Performance Progress Report - NA14NMF4270033

Title: **Demonstrating Shellfish Aquaculture Technology in Pilot and Commercial Scale Projects: Creating New Opportunities for Maine's Coastal Communities**

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Award Period: **July 1, 2014 through June 30, 2016**

Report Period: **January 1, 2016 through June 30, 2016**

The project proposed to: 1) demonstrate marine aquaculture technologies in pilot and commercial scale projects designed to create jobs in coastal communities, produce healthful, local seafood, revitalize working waterfronts and support traditional fishing communities; and, 2) provide training for fishermen and others in coastal communities in aquaculture production methods.

The proposed project goals were to: To increase soft-shell clam harvests locally in the face of increasing threats due to invasive green crab predation, warming seawater temperatures, and ocean acidification, and to create a model shellfish management program for coastal Maine communities facing unprecedented declines in clam landings.

The proposed project objectives were to:

1) Determine spatial and temporal variability of green crabs, *Carcinus maenas*, in the Harraseeket River and intertidal areas adjacent to the river using specially designed and tested traps in an attempt to remove crabs from the ecosystem that, otherwise, would remain to prey on soft-shell clams of all age-classes;

2) Test the efficacy of "green crab fencing" of intertidal areas using methods similar to those used in the middle of the last century to deter green crabs from preying on soft-shell clam juveniles and adults;

3) Examine whether sediment buffering, under the lowest sediment pore pH conditions, will result in enhanced numbers of soft-shell clam settlers and recruits compared to control areas where no buffering occurs.

4) Examine the interactive effects of stocking density of cultured soft-shell clam juveniles (10-15 mm shell length, SL) in netted plots and crab trapping on clam survival and growth.

5) Determine whether the use of predator deterrent netting, in combination with various densities of adult soft-shell clams, will result in an enhancement of wild clam recruits; and,

6) Train clammers and other interested fishermen in the use of aquacultural techniques to improve local clam harvests.

The third performance report did not include information about two field experiments conducted during 2015.

A. Field Experiment – Call's Cove (Fig. 1)

Methods:

During April 2015, a field experiment was conducted at Call's Cove (43° 49' 2.4348'' N; 70° 3' 52.8192'' W) to determine the interactive effects of two types of predator deterrent netting (flexible vs. extruded), stocking density (30 vs. 60 animals/ft^2), and aperture type (4.2 mm vs. 6.4 mm) on survival and growth of cultured soft-shell clam juveniles (20.6 ± 0.51 mm, n = 103)

(Table 1). Clams were added to wooden boxes (89 cm x 46 cm x 122 cm and lined with 4.2 flexible netting on the bottom) that were filled with ambient sediments. Wooden laths that were nailed to the top of the box were used to keep netting in place. Five replicates of each of six treatments were used. Boxes were placed in a 6 x 5 matrix (with 2 m spacing between rows and columns) and treatments assigned randomly to positions within the matrix (Fig. 2). During the first week of November 2015, netting was removed from each box and two benthic core samples ($A = 0.00811\ m^2$) taken randomly. Samples were washed through a 1 mm mesh, and all live and dead cultured clams (with undamaged valves and crushed/chipped valves) were enumerated and measured (two linear measurements – an initial shell length [SL] that appears on the valves of all cultured clams when added to sediments [see Beal et al., 1999], and the final SL) to the nearest 0.01 mm using Vernier calipers. In addition, all wild Mya from each core sample were counted and measured (as described above).

Table 1. Six treatments used in the field experiment at Call's Cove (April-November 2015)

Treatment	Aperture (mm)	Netting Type	Stocking Density (#/ft^2)
A	4.2	Flexible	30
B	4.2	Flexible	60
C	6.4	Flexible	30
D	6.4	Flexible	60
E	6.4	VEXAR	30
F	6.4	VEXAR	60

Figure 2. Intertidal boxes located at Call's Cove (Freeport, ME) on 16 July 2015.

Percent survival of clams in each sample was estimated by dividing the number of live clams by the total number of live and dead clams (% survival = # live clams/(# live clams + # dead clams). Prior to a one-way analysis of variance (ANOVA), percent survival data were arcsine-transformed to meet assumptions of variance homogeneity. Absolute growth data (Final SL – Initial SL) required no transformation; hence, ANOVA was performed on the raw growth data. A series of five a priori contrasts were conducted regardless of the overall F-test to better understand the nature of treatment means (Table 2).

Table 2. Five a priori contrasts used to determine treatment effects on mean survival and absolute growth.

Contrast	Description
I	Flexible vs. Extruded Netting (Trts A-D vs. Trts E & F)
II	For Extruded Netting – Density: 30 vs. 60 (Trt E vs. Trt F)
III	For Flexible Netting – Aperture: 4.2 mm vs. 6.4 mm (Trts A & B vs. Trts C & D)
IV	For Flexible Netting – 4.2 mm – Density: 30 vs. 60 (Trt A vs. Trt B)
V	For Flexible Netting – 6.4 mm – Density: 30 vs. 60 (Trt C vs. Trt D)

Results:

Survival

Live clams were recovered in 24 of 30 (80%) of the boxes. ANOVA detected significant differences in mean survival between treatments (Table 3; Fig. 3).

Table 3. ANOVA on the arcsine-transformed mean percent survival data (Call's Cove – April-November 2015).

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	5	5480.384627	1096.076925	2.24	0.0833
Flex vs. Vex	1	448.203187	448.203187	0.92	0.3482
Density for Vex	1	2620.931920	2620.931920	5.35	0.0296
4.2 F vs. 6.4 F	1	2359.054368	2359.054368	4.82	0.0381
Density for F @4.2	1	27.116237	27.116237	0.06	0.8160
Density for F @6.4	1	25.078285	25.078285	0.05	0.8229
Error	24	11751.93588	489.663990		
Corrected Total	29	17232.32051			

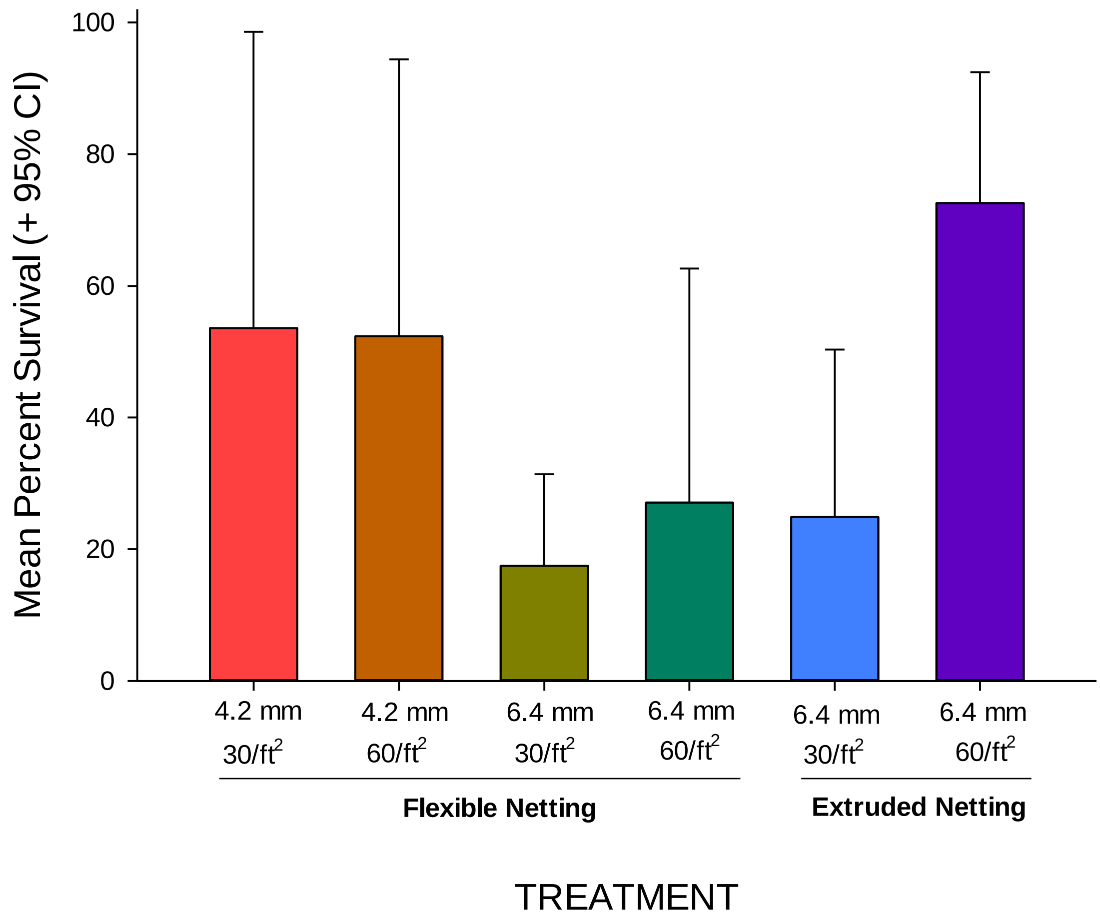

Figure 3. Mean percent survival (+ 95% CI) of cultured juvenile soft-shell clams at Call's Cove (April – November 2015).

No significant differences were observed between flexible (37.7 ± 7.1%, n = 20) vs. extruded (48.7 ± 21.8%, n = 10) netting. For boxes with extruded netting, survival was nearly 3x higher for clams planted at the higher vs. lower density (72.6 ± 19.9% vs. 24.9 ± 25.4%; n = 5; Table 4). Finally, among boxes with flexible netting , clam survival was significantly higher when protected with 4.2 mm vs. 6.4 mm netting (52.9 ± 23.7% vs. 22.3 ± 15.1%, n = 10). A significant proportion of animals were recovered dead with undamaged valves (Table 4). Mean SL of these dead clams was 32.1 ± 1.0 mm, suggesting that clams had died well after they had been planted in the boxes. It is likely that most of these animals succumbed to milky ribbon worms, *Cerebratulus lacteus*, that was found in 21 of the 30 boxes (70%). It is likely that most worms gained access to the boxes via the 4.2 mm aperture on the bottom of each box.

Table 4. Fate of hatchery-reared soft-shell clam juveniles at Call's Cove, Freeport (April-November 2015). Netting = Flexible (polypropylene) or Extruded (polyethylene). Alive = % of clams recovered alive; DU = % of clams recovered dead with undamaged valves; DC = % of clams recovered dead with crushed or chipped valves. Percentages are means ± 95% confidence intervals. Mean percent Alive, DU, and DC may not all add to 100% because some samples contained no live or dead clams. (n = 5)

Netting	Density	Aperture	Alive	DU	DC
Flexible	**30/ft^2**	**4.2 mm**	**53.6 (45.0)**	**31.4 (25.7)**	**15.0 (27.8)**
Flexible	**60/ft^2**	**4.2 mm**	**52.4 (42.1)**	**37.6 (22.8)**	**0.0 (0.0)**
Flexible	**30/ft^2**	**6.4 mm**	**17.5 (13.9)**	**37.5 (46.6)**	**15.0 (41.6)**
Flexible	**60/ft^2**	**6.4 mm**	**27.1 (35.6)**	**61.7 (35.4)**	**11.2 (13.8)**
Extruded	**30/ft^2**	**6.4 mm**	**24.9 (25.4)**	**71.8 (20.3)**	**3.3 (9.3)**
Extruded	**60/ft2**	**6.4 mm**	**72.6 (19.9)**	**24.1 (24.9)**	**3.3 (9.3)**

Growth

No significant differences in mean absolute growth were observed between treatments (Table 5). Mean absolute growth pooled across all treatments was 15.5 ± 1.3 mm (n = 24), and mean final SL was 36.2 ± 1.6 mm.

Table 5. ANOVA on mean absolute growth (Final SL – Initial SL) of cultured soft-shell clam juveniles at Call's Cove, Freeport (April-November 2015).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	**5**	**49.86870480**	**9.97374096**	**1.09**	**0.3981**
Flex vs. Vex	**1**	**38.13821023**	**38.13821023**	**4.18**	**0.0559**
Density for Vex	**1**	**0.46838093**	**0.46838093**	**0.05**	**0.8234**
4.2 F vs. 6.4 F	**1**	**1.97538763**	**1.97538763**	**0.22**	**0.6474**
Density for F @ 4.2	**1**	**9.19097084**	**9.19097084**	**1.01**	**0.3290**
Density for F @ 6.4	**1**	**0.37266696**	**0.37266696**	**0.04**	**0.8421**
Error	**18**	**164.3237369**	**9.1290965**		
Corrected Total	**23**	**214.1924417**			

Wild Clam Recruits

Wild soft-shell clams sampled in the benthic cores within the thirty boxes ranged from 1.83 mm to 20.16 mm, and the size-frequency distribution was strongly skewed to the right, with approximately 80% of the animals ≤ 8 mm (Fig. 4). A 6 x 4 (Treatment x Size) G-test of independence was statistically significant ($G = 61.5$, $df = 15$, $P < 0.0001$) indicating that size-frequency of wild recruits depended upon the treatments.

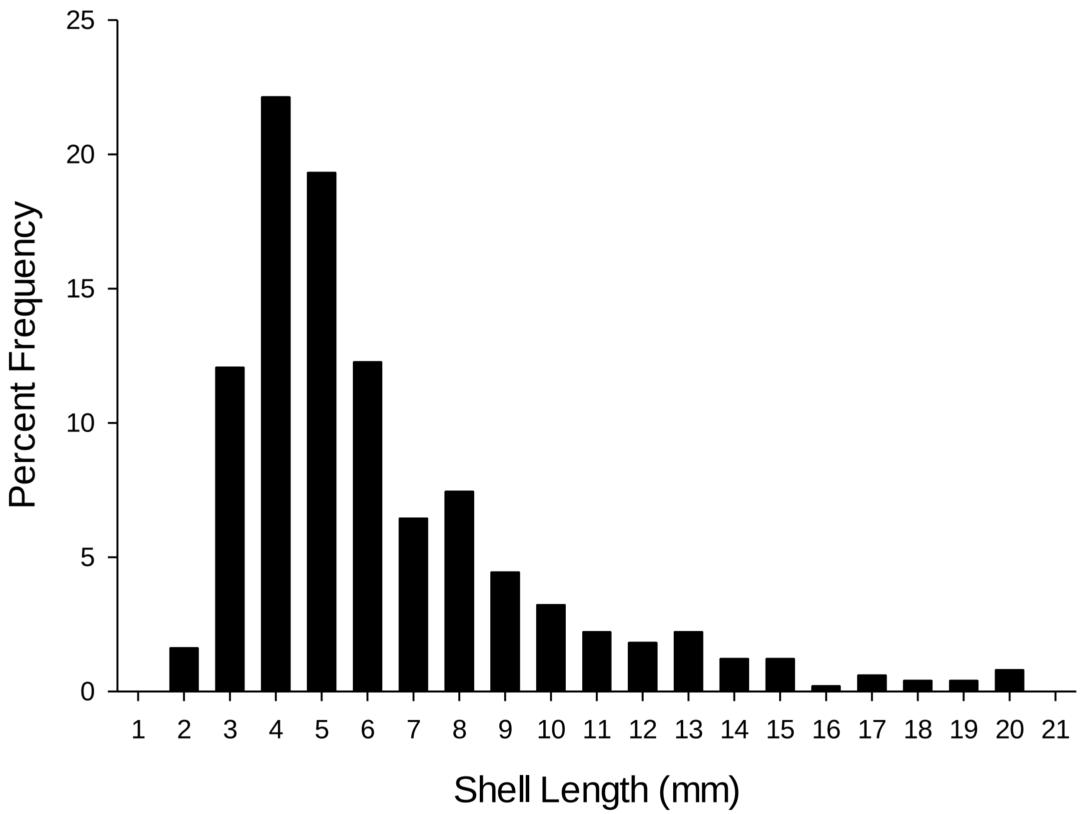

Figure 4. Size-frequency distribution of wild soft-shell clams in benthic samples from boxes taken at Call's Cove on 6 November 2015 (n = 497).

Analysis of variance on the square root-transformed mean count of wild recruits (Table 6) and on mean SL (Table 7) demonstrated no significant treatment effects. Mean number of wild recruits was 1976.9 ± 590.3/m^2 (81.7 ± 24.4/ft^2; n = 30).

Table 6. ANOVA on the square root-transformed mean count of wild soft-shell clam recruits at Call's Cove (6 November 2015).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	5	1058.134409	211.626882	1.31	0.2935
Flex vs. Vex	1	269.8843137	269.8843137	1.66	0.2088
Density for Vex	1	7.2757576	7.2757576	0.04	0.8339
4.2 F vs. 6.4 F	1	125.0000000	125.0000000	0.77	0.3882
Density for F @ 4.2	1	476.1000000	476.1000000	2.94	0.0990
Density for F @ 6.4	1	184.9000000	184.9000000	1.14	0.2957
Error	25	4052.833333	162.113333		
Corrected Total	30	5110.967742			

Table 7. ANOVA on the untransformed mean absolute growth data for the field experiment at Call's Cove (April – November 2016).

Source of variation	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	5	49.86870480	9.97374096	1.09	0.3981
Flex vs. Vex	1	38.13821023	38.13821023	4.18	0.0559
Density for Vex	1	0.46838093	0.46838093	0.05	0.8234
4.2 F vs. 6.4 F	1	1.97538763	1.97538763	0.22	0.6474
Density for F @ 4.2	1	9.19097084	9.19097084	1.01	0.3290
Density for F @ 6.4	1	0.37266696	0.37266696	0.04	0.8421
Error	18	164.3237369	9.1290965		
Corrected Total	23	214.1924417			

B. Field Experiment – Staples Cove (Fig. 1)

Because milky ribbon worms apparently are not deterred by protective netting placed on top of the mud flats, nor are they discouraged completely from entering boxes seeded with cultured soft-shell clam juveniles through the bottom of the box with a 4.2 mm aperture, we designed a field trial to attempt to discourage these nemerteans from entering boxes infaunally.

Methods:

On 7 May 2015, 21 boxes (4-ft x 2-ft x 6-inches deep) were deployed near the upper mid-intertidal at Staples Cove, Freeport, Maine. Boxes had one type of top: 4.2 mm flexible netting. One third of the boxes had bottoms covered with a 6.4 mm VEXAR (extruded polyethylene), one third of bottoms were covered with the 4.2 mm flexible netting (polypropylene, which was the same material as all the box tops), and one third of bottoms were covered with Pet screening (see: http://www.homedepot.com/p/Phifer-48-in-x-50-ft-Black-Pet-Screen-3004134/100614683). Ambient sediments were used to fill each box, and then soft-shell clams ranging in size from 6-20 mm SL were scattered onto the surface sediments of each box at a density of 100 individuals/ft^2. Tops were secured using rigid staples, nails, and laths. Seven replicates of each type of box bottom were used, and treatments were assigned randomly to positions within a 7 x 3 matrix (with 1 meter spacing between rows and columns).

On 10 November 2015, two benthic cores (Area = 0.00811 m^2) were taken from each box, and the contents of each washed through a 1 mm sieve. All cultured clams and wild recruits were enumerated and the final SL of each recorded to the nearest 0.01 mm using digital calipers.

Results:

No live clams were recorded from boxes that were covered on the bottom with the 6.4 mm or 4.2 mm mesh (Table 8). Approximately 55% of clams in boxes with Pet screen bottoms were recovered alive at the end of the study ($\bar{x}$ = 54.4 ± 21.3%, n = 7; Table 9). In addition, only samples from boxes with Pet screen bottoms contained wild soft-shell clam recruits (0-year class individuals) (Table 8).

Table 8. Fate of cultured clams planted in 4-ft x 2-ft wooden boxes at Staples Cove from 7 May to 10 November 2015. Cultured clams (6-20 mm) were seeded in each box at a density of 100/ft^2. Boxes were placed directly on top of the sediment surface and were filled with ambient sediments. Bottoms of boxes were covered in one of three types of mesh: 6.4 mm extruded (polyethylene) netting; 4.2 mm flexible (polypropylene) netting; or Pet screen. On 10 November 2015 two benthic cores (A = 0.008107 m^2) were taken haphazardly from each box. # Alive, # DU, and # DC refers to number of live clams, dead with undamaged valves, and dead with chipped or crushed valves per core, respectively. % Alive = (# Alive / (#Alive + #DU + #DC). # Recruits refers to the number of wild, 0-year class soft-shell clam individuals per core. # Crabs refers to the number of green crabs, *Carcinus maenas* per core. # Worms refers to the number of milky ribbon worms, *Cerebratulus lacteus*, per core.

Bottom	Rep	# Alive	# DU	# DC	%Alive	# Recruits	# Crabs	# Worms
6.4 mm								
	1a	0	7	0	0.00	0	1	0
	1b	0	5	0	0.00	0	0	0
	2a	0	3	1	0.00	0	1	0
	2b	0	7	0	0.00	0	0	0
	3a	0	15	3	0.00	0	0	0
	3b	0	12	4	0.00	0	0	0
	4a	0	12	5	0.00	0	0	0
	4b	0	8	4	0.00	0	1	0
	5a	0	3	0	0.00	0	0	0
	5b	0	1	1	0.00	0	0	0
	6a	0	3	0	0.00	0	0	0
	6b	0	7	2	0.00	0	0	0
	7a	0	5	2	0.00	0	0	0
	7b	0	4	1	0.00	0	0	0
4.2 mm								
	1a	0	13	0	0.00	0	0	0
	1b	0	12	2	0.00	0	0	0
	2a	0	4	3	0.00	0	0	0
	2b	0	3	4	0.00	0	0	0
	3a	0	13	0	0.00	0	0	0
	3b	0	13	5	0.00	0	0	0
	4a	0	13	5	0.00	0	0	0
	4b	0	3	1	0.00	0	1	0
	5a	0	13	1	0.00	0	0	0
	5b	0	11	0	0.00	0	0	1
	6a	0	18	0	0.00	0	0	0
	6b	0	15	1	0.00	0	0	0
	7a	0	5	6	0.00	0	0	0
	7b	0	16	3	0.00	0	0	0

Pet Screen

1a	16	2	1	84.2	0	0	0
1b	10	3	1	71.4	0	0	0
2a	2	7	3	16.7	0	0	0
2b	5	6	3	35.7	0	0	0
3a	3	12	0	20.0	0	0	0
3b	15	8	2	60.0	3	0	0
4a	0	2	1	0.00	0	0	0
4b	3	9	0	25.0	1	0	0
5a	5	1	1	71.4	0	1	0
5b	1	0	0	100.0	0	0	1
6a	12	3	2	70.6	0	0	0
6b	9	3	0	75.0	3	0	0
7a	21	3	0	87.5	8	0	0
7b	11	9	0	55.0	0	0	0

Table 9. Mean percent (± 95% CI) of living (Alive) and dead soft-shell clam juveniles (with undamaged and crushed valves) at Staples Cove in boxes lined on the bottom with various types of netting (Flexible = 4.2 mm aperture; VEXAR = 6.4 mm aperture; PET = 1.8 mm aperture). The tops of all boxes were protected with a flexible netting (aperture = 4.2 mm) (n = 7).

Net Type	Aperture	Alive	Dead Undamaged	Dead Crushed
FLEXIBLE	**4.2**	**0.0 (0.0)**	**82.3 (16.2)**	**17.7 (16.2)**
VEXAR	**6.4**	**0.0 (0.0)**	**83.4 (8.2)**	**16.6 (8.2)**
PET	**1.8**	**54.4 (21.3)**	**36.5 (19.8)**	**9.1 (7.7)**

Mean number of live clams in boxes lined with Pet Screen was 942.4 ± 602.3 per square meter (87.6 ± 55.9 per square foot; n = 7).

Wild clam recruits were found only in boxes protected with Pet screen on the bottom. Mean wild clam density was 132 ± 156.6 individuals/m^2 (12.3 ± 14.6 individuals/ft^2; n = 7)