Final Report
Developing improved management practices for mussel farming in Southern New England.
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PROJECT TITLE: Developing improved management practices for mussel farming in Southern New England.

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PARTICIPANTS:

Project Coordinator – Scott Lindell

Mary R. Carman
Michael Chambers
Alec Gale
Rick Karney
Richard Langan
Stanley Larson
Dale Leavitt
Michael Marchetti
Greg Mataronas
Robert Reynolds
Bill Silkes
Victoria Starczak

PROJECT OBJECTIVES:

1. Compare means and dedicated sites for collecting mussel seed for SNE.
2. Compare methods of tunicate eradication without compromising the survival of mussel seed.
3. Compare different types of socks and stocking densities to optimize growth and yield at harvest to improve management of mussel operations in SNE.
4. Develop training through hands-on workshops, publications, websites and conference/forums.

The beneficiaries of this research have been the fishermen/farmers in Southern New England (SNE) some who had already taken the first steps to establish and operate mussel longlines (via NOAA funding June 2009 – May 2011) and others who, over the course of this project period were encouraged to diversify and follow their success. The seafood-eating public, seafood processors, restaurants and retail outlets would benefit from locally produced seafood. The measurable benefits are sustainable new enterprises in MA and RI conducting best management practices for locally-produced mussels.

ANTICIPATED BENEFITS:

We can make projections about the potential impact of mussel farming on jobs in SNE based on economic studies of longline mussel farming in Prince Edward Island, Canada. A Department of Fisheries and Oceans report (2006) calculates the direct and total (including indirect and induced) impacts corresponding to $1 million of mussels sold by processors. Assuming the current processed sale price of $1.25/lb (Silkes, pers. comm.) and 10 tons of marketable product per

longline, SNE would need 42 longlines in operation to produce $1 million of processed sales. According to the DFO report, each million dollars of processed sales directly employs 6 full time workers, with a total direct and indirect economic impact of 13 workers.
Our goal is to attract the most likely entrants (commercial fishermen and shellfishermen) to this new aquaculture activity and lead the way for a new industry that can revitalize working waterfronts, and increase employment and economic activity in growing, processing, and distribution services in the Northeastern U.S. If our favorable growth and management assumptions are correct, at the project's conclusion the longlines will be adopted by fishermen as going concerns. According to an unpublished Business Planning Handbook by Hauke Kite-Powell at the Marine Policy Institute at WHOI, a $1.2 million investment in a 120 longline operation (yielding > $3 million in processed sales per year) could be paid back within in 5 years. That model and Langan and Horton (2005) assume a 2-year harvest cycle per longline and production costs of < $0.25/lb. With our projection of faster growth rates in SNE and 1 year harvest cycles, production could be much greater, and the payback could be sooner and larger.

Results of this work have been directly available and applicable for mussel farming operations in SNE. Commercial groups interested in mussel farming – American Mussel Harvesters, Salt Water Farms, and Sakonett Point Mussels, in Rhode Island and Martha's Vineyard Shellfish Group, Menemsha Fish House, Red's Best, and Menemsha Fish Market in Massachusetts have all worked directly on this project. As the project closed, we formally transfer ownership of the mussel product and grow-out structures to the trained fishers who have invested their boats, shore-side facilities and time to expand and maintain them as part of private mussel culture operations.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:
OBJECTIVE 1 – SEED COLLECTION
Implementation of our project and deployment of seed mussels was delayed during the first year relative to our original project timeline, due to delays until October 2011 for executing the funding contracts. We missed much of September and October, the important months for collecting seed and socking it for planting on the mussel longlines. Orders for the Aguin socking machine, accessories for a declumper/grader and Spanish ropes and socking materials were finally received in early November. Special spat collecting and grow-out ropes from New Zealand were also received in November. Jayco embedding anchors for the newly installed lines also arrived from China in mid-November as were locally made 2-ton concrete anchors for Vineyard Sound.

It took a couple of days of coordination to collect seed in RI, make socks, and transport them to Martha's Vineyard. Weather issues fouled our plans a few times in the fall of 2011, and prepared socks were planted on more protected sites in RI instead. Later in the season, the seed was exhausted from AMH sources. So we put our efforts into **Objective 1** – seed collection. On November 28th, PI Lindell and others hung about 200 feet of New Zealand special spat collecting rope (known as Xmas tree rope) at the Vineyard Sound site. We also hung about 50 feet of an experimental "fuzzy" nylon rope made for wastewater treatment bio-media in RI. We also tried dredging for seed nearby but without success.

Our seed collection efforts were conducted in 5 locations; in Narragansett Bay (AMH's Salt Water Farm site), offshore Newport RI (Sakonnet Mussel site), in Menemsha Harbor on

Martha's Vineyard, offshore in Vineyard Sound (reported above), and off the WHOI dock in Woods Hole . In November, MBL staff hung 3 different types of rope collectors off Woods Hole dock; 1) Used potwarp with pegs, 2) NZ spat collecting rope, and 3) RI-made nylon loopy rope (used in wastewater treatment). All 3 types showed colonization by mussels spat (< 1mm) by January. However by February most spat had disappeared. We put out more ropes then but few spat were found when we checked again in April although there was lots of fouling on the lines. Better spat collection was found inside Menemsha Harbor but before we could quantify it, the ropes were removed by the harbor master.

The collector materials put out in Menemsha Pond in early November 2011 were 1) the experimental looped material; 2) a fuzzy rope we had dipped in concrete and 3) pot warp with plastic stays. Tiny spat were first noted in Menemsha on March 16th . Spat seek sheltered nooks and crannies such as loops and knots, and timing corresponds to when mussel spat were first noticed in Katama Bay in early April at 1mm size. These collectors were observed again on October 3rd. Both the looped fiber and fuzzy rope caught and held the seed. The pot warp had fewer seed. Early observations of the pot warp in the Harbor in the spring showed a set that appeared equal or better to the other materials. However, the loss of larger seed was probably an indication of how poorly the pot warp retained seed. The greater surface area of the fuzzy rope and looped fiber appears to have held the seed better than the smoother pot warp. Offshore in MA, the ropes that we hung in November 2011 could not be found in one cursory inspection in the following summer, and boat mechanical trouble called an end to an inspection of the ropes summer long. Later in the fall (almost a year after deployment) these ropes were found to have collected so much fouling and tangled seaweed and very little seed. This underscored the need for consistent maintenance of the farm.

Various kinds of spat collecting ropes had great success when hung in Narragansett Bay during November 2011 and again in February 2012. It was a very good year for mussel seed and we measured seed on the collectors in May at densities ranging from 165 to 250 spat per centimeter. Offshore of RI, the seed collectors put out in the fall of 2011 got wrapped up and around the headrope and consequently collected seed very inefficiently. In future, these collectors must be sufficiently weighed down or set deeper in the water column to avoid that problem. More ropes were put out in February 2012 and set at a density (100 to 200 per foot) that Sakonnet Mussel staff felt would be good for letting them grow to market (anticipated this winter/spring).

As in the 2012, our seed collection efforts were conducted in 5 locations; in Narragansett Bay (AMH's Salt Water Farm site), offshore Newport RI (Sakonnet Mussel site), in Menemsha Harbor on Martha's Vineyard, offshore in Vineyard Sound (reported above), and off the WHOI dock in Woods Hole . In November, MBL staff hung 3 different types of rope collectors off Woods Hole dock; 1) Used potwarp with pegs, 2) NZ spat collecting rope, and 3) RI-made nylon loopy rope (used in wastewater treatment). None showed colonization by mussels spat by March. A few spat were found when we checked again in April although there was lots of fouling on the lines. There was poor spat collection at all our usual sites spring 2013 which may point to the need for a back-up hatchery supply in years with lean local wild seed supply.

Natural spat set was normally abundant winter/spring 2014 (in contrast to 2013) and provided ample seed for fall socking. American Mussel Harvesters put out special NZ spat collection ropes on their farm in Narragansett Bay and collected and stripped seed off those ropes. This was a much more efficient way of procuring seed compared to stripping it off oyster cages as in the past. American Mussel Harvesters invested in special mussel rope stripping, de-clumping and grading, and socking equipment from New Zealand to fully mechanize those operations. Seed was planted on a new farm in Narragansett Bay and the Newport Site. Small amounts of local seed were planted in the spring of 2015 at the Vineyard Sound site, and the first available seed from MVSG and MBL's mussel hatcheries were planted there in the June 2015.

OBJECTIVE 2. Tunicate eradication

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 short-term and long-term to juvenile mussels after they had or had not been exposed to tunicates. Acetic acid baths were lethal to juvenile mussels. Brine baths killed tunicates and less mussel death occurred in the short-term brine bath compared to the long-term brine bath. Both long-term and short-term freshwater baths were effective against tunicates but less mussel death occurred in the short-term bath. Long-term freshwater sprays were slightly more deadly to mussels than short-term freshwater sprays. Tunicates survived short-term freshwater sprays but not long-term freshwater sprays.

We presented our findings at the International Invasive Sea Squirt Conference V in October 2014 are have submitted a manuscript for publication in the on-line journal, *Management of Biological Invasions*. That manuscript is included as a separate appendix to this report – Appendix A.

OBJECTIVE 3. Socking experiments

While waiting for New Zealand and Spanish ropes and equipment to arrive in the Fall of 2011, American Mussel Harvesters (AMH) collected enough seed in October to sock up 256 droppers of the Canadian bisected cotton variety (Table 1) using simple existing equipment. We were successful at making about half of our socks low density and half high density and as close to the 600 and 900 per meter target as practical.

On November 15, 2011, we made our first attempt at grading and socking with the new machinery. It took about half an hour to grade 4 (hundred pound) totes of seed, and some product had to be run through twice. This suggests that it takes about 5 hours to sort enough declumped and graded seed for socking one 500 foot mussel line. AMH had saved some seed scraped from anchor lines but there was only enough to fill nineteen 100 pound totes not 40 as needed. We were able to sock 150 m of New Zealand Megaloop rope and 30 m of Spanish rope at about 900 seed/m, and to hang them on offshore lines in RI.

Table 1. Socking trials with Canadian cotton bisected socks - Fall 2011

		seed size		10'		type of
	Density	AVERAGE	#TOTES	# SOCKS	SEED/M	SOCK
SOCKING 10-6-11		mm				
GRADE 1	Low	22	0.5	11	462	5XL
GRADE 4	Low	42	4.5	24	442	9XXL
GRADE 2	High	25	2.5	23	796	7XXL
GRADE 3	High	30	7	49	666	8XXL
SOCKING 10-20-11 (seed harvested 10-18plant 10-22						
GRADE 1	Medium	16	0.33	8	683	4XL
GRADE 2	Medium	30	3	29	663	7XL
GRADE 3	Medium	37	7	41	709	9XL
SOCKING 10-25-11				PLANT 10-26		
GRADE 2	High	28	0.5	3	982	8XL
GRADE 3	Low	41	2.5	15	520	9XL
SOCKING 11-3-11 seed harvested 10-31-11						
GRADE 1	Low	18	0.5	6	536	5XL
GRADE 2	Low	29	1.5	14	692	7XL
GRADE 3	Low	38	5	29	582	9XL

Harvesting Spring/Summer 2012

Sakonnet Mussels monitored the socked ropes from November 2011 over the course of the winter. Harvesting was delayed until late June and unfortunately coincided with a heat wave. The farmers did not have enough ice to properly layer between the harvested socks, and the insulated fish vats were not sufficient without that. Almost 2,000 lbs. of product spoiled before it could be processed. This was a bitter lesson about preparation for harvest and market but one that was better to encounter early on a small scale than later on a larger scale.

From socks planted in the fall of 2011, Salt Water Farm had a series of harvests in the spring and summer of 2012 (Table 2). From this data, we can conclude that socking at higher seed density (900/m) versus lower seed density (600/m) yielded a similar average harvest density of product per meter of rope (4.6 – 4.7 kg/m), and lower yield of seed in general (21% vs. 34%). This suggests that more is not necessarily better and that seed densities of 400 to 600/m are sufficient with the Canadian socks.

Table 2. Mussel size, density and yield from harvests at Salt Water Farms

Mussel Harvest 2012							# Harvested
		Seed	Type of	Density	Harvest		/# of Seed
Harvested	Planted	Size (mm)	Rope	per m	Size (mm)	kg/m	Yield
5/17/2012	8/18/2011	25-35	8XL	988	60-65	4.5	16%
5/21/2012	9/27/2011	30-40	8XL	429	60-65	5.9	48%
5/23/2012	9/15/2011	15-24	5XL	676	60-65	4.9	25%
5/23/2012	9/15/2011	30-40	9XL	510	60-65	5.5	38%
5/31/2012	10/26/2011	41	9XL	510	50-60	5.6	45%
6/5/2012	9/27/2011	30-40	8XL	429	50-60	3.4	34%
6/11/2012	9/27/2011	30-40	8XL	429	50-60	4.2	42%
6/12/2012	9/15/2011	15-24	5XL	676	51-70	4.0	27%
6/18/2012	9/23/2011	24-Sep	4XL	673	55-65	3.0	20%
6/19/2012	9/13/2011	22-35	8XL	988	60-70	6.2	29%
6/26/2012	9/27/2011	22-35	8XL	988	60-70	5.3	24%
7/2/2012	9/27/2011	30-40	8XL	429	60-70	4.4	47%
7/5/2012	10/17/2011	30-40	8XL	562	60-70	3.5	28%
7/10/2012	10/17/2011	34-44	9XL	510	60-70	3.8	34%
7/25/2012	11/22/2011	35-45	NZ MEGA	975	60-70	4.2	20%
8/1/2012	11/22/2011	35-45	NZ MEGA	975	60-70	3.2	15%

Socking Experiments in Summer and Fall 2012

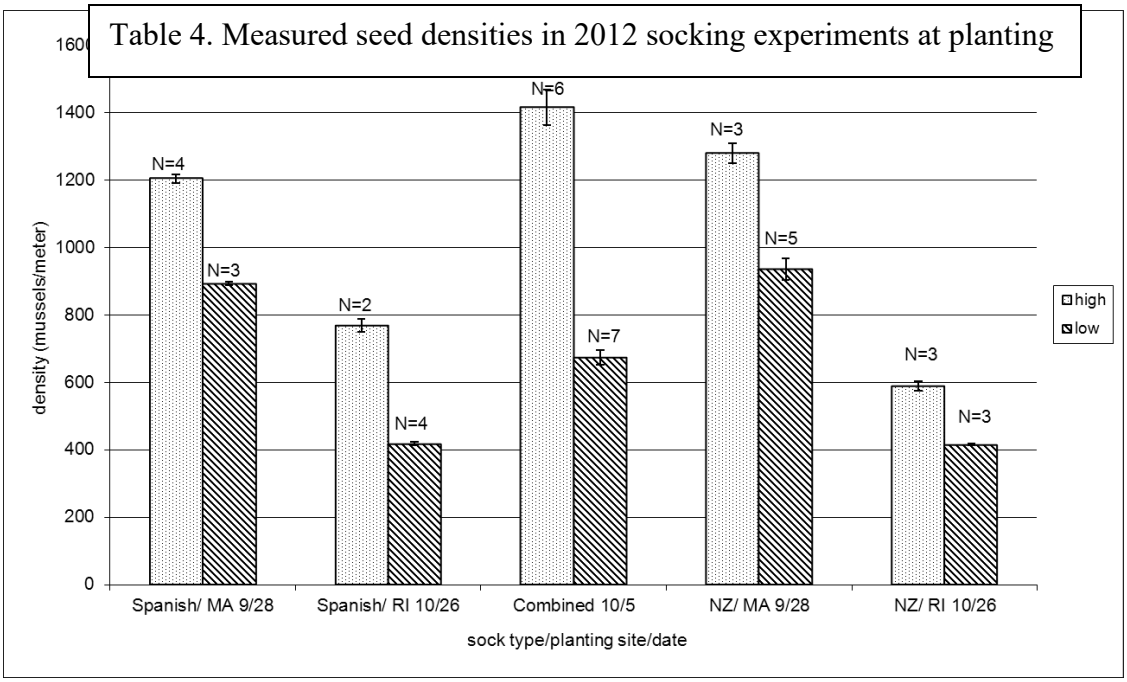
Our research plan was designed to test the optimal density for planting seed (or making socks) among the different types of ropes besides the Canadian ones described above. This was executed in fall 2012. A summary of our socking experiments is listed in Table 3, and the results of our manipulation of density are presented in Table 4.

Table 3. Results of Socking for NRAC Project Summer and Fall 2012

Date	Seed Av. size (mm)	No. of 100 lbs. Totes	No. of Socks*	Socks per Tote	Seed/m	Type of Sock	Site planted
8/29/2012	25	3	26	9	712	7XL	Narragansett
8/29/2012	34	4	25	6	575	8XL	Narragansett
9/11/2012	26	5	62	12.4	637	6XL	Newport
9/11/2012	31	13	81	6.23	861	8XL	Newport
9/18/2012	28	3.5	44	3	163	6XL	Narragansett
9/18/2012	35	5.5	34	4	138	8XL	Narragansett
9/26/2012	30	12	45*	3	1014	Spanish	Vineyard
9/26/2012	30	18	62*	4	1008	NZ	Vineyard
10/4/2012	32	15	55	3.5	980	Spanish	Newport
10/4/2012	32	22	92	4	980	NZ	Newport
10/25/2012	31	3.5	33	9.43	614	6XL	Newport
10/25/2012	31	4	30	7.50	855	8XL	Newport
11/27/2012	36	7	36	5.1	821	7XL	Vineyard

*Reported as number of 3-meter units in which Canadian socks are typically hung;
NZ are 23-meters and Spanish are 15-meters; mulitply by 3 above to calculate total meters

Table 4. Measured seed densities in 2012 socking experiments at planting



A detailed account of mussel seed socking activities are described below:

RI

On August 29 2012, seed were first collected and graded at AMH. Despite warm temperatures, a quick turnaround of handling and socking returned 51 Canadian socks to Salt Water Farms lines in Narragansett Bay. On September 5, there was a 3-day lag between handling, grading and hanging 134 Canadian socks. They were held in ambient rather than chilled flow-through seawater and by the time they were ready to be deployed most had died. This highlighted the need for close coordination between socking and the farm, and how easily a few days of bad weather could upset this. On September 11 2012, Sakonnet Mussel staff hung 143 Canadian socks on Line 4. On October 4th 20102, Sakonnet Mussel staff and Lindell loaded 37 totes of graded mussel seed and the Aguin mussel socking machine along with 12 lengths of NZ (23 m) and 11 lengths (15 m) of Spanish ropes. Socked ropes were made on the boat and deployed in alternating sequence immediately on the headrope Line 2. On October 26 2012, Sakonnet Mussel staff and Lindell worked on Line 1, and hung 58 Canadian socks and 6 NZ (23 m) socks and 6 Spanish (15 m) socks. Five additional Canadian socks were added to Line 4 to make up for what was missing from Septembers planting.

The disheartening news in the fall of 2012 was that Hurricane Sandy moved the anchors and tangled 2 of the 4 longlines in Rhode Island, and stripped much of the seed we had carefully deployed. The two tangled lines and anchors that moved were removed so as not to pose a hazard to navigation. The remaining two lines were checked and adjusted and the remaining tangled NZ socks were removed. Some of the Spanish socks had been re-colonized by naturally-set mussel seed in May, 2013, and were left to see how it would grow. By contrast the Martha's Vineyard site was undamaged probably because it is better protected from SE swells.

MA

On September 26, 2012, MVSG mussel farmer and Lindell hung 9 lengths of NZ (23 m) and 9 lengths (15 m) of Spanish ropes. Socked ropes were made at AMH, stored in vats in circulating cool water overnight at AMH and then shipped overnight to a seafood warehouse before finally reaching the dock in Woods Hole where they were picked up. Lindell joined the boat and then after steaming to the site we deployed the socks in alternating sequence on the headrope. The socks did not fare well by being held in the vats. The pegs on the Spanish rope tended to rip up both the Spanish and NZ socking that they were stored with as they were detangled and extracted from the vats.

On November 26 2012, a local fisherman was hired to dredge for mussel seed in Menemsha Pond and produced 14 totes. It took the morning to declump and grade this in our Spanish machine. It only resulted in 7 totes of graded and mostly singulated mussel seed, far less than we had anticipated. The rest of the afternoon was dedicated to rigging and operating a socking apparatus for preparing Canadian socks on the dock. It required fiddling with a water supply and compressed air but it worked, and in 3 - 4 hours we produced 36 socks.

Over the winter of 2012/2013, mussel grower/fisherman under contract to MVSG, Alec Gale sold his large dredging boat and bought a lobster-type boat. The boat is faster and more flexible in terms of ability to tend the lines in Vineyard Sound. Large seed (25 to 35 mm) from 2012's plentiful spat set was still in good supply on the pilings and margins of the Menemsha Harbor

and Pond in the spring of 2013. Lindell and Gale spent a couple of days collecting seed, declumping and grading and then socking in both NZ and Canadian socks in May 2013 for deploying offshore. Seed supply was very limited in Menemsha Harbor relative to the need for supplying grow-out offshore.

In June 2014 some of the socks planted the previous October were harvested from Vineyard Soound but only about 10% were market size. Many of those market-sized mussels were still trapped inside the Canadian socking mesh and a good proportion of the mussels were seed-size (67% < 20mm) at a density of 770 mussels per meter. Table 5 summarizes the results and shows that harvestable mussels were well below the generally acceptable density of 3 to 5 kg per meter. It may be that the mussel seed suffered some stress between being harvested and socked in RI and planted in MA, a 3-day process. This would explain why many of the larger mussels hadn't migrated out of the mesh of the sock and suffered poor growth. Another factor may have been that the lines were hanging lower in the water column than usual due to insufficient buoyancy. The summer water temperatures were too warm to harvest the mussels after June without special equipment that we do not have. This equipment is designed to catch mussels that would otherwise slough off when pulled with their ropes out of the water.

Table 5: June 2014 offshore MA

Summary of mussel rope samples

# of mussels	1385	
#/m	770	**kg/m**
% < 20mm	67%	0.02
% 20-50 mm	23%	1.72
% > 50mm	10%	1.25

Rhode Island

After planting mussel socks on longlines in October 2013, Sakonnet Mussel principal Mike Marchetti's schedule and boat troubles kept him away from essential maintenance of the longlines until February 2014. He reported then that anchors he had reset after "Storm Sandy" had moved again and that much of the product we had planted had been stripped by hitting bottom, and later riding too high near the surface. One of the 3 lines appeared to have some product but the tension on the line and the weather conditions prevented inspection until spring.

In May and June 2014, Marchetti was able to re-tension the lines at the site and provide mussel sock samples within a few days of comparable sampling at the MA site. What is apparent from this comparison (contrast Table 6 below with Table 5 above) is that most of the market-size mussels had been lost in RI, and that there was good recruitment of juvenile wild seed at that location.

Periodically, we measured the meat yields of our local New England farm grown mussels and compare them to the widely available farmed mussels from Prince Edward Island or wild mussels from Maine. In the examples presented in Table 7, the steamed meat yields (shown as a percentage of the total "wet weight" minus the shell weight) are substantially better for RI and MA grown mussels. This is another plus for growing and marketing local New England mussels.

Table 6: June 2014 offshore RI

Summary of mussel rope samples

# of mussels	2020	
#/m	2082	**kg/m**
% < 20mm	95%	0.26
% 20-50 mm	5%	0.56
% > 50mm	0%	0.06

Table 7. Summary of meat yields

% steamed meats/live wt.

dates	PEI	RI	ME	MA
Apr-14	30.3	40.6		
Aug-14	17.1	37.5		
May-15			32.26	49.09

In August of 2014, Marchetti and partner, Greg Mataronas, formally sold and signed over their offshore Newport lease and permits to American Mussel Harvesters/Salt Water Farms to own and operate. It became too much for these fishermen to manage along with their other family and fishing responsibilities. The Silkes family (father and 3 sons) has been farming oysters and mussels for over 40 years and one the sons, Adam, has recently been issued a permit for his own 8-acre mussel farm in Naragansett Bay. As mentioned above, they have invested substantially in some of the best equipment to make mussel farming efficient and profitable. They installed helical anchors on the site for more stable longlines since repeated movement of the drag/embedment type Jeyco anchors had been a repeated problem at this site.

In October of 2014, a new fisherman/farmer, Stanley Larson, owner of Menemsha Fish Market, took over the contract with MVSG (from Alec Gale) to manage the Vineyard Sound site. Stanley owns two large boats and retrofit one for mussel farming. He also owns his own declumper for mussels, and has some experience fishing for mussels. It took months to transfer the permits from the Town and the State before he could begin actual work. Meanwhile most of the farm had sunk below the surface. Stanley was able to resurrect the farm with a lot of grappling and hard work.

Outreach and Extension

There have been four formal workshops held in the Northeast with guest speakers from many of the major mussel farming regions around the world, and with broad attendance from all 6 New England coastal states. Below is a brief description of these:

Mussel Farming in New England Workshop – May 17, 2013

A workshop sponsored by Rhode Island Sea Grant, Northeastern Regional Aquaculture Center, Roger Williams University and the Woods Hole Marine Biological Laboratory at the Coastal Institute, University of Rhode Island Bay Campus.

This workshop was intended for those with practical experience working on the water and an interest in mussel farming. Speakers (8) and attendees (31 from 4 states, most of who also attended subsequent workshops and are listed below) at this workshop shared their experience with 4 different offshore sites in New England over the last 12 years. We were fortunate to have a veteran of the mussel farming industry in New Zealand, Joe Franklin, give a presentation with insights into developing the industry here. The powerpoint presentations, some of which are narrated, were made available to the public here:

http://www.mbl.edu/bell/scientific-aquaculture/2013-mussel-farming-workshop/

Anchor engineering and installation demonstration – January 20th, 2014
American Mussel Harvesters sponsored a one-day demonstration and workshop. Workshop leaders were Lindell and a guest from Iceland who is a leader in installing and servicing mussel line anchors and moorings. Ingvar Erlicson manages Hafbor that manufactures a rig for installing helical anchors at depths (> 100') that would not be feasible for divers to operate, and without the attendant risks. These depths are typical of many potential offshore mussel farming sites which otherwise require heavy, expensive deadweight anchors.

Participants met at the harbor in Port of Galilee in RI where we boarded the F/V Virginia Marise. On board was the 14' tall Hafbor anchor installation rig and a 4 m long helical anchor. While in the port, the Hafbor team demonstrated the ease of installing the anchors from start to finish. One advantage is that the attachment between rope and anchor may be made ahead of time, and frees divers from having to do it underwater. While the set up of the rig on and off the boat takes some time, once the boat is anchored on site, the anchor installations only take between 10 and 15 minutes each. The rig has 4 cameras that allow viewing of the bottom type and progress made while drilling which participants could watch. After the demonstration, participants gathered in a local coffee shop to ask questions, trade tips and networks.

Participants included:
Bill, Adam and Mason Silkes (American Mussel Harvesters)
Bernard Friedman (Santa Barbara Mariculture, CA)
Domenic Santoro (Santoro Fishing Co., and prospective mussel farmer, MA)
Vincent and Justin Prien, and Peter Flannigan (partners in NH offshore mussel farms)
Michael Marchetti (Sakonnet Point Mussel Farm, RI)
Scott Lindell and Emma Green-Beach (Marine Biological Laboratory, MA)

Socking demonstration and longline engineering presentation – March 26 and 27, 2014
American Mussel Harvesters sponsored and hosted a two-day demonstration and workshop. It was originally scheduled for one day but a snow-storm interfered and only the most local participants could make it the first day. The majority arrived the second day. An announcement was distributed on the ECSGA listserve and several newly interested parties participated. Workshop leaders were Lindell and two guests from New Zealand who are leaders in equipment and services for the mussel farming industry. Joe Franklin manages Quality Equipment which is a major supplier of ropes for the industry internationally. Graham Fielder manages Fielder Marine which installs and services mussel line anchors and moorings in NZ and overseas.

The draw for participants was the demonstration of a new compact (and lower cost) mussel socking machine from New Zealand as well as a chance to see new types of mussel hatchery and grow-out ropes such as "catch and grow" rope. After the socking demonstration, the group convened for lunch and a presentation and discussion was lead regarding different engineering designs of NZ offshore mussel farms and the US counterparts. The New Zealanders are testing what they call a semi-submerged design. This design relies on robust helical anchoring with 3:1

scope, and large surface floats from which the headrope is suspended only 4 to 5 m deep. This contrasts with US headropes that are typically 8 to 12m deep that depend entirely on submerged buoyancy.

Participants included:
David Alves (NOAA's Northeast Regional Aquaculture Coordinator)
David Beutel (RI Coastal Resources Management Council, Aquaculture Coordinator)
Michael Chambers (NH Sea Grant Extension)
Matthew Griffin (RWU Extension)
Bill, Adam and Mason Silkes (American Mussel Harvesters)
Dave Roebuck (Salt Pond Oyster Co., RI)
Mike and Isabel Osinkski (Widow's Hole Oyster Co., NY)
Matthew Moretti (Wild Ocean Aquaculture, ME)
Dylan Shaw (prospective mussel farmer, ME)
Domenic Santoro (Santoro Fishing Co., and prospective mussel farmer, MA)
Vincent Prien and Peter Flannigan (partners in NH offshore mussel farms)
Michael Marchetti (Sakonnet Point Mussel Farm, RI)
Scott Lindell and Emma Green-Beach (Marine Biological Laboratory, MA)

Northeast Aquaculture Conference & Exposition and the 35th Milford Aquaculture Seminar, Portland Maine - Mussel Farming Workshop – January 16, 2015

Lindell, with help from Extension agents, organized and chaired an outreach workshop which included 9 presentations on business planning tools, vertical integration of the mussel farming business from different perspectives representing the West Coast, Prince Edward Island, and East Coast mussel farming industries, and development of hatchery technologies for mussel seed. The complete program and abstracts can be found here:
http://www.northeastaquaculture.org/wp-content/uploads/2015/01/NACE-Program.pdf

IMPACTS:
Attendees of our workshops have continued to make progress in permitting and operations of new and expanded offshore mussel farms in Narragansett Bay and Newport RI, Vineyard Sound, Nantucket Sound and offshore Gloucester MA, and Rye NH. The prospective growers have consulted with project participants and have even volunteered to work alongside to get a better understanding of the farming routines. In August 2014, a new 28 acre mussel farm was permitted in the Federal waters off Massachusetts, the first such aquaculture permit on the East Coast. Lindell was the agent for the fisherman/farmer who holds the new permit, and was responsible for guiding the project through a lengthy 18-month review and permitting process. A couple of months later a second farm in Federal waters was permitted off Gloucester MA to Salem State University in which Lindell was also instrumental in advising.

As a result of working with new applicants for offshore leases for mussel farming, and meeting with State and Federal authorities, Lindell, Langan and Silkes from this project have joined a working group sponsored by NOAA's GARFO office to address real and perceived risks that offshore mussel farming pose to protected species. The working group is to review a white

paper being drafted by NOAA regarding the risk of protected species entanglement in aquaculture gear, and is invited to attend a 2 day workshop in September 2015.

Impacts Summary	Provide short statements (2-3 sentences) about each of the following: (pre-established fields for Researchers to complete short statement answers) 1. **Relevance:** Issue – what was the problem? The technology and management needs for mussel farming were unfamiliar to potential participants in Southern New England. Fishermen in the region are struggling to diversify, and mussel farming is an activity that fishermen could profitably conduct part or full-of thtime. 2. **Response:** What was done? Fishermen were given resources and training to expand their farms using advanced materials proven successful for mussel farming in other parts of the world. 3. **Results:** How did your work make a difference **(change in knowledge, actions, or conditions)** to the target audiences? We discovered that some of the materials and methods transfer well to our fishermen and offshore SNE environment, and some don't. Farmers learned lessons about the importance of regular maintenance for predictable mussel farming, and about how to use the tremendous labor saving equipment that is available for socking seeding. 4. **Recap:** One- sentence summary The current and proposed farms combined could employ dozens of employees and produce hundreds of tons of mussels worth in excess of $4 million dollars within the next two years

Publications, Manuscripts, or Papers Presented:

National Shellfish Association - March 29, 2012 in Seattle, WA
Mussel Farming Session, 9 talks organized and chaired by Scott Lindell
"Mussel Farming in Southern New England" presented by Lindell
"How to expand the mussel farming industry in the Northeastern U.S." presented by Silkes

"Research and Management for Offshore Mussel Farming in Southern New England" presented by Lindell at NACE/Milford Aquaculture Symposium in Groton CT, December 14, 2012.

"Offshore Mussel Farming in Southern New England; Research plans for optimizing economic yield" presented by Lindell at WAS/Aquaculture America Conference in Nashville, TN, February 2013.

"Mussel Farming in Rhode Island Waters" presented by Lindell to 50 people at the RISG Coastal State Forum for Shellfish Issues at URI Campus on March 28, 2013.

"Mussel Farming 101" presented by Lindell to an audience of 60 at the annual meeting of the Massachusetts Shellfish Officers Association – December 12, 2013 in Hingham, MA

"Do Mussel Farms Need Mussel Hatcheries?" present by Lindell to an audience of 100 at the Milford Aquaculture Seminar in Milford CT in February 2014.

"Opportunities in Farming Blue Mussels" presented by Michael Chambers (UNH extension) at WAS meeting in New Orleans, in February 2015

"Mussel Farming in State and Federal Waters of New England" presented by Lindell at NACE in Portland ME, January 2015.

"Facts and Figures for Mussel Farming Business Planning" presented by Lindell at NACE in Portland ME, January 2015.

IMTA/Offshore Aquaculture Session at National Shellfisheries Association Meeting, Monterey CA, March 2015.– co-organized and co-chaired by Scott Lindell and Sean Robinson with 8 presentations including, "Mussel Farming in State and Federal Waters" presented by Lindell .

Lindell, S. 2013. Offshore Mussel Culture – Biologists Refine Longline Methods In New England, USA. Global Aquaculture Advocate, July/August, p. 46 – 47.

Carman M.C., S.Lindell, E. Green-Beach, V.R. Starczak in press. Treatments to eradicate invasive tunicate fouling from blue mussel seed and aquaculture bags. Management of Biological Invasions.

Student participation:
Two undergraduate students have interned each summer of the project.

Appendix A: Manuscript submitted for publication to on-line journal

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

Mary R. Carman[1*], Scott Lindell[2], Emma Green-Beach[2], Victoria R. Starczak[1]

[1]Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[2]Marine Biological Laboratory, Woods Hole, MA 02543, USA
E-mail: mcarman@whoi.edu (MRC), slindell@mbl.edu (SL), emma.greenbeach@gmail.com (EG), vstarczak@whoi.edu (VRS)
*corresponding author

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 short-term and long-term to juvenile mussels after they had or had not been exposed to tunicates. Acetic acid baths were lethal to juvenile mussels. Brine baths killed tunicates and less mussel death occurred in the short-term brine bath compared to the long-term brine bath. Both long-term and short-term freshwater baths were effective against tunicates but less mussel death occurred in the short-term bath. Long-term freshwater sprays were slightly more deadly to mussels than short-term freshwater sprays. Tunicates survived short-term freshwater sprays but not long-term freshwater sprays.

Keywords
Ascidiacea, invasive species, *Mytilus edulis*, aquaculture, freshwater, brine, acetic acid

Introduction
Aquaculture of the blue mussel *Mytilus edulis* is still new on Martha's Vineyard, Massachusetts. There is an established shellfish market and demand for mussels in the area. Blue mussels are native to the North American coast and often occur abundantly in shallow water environments around Martha's Vineyard and elsewhere. Shellfish farmers can 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 diversity of non-native, invasive tunicates has been identified in southern New England coastal waters, including Martha's Vineyard (Carman and Roscoe 2003; Pederson 2005; Bullard et al. 2007), fouling artificial and natural substrates, including aquaculture gear and cultured and wild bivalve shellfish. 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 2003; Forrest et al. 2007), and elsewhere have been struggling to contain the cost of managing invasive tunicates that plague their farms. New England coastal habitats and aquaculture sites are frequently occupied by tunicates, including the non-native, invasive solitary and colonial species *Ascidiella aspersa* (D.F. Müller 1776)*, Botrylloides violaceus* Okra 1927*, Botryllus schlosseri* (Pallas 1766)*, Didemnum vexillum* Kott 2002*, Diplosoma listerianum*

(Milne-Edwards 1841) and *Styela clava* Herdman 1881 (Carman et al. 2010). Biological, mechanical, chemical and water (brine and freshwater) treatments, for ridding invasive tunicates from shellfish and gear without causing harm to shellfish, have had mixed results.

Biological Treatments

There are few to no natural predators of invasive tunicates. Biological control experiments have had little success. Rock crabs *Cancer irroratus* (Say 1817) and green crabs *Carcinus maenas* (Linnaeus 1758) consumed a limited number of an egregious, invasive species on PEI, the solitary tunicate *Ciona intestinalis* (Linnaeus 1767) (Carver et al. 2003). The periwinkle snail *Littorina littorea* (Linnaeus 1758) ate *D. vexillum* only when it was senescent (Carman et al. 2009). The neogastropods *Mitrella lunata* (Say 1826) and *Anachis lafresnayi* (P. Fischer and Bernardi 1856) preyed on larval recruits of *A. aspersa, B. schlosseri, D. listerianum* and *S. clava,* but did not readily consume adult forms of these species or *B. violaceus* recruits or adults (Whitlatch and Osman 2009). Switzer et al. (2011) found that the green sea urchin *Strongylocentrotus droebachiensis* (O.F. Müller 1776) was not successful at controlling tunicates.

Mechanical Treatments

Mechanical treatments employed to destroy tunicate fouling include exposure to air; scraping, scrubbing, sweeping, brushing or tumbling; and power washing. Air-drying is a common practice used by North American east coast aquaculturists to rid tunicates and other fouling organisms from gear and shellfish, often with some product loss (Carman et al. 2010). Exposure to sun and variations in air temperature 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 a longline. Non-lethal amounts of air drying can also stress tunicates to the point of spawning when returned to the water; when performed within the aquaculture lease site, this may only encourage new settling of larvae.

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 a lot of labor. Scrubbing with soft wire brushes did not completely remove *D. vexillum* from oysters because *D. vexillum* grew into oyster shell crevices making it difficult to remove 100% of the colonies (Switzer et al. 2011).

Power washing with seawater has been used with some success (Chou 1991; Arens et al. 2011; Paetzold et al. 2012). However, power washing *B. violaceus* and *D. vexillum* fragments colonies into pieces, and if these fragments are returned to the sea, may only exacerbate the problem because fragments of *Didemnum* and *Botrylloides* have the ability to reattach and grow (Stoner 1989; McCarthy et al. 2007; Valentine et al. 2007; Morris and Carman 2012). Often power washing is done boat-side or dockside and the logistics of collecting and disposing of tunicate fragments may be costly and infeasible.

Chemical Treatments

Chemical treatments that have been tried include bleach, hydrated lime, and acetic acid. In New Zealand, dilute bleach dips were effective against *D. vexillum* on green mussel *Perna canaliculus* (Gmelin 1791) (Denny 2008) but 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, to help control tunicates. However, long term hydrated lime immersions had little negative impact on the oyster boring sponge *Cliona celata* (Grant 1826) (Carver et al. 2010), and short term (4 min) hydrated lime (4%) was not 100% effective against *D. vexillum* and caused some oyster mortality (Switzer et al. 2011).

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 were 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 or less followed by 24 hours of air exposure also had mixed 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%, 20%) baths lasting 0.5, 3, and 6 hours were tried with 75-100% success against colonial and solitary tunicates on settling plates (Piola et al. 2010). Disposal of large quantities of spent lime may be a problem (Rolheiser et al. 2012), and disposal of spent acetic acid may or may not be a problem because of loss of acidity from use and dissipation in the sea (Locke et al. 2009).

Water Treatments

Water treatments, including brine and freshwater, have been somewhat successful. Tunicates naturally occur in full marine conditions and do not occur in hyposaline or hypersaline waters (van Name 1945). Mussels can tolerate exposure to freshwater for several days (Lützen 1999), but it is unknown if they can tolerate exposure to hypersaline conditions or brine. Brine baths (>90%) recommended for the control of the boring sponge in cultured oyster shells (Carver et al. 2010), may also be effective against tunicates. Hypersaline cold shock treatments destroyed soft-bodied organisms (flatworms) on oysters (Cox et al. 2012) and may also kill tunicates.

Some tunicate species can tolerate exposure to freshwater and air. Freshwater treatments lasting 5 or 20 minutes did not reduce *D. vexillum* fouling (Rolheiser et al. 2012). *D. vexillum* can tolerate exposure to air for up to 2 hours during low tide (Valentine et al. 2007), and if it is precipitating during low tide time, *D. vexillum* also tolerates exposure to freshwater. Effective air exposure and freshwater treatments against *D. vexillum* should probably last longer than 2 hours. *B. schlosseri* is a euryhaline species and may tolerate exposure to freshwater flux in upper estuary habitats (Brunetti et al. 1980). *S. clava* attached to boat hulls survived out of the water for 48 hours (Darbyson et al. 2009).

The US Natural Resources Conservation Service (2011) issued a practice standard for bivalve aquaculture gear and fouling control. The code states that environmentally appropriate fouling control methods to be used are: air-drying, brine, vinegar, freshwater, sweeping, or power washing. Specifics for treatments are not given nor the percentage of mussel loss for each treatment method. The main objective of our study was to test whether juvenile mussel mortality differed among some of these treatments. We conducted short-term and long-term chemical treatment (acetic acid) and water treatment (brine, seawater, freshwater) trials on socks of juvenile blue mussels with and without invasive species of colonial tunicates.

Methods

Pre-treatment

About 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 placed in flow through seawater tanks at the Marine Biological Laboratory (MBL) Loeb Lab. The ideal socking size for mussels is about 15-25 mm. On June 28, between 15 and 17 mL of small, healthy mussels between 3 and 25 mm in shell length were placed in 150 black plastic mesh aquaculture socks (8 cm^2; 3 mm mesh opening) with white plastic clasp closures. A diversity of tunicate species including *B. violaceus* and *D. vexillum* were present on the dock and we expected additional new tunicate growth to occur on some of the bags over the two-week period. Healthy-looking tunicates identified on the socks before treatment were *B. violaceus*, *D. vexillum,* and few volunteer recruits of *B. schlosseri, D. listerianum, A. aspersa,* and *C. intestinalis*.

Number of Mussels in the Treatment and Control Socks

The number of mussels in each sock varied, but the volume of mussels in each sock was held constant. The number of mussels is included in the analysis as it was assumed to give a rough idea of the size of the mussels. That is, higher number of mussels in a sock suggests that the average size of the mussels in that sock was smaller than in other socks.

On July 2, colonies of *B. violaceus* and *D. vexillum* were collected and placed in flow through seawater tanks at MBL Loeb Lab. About 3 cm^2 cut pieces of healthy-looking *B. violaceus* and *D. vexillum* colonies were placed in half of the socks with mussel seed. Socks were secured to two 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 the tunicates and mussels time to acclimate.

A total of 8,141 juvenile mussels were placed in 120 socks and the number of mussels per sock ranged from 14 to 126. Of the 120 socks, half were composed of mussels only and half were composed of mussels and tunicates. Eighty socks were arbitrarily assigned for treatment and 40 socks were assigned as experimental controls (10 to be left in Eel Pond, 10 to be air-dried for 1 hour, 10 for seawater bath and 10 for seawater spray). Socks were examined and assessed immediately before treatment.

Chemical and Water Treatments

An identification tag was secured to each of the 120 socks of mussels assigned for the experiment (60 socks with tunicates and 60 socks with no tunicates). Eighty socks of mussels (40 socks with tunicates and 40 socks with no tunicates) were exposed to one of 8 treatment types at the lab each day for 5 consecutive days (July 9-13, 2012). All treatments (long-term and short-term Acetic Acid Bath, Brine Bath, Freshwater Bath, Freshwater Spray) were followed by 1 hour of air-drying in the absence of sun and wind, in Loeb Lab.

Acetic Acid Bath

Room temperature white vinegar (5% acetic acid) was placed in a small plastic tub. Long-term (10 min) and short-term (5 min) Acetic Acid Baths were done on 10 socks of mussels (5 socks with tunicates and 5 socks with no tunicates).

Brine Bath

Commercial table salt was added to lab seawater and the salinity measured using a hand held refractometer. Long-term (20 sec) and short-term (10 sec) Brine Baths (210% salinity) were

conducted on 10 socks of mussels (5 socks with tunicates and 5 socks with no tunicates) in a small plastic tub.

Freshwater Bath

Long-term (24 hr) and short-term (8 hr) Freshwater Baths were done on 10 socks of mussels (5 socks with tunicates and 5 socks with no tunicates) in flow through fashion dripping fresh tapwater into a small plastic tub.

Freshwater Spray

Long-term (10 min) and short-term (5 min) Freshwater Sprays were conducted on 10 socks with mussels (5 socks with tunicates and 5 socks with no tunicates) using a garden hose and freshwater while the socks were in a small plastic tub. The rate of flow of Freshwater Spray was maintained at 5 liters/28 seconds (5.6 sec per liter).

Control Treatments

Forty socks of mussels (20 socks with tunicates and 20 socks with no tunicates) were designated as controls for the experiment. Control socks were treated in 4 ways: 1) Remained in Eel Pond for 1 week, 2) Seawater Bath for 24 hours, 3) Seawater Spray (5.6 sec per liter) for 10 minutes, and 4) Air-dried for 1 hour. After treatment, treated and control socks were randomly placed on one of 4 lines suspended between 0.5 m and 1 m water depth at the MBL dock on Eel Pond for 1 week.

Post-treatment

After exactly one week in Eel Pond, socks were retrieved and placed in seawater tanks at MBL Loeb Lab and opened each day for 5 consecutive days (July 16-20). Healthy looking tunicates ≥ 4 mm on socks and mussels were identified and considered survivors; tunicates < 4 mm were considered to be new, larval recruits (1 week old or less). Tunicates were considered dead if they were either absent, putrefying, or not attached to mussel or sock. The survival of the mussels was determined (dead/alive) by examining each mussel. In each sock, the lengths of the smallest and largest mussels were measured and the number of live and dead mussels counted.

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.

Results

Control Treatments

A comparison was made of the mean number of mussels per bag and of survival for those mussels that remained in Eel Pond versus Air-dried for 1 hour. Both of these control treatments had five socks with Tunicates or with No Tunicates.

Destruction of Tunicates

All tunicates that were placed in the small aquaculture bag with mussel seed survived the Control trials, Remained in Eel Pond and 1 Hour Air Dry.

Number of mussels per bag

A two-way analysis of variance (ANOVA) was run with the control treatment as one main effect and Tunicate as the other main effect to first determine whether the number of mussels per bag differed between treatments. The average number of mussels per bag ranged from 56 to 65, and no significant difference in average number of mussels per bag was detected between either of the two control treatments or in the tunicate treatments (Table 1).

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

Control Treatments	**Tunicates**	**Proportion mussels survived**		**Initial mussels/ bag**	
		Mean	**Std dev**		**Std dev**
Eel Pond	Tunicate	0.985	0.009	65.2	7.56
Eel Pond	No tunicates	0.993	0.009	56.8	7.01
Air Dry- 24 hr	Tunicate	0.956	0.030	63.2	5.74
Air Dry- 24 hr	No Tunicates	0.992	0.011	56.6	22.70

Table 2. Mussels per bag. ANOVA results testing whether the mean number of mussels per bag at the start of the experiment differed between treatments. Control treatments Eel Pond vs Air dried (1 hr).

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

Mussel survival

Average survival was over 95% in all Control treatments (Table 1) and there was no significant interaction between the main effects (Table 3; $p = 0.181$), and the average difference between the two control treatments was not significant ($p = 0.140$). The difference in survival among the tunicate treatments was slight, however, significantly more mussels survived in the treatments without tunicates (0.993 ± 0.001) than in the treatments with tunicates (0.971 ± 0.021).

Table 3. ANOVA results of mussel survival in Control treatments: Eel Pond vs Air dried (1 hr). The proportion of mussels that survived was arcsin square root transformed prior to analysis.

Source	Sum-of-Squares	df	F-ratio	P
Tunicate Treatment	0.0504	1	10.0077	**0.0060**
Control Treatment	0.0122	1	2.4183	0.1395
Control x Tunicate	0.0099	1	1.9592	0.1807
Error	0.0806	16		

Chemical Treatments

Mortality was complete among mussels in both long and short term chemical (acetic acid, 5%) treatments, therefore chemical treatments were excluded from all analysis.

Water Treatments

Destruction of Tunicates

The colonial tunicates placed in the aquaculture socks were destroyed in the 8 and 24 hour Fresh Water Baths, the 10 minute freshwater spray, but not in the 5 minutes freshwater spray. Tunicates were destroyed in both of the brine and acetic acid baths.

Mussel survival

A three-way ANOVA with no replication (randomized blocks design with days as the blocks) was run on the proportion of mussels that had survived in each sock. The arcsin square root transformation was used on the survival proportions to homogenize the variances. The main effects in the model were water treatments (Brine Bath – 10 and 20 seconds, FW bath 24 hours and 8 hours, FW Spray for 10 or for 5 minutes and SW Bath for 24 hours and SW Spray for 10 minutes), tunicate treatment (with or without the introduction of tunicates into the socks), and Day. On Day 1, a set of socks was used, with one sock of mussels for each treatment combination. On day 2, another set of socks was treated, and so on through Day 5.

Survival differed between days, tunicate treatment and water treatment (Table 4) with significant **Tunicate x Water Treatment** interaction ($p = 0.00099$) and a significant **Day x Tunicate** treatment interaction ($p = 0.00015$). The three way interaction, Water treatment x Tunicate Treatment x Day, could not be tested as there were no replicate socks on each day.

Table 4. Results of three-way ANOVA with no replication on testing whether mean survival differed between Tunicate treatments, Water treatment or Day of water treatments.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Tunicate	0.15679	1	0.15679	19.52143	**0.00014**
Water Treatment	0.91566	7	0.13081	16.28687	**0.00000**
Day	0.55198	4	0.13800	17.18186	**0.00000**
Tunicate x Water Treatment	0.27757	7	0.03965	4.93720	**0.00099**
Water Treatment x Day	0.28284	28	0.01010	1.25771	0.27405
Tunicate x Day	0.26678	4	0.06669	8.30412	**0.00015**
Error	0.22488	28	0.00803		

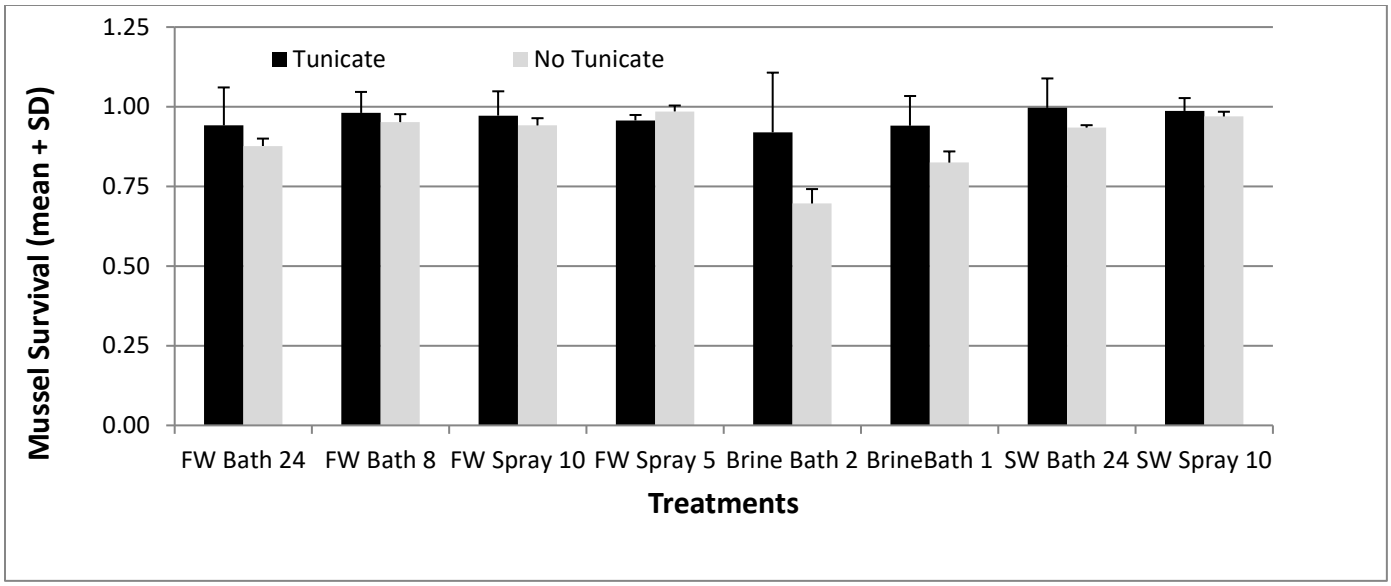


Figure 1. Mean and 1 Standard Deviation of mussel survival per sock in each water treatment and in each tunicate treatment (n =5).

In most water treatments, average survival of mussels with Tunicates was higher than for mussels in the No Tunicate treatment (Figure 1). Average mussel survival in the two Brine treatments was significantly lower for mussels that had No Tunicate (Brine Bath 20: 0.696 $\pm$ 0.187; Brine Bath 10:0.825 $\pm$ 0.093) than those with Tunicates (Brine Bath 20: 0.920 $\pm$ 0.0.45 and Brine Bath 10:0.940 $\pm$ 0.035). Mussels in the Brine Bath for 20 seconds with No Tunicate had significantly lower average survival than mussels in any of the other treatments except Brine Bath 10 secs with No Tunicates. Mussels in Brine Bath 10 sec spray with No Tunicate had significantly lower survival than mussels in all the treatments except Brine Bath 20 sec with or without Tunicates, or Fresh water Bath 24 with or without Tunicates.

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

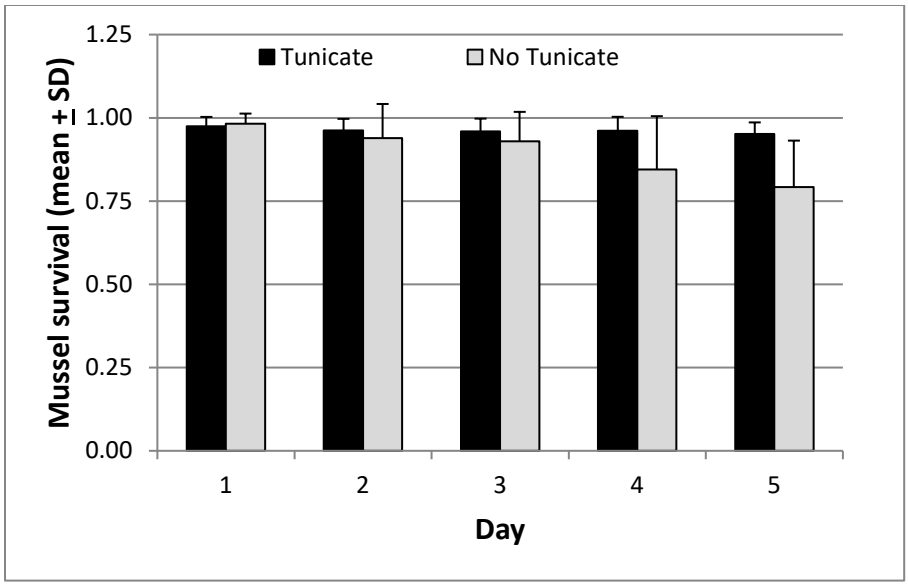


Figure 2. Average and Standard deviation of mussel survival on each experimental day (n=16).

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

Number of mussels per bag

A three-way ANOVA with no replication was run on the number of mussels in each sock at the start of the experiment. Data was -1/x transformed to homogenize the variances. The average number of mussels differed between tunicate treatments on certain days (Tunicate x Day, $p = 0.001$; Table 6). Pairwise comparisons (Tukey's HSD test) of Day and Tunicate treatment mean mussel sizes indicated that the No Tunicate treatment on Day 1 had significantly fewer mussels (54.4 ± 9.7) than in the No Tunicate treatment on Days 2, 3, 4 and 5 (Figure 4). This suggests that on average, mussels were larger in Day 1, No Tunicates treatment than on other days.

Number of mussels differed significantly between the Tunicate and No Tunicate treatments but not on the same day. On Day 4, the average number of mussels in the Tunicate treatment (57.5 ± 13.2) was less than the mussel number in the No Tunicate treatment on Days 2 (76.9 ± 10.1) , 3 (82.5 ±19.5) and 5 (86.5 ± 12.2) (Table 5).

Table 5. Mean and Standard deviation (SD) in the number of mussels per sock and mussel survival in each Tunicate treatment on each day (n = 16).

Tunicate Treatment	Day	Mean Mussel number per sock	SD	Mean Mussel Survival	SD
Tunicate	1	68.375	12.351	0.975	0.028

No Tunicate	1	**54.375**	9.709	**0.982**	0.030
Tunicate	2	70.625	12.153	0.962	0.035
No Tunicate	2	76.875	10.092	0.939	0.102
Tunicate	3	63.25	11.720	0.960	0.038
No Tunicate	3	**82.5**	19.516	0.929	0.089
Tunicate	4	**57.5**	13.245	**0.961**	0.042
No Tunicate	4	73.5	11.402	0.845	0.160
Tunicate	5	**64.13**	8.806	**0.952**	0.035
No Tunicate	5	**86.5**	12.177	**0.792**	0.140

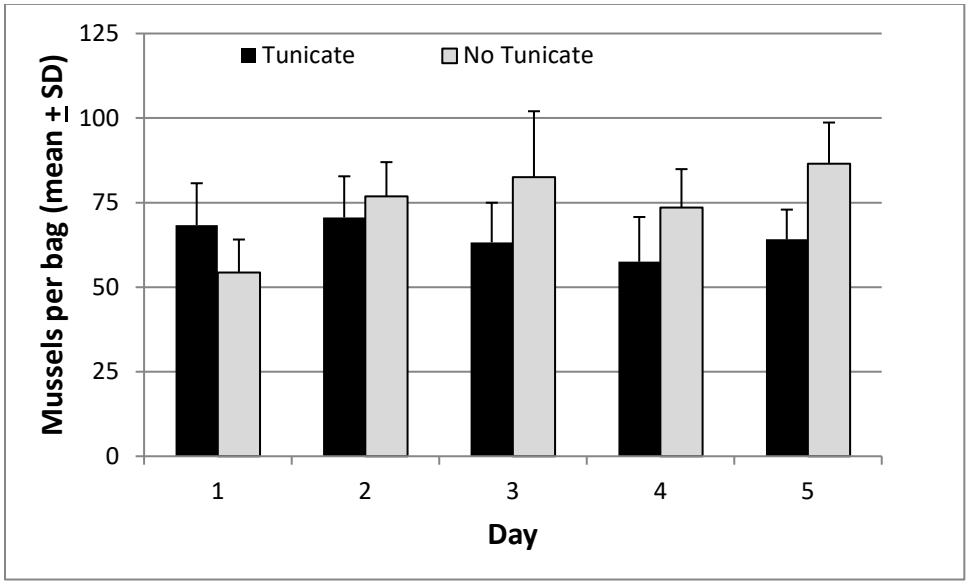


Figure 3. Mean and Standard Deviation of mussel number per sock on each date in the Tunicate and No Tunicate treatments.

The lower average mussel survival on Day 5 in the No Tunicate treatments (Mussel survival: 0.792 $\pm$ 0.14; number of mussels per sock: 86.5 $\pm$ 12.12) as compared to the Day 4 with Tunicates and Day 5 with Tunicates may have been a density-dependent or a size effect (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 the other Tunicate treatments.

Table 6. Analysis of variance results for testing whether average number of mussels differed between the tunicate and water treatments and day of experimental trial.

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Tunicate	0.00007	1	0.00007	9.5921	**0.0044**
Water Treatment	0.00004	7	0.00001	0.6768	0.6901
Day	0.00013	4	0.00003	4.3164	**0.0076**
Tunicate x Water Treatment	0.00008	7	0.00001	1.5510	0.1913
Water Treatment x Day	0.00013	28	0.00001	0.6231	0.8916
Tunicate x Day	0.00019	4	0.00005	6.2431	**0.0010**
Error	0.00021	28	0.00001		

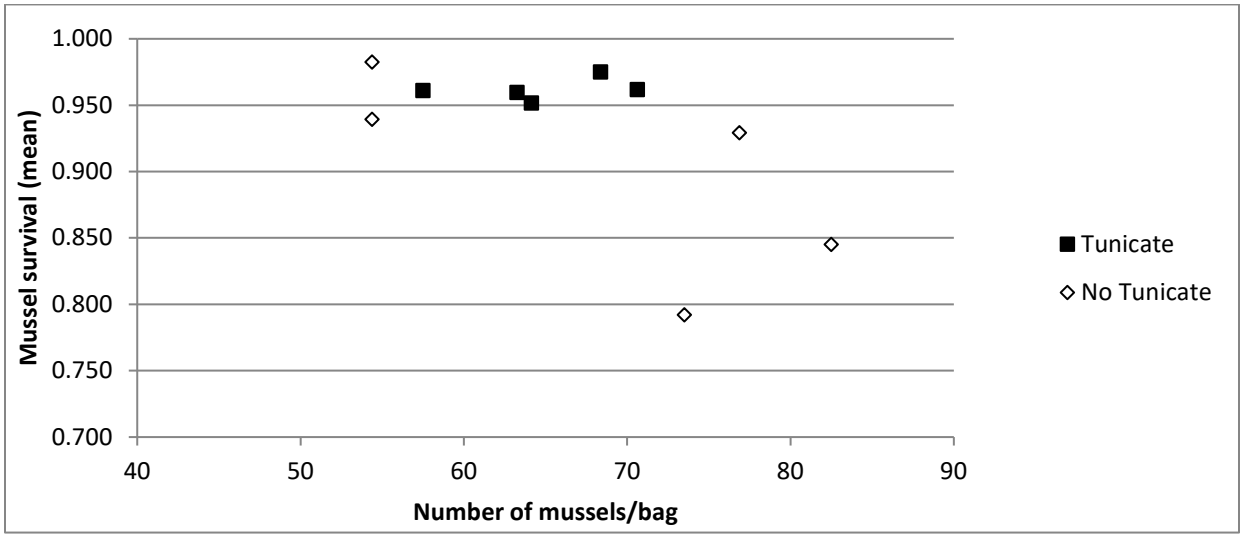


Figure 4. Mean mussel survival versus mean number of mussels per sock. Each point is the mean survival one of the experimental days either with Tunicates (black square) or with No tunicates (open triangle).

New tunicate recruits

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 24 experimental socks and 13 control socks. Other macro-invertebrate fouling on the socks was a bushy bryozoa *Bugula* sp.

Water temperature and salinity

At the start of the treatment trials, seawater temperature at the dock was 24.8°C and salinity was 31%; seawater temperature at the lab was 23.8°C and salinity was 31%; freshwater temperature at the lab was 22°C and salinity was 0%. At the end of the experiment, seawater temperature at the dock was 24.6°C and salinity was 31%; seawater temperature at the lab was 23°C and salinity was 31%; freshwater temperature at the lab was 22.3°C and salinity was 0%.

Discussion

We recommend freshwater baths and freshwater sprays to rid colonial tunicates from juvenile mussels and aquaculture socks. Overall, mussel survival was greater than 82% in the water and trunicate treatments suggeting that tunicates or water treatments did not have a large biological

effect on mussel survival. Short-term (8 hr) freshwater bath and long-term (10 min) freshwater spray treatments were the most effective against colonial tunicates while being the least lethal to mussels. Freshwater baths lasting less than 8 hours and more than 2 hours may be just as effective as the short-term freshwater baths. Freshwater bath and spray treatments may be effective against solitary tunicates such as *A. aspersa* and *C. intestinalis* but further work should be done to confirm this. Acetic acid bath treatments killed all the juvenile mussels and because of mixed results by other researchers, expense of the vinegar, and problems associated with the disposal of used vinegar, we do not recommend this treatment for juvenile mussels.

Brine baths with lower salinity than we used (210%) may be effective against tunicates and negatively impact fewer juvenile mussels. Brine baths >90% were effective against sponges in cultured oysters (Carver et al. 2010). Brine baths >90% and < 200% may be effective against sponges and tunicates and have no negative effect on oysters or mussels. Unexpectedly, in treatment trials where some mussels survived, the mussel survival rate was higher if tunicates were present. The difference in survival might be explained in part by differences in the number of mussels per sock. As volume was kept relatively constant and mussels were grabbed haphazardly, it is possible that on some days some treatments would have socks with more mussels than other treatments. High number of mussels in a sock might indicate that there were smaller mussels in the socks on average, and smaller mussels could be more susceptible to some of the treatments. Density dependent effects such as higher number of mussels competing for food might also have decreased survival, and this could have been confounded by the water treatment effect.

Our study did not examine the mussel tissue weight; further work could examine the effect of treatment on health of the mussels in ways other than length of shell and dead/alive parameters used in our study.

After shellfish aquaculture socks have been treated and to prevent tunicate fouling from re-occurring (due to tunicate larval recruits), treated socks should be returned to seawater in an area where there are no tunicates. This is not easy to do because 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 at Prince Edward Island and The Netherlands are located in the near shore where there tends to be an abundance of tunicates (Locke et al. 2009; Gittenberger 2009). Periodic freshwater bath or spray treatments during the growing season may provide the answer for keeping invasive tunicates off juvenile mussels and aquaculture gear that routinely become fouled by omnipresent tunicates.

Two control treatments, Air-dry and Remain in Eel Pond, were excluded from the analysis of all the rest of the chemical treatments because they were only done on the first day of the experiment. Hence, we do not know whether the controls would have shown the same results as seen in the other treatments which were run on days 1 through 5. See below, (i.e., decrease in survival over time of the mussels exposed to tunicates).

Size of mussels used in the experiments may have influenced the results if socks had different average or variance in mussel sizes. Maximum and minimum mussel size in each sock was measured, but these data are not sufficient to evaluate the effects of size. Future experiments should include measurements of a subset of the mussels in each sock. Size of dead mussels was only measured in a few socks and treatments, which was not sufficient to access whether dead mussel size was different from live mussel size or if size of dead mussels was correlated to treatment.

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