of shortening treatment on *C. intestinalis* length on four substrates. Differences between shortening-treated and control groups for each substrate were detected using two way ANOVA for spat collectors and Student's t test for all other substrates. * indicates $p < 0.05$, ** indicates $p < 0.001$. SD = standard deviation, N/A = data not available.

Substrate	Fouling category	Avg. wet weight (g) (SD)		Difference	p level
		Control	Shortening	(%)	
Buoys	Algae	59.4 (34.1)	1.3 (1.9)	-97.8	< 0.01
	Other tunicates	4.7 (1.9)	0.6 (1.2)	-87.2	< 0.01
Spat collectors	Algae	12.9 (4.6)	5.6 (2.6)	-56.6	< 0.05
	Algae (2009 data)	37.8 (10.7)	47.3 (33.8)	25.1	ns
	Other tunicates	0.3 (0.3)	0.4 (0.8)	33.3	ns
	Other tunicates*	4.6 (1.9)	3.6 (2.7)	-22.7	ns
Mussel sock sections	Algae	76.5 (40.6)	60.6 (29.5)	-20.8	ns
	Other tunicates	2.5 (1.6)	1.1 (1.3)	-56.0	ns

Table 1. Effect of shortening treatment on the biomass (g wet weight m^{-1}) of other tunicates (excluding *C. intestinalis*) and algae on three experimental substrate types. The % difference indicates ($weight_{(shortening)}$ - $weight_{(control)}$) *100/$weight_{(control)}$. Data presented here are untransformed irrespective of transformation for statistical purposes. Significance determined with Student's t test. SD = standard deviation, ns = not significant, i.e. $p > 0.05$.

While we observed a 38% decrease in *C. intestinalis* biomass on treated socks, the difference was not significant. This result is likely due to low statistical power (19.5%), a consequence of the high level of variation among replicates. One reason for the high variation may have been incomplete cleaning of mussel socks prior to treatment. Also, mussel socks were the only substrate that was not dry when treated, possibly affecting the adherence and efficacy of the shortening.

The shorter *C. intestinalis* found on treated collector plates suggest that the shortening is either retarding growth or delaying settlement of *C. intestinalis*. Future trials should study the settlement behaviour of *C. intestinalis* larvae on shortening covered substrates to determine whether the shortening acts as a repellent, affects growth of settled animals or affects *C. intestinalis* length through a combination of both.

Substrate	Year	Treatment		p level
		Control	Shortening	
Mussel sock sections				
Avg. mussel length (mm)	2008	47.6	45.5	ns
Avg. mussel growth (mm)	2008	5.4	3.3	ns
Avg. mussel density (no. per metre)	2008	426	379	ns
Avg. mussel biomass per metre (g)	2008	3878.9	2991.7	< 0.05
Spat collectors				
Avg. mussel spat biomass per metre (g)	2008	N/A	N/A	N/A
	2009	215.03	166.99	ns
Avg. mussel spat density (no. per metre)	2008	N/A	N/A	N/A
	2009	67.06	57.94	ns

Table 2. Effect of shortening treatment on mussel biomass, density and length on two substrate types. Mussel sock sections and spat collectors were either dipped in shortening or left untreated (control). Substrates were deployed on a mussel lease for 4 weeks in 2008 and for 17 weeks in 2009. Significance determined with Student's t test. N/A: not available because of little to no spat settlement on the lines in 2008; ns = not significant.

Effects of shortening on Mytilus edulis

In this study, mussel growth rates were not significantly reduced by treatment, though impact on growth would not be unexpected. Mudge et al. (1993) found that oil pollution increased fatty acid concentration in mussel tissue. Long term effects of shortening treatment on mussel growth need to be further evaluated to ensure that the treatment would not negatively impact the grow-out time or market value of the mussels. Decreased mussel growth resulting from heat stress due to the 81.9 ºC oil dip may have been a contributing factor to the decrease in mussel biomass seen on treated socks. Few linkages between mussel mortality and food grade oil have been identified in the literature. For example, following the sinking of the M.V. *Kimya* off the coast of Wales in 1991 and the spillage of 1500 t of its cargo of sunflower oil, no direct toxic effect on mussels was detected (Mudge 1995).

When spat collectors were deployed in 2009, mussel spat settled freely on both treated and control spat collectors, indicating that shortening does not deter spat settlement and could potentially be used to reduce tunicate fouling on spat collectors. We do not have an explanation for the observed difference in settlement of *C. intestinalis* larvae and *M. edulis larvae* on treated spat collectors. Potentially, mussel larvae can move around until they find a suitable settling spot, in this case a space not covered by shortening (Maas Geesteranus 1942). The reason for lack of mussel spat settlement on any of the spat collectors in 2008 is unclear since mussel larvae abundance in St. Mary's Bay was high during the study period (DFARD 2008) and spat was actually observed on adjacent ropes holding PVC collector plates. Due to the lack of consistency between the two field trials conducted, the use of shortening treatment on spat collectors needs to be further evaluated before any practical application can be proposed.

Suitability of different food-grade oils

Based on results from the field deployment, shortening is an effective inhibitor of biofouling on several aquaculture substrates. Shortening formed a solid layer in laboratory and field trials, similar to what was observed before with paraffin wax (N. MacNair, pers. comm.). However, the shortening appeared to be more persistent than paraffin wax. The thickness of the oil layer may not be solely responsible for the oil's anti-fouling properties documented in this study. The ability of an oil to soak into the substrate may also play a role in deterring fouling. Better methods of evaluating adherence of oils should be developed so that the

dip and drying times can be optimized. While results from the present study are promising, continued adherence and anti-fouling efficacy of shortening need to be evaluated over longer deployment periods. A range of other food grade oils could possibly be tested, though certain key characteristics such as high melting point, food grade, persistence and reasonable cost would still be regarded as relevant. Identifying the mechanism that precludes fouling settlement on oil-treated substrates is critical. This mechanism may be physical (the oil forming an unsuitable substrate for attachment of fouling organisms) or chemical (the oil deterring settlement of fouling organisms in a similar way as copper coatings act) (Borkow & Gabbay 2005).

Applicability of treatment in aquaculture operations

The shortening treatment worked particularly well at reducing fouling levels on buoys and could thus be beneficial for the aquaculture industry. Buoys can be easily treated before their deployment, avoiding the need for a heat source and other necessary equipment to melt the oil on boats. The cost of shortening application was CAN$0.10 per buoy, CAN$0.06 per metre of spat collector rope, CAN$1.29 per metre of mussel sock and CAN$0.04 per three collector plates. Treatment of spat collectors prior to spat collection is not suggested until further studies addressing the conflicting 2008-2009 results are completed. Treating spat collectors once mussels have settled may be useful pending studies on effects of oil and acute high temperature exposure on mussel spat. The addition of almost 1 kg *C. intestinalis* biomass per metre of mussel sock in the course of just four weeks emphasizes the need for fouling prevention rather than mitigation. The environmental impact of high concentration of shortening-coated gear in estuaries is yet another important aspect to consider before shortening is considered as an anti-fouling treatment on a larger scale.

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Bio-sketch

Jiselle A. Bakker

is interested in the management of invasive species.

S. Christine Paetzold

is a project manager in the field of invasive species mitigation research at the University of Prince Edward Island.

Pedro A. Quijón

works as Associate Professor of marine ecology working on coastal habitats of Prince Edward Island and Atlantic Canada.

Jeff Davidson

is a Professor in the Department of Health Management, Atlantic Veterinary College, University of PEI. His areas of interest include health and production medicine of molluscs and shrimp, aquatic invasive species and ecohealth.

Authors

BAKKER, Jiselle A. [(1)]

PAETZOLD, S. Christine [(1)]

QUIJÓN, Pedro A. [(2)]

DAVIDSON, Jeff [(1)]

[(1)] Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave., Charlottetown, PEI, Canada C1A 4P3

[(2)] Department of Biology, University of Prince Edward Island, Charlottetown, PEI, Canada C1A 4P3