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Research Article

Treatments to eradicate invasive tunicate fouling from blue mussel seed and aquaculture socks

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Abstract

Tunicates can foul blue mussels and negatively affect productivity on mussel farms. In New England and elsewhere, invasive species of colonial tunicates commonly foul wild and cultured blue mussels and aquaculture gear. Eco-friendly experimental treatments that meet industry guidelines were selected for trial application. Chemical (acetic acid) and water (brine and freshwater) treatments were applied in short-term and long-term applications to juvenile mussels that either were or were not exposed to tunicates. Acetic acid baths (5 mins and 10 mins) were lethal to juvenile mussels. Brine baths killed tunicates, but caused relatively high mussel mortality, though less mussel death occurred in the short-term (10 sec) brine bath (6–17%) compared to the long-term (20 sec) brine bath (8–30%). Both long-term (24 hr) and short-term (8 hr) freshwater baths were effective against tunicates, with less mussel mortality (2%) occurring in the short-term bath. Tunicates survived short-term freshwater sprays but not long-term freshwater sprays. Long-term (10 mins) freshwater sprays caused slightly more mussel mortality (4%) than short-term (5 mins) freshwater sprays. Each treatment demonstrated varying degrees of effectiveness, yet the freshwater short-term baths and sprays were able to remove tunicates while maintaining high survivorship among juvenile mussels. Additionally, freshwater treatments do not require the use or disposal of chemicals.

Key words: Ascidiacea, invasive species, *Mytilus edulis*, aquaculture, freshwater, brine, acetic acid

Introduction

Blue mussels *Mytilus edulis* are native to the North American coast and often occur abundantly in shallow water environments around Martha's Vineyard, Massachusetts, where the shellfish market and demand for mussels is established and aquaculture of the blue mussel is still new. Shellfish farmers take advantage of the wild mussel population by collecting mussel seed for socking, yet some of the most promising seed collecting sites tend to be associated with invasive species of tunicates (Ascidiacea, also called sea squirts). A suite of non-native tunicates have invaded southern New England coastal waters (Carman and Roscoe 2003; Pederson 2005; Bullard et al. 2007), including the solitary species *Ascidiella aspersa* (D.F. Müller, 1776) and *Styela clava* Herdman, 1881 and the colonial species *Botrylloides violaceus* Okra, 1927, *Botryllus schlosseri* (Pallas, 1766), *Didemnum vexillum* Kott, 2002, *Diplosoma listerianum* (Milne-Edwards, 1841) (Carman et al. 2010). Tunicates foul artificial and natural substrates, as well as aquaculture gear and cultured and wild bivalves. Invasive tunicates negatively impact mussel growth, feeding, and condition, and thus affect farm productivity and profitability. Tunicate fouling results in reduced shell growth and tissue weight in *Mytilus galloprovincialis* Lamarck, 1819 (Sievers et al. 2013), competition with blue mussels (and bay scallops) for the same phytoplankton nutrients (Colarusso et al. 2016, but see Lesser et al. 1992), and when tunicate growth

is very heavy, can make it difficult for bivalves to open to filter feed (M. Carman, personal observation). Additionally, because blue mussels are presented at market in the shell, shells fouled with tunicates are unattractive to consumers and reduce market value. Consequently, the removal of tunicates helps maximize shellfish growth and survivorship (Fitridge et al. 2012; Aldread and Clare 2014) and improves product appeal and market readiness.

Aquaculture permits in Massachusetts specifically prohibit transferring seed with invasive tunicates because they can have negative economic (Carman et al. 2010; Adams et al. 2011) and ecologic (Morris and Carman 2012) effects on shellfish resources. Aquaculturists in the Northeast US (Carman et al. 2010), Prince Edward Island, Canada (PEI) (Locke et al. 2009), British Columbia, Canada (Switzer et al. 2011), New Zealand (Coutts and Sinner 2004; Forrest et al. 2007) have struggled to contain the cost of managing invasive tunicates that plague their farms. Biological, mechanical, chemical and water (brine and freshwater) treatments have mixed results in ridding invasive tunicates from shellfish and gear without causing harm to shellfish.

Invasive tunicates have few natural predators and biological control experiments have had little success in controlling invasive tunicate populations. In PEI, rock crabs *Cancer irroratus* (Say, 1817) and green crabs *Carcinus maenas* (Linnaeus, 1758) consume a limited number of the solitary tunicate *Ciona intestinalis* (Linnaeus, 1767) (Carver et al. 2003). The periwinkle snail *Littorina littorea* (Linnaeus, 1758) eats *D. vexillum* when the tunicate is senescent (Carman et al. 2009). The neogastropods, *Mitrella lunata* (Say, 1826) and *Anachis lafresnayi* (P. Fischer and Bernardi, 1856), prey on larval recruits of *A. aspersa, B. schlosseri, D. listerianum* and *S. clava*, but do not readily consume adult forms of these species, nor do they eat the recruits or adults of *B. violaceus* (Whitlatch and Osman 2009). The green sea urchin *Strongylocentrotus droebachiensis* (O.F. Müller, 1776) did not successfully reduce tunicate populations in British Columbia (Switzer et al. 2011).

Mechanical treatments employed to destroy tunicate fouling include exposure to air, scraping, scrubbing, sweeping, brushing or tumbling, and power washing. Air-drying is a practice commonly used by North American East Coast aquaculturists to rid tunicates and other fouling organisms from gear and shellfish, often with some product loss (Bullard and Carman 2009; Carman et al. 2010). Exposure to sun and variations in air temperature and relative humidity are factors that contribute to shellfish survival after drying. Furthermore, air-drying is not possible for some types of aquaculture gear such as that suspended from longlines. Air-drying may also not be fully effective, as *S. clava* attached to boat hulls can survive out of the water for 48 hours (Darbyson et al. 2009).

Hand scraping or scrubbing tunicates off aquaculture floats (Chou 1991), scraping individual oysters with a knife or brush, or tumbling (using the sharp margins of the oysters shells to chip or cut pieces of tunicate off other shells), all require considerable labor. It may also not always remove the tunicates. For example, scrubbing with soft-wire brushes does not completely remove *D. vexillum* from oysters because the tunicate grows into oyster shell crevices (Switzer et al. 2011).

Power washing with seawater has been used with some success and is frequently used (Chou 1991; Arens et al. 2011; Paetzold et al. 2012). However, power washing breaks *B. violaceus* and *D. vexillum* colonies into fragmented pieces. If returned to the water, these tunicate fragments can reattach and grow (Bullard et al. 2007; McCarthy et al. 2007; Valentine et al. 2007; Paetzold and Davidson 2010; Morris and Carman 2012). As power washing is often done boat-side or dockside, collecting and disposing tunicate fragments to reduce return to seawater may be costly and infeasible.

Chemical treatments used on tunicates include bleach, hydrated lime, and acetic acid. In New Zealand, dilute bleach dips are effective against *D. vexillum* on the green mussel *Perna canaliculus* (Gmelin, 1791) (Denny 2008); however, bleach treatments are not permitted in US aquaculture. Morse and Rice (2010) recommend that New England blue mussel culture lines be lifted out of the water, sprayed with 5% hydrated lime-seawater solution and air-dried for a short period. But, short term (4 min) hydrated lime (4%) is not 100% effective against *D. vexillum* on oysters, and causes some oyster mortality (Switzer et al. 2011). Disposal of large quantities of spent lime may also be problematic (Rolheiser et al. 2012).

White vinegar (3–5% acetic acid) baths and sprays have been applied to aquaculture gear, shellfish, and tunicates with mixed results (Carver et al. 2003; Forrest et al. 2007; Locke et al. 2009; Piola et al. 2010). Acetic acid (5%) dips for 30 seconds have been found to be 95% effective against *C. intestinalis* (Carver et al. 2003). Tunicate-fouled ropes (as a surrogate for tunicate growth on green mussel seed) exposed to acetic acid (2% and 4%) for <4 minutes,

followed by 24 hours of air exposure has also had positive results (Forrest et al. 2007). Pieces of foam buoys used in blue mussel aquaculture dipped in acetic acid (5%) for 5 or 10 seconds followed by 10 seconds of air-drying resulted in 5–10% survival (10 sec dip) and 30% survival (5 sec dip) for *C. intestinalis* (Locke et al. 2009). Acetic acid (5%, 10%, and 20%) baths lasting 0.5, 3, and 6 hours have 75–100% success against colonial and solitary tunicates on settling plates (Piola et al. 2010). Disposal of spent acetic acid may or may not be a problem because of reduced acidity after use and dissipation in the sea (Locke et al. 2009).

Water treatments, including brine and freshwater, may be useful in controlling tunicates. Tunicates naturally occur in marine conditions (van Name 1945), and invasive tunicates can tolerate a broad range of salinities (Dijkstra et al. 2008). Mussels tolerate exposure to freshwater for several days (Lützen 1999), but it is unknown whether they can tolerate exposure to hypersaline conditions or brine. Brine baths (>32 ppt), which are recommended for the control of the boring sponge in cultured oyster shells (Carver et al. 2010), may be effective against tunicates. Hypersaline cold shock treatments destroy soft-bodied organisms (flatworms) on oysters (Cox et al. 2012) and may kill tunicates. During low tide, *D. vexillum* can survive up to 2 hours exposure to air and to freshwater precipitation (Valentine et al. 2007), and freshwater treatments lasting 5 or 20 minutes do not reduce *D. vexillum* fouling (Rolheiser et al. 2012). Effective air exposure and freshwater treatments against *D. vexillum* should probably last longer than 2 hours. *B. schlosseri* is a euryhaline species and may survive exposure to freshwater flux in upper estuary habitats (Brunetti et al. 1980).

The US Natural Resources Conservation Service (2011) issued environmentally appropriate fouling control standards for bivalve aquaculture gear and fouling control. The approved methods are air-drying, brine, vinegar, freshwater, sweeping, or power washing. However, the published standards lack details on duration of exposure and the percentage of mussel loss for any of the suggested methods. The goal of our study was to test whether juvenile mussel mortality differed after exposure to a variety of these treatments. We conducted short-term and long-term chemical treatment (acetic acid) and water treatment (brine, saltwater, freshwater) trials on socks of juvenile blue mussels with and without invasive colonial tunicates.

Methods

Approximately 10,000 juvenile blue mussels were collected from the bottom of a floating aquaculture platform in Menemsha Pond, Martha's Vineyard, on June 11, 2012, placed in buckets of seawater with aerators, transported by boat to Woods Hole, and put in flow-through seawater tanks at the Marine Biological Laboratory (MBL) Loeb Lab. On June 28, mussels that were 15–25 mm in shell length were placed into 150 black plastic mesh aquaculture socks (8 cm^2, 3 mm mesh opening) with white plastic clasp closures. The number of mussels per sock ranged from 14 to 126, but the volume of mussels in each sock was held constant (15 to 17 ml per sock).

On July 2, colonies of *B. violaceus* and *D. vexillum* were collected from Eel Pond, a saltwater pond adjacent to the MBL, and approximately 3 cm^2 pieces were cut from healthy-looking colonies of each species. One cut piece of each species was placed in 60 of the socks with mussels. The other 60 socks had no added tunicate pieces; the total number of mussels in the 120 socks was 8,142. All 120 socks were secured to lines attached to a MBL floating dock on Eel Pond and suspended between 0.5 and 1 m water depth for 2 weeks to give tunicates and mussels time to acclimate. The dock was fouled with a diversity of tunicate species including *B. violaceus* and *D. vexillum*. Thus, we expected additional new tunicate growth to occur on some of the socks over the two-week period.

Eighty socks, (40 with and 40 without added tunicate pieces) were arbitrarily assigned for chemical or water treatment (see below) and 40 socks (20 socks with and 20 socks without added tunicate pieces) were treated to test for handling effects (left in Eel Pond, air-dried for 1 hour, seawater bath for 24 hours, and seawater spray for 10 min). Socks were examined and assessed immediately before treatment for the presence of tunicate recruits. Before the application of chemical and water treatments, healthy-looking adult colonies of *B. violaceus*, *D. vexillum* were observed on the socks that we had inoculated with tunicates, and a few new recruits of *B. violaceus, D. vexillum, B. schlosseri, D. listerianum, A. aspersa,* and *C. intestinalis* were found on some of the socks.

Chemical and water treatments

To treat socks in an acetic acid bath, a sock was placed in a small plastic tub filled with white

vinegar (5% acetic acid) at room temperature for either 10 minutes (long-term) or 5 minutes (short-term).

To treat socks with a brine bath, baths (70 ppt salinity) were created by mixing commercial table salt to lab seawater in a small tub. Salinity was measured with a hand-held refractometer. For treatments, a sock was added to the Brine Bath for either 20 sec (long–term exposure) or for 10 seconds (short-term exposure).

A sock assigned to the freshwater bath treatment was immersed in a small plastic tub filled with fresh tap water for either 24 hours (long-term exposure) or 8 hours (short-term exposure). During exposure periods, freshwater was continually dripped into the tubs to ensure aeration.

For freshwater sprays, a sock was placed in a small plastic tub and sprayed with a garden hose for 10 min (long-term exposure) or 5 min (short-term exposure). The rate of flow of freshwater spray was maintained at 5 liters/28 seconds (5.6 sec per liter).

Experimental design

On July 9, 2012, we began an assay to compare the effects of added tunicates on mussel survival. A total of 20 socks were used, five for each treatment. Socks were either left suspended from the dock, or air dried for 1 hour and then returned to the water. Treatments included: 1) socks with added tunicates left in the water, 2) socks without tunicates left in the water; 3) socks with added tunicates air dried; 4) socks without tunicates air dried. Mussel survival and tunicate condition were evaluated after 1 week.

Because of the large number of chemical and water treatments, the second assay made use of a two-factor randomized block design. Each of 8 treatments was composed of one sock with tunicate pieces and one sock without tunicate pieces, with the assay being replicated over 5 separate days, starting on 9 July. For analysis, Day is the blocking factor, and the two fixed factors are the addition of tunicates and the chemical or water treatments.

On 9 July, eight socks with added tunicate pieces and eight socks without added tunicate pieces were removed from the dock. One sock from each group received one of the chemical (acetic acid, brine), freshwater treatments (bath or spray), or seawater (bath or spray) treatments. After treatment, all socks were air-died for one hour in the laboratory and were then placed on one of four lines suspended between 0.5 m and 1 m water depth at the MBL dock on Eel Pond. On 10–13 July, the same procedure was conducted on another 8 socks with tunicate pieces and 8 socks without tunicate pieces. The sock with the 24-hour freshwater spray treatment and the sock with the 24-hour seawater treatment applied on 13 July were returned to Eel Pond on 14 July.

After socks were suspended on lines in Eel Pond for one week (July 16–20 respectively, +1 day each for 24-hour freshwater bath treatments), socks were examined in the lab to assess mussel survival and tunicate condition. The largest and smallest mussel in each sock were also measured.

The survival of the mussels was determined by examining each mussel for signs of life. A mussel was considered dead if any of the following conditions was observed: shell was empty, the tissue was putrefied, the shell did not close upon touch, or the shell did not close when gently pried by a technician wearing gloves. Tunicates less than 4 mm were considered to be new larval recruits younger than 1 week old. Healthy looking tunicates greater than or equal to 4 mm on socks or mussels were identified to species and considered to have survived a given treatment. Tunicates were categorized as dead if they were absent, putrefying, or not attached to a mussel or a sock.

Water temperature and salinity

Seawater and freshwater temperature and salinity measurements were taken at the lab and dock at the beginning and end of the treatment trials to ensure that socks were kept in water of similar temperature during the experiment and that seawater at the lab and dock were similar salinity.

Statistical analysis

The proportion of mussels that survived in the 1 hour Air-dry versus Remain-in-Eel Pond assay, were analyzed using a two-way ANOVA with Tunicate Added as one fixed factor and Treatment as the second fixed factor. The number of mussels was also compared between treatments in a two-way ANOVA to determine whether some treatments had more mussels (likely smaller mussels) than other treatments. A higher number of mussels in a sock would suggest that the average size of the mussels in that sock was smaller than in other socks, and smaller mussels might be more vulnerable to some treatments.

Table 1. Mean and standard deviation of the proportion of mussels that survived per sock and of the initial number of mussels per sock in the control treatments and tunicate treatments.

Treatments	Proportion survived		Initial mussel number	
	Mean	Std dev	Mean	Std dev
Remain in Eel Pond – Tunicate	0.985	0.009	65.200	7.563
Remain in Eel Pond – No Tunicates	0.993	0.009	56.800	7.014
Air Dry- 24 hr – Tunicate	0.956	0.030	63.200	12.834
Air Dry- 24 hr – No Tunicates	0.992	0.011	56.600	22.700

Table 2. Mussel survival per sock. ANOVA results testing whether mussel survival differed between control treatments Remain in Eel Pond and Air dried (1 hr).

Source	Sum-of-Squares	df	F-ratio	P
Tunicate	0.051	1	10.008	0.006
Control	0.012	1	2.418	0.140
Control x Tunicate	0.010	1	1.959	0.181
Error	0.081	16		

Table 3. Mussels per sock. ANOVA results testing whether the mean number of mussels per sock at the start of the experiment differed between treatments Remain in Eel Pond and Air dried 1 hr and the tunicate treatments.

Source	Sum-of-Squares	df	F-ratio	P
Tunicate	0.109	1	1.991	0.177
Control	0.013	1	0.230	0.638
Control x Tunicate	0.000	1	0.006	0.939
Error	0.873	16		

Mean mussel survival was compared among the eight chemical or water treatments applied to socks with and without added pieces of tunicates with a three-way ANOVA (randomized blocks design with days as the blocks). An arcsin square root transformation on the survival proportions was used to homogenize the variances. The main effects in the model were the chemical/water treatments (Brine Bath – 10 or 20 seconds, Freshwater Bath 24 hours or 8 hours, Freshwater Spray for 10 or for 5 minutes, Saltwater Bath for 24 hours, and Saltwater Spray for 10 minutes), and the Tunicate Addition treatment (with or without the introduction of tunicate pieces into the socks).

Mussel number per sock was also compared among the water treatments and the tunicate treatments with the same three-way ANOVA model as used for mussel survival. Data was -1/x transformed to homogenize the variances.

Results

The difference in seawater temperature at the dock from the start (24.8°C) to the end (24.6°) of the experiment was minimal. Seawater temperature at the lab was 23.8°C at the start of the experiment and was 23°C at the end of the experiment. Freshwater temperature at the lab was 22°C at the start and 22.3°C at the end. Saltwater salinity was 31 ppt at the dock and in laboratory throughout the experiment, and was 0 ppt in the freshwater.

All tunicates that were placed in the small aquaculture socks with mussel seed survived in the Remained-in Eel Pond and 1 Hour Air-dry treatments. Average mussel survival was over 95% whether mussels remained in Eel Pond or were air dried for one hour (Table 1). There was no significant interaction between the main effects (Table 2; $p = 0.181$), and the average difference in survival of mussels that remained in Eel Pond and those that were air-dried was not significant ($p = 0.140$; Table 2). The proportion of mussels that survived without tunicates (0.993 ± 0.001) was significantly less (Table 2: $p = 0.006$) than the proportion of mussels that survived in the treatments with tunicates (0.971 ± 0.021), but the effect size for mussel survival among the tunicate treatments was slight (2.2%). The average number of mussels per sock ranged from 56 to 65 (Table 1), and no significant difference in average number of mussels per sock was detected between either of the two control treatments or in the tunicate treatments (Table 3).

Chemical and water treatment experiment

Mussel mortality was 100% in both the long and short-term chemical (acetic acid, 5%) treatments. Therefore, these chemical treatments were excluded from all statistical analyses, and only the water treatments were analyzed statistically.

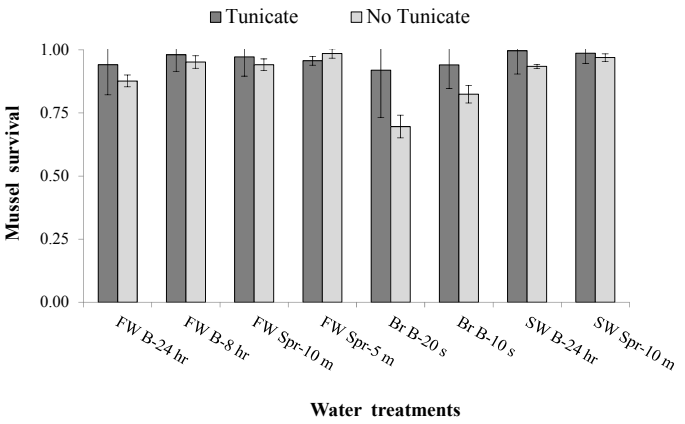

Figure 1. Mean proportion mussel survival per sock and standard deviation in each water treatment and in the Tunicate treatment (n = 5). Abbreviations: FW refers to Freshwater, Spr is Spray, Br is Brine, B is Bath and SW is Saltwater.

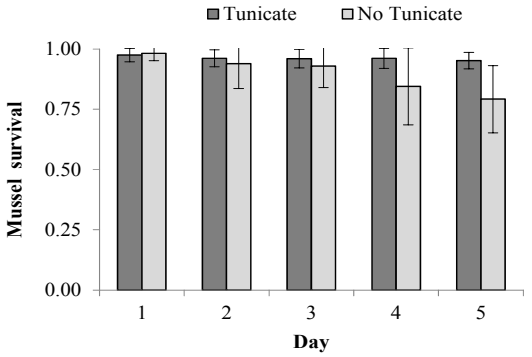

Figure 2. Mean proportion mussel survival per sock and standard deviation on each experimental day in the Tunicate treatments (n=8).

Mussel survival differed among tunicate treatments, among water treatments, and among days (Figure 1). The Tunicate × Water Treatment interaction ($p = 0.001$) and Day × Tunicate treatment interaction ($p < 0.001$) were both significant (Table 4).

In most water treatments, average survival of mussels per sock in the Tunicate Treatment was higher than for mussels in the No Tunicate treatment (Figure 1). Average mussel survival in the two Brine treatments was lower for mussels in the No Tunicate treatment (Brine Bath 20: 0.696 ± 0.187; Brine Bath 10: 0.825 ± 0.093) than those in the Tunicate treatment (Brine Bath 20: 0.920 ± 0.0.45 and Brine Bath 10: 0.940 ± 0.035) (Tukey's HSD test). Mussels in the Brine Bath for 20 seconds in the No Tunicate treatment had significantly lower average survival than mussels in any of the other treatments except Brine Bath 10 sec in the No Tunicate treatment. Mussels in Brine Bath 10 sec spray in the No Tunicate treatment had significantly lower survival than mussels in all the treatments except Brine Bath 20 sec with or without Tunicates, or Freshwater Bath 24 hours with or without Tunicates.

The treatment Saltwater Bath 24 hours with Tunicate (0.997 ± 0.008) had the highest mussel survival of all of the water treatments. Statistically, mean mussel survival per sock in the 24-hour SW bath was significantly higher than survival in either of the Brine treatments with or without added tunicates, or the 24-hour Freshwater bath with or without added tunicates. Average mussel survival in the long and short Freshwater bath and Freshwater spray treatments did not differ significantly among the No Tunicate or in the Tunicate treatments or among other water treatments.

Average mussel survival depended on the Day and the Tunicate treatments (Figure 2); average survival tended to decrease over experimental days in the No Tunicate Treatment. Mussels in the No Tunicate treatment on Day 4 (0.792 ± 0.140) and Day 5 (0.845 ± 0.160) had significantly lower average survival per sock than mussels in the No Tunicate treatment and the mussels in the Tunicate treatment on Days 1, 2 and 3 and 5 (Tukey's HSD test). Average survival of mussels on Day 5 in the No Tunicate treatment was not significantly different from average survival of mussels on Day 4 in the No Tunicate treatment. In contrast, in the Tunicate treatment, average mussel survival per sock was high and ranged from 0.952 to 0.975 and was not significantly different between any days.

Table 4. Mussel Survival per sock. Results of three-way ANOVA with no replication testing whether mean mussel survival differed between Tunicate treatments, Water treatments with Day.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Tunicate	0.157	1	0.157	19.521	<0.001
Water Treatment	0.916	7	0.131	16.287	<0.001
Day	0.552	4	0.138	17.182	<0.001
Tunicate × Water Treatment	0.278	7	0.040	4.937	0.001
Water Treatment × Day	0.283	28	0.010	1.258	0.274
Tunicate × Day	0.267	4	0.067	8.304	<0.001
Error	0.225	28	0.008		

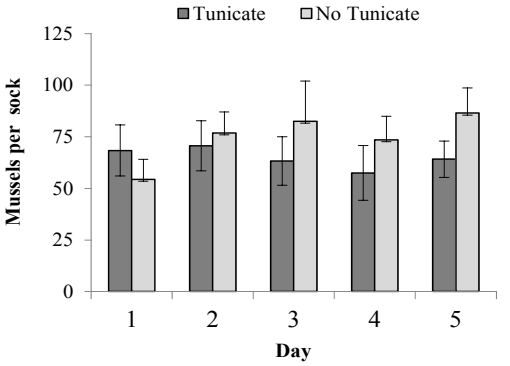

Figure 3. Mean and standard deviation of mussel number per sock on each day in the Tunicate treatments (n=8).

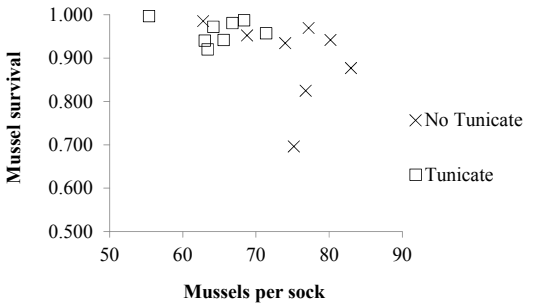

Figure 4. Mean proportion mussel survival versus mean number of mussels per sock. Each point is the mean mussel survival per sock in one of the experimental days in one of the tunicate treatments.

The average number of mussels per sock differed between tunicate treatments on certain days (Tunicate × Day, p = 0.001; Figure 3). Pairwise comparisons (Tukey's HSD test) of mean mussel number per sock between Day and Tunicate treatment indicated that the No Tunicate treatment on Day 1 had significantly fewer mussels per sock (54.375 ± 9.709) than in the No Tunicate treatment on Days 2, 3, 4 and 5 (Figure 3). This suggests that on average, mussels in socks used on Day 1 in the No Tunicates treatment were larger than on other days.

Number of mussel per sock differed significantly between the Tunicate and No Tunicate treatments but not on the same day. On Day 4, the average number of mussels per sock in the Tunicate treatment (57.500 ±13.245) was less than the mussel number per sock in the No Tunicate treatment on Days 2 (76.875 ±10.092), 3 (82.500 ±19.516) and 5 (86.500 ±12.177) (Figure 3).

Tunicate condition

All tunicates that were added to the treatments and tunicates that recruited to socks were dead after treatment in the brine and acetic acid baths. The colonial tunicates placed in the aquaculture socks were all destroyed (dead or shredded into fragments) in the 8- and 24-hour Freshwater Baths and the 10-minute freshwater spray, but not in the 5-minute freshwater spray.

New tunicate larval recruits (1 week old or less) identified on treated and control socks post-treatment were *B. violaceus*, *A. aspersa, B. schlosseri, D. listerianum* and *D. vexillum*. Recruits were observed on 37 of the experimental socks. Other macro-invertebrate fouling on the socks was a bushy bryozoan, *Bugula* sp.

Discussion

Freshwater baths and freshwater sprays can rid colonial tunicates from juvenile mussels and aquaculture socks. Short-term (8 hr) freshwater bath and long-term (10 min) freshwater spray treatments were the most effective against colonial tunicates. While these freshwater treatments were less lethal to mussels than other methods tested, some mussel loss did occur. Though not specifically tested, freshwater baths lasting between 2–8 hours may be just as effective as the 8 hour freshwater baths, because 2 hours is the amount of time that *D. vexillum* can tolerate exposure to

Table 5. Number of mussels per sock. Analysis of variance results for testing whether the average number of mussels per sock differed between the tunicate and water treatments and day of experimental trial. Values for Sum of Squares, and Mean Squares are x E -05.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Tunicate	7.109	1	7.109	9.592	0.004
Water Treatment	3.511	7	0.502	0.677	0.690
Day	12.796	4	3.199	4.316	0.008
Tunicate × Water Treatment	8.046	7	1.149	1.551	0.191
Water Treatment × Day	12.930	28	0.462	0.623	0.892
Tunicate × Day	18.508	4	4.627	6.243	0.001
Error	20.752	28	0.741		

precipitation while out of seawater (Valentine et al. 2007). Freshwater bath and spray treatments may also be effective against solitary tunicates such as *A. aspersa* and *C. intestinalis,* but further work is needed to confirm this.

Acetic acid bath treatments killed all juvenile mussels during our assays. Because of these results, the mixed success reported by other researchers (Carver et al. 2003; Forrest et al. 2007; Locke et al. 2009; Piola et al. 2010), and because of the expense of vinegar and problems associated with vinegar disposal, acetic acid does not appear to be useful for eliminating tunicates on juvenile mussels. If future work is conducted with acetic acid, investigators may want to use treatment times that are less than 5 minutes, and acetic acid mixed with seawater (e.g., Forrest et al. 2007).

Brine baths with lower salinity than we used (70 ppt) may be effective against tunicates and negatively impact fewer juvenile mussels. Brine baths >32 ppt were effective against sponges in cultured oysters (Carver et al. 2010). Brine baths >32 ppt and < 70 ppt may be effective against sponges and tunicates and have no negative effect on oysters or mussels. Unexpectedly, in brine treatments where some mussels survived, mussel survival rate was higher in tunicate added socks. It is unclear why this was the case. The difference in survival might be explained in part by differences in the number of mussels per sock. As mussel volume was kept relatively constant and mussels were loaded into socks haphazardly, it is possible that on some days some treatments had socks with more mussels than other treatments. Alternatively, perhaps the presence of tunicates created micro-habitats with lower brine concentrations that helped protect mussels. Additional work would be needed to tease out the role of these, and other factors.

Our study did not examine mussel tissue weight; further work could examine the effect of treatments on health of the mussels in ways other than survival and shell length. The lower average mussel survival on Day 5 in the No Tunicate treatments (Mussel survival per sock: 0.792 ± 0.140; number of mussels per sock: 86.500 ± 12.177) as compared to the Day 4 with Tunicates and Day 5 with Tunicates effects may have been density-dependent or size-dependent (Table 5 and Figure 4). Smaller mussels could have had lower survival in the Day 5, No Tunicate treatments because they were younger or more numerous than the mussels in the Tunicate treatments on those or other Days or in the other Tunicate treatments. The two control treatments, Air-dry and Remain in Eel Pond, were excluded from the statistical analyses of the other water treatments because these control treatments were only done on the first day of the experiment. Hence, we do not know whether the controls would have shown the same results among days as seen in the other treatments run on days 1 through 5.

After treatment, socks should be returned to seawater in an area where there are no tunicates to prevent tunicate fouling from re-occurring. Unfortunately, tunicates inhabit most of the New England coast (Dijkstra et al. 2007; Valentine et al. 2007; Carman et al. 2010), and collectively they release larvae from early spring to late fall (Bullard and Whitlatch 2004; Valentine et al. 2009). The majority of mussel aquaculture on PEI and in the Netherlands is located in the nearshore where tunicates are abundant (Locke et al. 2009; Gittenberger 2009). Freshwater baths and sprays on mussel seed being transferred from inshore collections sites to offshore farms may be used to avoid the inadvertent spread of invasive tunicates. Periodic freshwater bath or spray treatments during the growing season may prevent invasive tunicates from fouling mussels and aquaculture gear with their expanding omnipresence.

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