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Cover Photo: Dry harrow treatment in process, Grassy Island WA 2018

Willapa Bay Mechanical Management of Burrowing Shrimp Supplement

July 2018 Aquatic Assessment and Monitoring Team Aquatic Resources Division



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This project was completed only with the collaboration and hours pent from many dedicated contributors from DNR Aquatic Resources staff. Contributors are staff of the Washington State Department of Natural Resources (DNR) unless otherwise indicated.

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Washington Department of Natural Resources (WDNR) Willapa Grays Harbor Oyster Growers Association (WGHOGA) Rural Communities Partnership Initiative (RCPI)

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Copies of this report may be obtained from (WDNR Aquatic Resources Division, Olympia WA.).

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EXECUTIVE SUMMARY

The deposit feeder *Neotrypaea californiensis* (burrowing ghost shrimp), is a native benthic invertebrate that burrows into sandy sediment along marine shorelines in Washington State. *N. californiensis* can live up to ten years, and provides an important food source for Dungeness crab, Green sturgeon, and other intertidal species (Dumbauld et al. 2008, Moser et al. 2017). The shrimp suspends sediment particles when maintaining its burrows and feeding. Sediment that is softened and re-suspended in this act affect farmed shellfish, which can sink into the sediment and suffocate - especially as settling larvae and spat (Stevens 1928).

Burrowing shrimp were treated with the insecticide, Carbaryl, until 2013. Following this, an alternative compound, Imidacloprid, was tested for use to control burrowing shrimp on tidelands (Felsot and Rupert 2002, Patten 2016). In 2018, The Washington Department of Natural Resources' (WDNR's) Aquatic Assessment and Monitoring Team (AAMT) designed a study to assess the feasibility of mechanical control for burrowing shrimp in Willapa Bay. This study is part of the Rural Communities Partnership Initiative (RCPI) - a partnership with the goal of sparking economic development in rural communities of Washington State. The partnership aims to bolster the shellfish economy on Washington's outer coast. Partners of this group include WDNR, Willapa Grays Harbor Oyster Growers Association (WGHOGA), Washington State Department of Agriculture (WSDA), and Washington Sea Grant.

Results from an initial Proof of Concept (POC) study at Grassy Island indicate significant reduction of burrowing shrimp densities post treatment using a large roller-harrow implement towed behind an amphibious vehicle. We refer to this treatment as "Dry Harrowing". The purpose of this study and supplemental monitoring was to assess the longevity of treatment, test effects of more intensive dry harrow treatment, and to determine if and how shrimp recolonization occurred by the end of the season. Results indicate that more intensive methods of dry harrowing (4 passes) reduced shrimp density by 89%, and biomass by approximately 79%. We found impacts to the smallest, and likely 1 to 2 year old size class with 4 passes, where a less intensive method, (2 passes), did not. No evidence of recolonization from the edges of treated plots was observed over a six week period.

INTRODUCTION

Results of a burrowing shrimp mechanical management Proof-of-Concept (POC) study indicate that of three mechanical treatment methods applied; wet harrowing, flooding and dry harrowing, only the dry harrow treatment showed promise in effectively reducing shrimp number and density (WDNR 2018). Dry Harrowing entailed driving a Marshmaster 2 XL (manufactured by Coast Machinery LLC) with attached rolling implement called a "roller-chopper" harrow across tideflats at low tide.

This POC supplemental study was carried out with three overall goals:

- 1. To examine whether the reduction in shrimp density and biomass from dry harrowing was sustained.
- 2. To assess whether more intense application of the dry harrow treatment reduced shrimp numbers further.
- 3. To document any recolonization or movement of shrimp back into treated plots from adjacent non-treated controls.

Site Description

This study was carried out on state-owned aquatic land (SOAL) on the Long Beach Peninsula in Willapa Bay (Figure 1), accessed from the Northern Leadbetter State Park lot. This area - known as "Grassy Island," or "Stackpole" is characterized by a large sandflat that extends approximately one mile to the water. It is extremely dynamic and exposed to winter storms - which dissuade shellfish aquaculture throughout the year. Grassy Island is also known for its high density of burrowing shrimp. It has a winding slough that remains full throughout the tidal cycle. Dense beds of the native eelgrass *Zostera marina*, as well as the non-native species *Zostera japonica* are distributed throughout the site.



Figure 1. Grassy Island - Willapa Bay WA. Dry Harrow and subsequent control plots in supplemental monitoring study. The dashed line indicates plots DH4 through DH6 - established for supplemental monitoring.

METHODS

Dry Harrow Treatment

Dry harrowing involved towing a robust steel roller behind an amphibious tracked vehicle called the "Marsh Master-2LX" (Figure 2). The roller is a product from Coast Machinery LLC., weighs 700 pounds empty, and is designed to cut an 8-foot wide swath through marsh and wetland cattail (Coast Machinery LLC. 2018). It has a series of flat plates welded to it, which penetrate into the sediment approximately 30 cm. The implement can be either hooked up to a 4 point hydraulic hitch, or towed with load bearing rope. It both crushes and forces shrimp out of their burrows where they can be consumed by birds (see Figure 2).

Dry harrowing showed immediate reduction of burrowing shrimp on fallow land at Grassy Island of 61% for density, and 67.5% for biomass during WDNR's POC. Where dry harrow treatment plots (DH#) were treated with two separate passes for DH1, DH2 and DH3 (WDNR 2018). In the present study, three additional treatment plots (DH4, DH5 and DH6), were harrowed a total of four passes each. Treatment plots were bordered on two sides by control plots (a total of 8 plots), where DH5 and DH6 shared a control plot between them (Figure 1). Pre- and post-treatment shrimp density was sampled with pumped and manual cores. Sediment penetration, grain size, and shrimp recolonization was also monitored. Surveys spanned a period of six weeks from July 2018 to September 2018. Table 1 is a schedule of sampling and harrowing in 2018.



Figure 2. WDNR Marshmaster-2XL towing the roller – chopper "dry harrow" implement at Grassy Island, treating a dry harrow plot.

Experimental Design

For each dry harrow plot and adjacent controls, 21 pumped cores were collected for shrimp density and biomass. Every treatment plot and adjacent controls had 12 pumped cores within control plots, and 9 pumped cores within treated plots. As a relative measure of shrimp density, 143 manual cores were sampled, and 27 sediment penetration readings as well as sediment samples were taken.

Transects were established along a distance gradient at nine, eighteen, and thirty-six meters between control and treatment plots. These transects extended into each control and treatment plot and were sampled at the three, six, and nine meter mark moving into each plot. This design allowed us to assess whether shrimp moved from untreated plots to recolonization treated plots (Figure 3).



Figure 3. Schematic of sampling locations for one set of dry harrow plot and subsequent controls. Gradient transects are encircled by dotted lines.

Experiment Timing and Monitoring

For the Proof of Concept (POC) study initiated in April 2018, dry harrowing was conducted on three half-acre plots (DH1, DH2, and DH3) with two passes of the WDNR Marshmaster-2X towing the roller -chopper implement. Initial results (post treatment two weeks) indicated up to 62% control of burrowing shrimp (WDNR 2018). These plots and their subsequent controls were revisited in July 2018 (post treatment 12.4 weeks) and for just plot DH1, in September 2018 (post treatment 20 weeks). For the Supplemental study, pre-treatment plots were established and sampled in July (t0). Dry harrowing was conducted on three half-acre plots (DH4, DH5, and DH6) with four passes of the roller-chopper, then monitored in August (t1) and September (t2) (Table 1).

2 passes Dh1-3	t0	tl	t2		t3 (Dh1 only
			20 Weeks		
4 passes Dh 4-6			tO	tl	t2
				6 Weeks	
	April	May	July	Aug	Sept
			time \rightarrow		

Table 1. Field surveys conducted for treatment plots DH1 through DH6, and associated control plots spring andsummer 2018.

Pumped Core Shrimp Sampling

To assess shrimp density and biomass at each plot, we liquefied the entire contents of a 1/8 meter² (m²) surface area core. The core - one meter (m) tall and constructed of stainless steel, is pushed into the sediment approximately 0.75 m. Handles are welded to each side, and a capturing net of 2 millimeter (mm) mesh with PVC support encloses its top (Figures 4 and 5 show the system in use). The capturing net adds approximately 0.5 meters (m) to the top of the core, and retains all but the smallest recruited shrimp (< 2mm carapace length) that float to the surface. Honda water pumps mounted on the Marshmaster pull water from a nearby slough into the core. We used three-inch Tigerflex[®] hose on the suction end of the pump and two inch rubber jacketed fire hose for the outflow. Firehose was cut into 100-foot sections and fitted with camlock quick links -it was important to have abundant hose on hand to reach plots farthest from the slough. A custom PVC stinger was attached to the end of this firehose - which was used to

penetrate into the sediment (Figure 4). Shrimp are buoyant and float to the surface of the core, where they are scooped up, placed in site-specific labelled bags then frozen for later lab processing. Nine pumped cores were randomly taken per set of treatment and control plots (three inside each plot), as well as six along each gradient transect at three, six, and nine meters - another 12 cores (Figure 3). We were careful to avoid sampling the same core locations in subsequent post treatment surveys. All shrimp collected were measured for total length (TL), carapace length (CL), and biomass (g).



Figures 4 and 5. Large core hydraulically pumped out and used for assessing shrimp density. The core is one meter deep with a surface area of $1/8 \text{ m}^2$.

Manual Core Shrimp Sampling

Clam guns were used to excavate sediment cores of one meter in depth and approximately .078 m² in surface area (Figure 6). While not an absolute measure of shrimp density, manual cores can be sampled rapidly, at a greater scale than pumped, and provide a measure of relative density. For each set of dry harrow treatment and controls we sampled 133 manual cores. Of these 133 cores, 5 were taken surrounding each of the 21 locations that pumped cores were sampled, and another 28 along the four different gradient transects at zero, three, six, and nine meter sampling

locations



Figure 6. Manual coring method

Burrow Counts

While burrow counts exist as a relatively simple and easy method of assessing indirect burrowing shrimp density, they are subject to inaccuracies due to increased burrowing activity at various times of year, and misidentification of holes created by other organisms as shrimp burrows (McPhee and Skilleter 2002). For these reason it is imperative that this indirect method of population assessment is validated to ensure that estimates of density accurately reflect the actual density of shrimp. For burrow counts to be a reliable and accurate method of estimating shrimp density, the burrows must be clearly distinguishable from burrows created by other benthic animals, and the relationship between burrow count and animal density must be known and predictable, even if it varies seasonally (McPhee and Silleter 2002). Shrimp burrow counts vary both temporally and spatially variable due to shrimp population dynamics and environmental conditions (Dumbauld et. al 1996, Dumbauld et al. 2006). It is therefore recommended that counts are taken as close to treatment periods, and during summer months when shrimp are active and storm activity is low (Dumbauld et al. 1996).

Two methods for counting burrows were implemented: an extrapolated estimate made from counts in the pumped core sampling, and a true count of burrows within a square meter quadrat for the manual core sampling. For the pumped core sampling, shrimp burrows were counted within the perimeter of each 0.125 m^2 pumped sampling core. This value was then multiplied by eight to estimate the number of burrows that would be contained within a square meter. Burrows were identified as 1 to 3 cm diameter holes with upturned sediment mounded around the

periphery. Burrows became more evident as the sampling core was inserted into the sediment and water was observed exiting them. For the manual core sampling, a square meter quadrat was placed on the tideflat and all burrows within the quadrat were counted and recorded. The manual cores were sampled from within this square meter quadrat.

Sediment Compactness and Grain Size

Sediment compactness was measured pre- and post-treatment with a custom "penetrometer". The penetrometer is a 159 cm long stainless steel rod with a base plate welded to it (Figure 7). For each measurement, the rod was placed on the sediment surface. A five pound drop weight was then released from the top of the rod and contacted the base plate a total of five times. Penetration of the rod was measured after each drop of the weight.

Sediment grain size was estimated from the analysis of grab samples scooped from the top 10 cm of sediment surface at every penetrometer reading site. Samples were placed in labeled Ziploc bags and frozen until prepared for grain size analysis using ASTM International standard methods (STP S447B, 1998). Samples were washed and dried in a standard convection oven, removed from the oven and weighed. They were then added to a stack of sieves ranging from 2.0 mm to less than 0.063 mm. The sieve stack was shaken with a Gilson sieve shaker for 10 minutes. Shaking allowed each sieve to capture the portion of the sediment with grain size too large to fall through its mesh. Mass of sediment grain size and sorting was calculated percentage of the total sediment weight captured in each sieve.



Figure 7. Penetrometer used to measure sediment compaction before and after treatment

Processing of Shrimp Samples

All shrimp were measured for total weight in grams (g), total length in millimeters (TL) (mm), carapace length (CL) (mm), species, and sex. Partial body parts were counted as individual shrimp if they could not be matched to complete shrimp. Partial shrimp were not measured for CL, TL, mass, or sex. Shrimp were then classified into one of four size classes based on their carapace length (Table 2 indicates ranges for each size class). Size classes were established from the size frequency distribution. Similar size classes have been established based on carapace length in other studies within Willapa Bay (Dumbauld et al. 1996, Bosley and Dumbauld 2011). Average size classes were 20.26 mm CL Large, 14.58 mm CL Medium, 10.40 mm CL Small, and 6.16 mm CL Extra Small. Size ranges were based off the mid-point between size class averages.

Shrimp Size Classification

Shrimp were separated into four classes based on carapace length in treated and control plots at

t0, t1 and t2. Frequency and density plots for carapace length were created to assess population change post treatment. Analyses were completed for both proportional population change over time as well as total population change within separated size classes. Control plots were standardized based on the number of pumped control samples (33), and the total number of pumped treated plot samples (27). Due to the nature of how the manual core samples, small shrimp (generally less than 8 mm carapace length) are not detected. To eliminate any bias that manual cores may influence, size classification analysis was only performed on shrimp collected from pumped cores.

Size Class Ranges	Large	Medium	Small	Extra Small
Carapace Length (mm)	> 17.42	17.42 - 12.49	12.49 - 8.28	8.28 >
Total Length (mm)	> 69.95	69.96 - 49.25	49.25 - 30.94	30.95 >
Mass (g)	> 6.85	6.85 - 2.41	2.41 - 0.62	0.62 >

Table 2. Range used to classify shrimp in each size class

Mean size								
class (mm)	Large		Medium		Small		Extra Small	
		Bosley &		Bosley &		Bosley&		Bosley &
Source	WDNR	Dumbauld	WDNR	Dumbauld	WDNR	Dumbauld	WDNR	Dumbauld
	20.33	13.26	14.58	10.75	10.32	8.55	6.16	6.28
CL±	<u>+</u>	±	<u>+</u>	<u>±</u>	<u>+</u>	<u>+</u>	<u>+</u>	±
SD (mm)	2.36	1.97	1.69	0.31	1.41	1.49	1.60	1.78

Table 3. Size classes collected in WDNR 2018 Supplemental compared to average values from Bosley and Dumbauld (2011).

Shrimp Density and Biomass Statistical Analyses

t-Tests assuming unequal variances (p < 0.05) were run for control and treated groups over time. Single factor ANOVAs (p < 0.05) were run using 2016 Microsoft Excel's Data Analysis Package for pumped core shrimp density and shrimp biomass over time as well as for distance gradient transects over time. Two-way and three- way ANOVAs were performed using AnalystSoft StatPlus statistical software package for manually cored shrimp density and biomass, for sediment compaction and sediment grain size data. In all statistical analyses performed, the null hypotheses were no difference existed between the means of the dependent variables grouped by plot type or time. Density plots for population carapace length (mm) were created with R 3.5.2 statistical analysis software using the ggplot package (R Core Team 2013).

RESULTS

Pumped Core Shrimp Density and Biomass

Shrimp Density Pumped Cores

Before treatment, shrimp densities in control and treated plots were not statistically different in a 2 sample t-Test assuming unequal variances t(60) = 0.76, p = 0.45. Mean shrimp density collected within control plots at t0 was $10.97 \pm$ standard error (SE) 1.14 shrimp/core. Mean shrimp density collected within treatment plots at t0 was $9.77 \pm$ SE 1.09 shrimp/core.

Post treatment, shrimp density measured in treated plots dropped by an average of 89% (Figure 8). Sample t-Tests (at p < 0.05) indicated significant differences in shrimp density between control and treated plots at t1 (t(48) = 5.38, p <0.001), (control density = $10.21 \pm SE 1.41$, treated plot density = $1.59 \pm SE 0.75$ shrimp/core). Six weeks later at t2, control plots retained significantly higher mean shrimp densities ($8.00 \pm SE 1.17$ shrimp/core), compared to densities in treated plots ($0.73 \pm SE 0.23$ shrimp/core) (t(38) = 6.18, p <0.001).

Control plots did not show a statistically significant change in density over the entire time period from t0 to t2 (10.97 ± SE 1.14 shrimp/core and 10.21 ± SE 1.41 shrimp/core and 8.00 ± SE 1.17 shrimp/core), respectively (Figure 8) (Single factor ANOVA, F(2, 105) = 1.36, p = 0.26). Treated plots differed significantly from t0 (9.77 ± SE 1.09 shrimp/core) to t1 (1.59 ± SE .75 shrimp/core) and t2 (0.73 ± SE 0.23 shrimp/core) (Single factor ANOVA, F(2, 77) = 40.03, p <0.001).



Figure 8. Mean shrimp density collected per pumped core at Control and Treated plots at t0, t1, and t2 (shrimp/0.125m²). Dotted line indicates timing of treatment.

Shrimp Biomass Pumped Cores

Before dry harrowing (t0), no difference was detected between Control and Treatment Plots $(49.59 \pm \text{SE} 7.83 \text{ and } 44.83 \pm \text{SE} 6.98 \text{ g/core respectively})$ (t-Test t(60) = 0.49, p = 0.62). After treatment, a statistically significantly lower mean biomass was observed in harrowed plots (t-Test t(53) = 4.27, p <0.001). Biomass in treated plots dropped by 79% (to 9.51 ± SE 4.4 g/core) at t1, and remained significantly lower through t2 (ANOVA F(2, 77)= 20.63, p <0.001). No statistically significant differences were detected in biomass within control plots measured over time from t0 to t1 to t2 (ANOVA F(2, 105) = 2.95, p = 0.06).



Figure 9. Mean shrimp biomass collected at Control and Treated plots $(g/0.125m^2)$ at t0, t1 and t2. Dotted line indicates treatment timing.

Declines in biomass in both control and treated plots over time from early spring to early fall can be seen in Figures 10 and 11, however the declines in control plots are not statistically significant. Fluctuations in population density within Willapa Bay have been observed at monitoring stations and are thought to be associated with variable recruitment, predation, and habitat loss (Dumbauld et al. 2012).



Figure 10. Mean biomass for DH4 - DH6 control plots over time. A 41% drop in mean biomass can be seen from t1 to t2.

Control plot biomass and density remained statistically the same from t0 to t1, but experienced a drop from t1 to t2. This decline was seen in treated plots from t1 to t2 as well. Treated plots that were reduced to 21% of their previous biomass in July were reduced another 12.8% from their t0 values in September. Control plot biomass are not statistically different from t0 (49.78 ± SE 7.18 g/core) to post treatment t1 (48.11 ± SE 8.01 g/core) (t-Test t(69) = 0.16, p = 0.87), was reduced between August (t1) and September (t2) surveys to 59% of previous mean biomass (mean biomass at t2 = $28.53 \pm SE 5.51 \text{ g/core}$) (t-Test t(62) = 2.04, p = 0.05).



Figure 11. Mean biomass for treated plots DH4 DH5 and DH5 over time. A 61% drop in mean biomass can be seen from t1 to t2.

Manual Core Shrimp Density and Biomass

Shrimp density and biomass data collected through manually cored sampling before and after treatment were analyzed using two-way analyses of variance. The independent variables were plot type (untreated control or dry harrowed) and time (before and after harrowing).

Shrimp Density Manual Cores

The shrimp density data analysis results indicate that prior to dry harrowing (at t0), mean shrimp densities between control and treatment plots were not significantly different (5.19 \pm 0.81 and 5.96 \pm 0.76 respectively, @ 95% confidence interval, p = 0.315). The analysis however, did show statistically significant differences in mean shrimp density between control and treatment plots after dry harrowing (@t1 control 4.02 \pm 0.58 and dry harrow 0.87 \pm 0.18; @ t2 control 1.35 \pm 0.33 and dry harrow 0.133 \pm 0.06) @ 95% confidence interval, p < 0.001). Mean shrimp densities were significantly reduced in treated plots before and after dry harrowing (5.96 \pm 0.76 @ t0, to 0.867 \pm 0.181 @ t1), @ 95% confidence interval, p < 0.001. Although shrimp density in treated plots decreased to 0.133 \pm 0.056 @ t2, this difference was not significant (@ 95% confidence, p = 0.997. The two-way ANOVA indicated a significant interaction between factors of plot type and time F(2, 366) = 7.49, p < 0.001. Changes in manually cored shrimp densities in treated versus control plots over time are presented in Figure 12.



Figure 12. Mean shrimp density collected by manual core in Control and Treated plots at t0, t1, and t2. Dashed line indicates relative timing of treatment.

Shrimp Biomass Manual Cores 14

The two-way ANOVA performed on the biomass data indicate no significant difference in mean biomass in samples collected from control $(15.2 \pm 4.02 \text{ (g/0.4m}^2))$ and treatment $(17.81 \pm 4.95 \text{ (g/0.4m}^2))$ plots prior to dry harrowing (at t0). The analysis does however indicate statistically significant differences comparing mean shrimp biomass between control and treatment plots after dry harrowing (@ t1 control = 25.5 ± 4.91 , dry harrow = 8.89 ± 1.79 , and @ t2 control = 11.12 ± 1.78 , dry harrow= 08.03 ± 2.31). After dry harrowing, mean shrimp biomass in treated plots was significantly reduced from t0 to t1, however mean biomass in treated plots from t1 to t2 did not change significantly F(2, 226) = 3.45, p > 0.05. Change in shrimp biomass within control and treated plots over time is plotted in Figure 13.



Figure 13. Mean shrimp biomass $(g/0.4 m^2)$ collected by manual core in Control and Treated plots at t0, t1, and t2. Dashed line indicates relative timing of treatment.

Shrimp Size Distribution Pumped Cores

Shrimp Carapace Length

Carapace length (CL) of all collected shrimp is the dimension used to estimate shrimp body size. From the size frequency distribution plot, it is clear that the pumped core sampling method is capturing shrimp down to 2 mm CL size. Shrimp of all sizes were effectively reduced from t0 to t1 within treated plots. Shrimp size remained reduced into our t2 survey six weeks after treatment. Figure 14 shows the distribution of carapace lengths for shrimp collected at control and treated plots. Control plots were standardized by a factor of 0.81 based on the number of pumped control samples (33), and the total number of pumped treated plot samples (27). From t0 to t1, total magnitude of the small and extra small size classes were reduced to 8% and 3% of their previous size (Figure 14). Large and Medium size classes were reduced as well, down to

23% and 22% of their previous size. Impacts from dry harrowing were sustained, and evident in our September surveys (Figure 14). September surveys however, found an increase in overall magnitude of the small and extra small sized shrimp, where control plots indicated a 56% increase, and 69% increase from t0 to t2 respectively (t2 Figure 14). Treated plots saw a small increase of the extra small sized shrimp from t1 to t2 of 7%.



Shrimp Size Distribution at Each Sample Time

Figure 14. Carapace lengths (CL) for treated and control plots - shrimp at t0, t1, and t2 collected from pumped density cores for plots DH4, DH5 and DH6. An increase in the magnitude of the extra small class can be seen within circled region at t2.

Proportional Size Class Changes

Prior to treatment, large, medium, and small size class proportions differed by no more than 7% between control and treatment plots. Table 4 shows the percent composition of each size class at t0, t1 and t2 for both control and treated plots.

Treatment of plots influenced a shift in community structure and a proportional reduction in both small and extra small size classes (7.98 and 19.27 % declines respectively from treated plots t0 to t1). Control plots on the other hand experienced little proportional change in size classes from t0 to t1 (less than 1%). From t1 (August) to t2 (September) a proportional shift in the small and extra small size classes occurred within treated plots. The small size class was reduced significantly, while the extra small size class increased by 13.42%. In control plots, a decrease of the large size class by 12.4% from t1 to t2 allows the extra small size class to proportionally increase by 15.7%. Figures 15 and 16 are distribution plots for the population of shrimp by carapace length collected within DH4 -DH6 control plots and treated plots over time. A shift from larger shrimp dominating the population in control plots to the extra small size class at t2 can be observed in Figure 15.

	tO		t1		t2	
	Control	Treatment	Control	Treatment	Control	Treatment
L %	30.13	23.13	29.45	40.48	17.05	39.39
M %	33.47	33.75	32.36	45.24	27.75	42.42
S %	15.48	17.50	18.91	9.52	20.23	0.00
XS %	20.92	25.63	19.27	4.76	34.97	18.18

Table 4. Size class distribution (percentages calculated from # of shrimp collected from specified size classes at t0 t1 or t2) from Control and Treated plots pre- and post-treatment. A dashed line indicates at what point treatment plots were treated.



Figure 15 and 16. Frequency distribution of shrimp carapace length (mm) sampled from DH4-DH6 plots over time July (t0) through September (t2).

Distance Gradient Pumped Cores

We found no differences between shrimp density or biomass at 3, 6, 9, and Random Treated (RT) meters extending from control into treated plots through time (t0, t1, and t2, Single Factor ANOVAs F(3, 17) = 1.68, p = 0.21, F(3, 16) = 0.49, p = 0.69, and F(3, 17) = 0.65, p = 0.59 for density, and F(3, 16) = 1.06, p = 0.39, F(3, 17) = 0.35, p = 0.79, and F(3, 17) = 0.85, p = 0.49 for biomass. These results show no evidence of movement of shrimp from the higher densities remaining in the control plots to recolonize the sparsely populated treated plots. Figure 17 shows average shrimp density at t0, t1, and t2 for transects 3, 6, 9, and randomly located core along transect (indicated as R).



Shrimp Densities: Distance from treatment edge (pumped cores)

Figure 17. Mean shrimp densities from pumped cores along a distance gradient from treatment edge at times t0, t1, and t2 for transects moving into treated plots. Error bars indicate standard error.

Distance Gradient Manual Cores

Shrimp density collected through manually cored sampling before and after treatment were analyzed using a three-way analysis of variance. The independent variables were plot type (untreated control or dry harrowed) time (before and after harrowing), and distance from treatment edge (3, 6, or 9 meters). The shrimp density ANOVA results indicate no statistically significant differences along the distance gradient prior to dry harrowing (at t0), or post treatment (t1 and t2) between the control or treatment plots. F(6, 338) = 0.743, p = 0.615.



Shrimp Densities: Distance from treatment edge (manual cores)

Figure 18. Mean shrimp densities from manual cores along a distance gradient from treatment edge, at times t0, t1 and t2. Error bars indicate standard error.

There is no evidence that shrimp from the high density control plots are moving across the edge into the treated plots to recolonize the area. Mean shrimp densities measured along the distance gradient from the edge of the treatment into the dry harrowed and adjacent treatment plots over time (at t0, t1 and t2) are plotted in Figure 18.

Burrow Counts

Relationship Between Burrow Counts and Shrimp Density

Linear regressions were performed on both types of burrow and shrimp density sampling methods: 1) burrows counted within the 20 cm diameter core and shrimp density within the pumped core; and 2) burrows counted within a meter square quadrat and shrimp densities counted from five 10 cm, 70 cm long cores collected from within the quadrat. The pumped core regression is plotted in Figure 19. It has a coefficient of determination $R^2 = 0.51$ (p = 0.001, n = 243). Such an R^2 value given this sample size and p value is indicative of a weak, or low effect relationship (Moore et al. 2013). The linear regression between burrow count and shrimp biomass shows a slightly weaker relationship, $R^2 = 0.47$ (p = 0.001, n = 243). These data indicate approximately 50% of the variance in the dependent variable (shrimp density) can be explained by the independent variable (burrow counts).



Figure 19. Burrow counts compared with shrimp density collected from within pumped cores (p = 0.001, n=243). Pumped cores were 20 cm diameter or 0.125 m^2 , $(1/8\text{m}^2)$ in surface area.

The shrimp to burrows/m² regression from quadrat counts and manual coring is plotted in Figure 20. Although these are absolute burrow counts within the square meter quadrat, the shrimp density is a sub sample from five 10 cm diameter cores collected from within the quadrat, which is likely an underestimate of total shrimp within the entire quadrat. This regression shows a coefficient of determination $R^2 = 0.32$ (p = 0.001, n = 415). Given this sample size and p value, this value of R^2 indicates the relationship between burrow counts and shrimp density to range from very weak, to no relationship at all (Moore et al. 2013).



Figure 20. Burrow counts compared with shrimp density collected from within a square meter quadrat (p = 0.001, n = 415).

Pre- and Post-treatment Burrow Counts

Estimated mean burrow density extrapolated from counts within the 20 cm diameter pump core declined significantly by 77% (49 burrows/m² ± SE 0.59 to $11 \pm$ SE 0.53 burrows/m²) in treated plots from t0 to t1, and another 5% (to $8.88 \pm$ SE 0.35 burrows/m²) from t1 to t2 (Figure 21). Control plots declined by 25% (54 ± SE 0.39 burrows/m² to 41 ± SE 2.33 burrows/m²) from t0 to t1, and another 21% (to 29 ± SE 0.42 burrows/m²) from t1 to t2 (Figure 21). Significant differences were found over time from t0 to t1, but not from t1 to t2 for both control and treated plots (t-Test assuming unequal variances t0 vs t1, t1 vs t2 at p < 0.05)



Figure 21. Estimated mean burrow density (#burrows/m²) from pumped core locations for treatments and control plots (DH4 to DH6). Dashed line indicates when treatment occurred relative to sample times.

Mean burrow counts from within square meter quadrats conducted during manual coring declined significantly by 95 % (22.7 burrows/m² ± SE 1.97 to $1.24 \pm$ SE 0.16 burrows/m²) from t0 to t1 in treated plots. Burrow count declined again from t1 to t2 by 15% (to $1.05 \pm$ SE 0.14 burrows/m²) (Figure 22). Control plots declined by 25% (54 ± SE 0.39 burrows/m² to 41 ± SE 2.33 burrows/m²) from t0 to t1, and another 21% (to 29 ± SE 0.42 burrows/m²) from t1 to t2 (Figure 22). Significant differences were found over time from t0 to t1, for both control (two-sample t (133) = 2.27, p = 0.024), and treated plots (two-sample t (64) = 10.75, p < 0.001). The difference between burrow counts at t1 and t2 for the control plots was also significant (two-sample t (88) = 4.09, p < 0.001). There was not a significant difference found, however, between burrow counts in treatment plots from t1 to t2 (two-sample t (145) =0.84, p = 0.40). The burrow counts in the treatment plots dropped significantly following the treatment and remained low.



Figure 22. Mean burrow counts (#burrows/m2) counted within square meter quadrat for treatments and control plots (DH4 to DH6). Dashed line indicates when treatment occurred relative to sample times.

Sediment Compactness and Grain Size

Sediment Compactness

Two- and three-way ANOVAs were conducted to explore whether dry harrowing influenced compaction at surface and depth. The independent factors were (1) plot type (untreated control, and dry harrowed plots), (2) time (before and after harrowing and (3) weight drop. While results from the three-way ANOVA do not indicate statistically significant interaction among the three factors F(4, 764) = 1.10, p-value = 0.54, simple comparisons among groups does reveal statistically significant differences. Mean sediment penetration is higher in treated plots before (t0) versus after dry harrowing (t1). The difference of 31.64 mm is significantly different @ 95% confidence interval, and p-value = 0.001. The difference in mean sediment penetration measured for treated plots is significant for each weight drop (surface drop 1, drop 1+2 and drop 1+2+3) from t0 to t1. Mean sediment penetration in treated plots at t2 remained low for all weight drops, and does not differ statistically from values measured at t1. Surface and cumulative sediment penetration consistently increased with each consecutive weight drop, and the difference in sediment penetration among drop levels (weight drop 1, 2 and 3) was significant for both control and treated plots. Mean sediment penetration over time in control and treated plots is presented in Figure 23 a-d.











Figure 23. Sediment penetration depth pre- and post- dry harrow treatment measured in DH4-DH6 and control plots after a) first weight drop, b) cumulative penetration after first and second weight drop, c) cumulative depth penetrated after all three weight drops, and, d) mean penetration for all weight drops.

Sediment Grain Size

Grain size analysis was performed on 190 grab surface sediment samples collected from dry harrowed and control plots, beginning pre-treatment through the post treatment monitoring. Two-way ANOVAs indicated no statistically significant difference in mean grain size in sediment collected from treatment or control plots over time F(2, 189) = 0.354 @ p value = 0.702. Mean grain size of control and treatment samples for times t0 through t2 are plotted in Figure 24. These data indicate that the change in sediment compactness is not attributable to a



change in sediment grain size by erosion of native sediment from, or deposition of new sediment to the area.

Figure 24. Mean grain size of sediment surface grab samples from control and treatment pre- and post-dry harrowing.

Return monitoring of POC experiment: dry harrowed and control plots

All POC dry harrowed (2-passes with roller-chopper), and adjacent control plots were monitored in July. This is indicated as time 2 (t2) for the DH1, DH2 and DH3 plots and controls. As the Supplemental Experiment was initiated at this same field sampling event, this is time 0, (t0) for DH4, DH5 and DH6 plots and controls. Just one of the dry harrowed plots, DH1 and controls was sampled again in September. This is indicated as time 3 for plot DH1 and controls, and, at twenty weeks is the longest post treatment monitoring that occurred (refer to Table 1 in Methods section - experimental timing and monitoring).

Shrimp Density

Mean shrimp density within control plots did not differ significantly over time from t0 (April) to t2 (July) (maximum mean change 2.34 shrimp/core) (ANOVA F(2, 57) = 0.40, p = 0.67).

Shrimp density that was significantly reduced from a mean of $20.3 \pm SE \ 0.24$ shrimp/core to a mean of $7.8 \pm SE \ 0.21$ shrimp/core after initial treatment (April and May 2018 POC) (t-Test t(15) = 5.39, p < 0.001) remained low until t2 (July) (9.7 ± SE 0.09 shrimp/core) (Figure 25). Mean shrimp density within treated plots did not change significantly from t1 to t2 (t-Test t(25) = -1.03, p = 0.32).

Shrimp Biomass

Biomass within control plots did not differ significantly throughout all time periods (ANOVA F(3, 68) = 0.27, p = 0.85), from a mean of 87.74 ± 9.56 SE g/core @ t0 to a mean of $90.44 \pm 8.73 \pm$ SE shrimp/core at @ t2 (July).

Mean biomass changed significantly at treated plots DH1, DH2 and DH3 from t0 to t1 (t-Test t(13) = 1.77, p < 0.001) (Figure 26). Shrimp biomass was reduced from pre-treatment levels $104.74 \pm SE 9.91$ g/core @ t0, to $34 \pm SE 5$ g/core @ t1 post treatment. Mean shrimp biomass at t2 remained low in treated plots, and did not differ significantly from the first post-treatment biomass mean collected at t1 (t-Test t(16) = 0.93, p = 0.37).

Post treatment biomass was reduced 67% and 72% from pre-treatment levels for May and July surveys respectively.



Figure 25. Mean burrowing shrimp density for control and treatment DH1-DH3 plots from POC experiment. Plots dry harrowed with two passes of the roller-chopper, and revisited for monitoring in May and July.



Figure 26. Mean burrowing shrimp biomass for plots treated in WDNR's POC. Plots were treated with two passes of dry harrow treatment, and revisited in May and July.

Shrimp Size Distribution @ Return Monitoring POC (DH1-DH3) Plots

Frequency distribution of shrimp population density by carapace length collected within DH1 - DH3 control and treated plots over time are presented in Figures 27 and 28 (t0 - April, t1- May, t2- July, and t3 for DH1 only in September). Control plots generally had a normal distribution skewed towards shrimp of larger carapace length. This is with the exception of the September DH1 survey, where the peak population shifts to smaller shrimp with a mean carapace length of 8 mm (Figure 28).

Treated plot population distribution shifted from a normal distribution pre-treatment to a multimodal distribution post treatment. The multimodal distribution indicates that while all sizes of shrimp were impacted by dry harrow treatment, the medium and small size classes were most heavily reduced. Again, the September (DH1 only) survey shows that the majority of shrimp collected were in the extra small size class.



Figure 27. Frequency distribution of shrimp population by carapace lengths of shrimp collected from t0 - t3 from control plots associated with DH1 - DH3



Figure 28. Frequency distribution of shrimp population by carapace lengths from t0 - t3 from treated DH1 - DH3 plots

Estimated Burrow Densities

Estimated burrow densities (from pumped cores) at treated plots continued to decrease after initial treatment in April. Mean burrow density within treated plots was $72 \pm SE 1.36$ burrows/m², $54 \pm SE 1.27$ burrows/m², and $47 \pm SE 0.56$ burrows/m² for t0, t1, and t2 respectively. Mean burrow density within control plots, however, increased from April to July, then declined markedly between July and September. Burrow density at control plots averaged $68 \pm SE 1.36$ burrows/m², $74 \pm SE 1.27$ burrows/m², and $80 \pm SE 0.48$ burrows/m² respectively at t0, t1, and t2. Burrow density at control plots for time periods t0 to t2 showed no significant change (ANOVA, F(2, 55) = 0.8, p = 0.45). Treated plots, however showed significant differences between t0 and t2 (ANOVA, F(2, 41) = 4.05, p = 0.03).



Figure 29. Burrow density (burrows/m²) over time in the POC treated plots (two passes of the roller-chopper).

DISCUSSION

Dry harrowing treatment effects (plots DH4 - DH6 and associated controls)

From visual observation of the roller-chopper harrowing method, it appeared that towing the implement over shrimp-inhabited mudflats created hydrologic pressure that forced water contained in the intertidal up and out of the sediment surface through shrimp burrow openings. Shrimp were pushed out with the water and floated to the surface. The combined weight of the Marshmaster 2 XL towing the roller-chopper across the intertidal collapsed shrimp burrows and displaced water. Tines on the roller also penetrated to 15 cm. and turned over the top layer of sediment. After one dry harrow pass, pressurized water could be observed continuing to push up and out of burrow openings, where displaced shrimp were deposited. If these shrimp were not consumed by birds immediately, they were crushed by the Marshmaster and towed implement during its second pass.

The first post treatment survey (approximately 20 days later), showed that shrimp density in dry harrowed plots dropped significantly from pre-treatment densities (by an average of 89%) (t-Test t(48) = 5.38, p < 0.001). After another four weeks (six weeks post-treatment, t2) this low shrimp density ($0.73 \pm SE \ 0.23$) shrimp/core persisted. While shrimp densities in control plots also decreased from early spring to fall, the difference was not statistically significant (ANOVA F(2, 105) = 1.36, p = 0.26). These data indicate the dry-harrowing impacts shrimp densities beyond the natural shrimp population variability.

Similar mechanical experiments with heavy crushing vehicles called the "Rolligon" and "Argo" have been carried out which did not result in effective shrimp control. The results from the current experiment could differ due to the fact that the Rolligon and Argo trials did not include a roller - harrow component, and instead only utilized the "crushing" capacity of the vehicle. They produced results that were preferentially effective against larger male shrimp, but not against shrimp of other size classes (Booth 2007). While it looks like there is approximately 10% more effect of control for male shrimp than female shrimp, the current study indicates that treatment with the Marshmaster 2X and roller chopper implement remain an effective combination for control of all size and sex of burrowing shrimp collected. Figure 14 displays size classes collected over pre- and post-treatment surveys. All size classes collected were effectively reduced post dry harrow treatment.

One negative aspect of roller-chopper dry harrowing is the inability to apply it when a shellfish aquaculture bed is actively planted. Performing a dry harrow treatment in this situation would crush and kill shellfish product as it destroys shrimp burrows. For this reason, treatment timing plays a key role in determining whether dry harrowing has any potential as a shrimp population management tool. Dry harrowing may also impact other benthic organisms. Further investigation into the method will be necessary to assess the extent.

Gradient Transects

Reports from previous field observations suggest that larger juvenile or adult shrimp may move in from adjacent areas to recolonize treated areas (Dumbauld et al. 2006). To explore this

potential shrimp migration and resettlement phenomena, we sampled along a distance gradient perpendicular to the edge of a treated plot. We established three perpendicular transects on two sides of each treatment plots (six transects per treatment plot). Transects from within the treated plots, cut across the shared treatment-control plot edge, and into the adjacent control plot. We sampled at three, six, and nine meters from the edge into both the treated and control plots (Figure 3). We hypothesized that if shrimp were indeed migrating from the control plots, into the treated plots, we would see higher shrimp numbers at the plot edges compared to the treated interior. We observed no evidence of lateral movement of shrimp from adjacent plots. This finding was consistent for all sampling transects post treatment at 11 and t2. Shrimp density and biomass from transects at three, six, and nine meter distances from the edge of control - treatment threshold showed no significant differences among distance position grouping over time. The same finding was consistent for long-term plots surveyed at 12 weeks (t2) post treatment at DH1, DH2 and DH3. Seasonal larval shrimp recruitment that occurs in late fall may be the main mode of recolonization of the treated plots.

Treatment Intensity Compared

Plots that were treated with four dry harrow passes (DH4, DH5 and DH6) took an average of 41.5 minutes/half acre to treat, and experienced 79% initial reduction in biomass (g/core) from t0 to t1. During the POC experiment completed in May, two passes of the dry harrow treatment took an average of 28 minutes/half acre, and yielded 67% shrimp control from biomass (WDNR 2018).

This 12% reduction in biomass required a 33% increase in field time - which may raise questions regarding efficiency, however, four passes had significantly greater impact on the XS size class shrimp (4.5 - 8.5 mm CL), compared with the 2 passes applied for the POC. This is evident in the population density distribution plots (Figures 15, 16, 27 and 28) provided in the Results section. Plots treated by 4 passes of the dry harrow experienced the smallest increase (from all treated and control plots DH1 - DH6) of the XS size class found in September surveys. This may support treatment of 4 rather than just 2 passes of the dry harrow as a more effective method at removing shrimp of the smallest size.

Burrow to Shrimp Density Relationship

Burrow density has been the most common metric used to estimate shrimp density. Yet, burrow density varies seasonally, with wave climate and temperature. The relationship between burrow openings and shrimp may be seasonably dependent. Shrimp density and biomass were plotted against total burrows counted within a sampling core. While shrimp biomass and burrow density have a positive linear association (approximately 8.39 g shrimp, or 2.8 shrimp per burrow), the relationship is weak and provides no predictive capability.

This study and others have found that burrow counts are largely unreliable as a measure of absolute shrimp density (Dumbauld et al. 1996, McPhee and Skilleter 2002, WDNR 2018). Burrow counts collected in spring and summer months when shrimp are most active are possibly more reliable (Dumbauld et al. 2006). Our data supports this suggestion: a mid to late summer burrow count to biomass regression using data from this experiment yields an $R^2 = 0.47$. While

this is not a very robust relationship, it is better than the than the regression of $R^2 = 0.06$ created using data collected from the same site in spring, 2018 for the POC study (WDNR 2018).

Burrow density was reduced 77% from 49 burrows/m² to 11.38 burrows/m² pre- and postsupplemental treatment (25% within control plots - 54.3 to 40.68 burrows/m²). Burrow density fell another 5% - 11.38 to 8.88 burrows/m² in treated plots when sampled in September at t2 (21% within control plots - 40.68 to 29.33 burrows/m²).

Substantial loss of oysters occur when burrow density exceeds 20 to 40 burrows per m² (Dumbauld et al. 2006). Ten burrows per m² has been used as a minimum threshold value for justifying treatment for shrimp control in Willapa Bay (Booth 2007). Treatment with four passes of the dry harrow treatment reduced burrow counts to a suitable density based on this information.

Duration of treatment effect on POC (DH1 - DH3) plots

Shrimp density within plots treated with 2 passes of the roller-chopper remained at low levels from April (t0) until July (t2) surveys (12.42 weeks). By September, 20 weeks after treatment (t3 surveys completed for only the DH1 treated plot and associated control plots), a higher proportion of extra small shrimp were observed to dominate the population distribution. This can be seen in Figures 27 and 28, where the population distribution changes from early and mid summer 2018 to September (t3), when the XS size class comprises the majority of the population of shrimp sampled. These XS shrimp collected in September surveys were not found in surveys completed in May or July. It is probable that shrimp recruited in Fall 2017 were too small to be detected in April 2018 (t0) or May 2018 (t1), but had grown large enough by the end of the summer (September 2018) to be detected in our pumping surveys. Other studies within Willapa Bay have shown that shrimp collected and classified into the XS size class (CL 4.5 to 8 mm) likely fit into a 1 to 2 year old age class (Dumbauld 2012).

Combination of Mechanical Treatments for Shrimp Population Control

An understanding of annual shrimp recruitment is important for long term management on farmed intertidal lands (Dumbauld et al. 1996). *Neotrypaea californiensis* reproduces annually. Pelagic larvae leave the estuary, develop in nearshore coastal waters, and return to the estuary to settle or "recruit" to the lower and sub-tidal beaches in late summer or early fall (Dumbauld 2012). In years where recruitment has been high, shellfish growers have observed shrimp population re-establishes back into previously treated areas. Shrimp burrows appeared to be as abundant one year after Carbaryl application as they were pre-treatment (Feldman et. al. 2000, Dumbauld et al. 2001). High recruitment in the early 1990's, followed by a period of relatively low recruitment (late 1990's to early 2000's) influenced a boom, and subsequent decline of burrowing shrimp populations in Willapa Bay. These steep shifts in recruitment abundance are thought to be influenced by climatic conditions, and more specifically El Niño. High shrimp recruitment tends to occur in strong El Niño years (Dumbauld et al. 2012b).

While results from plots treated with four passes of mechanical treatment indicate the smallest classes of shrimp (0 to 2 years old) are effectively managed, supplementing with wet harrow

treatment could be an effective solution for managing the XS size class (WDNR 2018). In particular, wet harrowing in active aquaculture beds could control for new recruits that arrive to the site that has been previously dry-harrowed when fallow. New recruits – shrimp with CL equal to or less than 6mm in length, generally fit into the 0 to 2 year age class, and due to their body size are unable to burrow as deep - often inhabiting the top 10 to 30 cm. of sediment (Dumbauld 1996, Bosley and Dumbauld 2011).

Preliminary results from wet harrowing by dragging harrows with 15 cm- long tines by vessel suggest that the method seems to control for this smallest size class and does not appear to damage shellfish product (WDNR 2018). While the method may reduce shrimp density, the time required to treat plots is high, and perhaps not realistic for large scale commercial operations. With this in mind, a combination of high tide wet harrowing of actively farmed land that was dry-harrowed prior to planting may be feasible.

Timing of Treatment

Treatment timing is an important factor in the management of burrowing shrimp for the benefit of oyster ground culture (Dumbauld et al. 1996). Our surveys indicated an influx of small shrimp (4.5 to 8.5 mm CL) in September 2018. To effectively manage this smallest size class as well as newly recruited shrimp, it has been suggested that late summer to early fall is the most effective time for treatment (Dumbauld et al. 1996). This timing provides decent windows of daytime low tides that can be utilized for dry harrowing. Shrimp burrowing activity is also near its peak for the year, with *N. californiensis* closer to the substrate surface, and is more easily impacted by methods of control (Booth 2007, NOAA 2018).

New recruits, due to their small size, do not harm oysters for at least 2 years (Dumbauld et al. 2006). During these years, fattening and seed beds (which are typically used for one to two years) could remain untreated after initial control. If the majority of adult shrimp are removed before planting, the site could still successfully grow shellfish product for up to two years. This however, would not be the case for seed to harvest beds, or other growing plans that require the crop to be present for three years or more (Dumbauld et al. 2006).

Testing in 1999 of Carbaryl on *N. californiensis* in Willapa Bay saw treatment efficacy of 85 to 95% from shrimp burrow counts. Due to low recruitment in following seasons, this treatment effect lasted for 2 to 3 years. Alternatively, reports from years of high recruitment describe immediate recolonization of plots treated with Carbaryl one year prior (Dumbauld et al. 2006).

Because *N. californiensis* recruitment can be extremely variable, monitoring of recruitment trends in accordance with climatic conditions is essential for developing shrimp management plans (Dumbauld et al. 2006, Dumbauld et al 2012b). Results from 4 passes of dry harrow treatment (77% efficacy from burrow counts) suggest that (with the variability of burrow counts in mind) the effectiveness of mechanical management warrants further investigation.

CONCLUSION

While the POC and supplemental studies have provided useful information regarding the potential for mechanical management of burrowing shrimp in Willapa Bay, a number of relevant questions remain unaddressed. Some of these are technical questions that focused field studies can be designed to answer, such as: *Can a sediment compaction threshold that allows for productive ground culture be identified? Can a reliable relationship between shrimp density and sediment compaction be determined? What is the longevity of a specified range of various mechanical treatments (e.g. number of dry harrow passes per treatment event, number of dry harrow treatment events per farm plot, combination of dry and wet harrow treatments)? What is the timing with respect to shrimp life cycle, weather, tide and farming cycle that most effectively controls shrimp?*

Broader issues, however, complicate the viability of mechanical methods for burrowing shrimp control: *Are the methods viable on a larger, commercial scale? Are they economically realistic? Are they compatible with shellfish ground culture?* The POC and Supplemental study results provide evidence that dry harrowing warrants further testing, as it may have some potential to play a role in the management of burrowing shrimp.

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