Final Report
Northeast SARE Program
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1. TITLE:
Exploring Husbandry and Equipment Solutions to Infestations of Polydora sp. on a Maine Oyster Farm - FNE09-663

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2. GOALS
1. To evaluate the effect of equipment type, air drying, and salt brine dips on reducing the presence of mud blister worms (*Polydora sp.*) in eastern oysters (*Crassostrea virginica*) on my farm.
2. To evaluate the effects of the above treatments on growth and survival of oysters.
3. To transmit the results of our project to relevant stakeholders.

3. FARM DETAIL
My farm on the Bagaduce River in eastern Maine covers 4.3 acres, and includes several hundred thousand eastern oysters, of various sizes. I use part of the site as a nursery area, and part is for growout. Virtually all of my oysters are grown in containment, as opposed to sowing them directly to the seabed. The location of our lease and the typical gear we use are shown in Appendix I. Over the last several years, the presence of mud blister worms has had a strong negative effect on the income from my oyster farm, and I have to find a solution to this problem. I've operated the oyster farm for over 10 years now, and generally I have a very strong market for my product, both in terms of volume and price. As a direct result, I have also had to pursue other locations to grow my oysters, in areas that do not support populations of blister worms.

I usually buy oyster seed from one of the hatcheries in Maine; receiving seed in May or June of the year, at about 1.5mm shell height. Depending on my farm production and the seed I hope to sell to others, it is not uncommon to purchase 1,000,000 oyster juveniles. Oyster seed first go into a piece of equipment called an upweller, which provides strong flow for the oysters, and good access to planktonic food. For a good review on upwellers, visit the Maine Sea Grant website:
www.seagrant.umaine.edu/extension/shellfish-upweller

Once oysters reach at least 15mm, I may set the oysters out into floating bags, as shown in Appendix I. Sometimes I keep the oysters in the upweller, but the floating bags are easy to clean and handle, and allow the oysters access to warm temperatures and plenty of feed. My oysters will often grow to larger than 2" in the first year, and since the farm

site ices over, I put oysters on the bottom in cages or in moist air storage for overwintering (Hidu et al. 1988) During the second growing season, oysters will reach market size (generally 2.5" and above), for sales to my markets throughout the northeast and the US.

4. COOPERATORS

My collaborators include Eric Moran, who has been a long-time partner in the business, and Dana Morse, who acted as the Technical Advisor. Eric participates in all aspects of farm operations, and Dana helps with the project design, data collection/analysis, and reporting. For this project, we also had help from Dr. Nick Brown, the Director of the Univ. of Maine Center for Cooperative Aquaculture Research (CCAR) in Franklin, Maine, and Dr. Brian Beal, a faculty member at the University of Maine at Machias. Dr. Brown provided us with urchins for one of the tests in the study, and Dr. Beal provided assistance in data analysis. We also received advice from Dr. John Kraeuter of the Haskin Shellfish Lab in Bivalve, New Jersey, regarding the use of indices to evaluate the worm burrows in the final samples.

5. Project activities

Major activities in this project include:
- Project setup with appropriate equipment, sampling and data acquisition/analysis (May-June, 2009)
- Weekly and monthly treatments as written under the initial proposal (2009, 2010)
- Sampling at end of first year and end of project (Oct 2009)
- Adjustment to changes in planned activities (see below)
- Outreach to shellfish growers and others (2009-2011)

In more detail, the project involved the following.
1 - Preparation our cage variables: Floating Bags, Oyster Gro cages, Dark Sea Trays, and Bottom Racks. Photos of these are shown in Appendix 1, with descriptions below. To begin the experiment, young oysters were stocked into our experimental units, in June of 2009. The oysters were produced in the hatchery by Muscongus Bay Aquaculture, in Bremen, Maine, and subsequently held in our upweller on the Bagaduce River. We stocked 2 US gallons of seed to each experimental unit, as a volume that allowed reasonable space for growth, and which approximated commercial conditions.
2 - Implementation of our treatment schedule. We had planned to have the following treatments:
Cage flipping of bags and Oyster-Gro units, once per week. By flipping the bag or OG unit, we were able to allow the structure to air dry and to be cleaned if necessary. Both drying and scrubbing help to keep the structure clean, so that water can flow to the oysters. Oyster Gro units were flipped for 24 hours, then re-submerged.

Salt brining of oyster bags, Oyster Gro cages, and bottom racks, once per month. This treatment was performed to kill fouling organisms, and was intended to kill or reduce mud blister worm populations as well. To perform this treatment, salt is mixed into seawater to saturation, in a tank large enough to hold several oyster bags. The

oysters and bags are submerged in the solution for a specified time (5,10 or 15 minutes), then removed and allowed to dry in the air for at least 1 hour, at which point the oysters are returned to the water.

Submersion in an iodine bath, once per month. This treatment was to have been investigated, modeled after the suggestion of Nell (2007); essentially to dip the oysters and cages in a low-concentration iodine solution, to be followed by an air dry. However, as we describe below, we were unable to obtain the necessary permitting to try this treatment, and the cages we set up for this purpose were considered as extra controls.

Urchins as epiphytic grazers. We speculated that since larval blister worms have to settle on the oysters before they create their burrows, that small Green Sea Urchins (*Strongylocentrotus droebachiensis*) would feed on newly settle worm larvae, and therefore reduce the infestation rate. Juvenile urchins were obtained from the University of Maine's Center for Cooperative Aquaculture Research (CCAR), in Franklin, ME. However, while the urchins were in a protective cage and provided with sufficient food prior to deploying into oyster bags, they all died an rapidly decomposed, leading us to believe that the salinity and/or temperature were not appropriate for urchin survival.

Adjustable Longlines were to have been implemented in the project, but this depended on the acquisition of a new site that would have the right depth and bottom time to support this gear. The new site was not approved during the length of the study, and we were not able to investigate this approach. However, given the observations of Littlewood et. al. (1991), we feel that the ALS system might be a valuable one, since regular air drying on a per-tide cycle appears to have some benefit.

We also planned for each gear type to have a **Control**. Controls received only the normal handling, which included a weekly flipping for Bags and Oyster Gro units. Dark Sea tray and Bottom Rack controls were removed from the water during the time that some portions of the equipment (ie: bags within a bottom rack, or levels within a stack of Dark Sea Trays) received the salt baths, but otherwise were untreated.

Stocking volumes for all treatments were adjusted to 2 gallons per bag at the start of year 2 activities, to reflect the stocking volume used at the start of the project, and to minimize the effects of competition and density on growth and infestation.

3 - Overwintering of oysters on the bottom of our lease site in the Bagaduce River, in November/December of 2009 through April of 2010.

<u>Gear Descriptions:</u>
Floating Bags
Floating bags, often referred to as 'shellfish bags' or 'tray's consist of a plastic mesh bag with two floats attached, to either side. The bag itself is made of black extruded polyethylene, and can come in varying sizes; ours had meshes of 3/8" bar space (not diagonal). The floats are made of either blue Styrofoam insulation or Styrofoam 'logs'; either allows the bag to float partly out of the water. When being dipped, the entire bag is

set into the salt bath, together with the oysters inside it. Different bag treatments received dips of different length, reflected in the treatment name.

Oyster Gro

The Oyster Gro system (www.oystergro.com) combines large plastic floats with a cage, which holds 6 shellfish bags. The normal position is where the floats are on the top position with the cage hanging submerged below it, and the air-drying position is where the cage is flipped so that the oyster bags are exposed to air, and the whole unit floats on the waters' surface. During out dipping trials, bags were removed from the Oyster Gro cage, and dipped/dried according to the specified length.

Dark Sea Trays

These are rigid plastic trays that stack one above the other, such that a rope or post can be set up through the middle of the stack of trays, and either placed on the seabed or hung from a structure like a raft. During dipping trials, the whole tray was set into the salt bath, oysters and all. (http://www.bannertown.com/darksea.html, for more information)

Bottom Cages

Our cages were simply wire mesh structures that held plastic shellfish bags, one above the other. In our case, the bottom cages were one bag wide, and four levels wide. Bags were removed from their cage and dipped for the time specified by the treatment name.

Data collection:

Sampling for initial/final size and growth were conducted on June 26, 2009, August 13, 2009, October 22, 2009 (end of first year), May 5, 2010, July 27, 2010, and November, 2010. A sample of 30 individuals was drawn haphazardly from each experimental unit, with shell lengths taken to the nearest millimeter, using a manual caliper. Shell length data was recorded in an Excel spreadsheet for later analysis.

The end-of-first-year sampling focused only on larger individuals, and was to examine the number of worm burrows in the shells. We selected larger individuals because we anticipated greater settlement inside them, compared to smaller individuals. Infestation rates were low enough so that the burrows could be counted separately.

End of project data collection:

Final sampling focused on evaluating the number of burrows and on the sizes of the oysters in the different treatments, and the analysis for worm burrows proved to be more difficult that we anticipated. Specifically, the end-of-project sampling revealed that many oysters had so many burrows (Appendix II), that an outright count was not possible to attain – the burrows overlapped so much as to become individually indistinct. After consulting with experts in shellfish aquaculture and statistics at the Univ. of Maine and the Haskin Shellfish Research Lab (New Jersey), data on infestations was collected as a relative index, calculated as follows:

Shell heights were taken on 30 individuals for each treatment, and an index was provided for each individual in the sample, on both the left and right valves. The index was as

follows:
1 = Zero burrows
2 = 1 to 10 burrows, with minimal coverage of the inner surface of the shell
3 = 1 to 10 burrows, with major coverage of the inner surface of the shells
4 = More than 10 burrows, or so many that they could not be counted accurately

Data analysis for the indices in the final samples was as follows, with a specific acknowledgement to the assistance of Dr. Brian Beal in this area.
- Unreplicated treatments were pooled, yielding 11 treatments: Oyster Bag Control, Oyster Bag Brine, Oyster Bag Iodine, Urchins, Dark Sea Trays, Oyster Gro Control, Oyster Gro Brine, Oyster Gro Iodine, Rack Control, Rack Brine, Rack Iodine. This did not allow us to investigate the differences in dip time, but did allow for some power with respect to evaluating gear type.
- Shapiro-Wilk analysis indicated that the data were distributed normally, and Levene's test (p-0.0564) indicated that variances in the data were homogeneous.
- Mean difference in the infestation indices for the left and right valves was 0.190476, and not significantly different from zero, so ANOVA analysis could be performed on either set of valves (in this case, the right valve was used).

ANOVA Analysis
Six contrasts were performed:
1. All controls (Bags, Urchins, Racks and Iodine) except Oyster Gro controls were contrasted with all other treatments
2. Oyster Gro vs. all Racks, Bags, Urchins, Dark Sea trays
3. Oyster Gro Control (including Iodine) v. Oyster Gro Brine
4. Dark Sea and Rack Controls vs. All brined treatments
5. Dark Sea Trays vs. Rack Controls
6. Brined bags and brined Oyster Gro vs. Brined Rack

Shell Heights:
We compared the growth of the oysters in the different treatments very simply, by calculating the means and the 95% confidence intervals for each group. Overlapping intervals indicate a significant difference, so we were able to graph out a basic way to view average shell size, and whether or not a statistically significant difference existed, from one treatment or group to the next. Means were calculated for a whole treatment in some cases (such as the three replicates comprising the Control Bags treatment) or for a single replicate in those cases where there was only one replicate, such as the Dark Sea Tray, High position.

6. Results

Shell Heights and Worm Burrows – Initial stocking and mid-test sampling

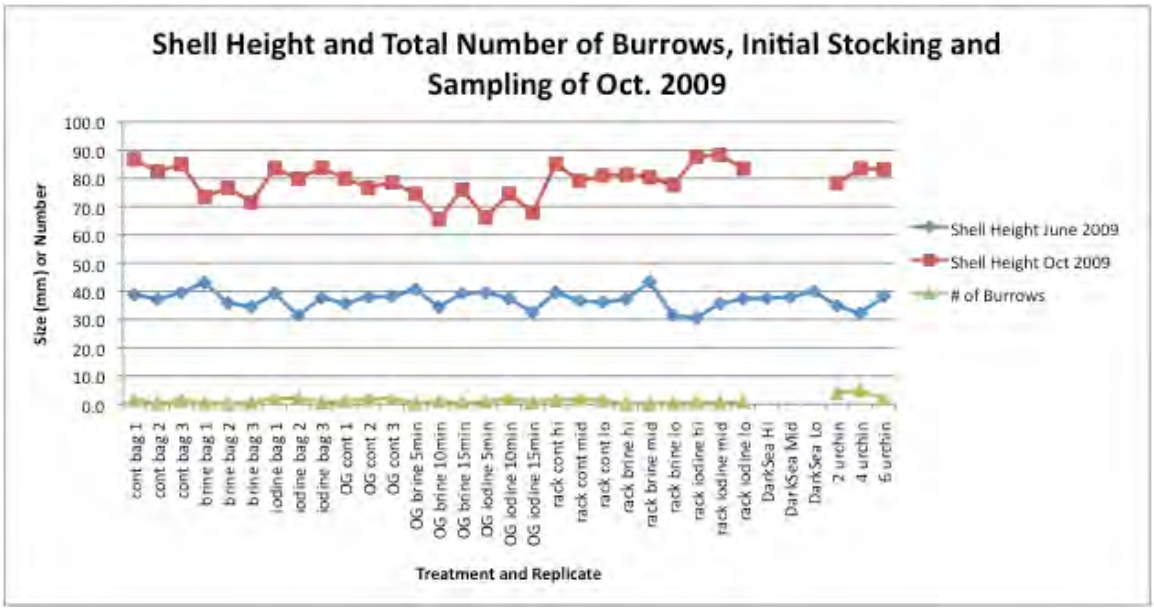
The initial sizes of oysters stocked into experimental units ranged from 30.6mm to 43.4mm, although they were taken randomly from the same stock. All experimental units received 2 gallons of seed to begin. By the end of the first years' program, growth increment ranged from 26.6 mm (Oyster Gro Iodine 5 min) to 57.1mm (Rack/Iodine, high position). Interestingly, the number of worm burrows in both the left and right

valves of the oysters was extremely low, rarely above a total of 2 burrows per oysters. Initial and mid-term shell heights are shown in Table 1, together with the mean numbers of worms per oyster. The data for growth and number of burrows is also displayed graphically in Figure 1.

Table 1. Shell heights at initial stocking (June 2009) and sampling at end of first year (October 2009), growth achieved, and number of mud blister burrows, by treatment and replicate. SH = Shell Height, N = sample size.

Treatment/Replicate	Initial SH	Initial N	Mid-test SH	Mid-test N	Growth	Left	Right	Total
cont bag 1	38.8	30	86.8	10	48.0	0.7	1.0	1.7
cont bag 2	37.2	30	82.4	10	45.2	0.0	0.3	0.3
cont bag 3	39.5	30	85.0	10	45.5	0.7	0.7	1.4
brine bag 1	43.1	30	73.5	10	30.4	0.4	0.1	0.5
brine bag 2	35.9	30	76.7	10	40.8	0.0	0.2	0.2
brine bag 3	34.5	32	71.6	10	37.1	0.2	0.4	0.6
iodine bag 1	39.3	32	83.6	10	44.3	0.9	1.1	2.0
iodine bag 2	31.4	32	79.9	10	48.5	0.8	1.4	2.2
iodine bag 3	37.8	32	83.7	10	45.9	0.4	0.3	0.7
OG cont 1	35.7	31	80.0	9	44.3	0.6	0.6	1.1
OG cont 2	38.1	31	76.7	10	38.6	0.5	1.3	1.8
OG cont 3	38.2	31	78.6	10	40.4	1.1	1.1	2.2
OG brine 5min	40.7	30	74.5	10	33.8	0.1	0.2	0.3
OG brine 10min	34.5	30	65.5	10	31.0	0.4	0.7	1.1
OG brine 15min	39.2	30	76.0	11	36.8	0.1	0.5	0.6
OG iodine 5min	39.6	30	66.2	10	26.6	0.0	0.8	0.8
OG iodine 10min	37.5	30	74.6	10	37.1	0.9	1.2	2.1
OG iodine 15min	32.5	30	67.9	10	35.4	0.7	0.0	0.7
rack cont hi	39.6	30	85.2	10	45.6	0.5	1.1	1.6
rack cont mid	36.6	30	79.1	10	42.5	0.3	1.5	1.8
rack cont lo	36.2	30	81.0	10	44.8	0.5	1.1	1.6
rack brine hi	37.2	30	81.4	10	44.2	0.0	0.1	0.1
rack brine mid	43.4	30	80.4	10	37.0	0.1	0.1	0.2
rack brine lo	31.4	30	77.6	10	46.2	0.4	0.0	0.4
rack iodine hi	30.6	30	87.7	10	57.1	0.1	0.5	0.6
rack iodine mid	35.7	30	88.3	10	52.6	0.1	0.5	0.6
rack iodine lo	37.4	30	83.5	10	46.1	0.1	0.7	0.8
DarkSea Hi	37.5	32		0				
DarkSea Mid	38.0	32		0				
DarkSea Lo	40.0	32		0				
2 urchin	34.8	31	78.3	10	43.5	1.9	2.2	4.1
4 urchin	32.2	30	83.5	10	51.3	2.4	2.6	5.0
6 urchin	38.3	30	83.2	10	44.9	0.6	1.3	1.9

Figure 1. Mean shell heights for all treatments and replicates at initial stocking and mid-term sampling, and mean total worm burrows per oyster at the end of the 2009 experimental season.



Worm infestation at Final Sampling, November, 2010

The Analysis of Variance test revealed some differences between gear types and treatments, at the 95% confidence level. Specifically, contrast Numbers 1, 4 and 6 returned significant differences. Details of the ANOVA procedure are shown in Table 2.

Table 2. Results of ANOVA test on the shell infestation indices, concerning treatment and equipment type.

The GLM Procedure
Dependent Variable: rigtbur

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	4.64741882	0.46474188	3.77	0.0045
Error	22	2.71465529	0.12339342		
Corrected Total	32	7.36207411			

R-Square	Coeff Var	Root MSE	rigtbur Mean
0.631265	10.32306	0.351274	3.402808

Contrast	DF	Contrast SS	Mean Sq.	F-Value	Pr>F
1. Controls vs. Rest	1	1.69300108	1.69300108	13.72	0.0012
2. OysterGro vs. 4 Non-Oyster Gro treatments	1	0.09294100	0.09294100	0.75	0.3948
3. OysterGro - (Controls vs. Brine)	1	0.09083007	0.09083007	0.74	0.4002
4. Dark & RackCon v. Brine & Rack Brine	1	0.70163743	0.70163743	5.69	0.0261
5. Dark v. RackCon	1	0.22218945	0.22218945	1.80	0.1933
6. Brine v. Rack Bri	1	0.58139268	0.58139268	4.71	0.0410

These tests indicate the following:
First, the control groups, when tested against other treatments and gear types, had significantly higher worm infestations. Second, brining had an inhibiting effect on infestation by Polydora, generally. Third, the use of bottom racks, which became exposed to air during each tidal cycle, also has an inhibiting effect on the infestation rates.

A more basic evaluation was a simple means testing of the burrow indices for those treatments that used brine, vs. all other treatments. For this test, all the simple means were calculated for the indices on both valves, for brined samples vs. all non-brined samples. 95% confidence intervals on these basic averages supported the ANOVA results, with brine treatments having an average index of 2.982 (Std. Dev = 0.4125, 95% CI = 0.2695, N = 9), vs. an average index of 4.4135 (Std. Dev. = 0.3906, 95% CI = 0.1596, N = 23). The 95% confidence intervals do not overlap, indicating a statistical difference, at the 95% level of confidence. A similar evaluation on floating bag controls (planned controls, plus the urchin, and iodine groups) vs. all others indicated that floating bag controls had significantly higher infestation rates than other treatments, with burrows being too numerous to count individually: floating bag controls average index was 3.73 (Std. Dev. = 0.1419, 95% CI = 0.0927), while all others combines had an average index of 3.1482 (Std. Dev. = 0.4048, 95% CI – 0.1620).

Even given the results of the ANOVA and means analyses, the treatments and equipment that we tried in this experiment had little positive impact in the sense of achieving a marketable product. For example, even though the salt brine had an apparently positive effect in reducing worm infestations relative to the other groups, there were still so many that most of the oysters we sampled would have been effectively unmarketable, or at least not as prices necessary for profitability.

The results can be seen in the data output that summarizes the indices for left and the right valves for all treatments and replicates, as shown in Table 3. Note that indices are commonly well above 2.0, and frequently above 3; this means that it is common that each valve frequently had a significant portion of the inner surface occupied with blisters, or that there were so many as to be indistinguishable.

Table 3. Mean infestation indices for replicates at final sampling, November, 2010. R = replicate, N = sample size, SH = Shell Height, Diff. = difference in index between left and right valves (determined to be not significantly different from zero).

Obs	treatmen	rep	N	S.H.	Rt. valve	L. valve	Diff.
1	ControlB	1	30	81.60	3.93	3.80	0.13
2	ControlB	2	26	80.54	3.81	3.42	0.38
3	ControlB	3	30	82.93	4.03	3.87	0.17
4	DarkSeaH	1	26	71.19	3.77	3.58	0.19
5	DarkSeaH	2	28	76.68	3.79	3.68	0.11
6	DarkSeaH	3	26	66.50	3.62	3.35	0.27
7	OGroBr5m	1	30	75.53	3.60	3.40	0.20
8	OGroBr5m	2	28	77.50	2.57	2.68	-0.11
9	OGroBr5m	3	30	79.10	2.87	2.90	-0.03
10	OGroCont	1	30	76.53	3.77	3.53	0.23
11	OGroCont	2	29	74.93	3.00	3.07	-0.07
12	OGroCont	3	31	76.87	2.77	2.84	-0.06
13	OGroI05m	1	28	74.32	3.50	3.25	0.25
14	OGroI05m	2	31	77.10	2.58	2.39	0.19
15	OGroI05m	3	30	73.90	3.73	3.40	0.33
16	RackBriH	1	28	73.64	2.79	2.64	0.14
17	RackBriH	2	28	65.71	2.96	2.43	0.54
18	RackBriH	3	24	60.71	2.46	2.42	0.04
19	RackConH	1	28	82.93	3.54	3.54	0.00
20	RackConH	2	27	61.07	3.00	2.85	0.15
21	RackConH	3	25	63.56	3.48	3.04	0.44
22	RackIoHi	1	28	73.82	3.39	3.39	0.00
23	RackIoHi	2	25	71.68	3.04	2.80	0.24
24	RackIoHi	3	27	64.22	3.00	2.70	0.30
25	Urchin2	1	28	72.61	3.86	3.25	0.61
26	Urchin2	2	25	74.24	3.92	3.76	0.16
27	Urchin2	3	31	75.94	3.87	3.71	0.16
28	brine	1	29	72.10	2.86	3.17	-0.31
29	brine	2	31	68.84	3.58	3.29	0.29
30	brine	3	30	77.03	3.63	3.40	0.23
31	iodine	1	31	72.52	3.68	3.45	0.23
32	iodine	2	30	72.57	4.00	3.63	0.37
33	iodine	3	29	82.10	3.90	3.38	0.52

Shell Height, Final Samples:

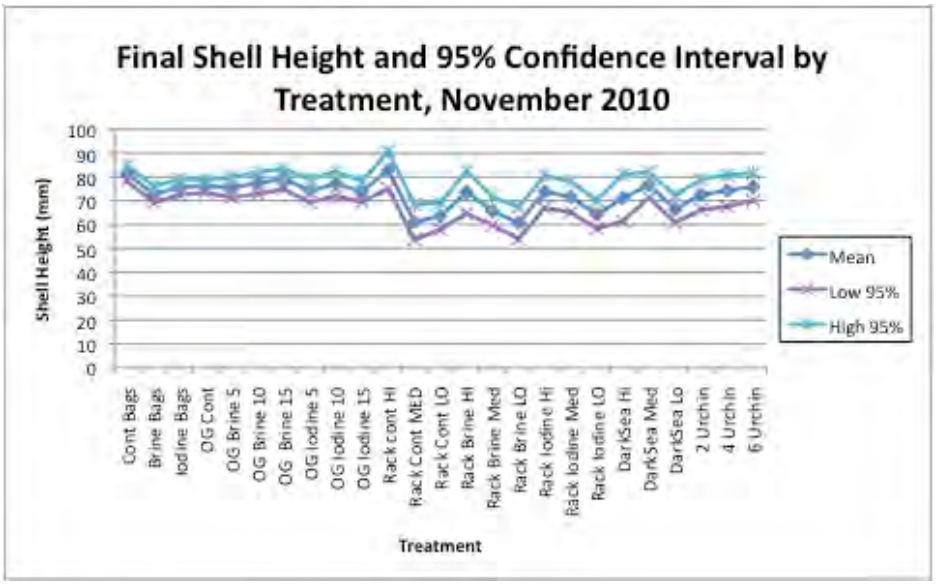
Shell height means and confidence intervals are listed in Table 4, and graphically in

Figure 2; non-overlapping confidence intervals indicated a statistically significant difference at the 95% confidence level. Overall, there was a good deal of overlap between many groups. Control bags did significantly better than several treatments, including the Brine Bags and the lower positions in the Racks and the Dark Sea trays. Treatments in the lower positions for the bottom racks and the Dark Sea Trays did noticeably worse overall, with significantly lower growth than several other treatments. This lack of growth might be attributed to higher degrees of fouling, or reduced rates of water flow, or to slightly reduced temperature. However, many treatments did not perform significantly different from one another, indicating that treatment or equipment had limited effect on growth during this experiment, again with the exception of the lower levels of stacked equipment, such as the Rack and Dark Sea Treatments.

Table 4. Shell Heights (in millimeters) for groups at final sampling, with boundaries for 95% confidence intervals.

Treament	Mean	Count	Conf Int	Low 95%	High 95%
Cont Bags	81.54	85	3.26	78.28	84.80
Brine Bags	72.62	90	3.47	69.15	76.09
Iodine Bags	75.75	89	3.26	72.49	79.01
OG Cont	76.13	90	2.64	73.49	78.78
OG Brine 5	75.53	30	4.13	71.41	79.66
OG Brine 10	77.50	28	4.61	72.89	82.11
OG Brine 15	79.10	30	4.28	74.82	83.38
OG Iodine 5	74.32	28	4.91	69.41	79.23
OG Iodine 10	77.10	31	5.07	72.02	82.17
OG Iodine 15	73.90	30	4.36	69.54	78.26
Rack cont HI	82.93	28	7.75	75.18	90.68
Rack Cont MED	61.07	27	7.44	53.63	68.52
Rack Cont LO	63.56	25	5.57	57.99	69.13
Rack Brine HI	73.64	28	8.95	64.69	82.59
Rack Brine MED	65.71	28	5.89	59.83	71.60
Rack Brine LO	60.71	24	6.70	54.00	67.41
Rack Iodine HI	73.82	28	6.78	67.04	80.60
Rack Iodine MED	71.68	25	6.49	65.19	78.17
Rack Iodine LO	64.22	27	5.93	58.29	70.15
DarkSea Hi	71.19	26	9.73	61.47	80.92
DarkSea MED	76.68	28	5.31	71.37	81.99
DarkSea LO	66.50	26	5.72	60.78	72.22
2 Urchin	72.61	28	6.35	66.25	78.96
4 Urchin	74.24	25	6.63	67.61	80.87
6 Urchin	75.94	31	5.85	70.09	81.79

Figure 2. Shell heights and 95% confidence interval boundaries for all groups, during final sampling, November, 2010.



Mortality observed during the experiment:
Although we did not keep especially detailed notes on mortality, we did observe that mortalities appeared higher in the brine treatments of 10 minutes and especially 15 minutes. We estimate that in some cases, mortality up to 25% was occurring. This result came as a surprise, since reports from other oyster growers indicated little impact from a bath of this duration, and consequently, we have no explanation as to the actual cause.

Conditions
No unusual conditions were experienced that would have contributed to the results we saw. The growing season was productive, and overall shellfish health was good.

8. Economics
This study was unable to provide information that would be benefit the bottom line of a suspension-culture oyster operation, either through reduced labor, or through maintaining the sale price of the product. As mentioned above, even those oysters that had a statistically lower infestation rate were still infested to such an extent that their marketability was questionable. With that said, the freezing experiment mentioned below did appear to have some promise, and the current project may have provided the insights necessary to achieve an eventual solution.

9. Assessment
This project did stimulate a pre-proposal to the Northeast Regional Aquaculture Center, with collaborators from ME, MA, RI, NJ, NY and NC, and a full-proposal was requested, although the final proposal was not funded. It did however shed light on the fact that Polydora infestation is a problem of interest to many growers along the US East Coast.

Secondly, this project fostered another, involving staff at the Darling Marine Center and several undergraduate students. Michael Devin, Hatchery Manager at the DMC, worked with four undergraduates to examine the effects of freezing on the survival of Polydora vs. that of the oysters. Freezing appears to be a treatment with some promise, though more work is needed to examine the possibilities more fully. A summary of the project is provided in Appendix 2, which is a presentation used as part of our outreach, during the 2010 Northeast Aquaculture Conference and Expo, in Plymouth, MA.

Last, a side experiment conducted at the Center for Cooperative Aquaculture Research in Franklin, Maine, yielded encouraging results. A sample of over 100 oysters was put into a cooler at approximately 38deg. F. A small sample (10-20 individuals) was looked at after 4 weeks, 6 weeks and 8 weeks, to see if the oysters were alive, and if the worms were as well. Our opinion after looking at the samples is that 4 weeks in moist storage was enough to kill all the adult worms, while keeping the oysters alive. It is unknown what effect the storage will have on the eggs of the worms, which are laid and retained inside the burrows.

10. Adoption
This experiment, and the additional experiments that it generated, gave us some hope as to how to break the cycle of worm infestation. Using the moist air storage technique, we hope to create conditions over winter where we can kill all the worms (and maybe the eggs too), so that when we put our oysters back in the water, they have a chance to cover over the burrows, and resume normal growth.

Update - November 2011: Of the oysters brought to CCAR for moist air storage, a subsample was removed at 1 week, 2 weeks and 3 weeks, to assess the viability of worms inside the shell, as well as the oysters themselves. After 3 weeks, we have found that virtually all of the worms were dead, with no mortality of oysters. At this point, we now feel that we have a tool that we can use, to break the infection cycle and allow our oysters to repair their shells, and to regain some of the condition they may have lost. This is a significant development, and while it was not part of the original proposal, the SARE-funded work allowed us to develop the hypothesis and test the treatment.

11. Outreach
Outreach activities for this project included:
- Presentation at the 2010 Northeast Aquaculture Conference and Expo, December, Plymouth, MA.

- Presentations at two meetings of the Shellfish Working Group; February 2011 and February 2010.
Information from this work will be included on the shellfish pages of the Maine Sea Grant website, and will be incorporated into a project flyer ('Research in Brief') as part of an occasional series summarizing research details produced by Maine Sea Grant.

12. Report Summary

A two-year study was conducted on a Maine oyster farm, to investigate the influences of gear type, air drying and brine dipping, on the infestation rates of the marine polychaete *Polydora websterii*. A combination of floating shellfish bags, Oyster-Gro cages, Dark Sea Trays and bottom racks were used in conjunction with brine dips and subsequent air drying, and the oysters were sampled periodically for infestation rates and growth, with notes taken on mortality. After the first season, infestation rates were very low, often below two burrows per oyster. After two seasons of growth, the Control group of floating oyster bags had significantly higher infestation rates than other treatments. However, the infestation indices for both the Controls and all others was in excess of 3.0, which meant that a significant portion of the inner shell was occupied with burrows, and likely to receive a negative impression in the marketplace. Brine dipping was also found to have an inhibitory effect on worm infestation, as did the use of bottom racks that were exposed to air at low tide. That said, our treatments of brine and air exposure in this experiment did not have enough of an effect as to dramatically improve the marketability of the oysters.

Subsequent work at the Darling Marine Center and the Center for Cooperative Aquaculture Research did however shed some light on a treatment that could be useful for oyster producers, and was a direct result of the SARE investigation. The use of moist air storage is suggested as one potential approach that could allow oyster farmers to break the cycle of infestation.

Literature Cited:
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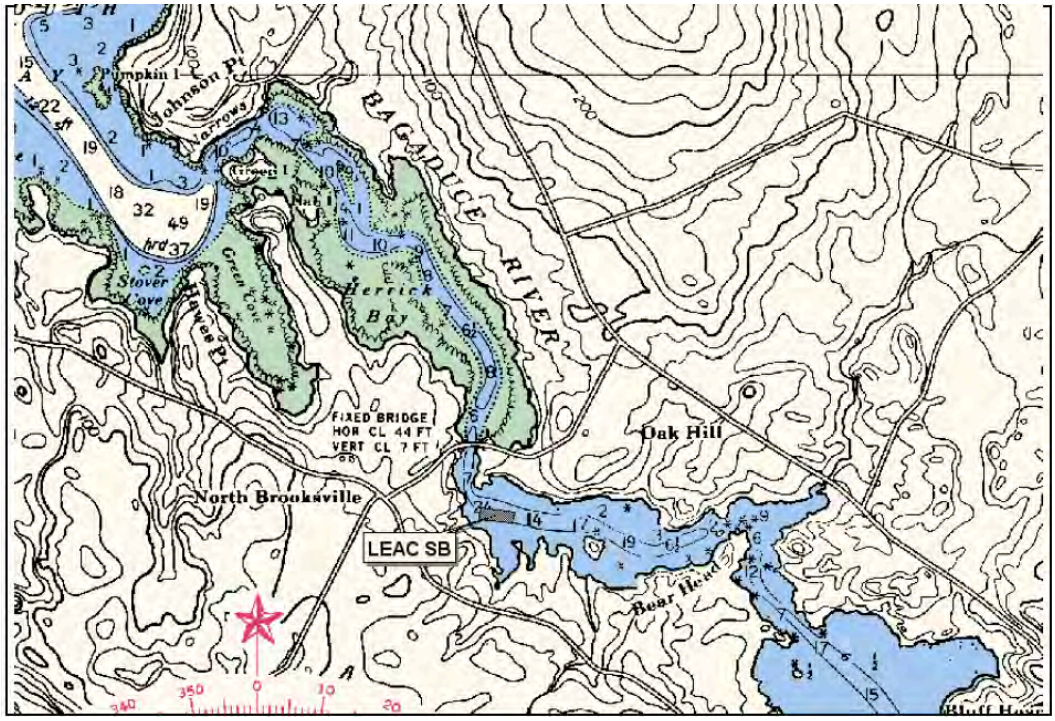
Littlewood, DTJ; Wargo, R N; Kraeuter, J N; Watson, R H. (1992). The influence of intertidal height on growth, mortality and *Haplosporidium nelsoni* infection in MSX mortality resistant eastern oysters, Crassostrea virginica (Gmelin, 1791). *J. Shell. Res.* 11(1): 59-64.

Nell, J. 2007. Controlling mudworm in oysters. Primefact #590. New South Wales Dept. of Primary Industries; Nelson Bay, New South Wales, Australia. 4pp.

Appendix I: Farm Location and Equipment

Lease site location on the Bagaduce River – Figure courtesy of Maine Dept. of Marine Resources



Oyster bags (left) and Oyster Gro units on the lease site.

Single level of a Dark Sea Tray – these are stacked one above the other.

Bottom cage, typical of those used in the project. Shellfish bags are placed into each level of the 4-tier cage, which rests on the bottom.

Appendix II. Polydora websterii

Polydora websterii, removed from its burrow, in an oyster from the Bagaduce River Oyster Company, October 2009.

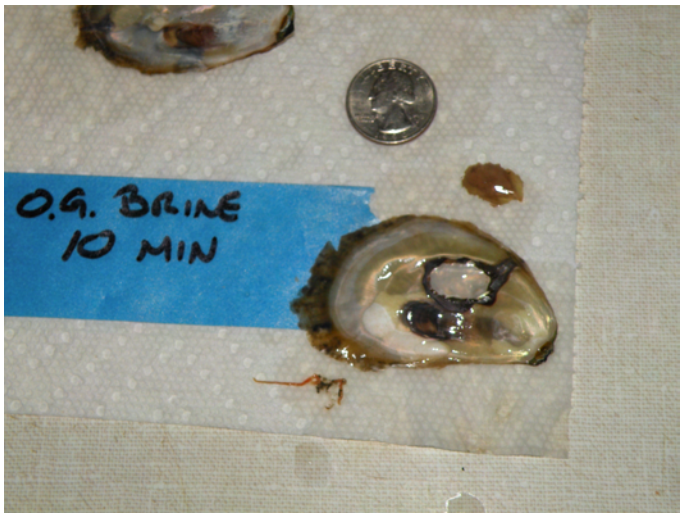


Photo of some representative oysters at the end of sampling. No oysters in this sampled rated an index of 1,which would have indicated zero burrows. The oyster in the left column, at the top, would have rated an index of 2: between 1-10 burrows, minimal shell coverage. The oyster in the right column, third one down, would have rated an index of 3 – between 1 and 10 burrows, with significant valve coverage. The oyster at the bottom right, actually a small individual, would have rated an index of 4 – too many burrows to count, and significant valve coverage.

Other documents:
Accompanying this report are a full set of photographs taken during the experiment, and the database files showing the raw data and the various manipulations used during analysis.