N A T U R A L   R E S O U R C E S

Proof of Concept (POC) Willapa Bay: Mechanical Management of Burrowing Shrimp

April 2018

WASHINGTON STATE DEPT OF NATURAL RESOURCES

Cover Photo: Burrowing shrimp displaced by Dry Harrow treatment, Grassy Island WA.

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Aquatic Assessment and Monitoring Team
Aquatic Resources Division

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Acknowledgements

This project was completed only with the collaboration and hours spent 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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EXECUTIVE SUMMARY

Two native shrimp species, *Neotrypaea californiensis* (ghost or sand shrimp) and *Upogebia pugettensis* (mud shrimp) are benthic invertebrates that excavate extensive burrows in the intertidal and high subtidal marine shores of Washington's Pacific and Puget Sound coasts. *N. californiensis* is the more predominant of the two species. It is widely distributed on sandy beaches, can live more than 10 years, and provides an important food source for Dungeness crab, Green sturgeon, Gray whales, shorebirds, and other mesopredators (Dumbauld et al. 2008, Moser et al. 2017).

In the Willapa Bay and Grays Harbor estuaries, qualitative assessments note that the deposit feeding and burrowing of *N. californiensis* loosens the sediment, affecting the productive culturing of clams and oysters. Shellfish growers have used many methods to attempt to control burrowing shrimp populations in the past, including chemical (i.e., pesticides) and non-chemical methods (i.e., from covering to mechanical disruption).

The Washington Department of Natural Resources (WDNR), Aquatic Resource Division was in a unique position to study a suite of mechanical methods to control shrimp, because its Aquatic Assessment and Monitoring Team (AAMT) was just finishing a study of burrowing shrimp in Willapa Bay and because it has existing equipment in the bay to manage invasive species, primarily Spartina. Therefore, AAMT designed a study to assess the feasibility of mechanical control for burrowing shrimp in Willapa Bay. Three mechanical methods for controlling burrowing shrimp were tested in the spring of 2018. **Dry Harrowing** - towing a large roller - chopper with an amphibious vehicle; **Flooding** - hydraulically liquefying sediment with pumped sea water and **Wet Harrowing** - dragging a modified farm harrow at high tide by boat. Test plots were located at Grassy Island, Willapa Bay and Southern Grays Harbor. Of the three mechanical methods tested, Dry Harrowing emerged as a method worthy of further study for controlling Small, Medium and Large sizes of burrowing shrimp. It was also the most economically viable of the three options. Data collected from Grays Harbor appears to demonstrate Wet Harrowing has potential as a mechanical method to reduce population of the smallest and youngest size class (Carapace Length < 8.28 mm) at high tides when Dry Harrowing is not possible. Further investigation into the duration of treatment effect, trend of recolonization by shrimp, and practicality in use with commercial growing is needed to assess whether Dry Harrowing could be utilized on a commercial scale in Willapa Bay.

INTRODUCTION

Two species of burrowing thallasannid shrimp the "Ghost Shrimp" *Neotrypaea californiensis*, and "Mud Shrimp" *Ubogebia pugettensis* inhabit Pacific Coast estuaries. Populations of *U. pugettensis* have seen sharp declines due to parasitism by the introduced isopod *Orthione griffenis,* and have largely disappeared (Dumbauld et al. 2011). *N. californiensis,* on the other hand is widely abundant and is a significant food source for the threatened green sturgeon, crabs, salmonids, and other higher trophic species that utilize Willapa Bay (Dumbauld, Holden and Langness 2008, Moser et al. 2017, Borin et al. 2017). While studies show that overall populations of *N. californiensis* have also been on the decline since 2002 (Dumbauld 2012), reports from Willapa Bay have noted populations on the rise (Dumbauld, pers comm.). Shellfish aquaculture in Washington State may be negatively influenced by *N. californiensis,* which excavates extensive burrow systems 60 to 90 centimeters deep, and re-suspends sediment while deposit feeding and ventilating its burrows (Posey 1986, Berkenbusch and Rowden 2003, Ferrararo and Cole 2004, Bosley and Dumbauld 2011). These activities liquefy sediment and can destabilize portions of mudflat that are used to farm shellfish – potentially causing shellfish to sink and suffocate. An estimated 250 liters per m^2, or roughly all of the pore water contained in upper 50 cm of sediment is cycled every few days due to *N. californiensis* deposit feeding and ventilating its burrows alone (Vokenborn et al. 2012). This significantly contributes to oxygenating and de-nitrifying sediment.

Because of the purported impact to shellfish ground culture that shrimp pose, shellfish farmers and resource managers in Willapa Bay have been working to find a solution for control of the pest species for decades (Stevens 1928, Patten 2017). Beginning in 1960 when populations of *N. californiensis* expanded rapidly, many growers applied an insecticide called Carbaryl (marketed as Sevin) to the surface of the intertidal (WDFW 1970, Feldman et al. 2000, Felsot and Ruppert 2002). Carbaryl was restricted and completely prohibited as an option by 2013. Efforts to find an alternative control method began to focus on another compound called Imidacloprid. Both chemicals produce tetany in insects and invertebrates as well as muscle paralysis and death. A number of studies were conducted to investigate Imidacloprid's effectiveness (Felsot and Rupert 2002, WSU 2014, Patten 2016).

WDNR's Aquatic Resources Aquatic Assessment Monitoring Team (AAMT) staff began an investigation in 2013 to estimate quantity and distribution of burrowing shrimp available for predation by migrating gray whales in northern Puget Sound (Pruitt and Donoghue 2016), and the energetic requirements of green sturgeon from the threatened southern distinct population that feed on the shrimp during their annual summer residency in Willapa Bay (Borin et al. 2017). In 2018, DNR AAMT began an effort to explore non-chemical methods of managing burrowing shrimp populations in Willapa Bay. Three mechanical methods were tested for their effectiveness at reducing burrowing shrimp density. This work was part of a broader DNR effort, the Rural Communities Partnership Initiative (RCPI), an initiative with the goal of supporting economic development in rural communities of Washington State.

1. Dry Harrowing (DH)
2. Wet Harrowing (WH)
3. Flooding (F)

These methods were chosen from a number of other options because they had shown to have some effectiveness, and were the most cost effective alternatives to chemical control with available resources.

Site Description

Willapa Bay

Located between the Columbia River to the South and Grays Harbor to the North, Willapa Bay is the second largest coastal estuary on the Pacific Coast. Tidal flats and shallow channels characterize the estuary with more than 85% of its area never reaching a depth of greater than 7 meters (Troiano and Grue 2016). There are no major ports, and it is considered one of the least human-altered coastal estuaries in the United States (US. Fish and Wildlife Service 2012).

Grassy Island

This proof of concept (POC) was located on State Owned Aquatic Land (SOAL), an area not historically cultivated for shellfish, just south of Grassy Island on the Long Beach Peninsula in Willapa Bay (Figure 1). For our purposes, we will refer to the site as "Grassy Island," but it is also known as "Stackpole."

The site at Grassy Island was chosen for its abundance of burrowing shrimp. The sediment here is composed primarily of medium grain sand, with some native and non-native eelgrass (*Zostera marina* and *Zostera japonica*). Grassy Island is a dynamic site that is exposed to storms in the winter when Southern winds blow up the bay. At low tide, the flat extends approximately one mile out. A single main slough retains water throughout the tidal cycle. We positioned our replicate plots along this slough to take advantage of water availability for density assessments. The majority of intertidal to the South of Grassy Island is privately owned (Figure 1). A few landowners use this area for shellfish aquaculture.

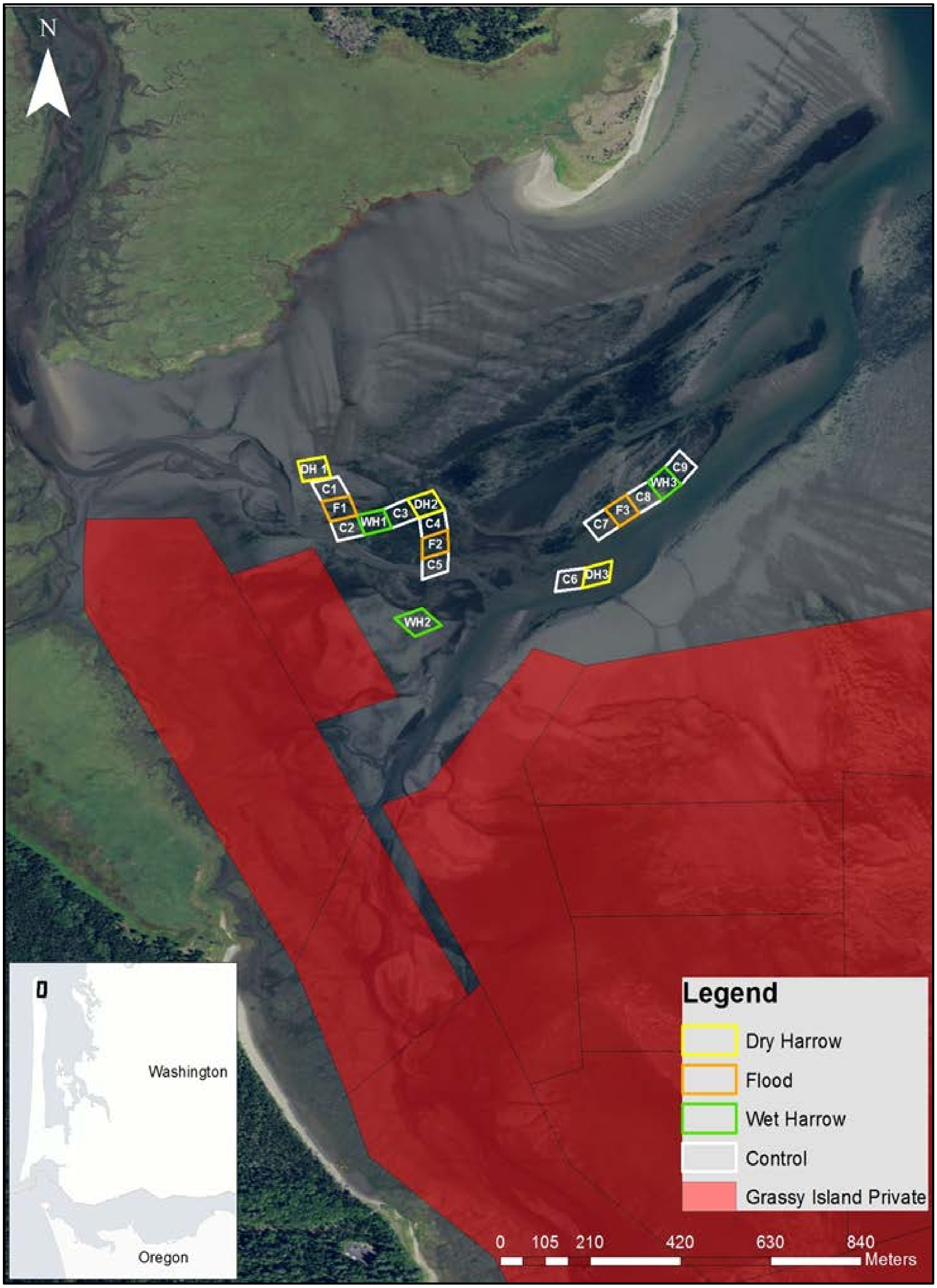


Figure 1. *Site Location Proof of Concept Mechanical Management Study in Willapa Bay Grays Harbor*

Located approximately 30 kilometers (km) north of our site at Grassy Island, four separate acre sized plots were additionally set up on the Southern side of Grays Harbor. Grays Harbor is Washington State's second largest coastal estuary behind Willapa Bay, located just to the North (Figure 2). The site we chose on SOAL was an area not historically cultivated for shellfish. The site was flanked to its Westside by a steep channel and to its east by flat tidelands utilized for aquaculture (Figure 2). Like Willapa Bay, Grays Harbor is largely undeveloped and used for shellfish production. We contracted with a local grower to Wet Harrow three separate one - acre sized treatment plots. One control plot separated all three treated plots. This separate Wet Harrow study in Grays Harbor was conducted to assess positive results the shellfish grower has seen with a larger harrow and oyster dredge – equipment we could only replicate on a smaller scale in Willapa Bay.

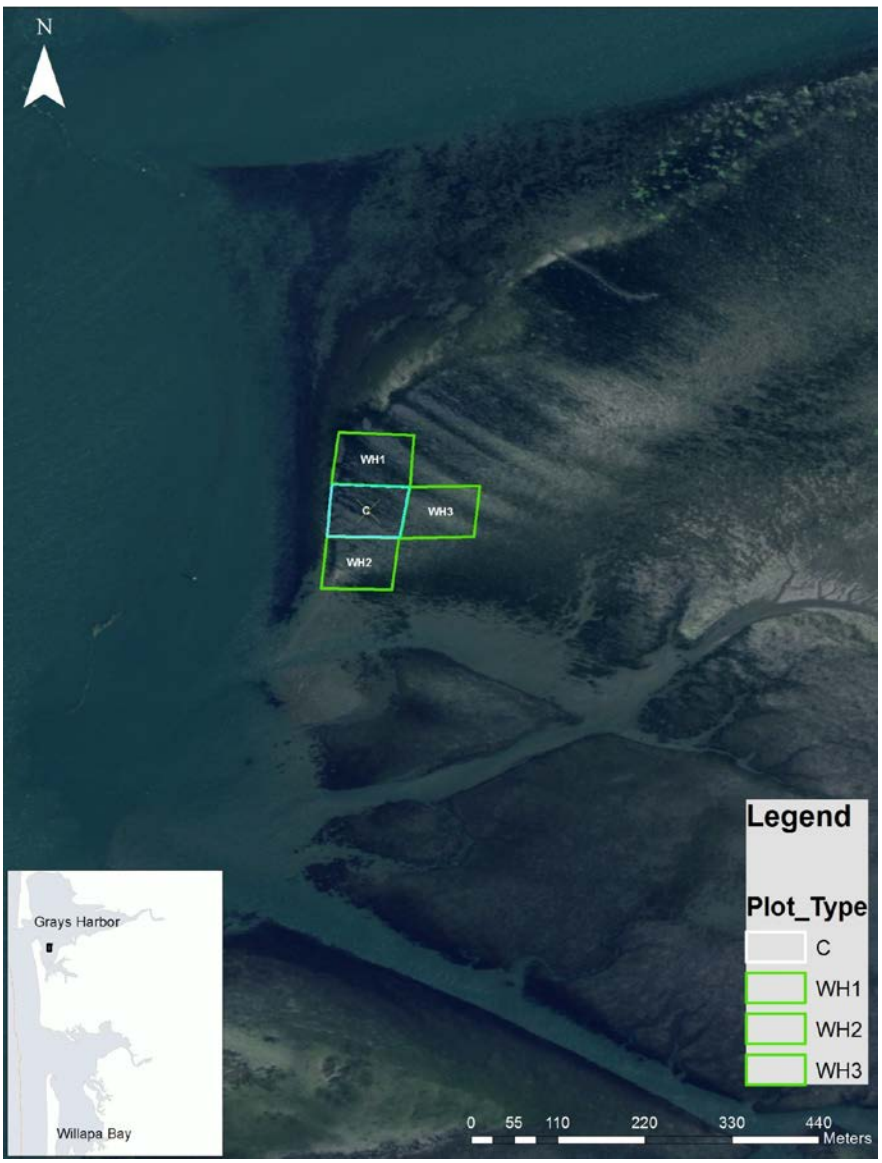


Figure 2. *1-acre wet harrowing plots in Grays Harbor*

METHODS

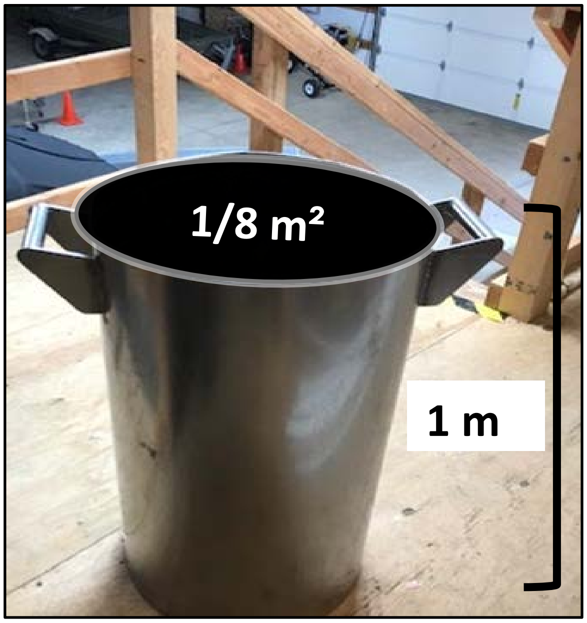
Experimental design

Eighteen half-acre plots were established along the slough at Grassy Island (Figure 1). These 18 plots included three Dry Harrow plots, three Wet Harrow plots, three pumping plots, and nine control plots separating each of the treatment plots. Control plots were left untreated. Measurements of shrimp density, burrow counts, grain size, and compactness were taken before treatment of plots (March and April). This first establishment and survey of plots we call time zero (t0). Plots were treated in late April, and surveyed for metrics mentioned above “post treatment” were two weeks later. The second “post treatment” survey is known as time one (t1).

With the exception of site access and operability, which were inherent to completing the study, other factors relevant to natural resource managers and growers were considered beyond the scope of this study. These parameters included other sediment characteristics, wave climate, currents, fresh water influence, nutrient availability, water temperature, pH, and predation, among others.

Shrimp Density

To assess shrimp density at each plot, we liquefied the entire contents of a 1/8 meter^2 (m^2) surface area core pushed one meter into the sediment. The core is a custom design made of stainless steel (Figure 3). It has two handles, with a capturing net built of 2 millimeter (mm) mesh and PVC supports that enclose its top. The capturing net adds approximately .5 meters (m) to the top of the core, and retains any shrimp that float to the surface where they can be collected. Honda water pumps mounted on a Marsh Master 2XL from Coast Machinery brought 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 firehose for the outflow. The 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). Positively buoyant shrimp float to the surface of the core, where we collected, bagged and froze them for later measurement. Three cores were randomly taken per plot, with care to not resample the same location pre and post treatment. Total biomass and total number of shrimp from each core were counted respectively. Because shrimp density has been used as the preferred metric explaining populations of *N. californiensis* (Dumbauld 1996, Dumbauld et al. 2014), we focus our results on shrimp number first. Biomass, however, is a more holistic metric of shrimp quantity, which accounts for differences in shrimp size, and is thus noted secondarily.



Figures 3 and 4. *Burrowing shrimp sampling core, and sampling method for burrowing shrimp density.*

Lab Processing of density samples

All shrimp were measured for total weight in grams (g), total length in millimeters (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 1 indicates ranges for each size class). Size classes were established from previous size distribution analyses. (Pruitt and Donoghue 2016). Four to six similar size classes have been established based on carapace length in other studies within Willapa Bay (Dumbauld et al. 1996, Bosley and Dumbauld 2011). Carapace length of burrowing shrimp is not well correlated to shrimp age. However, research does support shrimp of 6.16 mm CL (our XS size class) as roughly two or less years old (Dumbauld et al. 1996, Bosley and Dumbauld 2011). *N. californiensis* recruit in late summer to early fall, so it is not likely that we are observing new recruits (0 to 1 yr.) in any of the shrimp we collected (Dumbauld et al. 1996). Average size classes were 20.33 ± SD 2.36 CL Large, 14.58 ± SD 1.69 CL Medium, 10.41 ± SD 1.41 CL Small, and 6.16 ± SD 1.69 CL Extra Small. Size ranges were based off the mid-point between size class averages.

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 1. *Range used to classify shrimp in each size class*

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

Table 2. *Size classes collected in WDNR 2018 Supplemental compared to average values from Bosley and Dumbauld (2011).*

Data Analysis of shrimp density samples

Pre and post treatment surveys were compared at Control, Dry Harrow, Flooding, and Wet Harrow Plots. t-tests ($p<0.05$) assuming unequal variance were conducted and run using Microsoft Excel 2016's data analysis package. t-tests had null hypotheses that there was no difference in shrimp density or biomass between groups. Community size structure was further investigated by classifying shrimp size into four classes – Large (L), Medium (M), Small (S), and Extra Small (XS), based on Carapace Length (CL) (mm). Density of carapace length was plotted and analyzed using R version 3.4.3's ggplot package.

Burrow Counts

Shrimp burrows were counted within each 0.125 m^2 (20 cm diameter) core at every sampling location. Burrow number was then multiplied by eight to estimate the number of burrows within a square meter area. This is likely an overestimate of the actually number of burrows per square meter as the burrows are patchily distributed. Burrows were identified by mounded sediment with a burrow opening in the middle. Burrow shows were often indiscernible until the core was pushed into the sediment, when water expelled from openings.

Sediment Samples and Analysis

Surface grab sediment samples were collected from the top 10 cm at every plot and placed in Ziploc bags. Sediment samples were frozen until prepared for grain size analysis. Samples were washed with tap water and placed in individual metal tins in a 100°C oven for 24 hours to dry. Samples were removed from the oven and weighed initially, then added to a stack of sieves ranging from 2 mm to less than 0.063 mm. The sieves were shaken with a Gilson sieve shaker for 10 minutes. Sample size in each sieve post-shaking was weighed and recorded.

Sediment Compactness

Compactness was measured pre and post treatment with a custom "penetrometer". The penetrometer is a 159-cm. stainless steel rod with a base plate welded to it. For each measurement, a five pound drop weight was released, and allowed to free-fall until it contacted the base plate, pushing the rod into the sediment. Rod penetration depth was measured and recorded after the weight drop. The weight was raised and released a second and third time at the same sample point, and cumulative penetration depth was measured and recorded. Five random sample points were selected per plot.

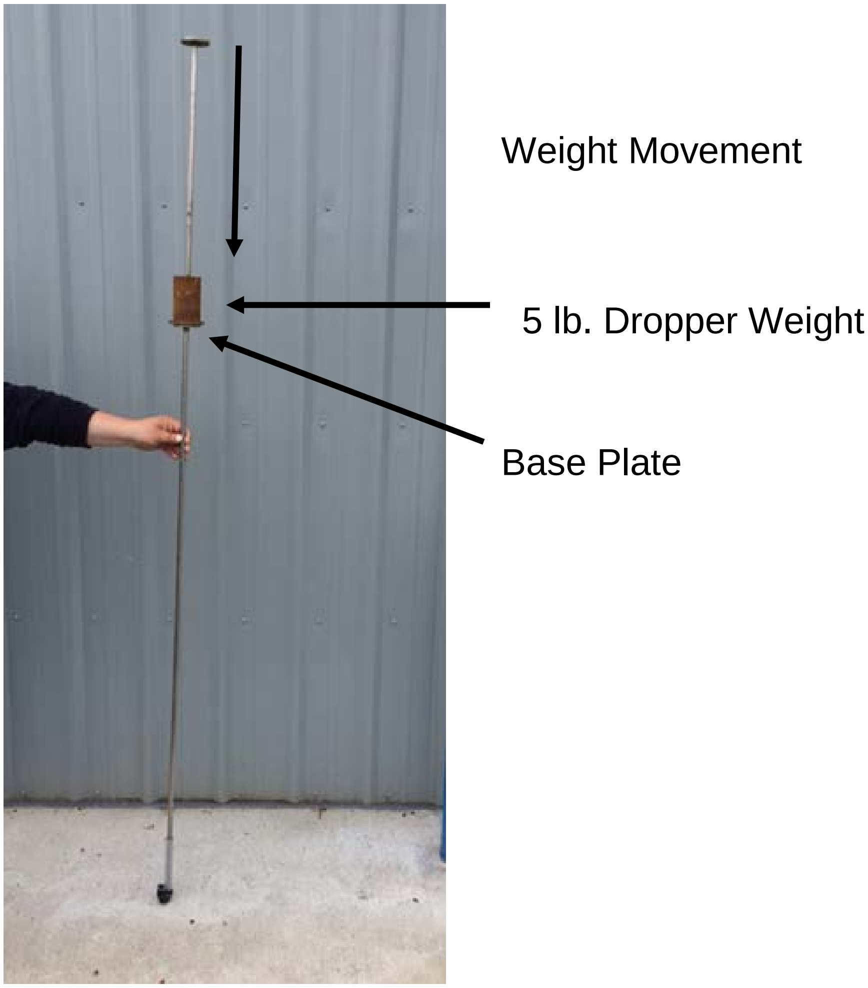


Figure 5. *Penetrometer used for assessing sediment compaction pre and post treatment.*

Treatment Effort

Human hours (Human hours = hours worked treating * crew size) were recorded as a metric to gauge effort put into each treatment type.

GPS

Positions were recorded with a handheld Trimble XH GPS for every core, penetrometer, and sediment sample. GPS track line data was collected for DH, F, and WH treatments to measure total area treated. GPS data was post processed and corrected with Trimble GPS Pathfinder Office version 5.8.

TREATMENTS

<u>Dry Harrowing:</u> This treatment involved towing a robust steel roller (manufactured by Coast Machinery LLC) behind an amphibious tracked vehicle called the "Marshmaster-2LX" (Figure 6). This roller weighs 700 pounds, and is designed to cut an 8-foot wide swath through marsh and wetland (Coast Machinery LLC. 2018). It has a series of flat plates welded onto it, which penetrate into the sediment approximately 30 cm and thus referred to as a "roller-chopper". 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 are consumed by birds. Treated plots were Dry Harrowed with two passes of the roller-chopper.

Figure 6. *WADNR Marshmaster-2XL towing a roller-chopper, the implement used for "Dry Harrowing"*

Flooding: Water pumps were used to liquefy sand to a depth of one meter (Figure 7). The shrimp - which are positively buoyant, float to the surface and are consumed by birds. The field crew worked back and forth across treatment plots to ensure the entire ½-acre flooding plot was covered once. Care was taken to avoid eelgrass within and near experimental plots.

Figure 7. *Liquefying an entire flooding treated plot*

Wet Harrow - Grassy Island: A custom harrow with 15 cm long tines was developed and used to harrow intended plots by towing the harrow at high tide by skiff (Figure 8). The harrow developed for this study was designed similar to a cockle-collecting rake, which uses a flat plate to provide downward pressure while moving along the bottom. Shrimp are consumed by fish and other predators as they are exposed by the harrow. Each ½ acre wet harrow plot was covered once.

Wet Harrow - Grays Harbor: Wet Harrowing in Grays Harbor was conducted with much larger equipment; plots were 1 acre in size (Figure 2), and covered by a 21 foot oyster dredge with harrows to each side. The harrows used were modified drag harrows with 15 cm tines (Figure 9). The vessel and equipment used for these trials was contracted through a local shellfish company who regularly treats their beds for shrimp by harrowing them (E. Buck, personal communication). The company is able to complete this treatment on beds where oysters are on the sediment during the grow-out cycle (E. Buck personal communication Feb. 9, 2018).

Figure 8. *Harrow used at Grassy Island site - towed by skiff at high tide.*

Figure 9 (at right). *Harrow used in Grays Harbor, towed by large skiff. Harrows extended off booms on both sides of the skiff*

RESULTS

Treatment Effort

Treatment of Dry Harrow plots required one marsh master operator, and took one day on April 23, 2018 to complete. Flooding required a larger crew (3 to 7), and took four days to complete treatment (April 16, 17, 18, and 19, 2018). Like Dry Harrowing, Wet Harrowing at Grassy Island was accomplished in one day (April 26 2018), and took a crew of two to accomplish by boat. Wet Harrowing at Grays Harbor was conducted three times over each acre plot.

Dry Harrow treatments took significantly less time to complete than either the Flooding or Wet Harrow treatments. Treating one Dry Harrow plot (.47 ± SE .08 human hours) took 1.9% of the time it took completely Flood one plot (23.95 ± SE 1.4 human hours), and 30%

of the time it took to harrow (30 passes by boat) one Wet Harrow plot (1.54 ± SE .28 human hours). Figure 10 shows average human hours to treat half-acre plots of each treatment type.

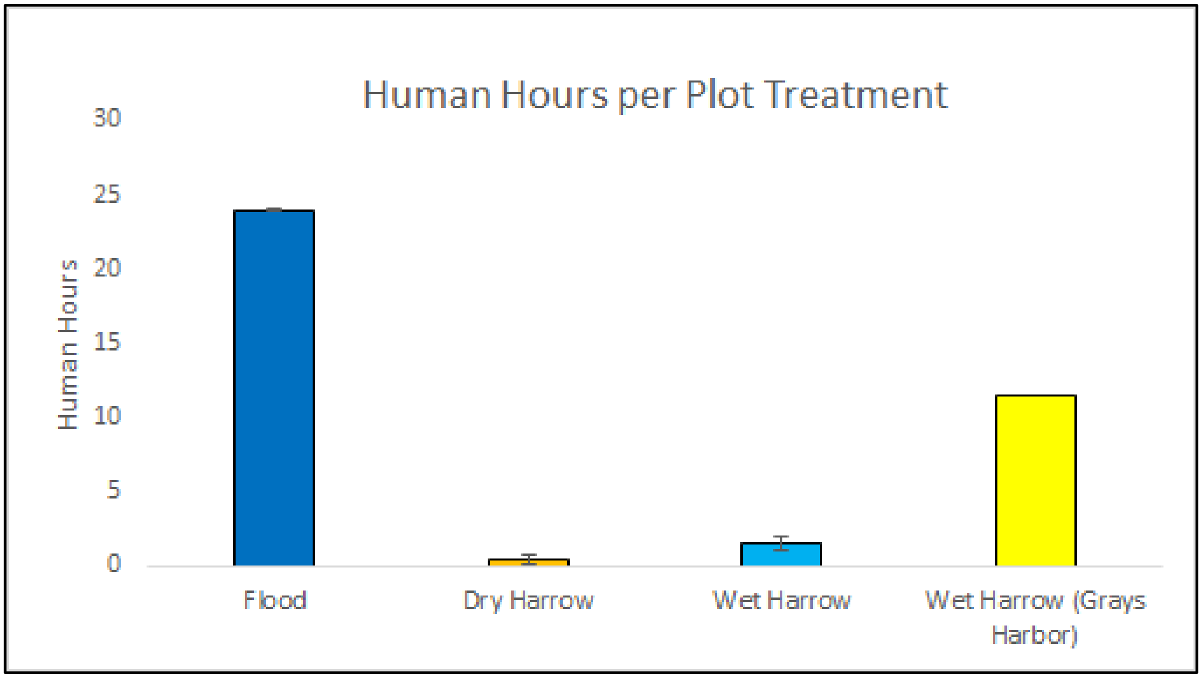


Figure 10. *Mean human hours per plot. (Human hours = Hours worked treating * crew size). Values are for 0.5-acre plot size. Error bars indicate standard error.*

Burrow Counts

Although burrow counts have been previously relied on to assess shrimp density (Dumbauld 1996, Dumbauld et al. 2014, Dewitt et al. 2004), data collected from this POC study show no relationship between shrimp density and burrow count (Figure 11).

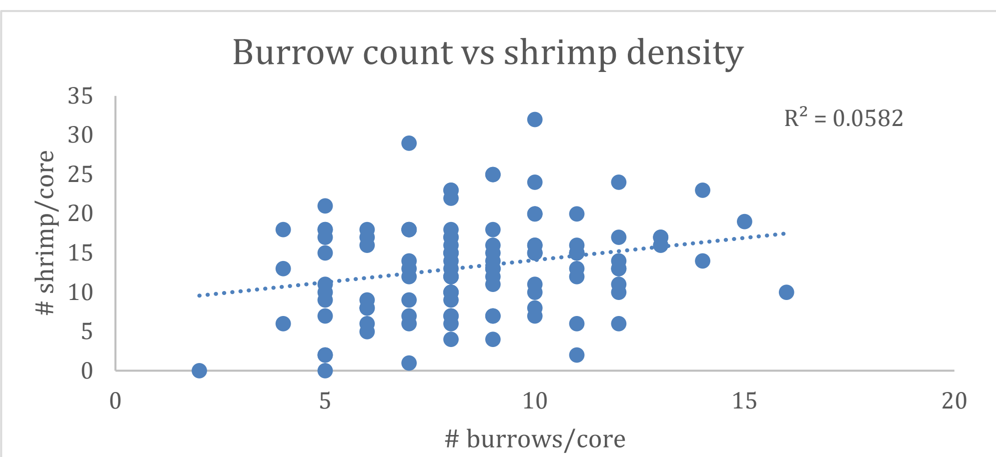


Figure 11. *Burrow count within core area plotted against shrimp collected within pumped core.*

This relationship is consistent with our previous five years of shrimp sampling and burrow counts throughout Willapa Bay as well as Puget Sound. At best, our data collected over 15 seasons demonstrates a weak to very weak relationship in the summer months. Multiple studies have found that burrow counts are better suited to indicate trends in shrimp abundance at other periods (mid to late summer), when shrimp activity is at its peak, and a better correlation between shrimp population and burrow count is obtained (Dumbauld et al. 2006, Dumbauld et al. 1996, McPhee and Skilleter 2002).

Grassy Island - Shrimp Density

939 shrimp were collected in all pretreatment surveys. Of those 939 shrimp, only one was identified as the mud shrimp species *Ubogebia pugettensis.* The remainder of shrimp were *N. californiensis*. 413 (50.3%) shrimp were classified as female, and 408 (49.6%) were classified as male. 118 shrimp (12.5%) were too small to classify as a certain sex. 180 (44%) of the 413 female shrimp were egg bearing. Zero of those shrimp carried a parasitic isopod common on ghost and mud shrimp.

717 shrimp were collected in all post treatment surveys. All of the shrimp collected in post treatment surveys were *N. californiensis.* 309 (43%) of those shrimp were female, 254 (35%) were male, and 154 (21%) were too small to classify as a certain sex. Of female shrimp collected, 174 (56%) were egg bearing. No parasites were found on shrimp collected in post treatment surveys.

1. Controls

Burrowing shrimp density averaged 14.55 ± Standard Error (SE) of 1.67 shrimp per core at control plots pre-treatment and averaged 14.44 ± SE 1.06 shrimp per core post treatment. No statistical difference was detected among controls in a two sample t-test assuming unequal variances. (n=56, t=0.02, p=0.99).

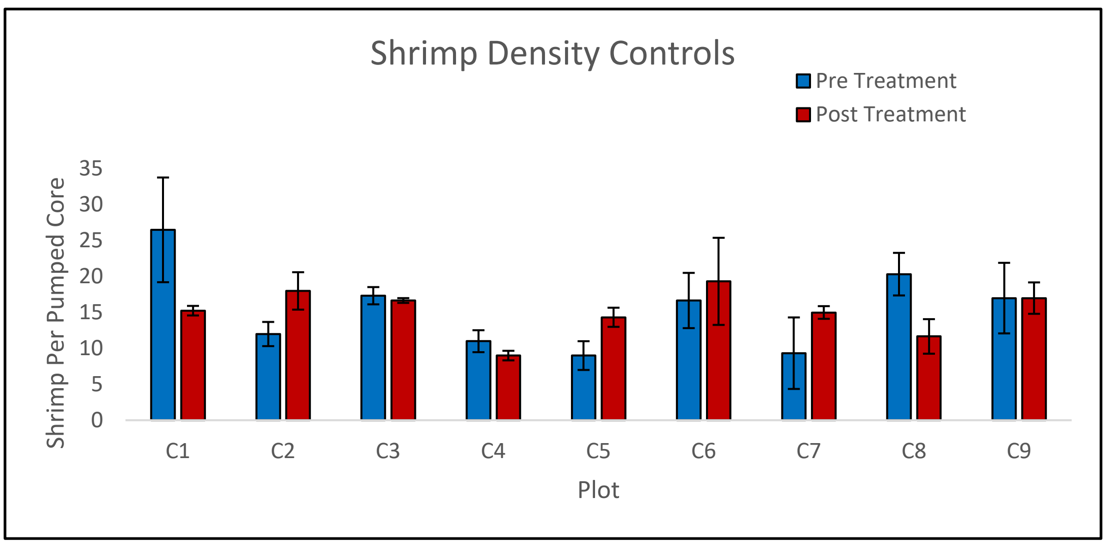


Figure 12. *Shrimp density (shrimp/core) at control plots t0 and t1. Error bars indicate standard error.*

2. Dry Harrow Treatment

Shrimp density post Dry Harrow treatment averaged 7.88 ± SE 1.28 shrimp per core. Significant differences were detected in a two sample t-test assuming unequal variances (n=19, t=5.39, p<0.001) for pre and post Dry Harrow groups. Dry Harrow treatments provided the best control of *N. californiensis*, where a mean 61.2% ± SE 6.9% reduction in shrimp was observed (Figure 13). [Mean shrimp density measured at Dry Harrow plots pre-treatment (20.3 ± SE 1.91 shrimp per core) was higher than that at the Flooding, Wet Harrow, and control plots pre-treatment. We estimate this to be attributed to slight differences in plot elevation].

3. Flooding Treatment

Mean shrimp density pre Flooding treatment was 10.60 ± SE 1.26 shrimp per core. Mean shrimp density post Flooding treatment for all flooded plots was 6.77 ± SE 2.02 shrimp per core. We used t-tests assuming unequal variances, to assess this difference and it was not significant (n=19, t=1.8, p=0.10). Control of *N. californiensis* with Flooding had variable results correlated with treatment intensity. When flooded plots were analyzed separately, plot F2 did have a significant decline in shrimp density pre and post treatment (two sample t-test assuming unequal variances (n=6, t=4.97, p=0.04)), however, the human-hour investment required to apply the flooding treatment determined this method to be impractical (Figures 13 and 10).

4. Wet Harrow Treatment Grassy Island

Mean burrowing shrimp density was 13.70 ± 4.00 shrimp per core pre Wet Harrow treatment and 15.66 ± 4.00 shrimp per core post Wet Harrow treatment. While the post-treatment mean appears to be slightly higher than pre-treatment, there was no statistically significant differences detected in shrimp density pre and post Wet Harrow treatment (two sample t-test assuming unequal variances (n=19, t=-0.96, p=0.35).

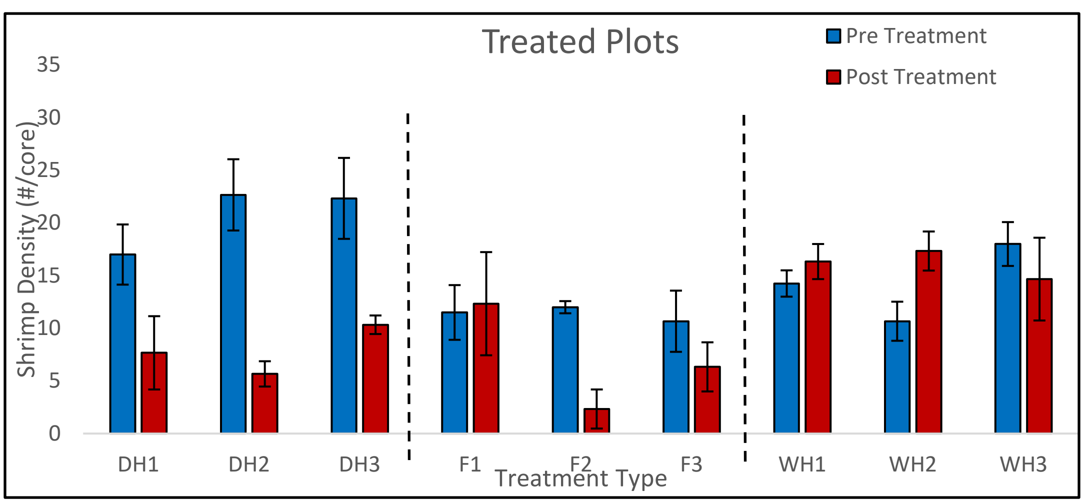


Figure 13. *Density of shrimp collected in Dry Harrow (DH), Flooded (F), and Wet Harrow Treatment (WH) Plots. Error Bars indicate standard error.*

Grassy Island - Shrimp Biomass

1.Controls

Mean biomass assessed pretreatment at control plots was 81.14 ± SE 7.72 g per core before treatments. Post-treatment control plot biomass averaged 80.25 ± SE 7.44 g per core. No statistical differences were detected across all nine control plots pre and post treatment in a two sample t-test assuming unequal variances (n=56, t=0.08, p=0.94).

2. Dry Harrow

67.4% mean reduction in biomass was observed pre and post Dry Harrow treatment. Shrimp biomass pretreatment at Dry Harrow plots averaged 104.77 ± SE 9.91 g per core, and 34.08 ± SE 5 g post-treatment per core. Significant difference was detected with a two sample t-test assuming unequal variances (n=19, t=3.26, p=0.005) between pre and post treatment.

3. Flooding

Biomass in pre Flooding plots averaged 60.66 ± SE 6.42 g per core. Biomass post Flooding treatment averaged 49.34 ± SE 14.82 g per core. There was no difference detected in t-tests between pre and post Flooding treatments (two sample t-test assuming unequal variances (n=19, t=1.7, p=0.11)).

4. Wet Harrow

Mean shrimp biomass at pre Wet Harrow treatment sites was 79.09 ± SE 9.80 g per core. Shrimp biomass post Wet Harrow treatment averaged 72.73 ± SE 6.51 g per core. There was no significant difference in shrimp biomass pre and post Wet Harrow (two sample t-test assuming unequal variances (n=19, t=0.53, p=0.60)).

Grassy Island - Shrimp Population Structure

Control plots retained nearly the same community proportions pre and post-treatment. Size classes for control plots pre-treatment were split 41.0%, 26.2%, 21.2%, and 11.5% for L, M, S, and XS shrimp. Post-treatment control plot proportions were 39.0%, 26.5%, 16.7%, and 17.7%.

Plots that were treated with the Dry Harrow treatment saw reductions in shrimp density within Large, Medium, and Small Size classes (59% reduction, 73% reduction, and 72% reduction respectively). Proportional size of the L class remained consistent pre and post Dry Harrow (30.6% to 32.3%) while the M and S classes shrunk proportionally 10% and 5% respectively (35.7 to 25.0% and 21.0 to 14.7%). In post Dry Harrow treatment surveys, and most likely due to the shrinking of M and S size classes, the XS class proportionally increased nearly 16.0 % (12.5 to 29.0 %).

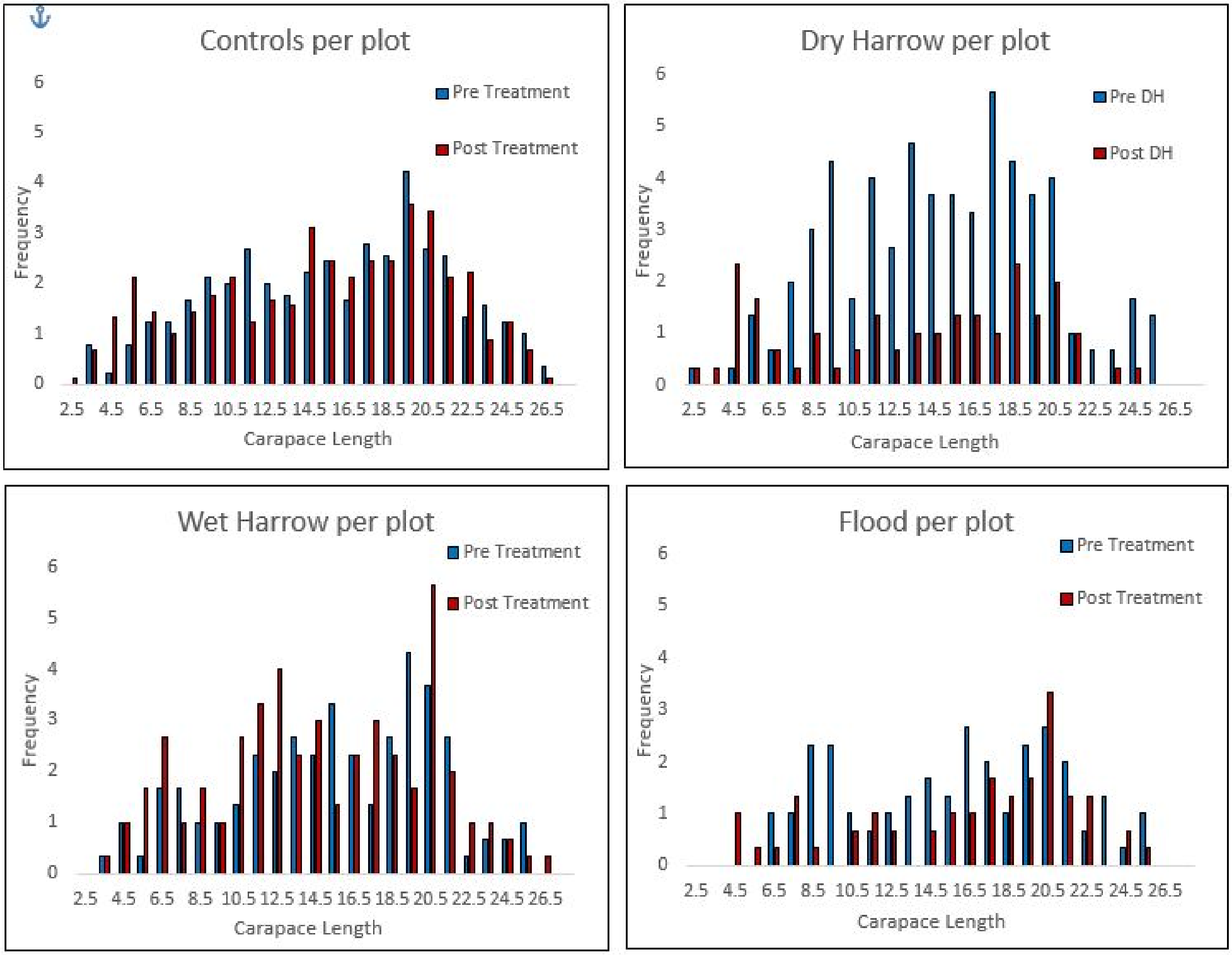
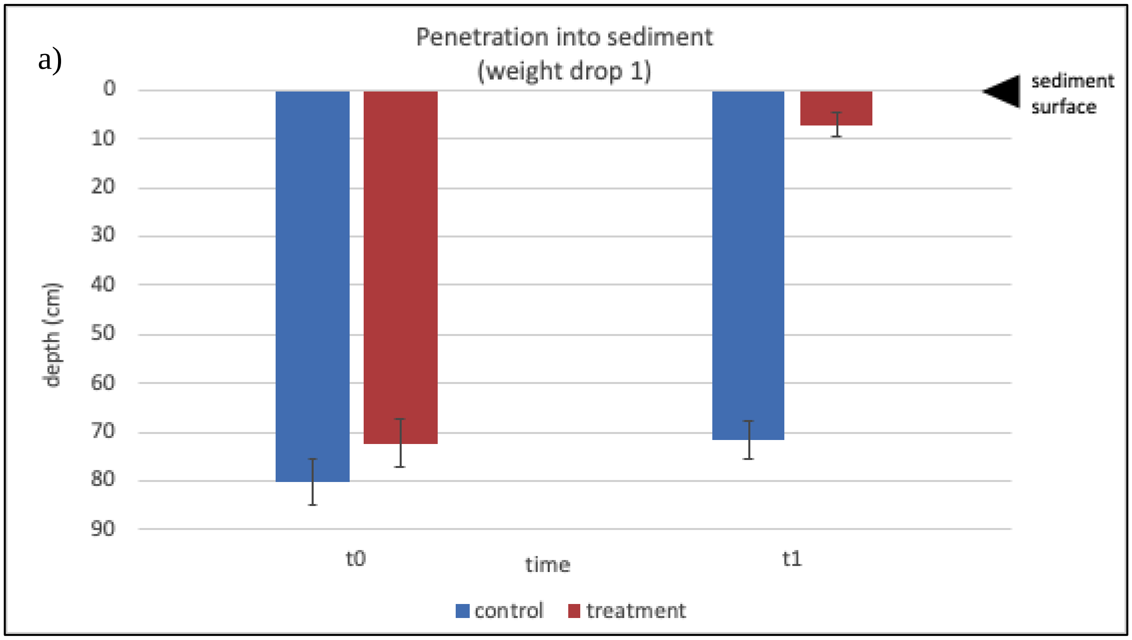


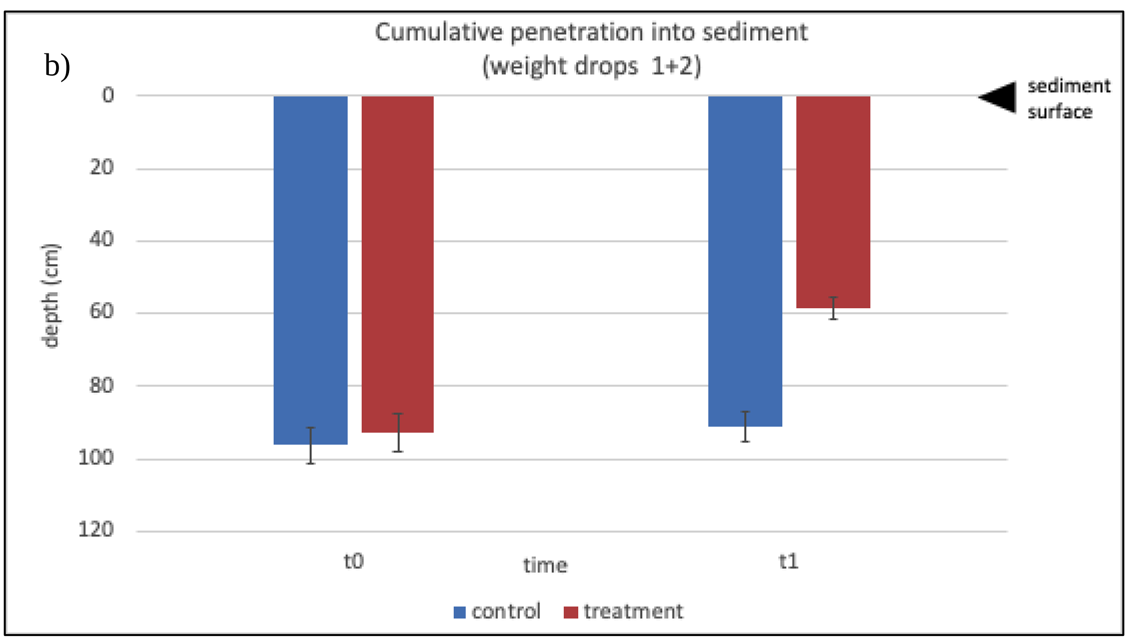
Figure 14. *Carapace length frequency per plot for shrimp collected at t0 and t1 for control, Dry Harrow, Wet Harrow, and Flood treatments.*

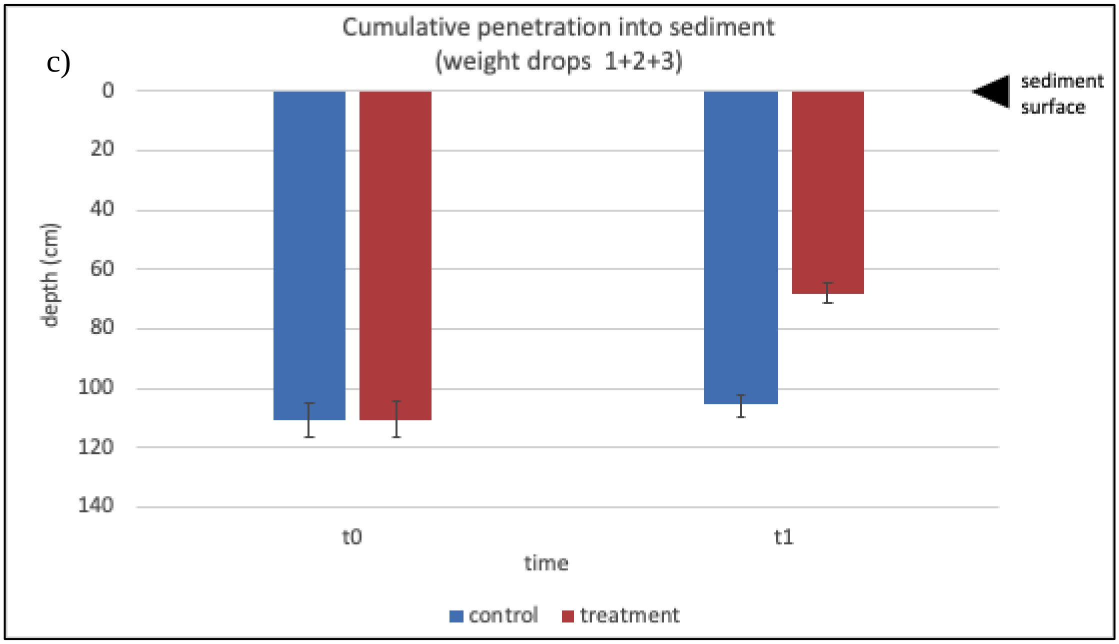
F1, F2, and F3 experienced 58% and 59% reductions in shrimp density that were in the M and S size class respectively. Flooding plots proportionally saw shrinkage in Medium and Small size classes pre-treatment to post-treatment of roughly 10%, and slight increases in the large and extra small size classes at 13% and 4%.

Grassy Island - Sediment compaction

Sediment penetration was measured as an indication of the compaction or firmness of the sediment. Two-way analysis of variance (ANOVA) were conducted to explore whether treatment type influenced sediment compaction. The independent factors were *(1) plot type* (untreated control, Dry Harrowed, Flooded and Wet Harrowed plots) and *(2) time* (before and after harrowing. Statistically significant differences in mean sediment compaction before and after treatment were found only in the Dry Harrowed plots $F(3, 539) = 16.14$, $p<0.001$. A two-way ANOVA was then run on the three Dry Harrow treated plots and three adjacent control plots. The results indicate sediment penetration measured post treatment was significantly different between the control and treatment plots after harrowing. The factors of 'plot type' and 'time' are responsible for the main effects with $F(1, 314) = 88.43$, $p<0.001$ and $F(1, 314) = 58.34$, $p<0.001$ respectively. The sediment penetration did not differ significantly in the control plots before and after treatment. However, a significant difference in mean sediment penetration was detected in treatment plots before and after Dry Harrowing.







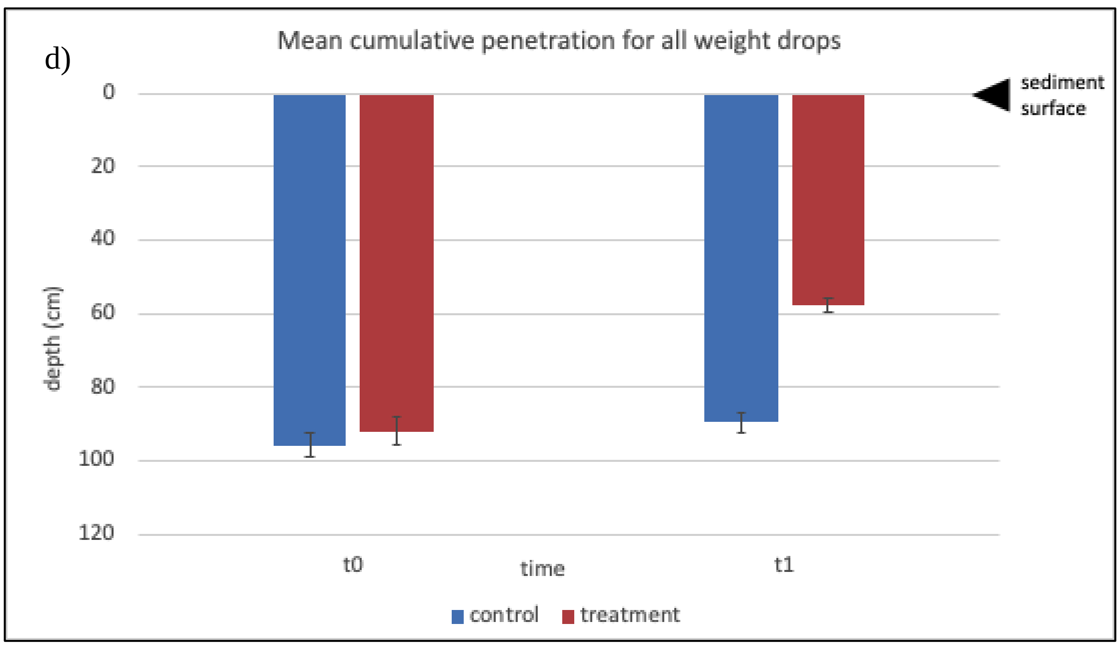


Figure 15. *Sediment penetration depth pre- and post- Dry Harrow treatment measured in DH1-DH3 and control plots after a) first weight drop, b) cumulative penetration after first and second weight drop, c) cumulative depth penetrated after all three weight drops, and, d) mean penetration for all weight drops.*

Grays Harbor - Shrimp Density

Mean shrimp density in Grays Harbor Plots did not change significantly from pre to post treatment within treated plots (two sample t-test assuming unequal variances ($p<0.05$)). Mean shrimp density pre-treatment was 15.50± SE 0.029 shrimp/core, and mean shrimp density post-treatment was 19.33 ± SE 0.29 shrimp/core. Control plot shrimp density did not change pre-treatment to post-treatment in a two sample t-test assuming unequal variances ($p<0.05$). Mean pre-treatment control plot density was 15.50 ± SE 0.82 g/core, and mean post-treatment density was 23.00 ± SE 0.79 shrimp/core.

Grays Harbor - Shrimp Biomass

Mean shrimp biomass did not change from pre to post treatment within treated plots (two sample t-test assuming unequal variances. ($n=18$, $t=-0.83$, $p=0.42$)). Mean shrimp biomass pre-treatment was 69.18 ± SE 0.64 g/core, and 84.05 ± SE 0.72 g/ core post-treatment. Biomass within the control plot did not change from pre-treatment to post-treatment (two-sample t-test assuming unequal variances. ($n=6$, $t= -0.41$, $p=0.70$)). Mean pre-treatment biomass within the control plot was 63.90 ± SE 1.56 g/core, and 72.27 ± SE 1.75 g/core post-treatment.

Grays Harbor - Population Structure

Large, Medium, and Small size classes saw no impacts from the Wet Harrow treatment in Grays Harbor. The Extra Small size class, however, saw a proportional and total decline (64.6%) pretreatment to post treatment (Figure 16). This removal of the XS class can also be seen in Figures 17 and 18, where an obvious shift is observed in the post-treatment density distribution between control and treated plots - with treated plots missing the XS size class at t1.

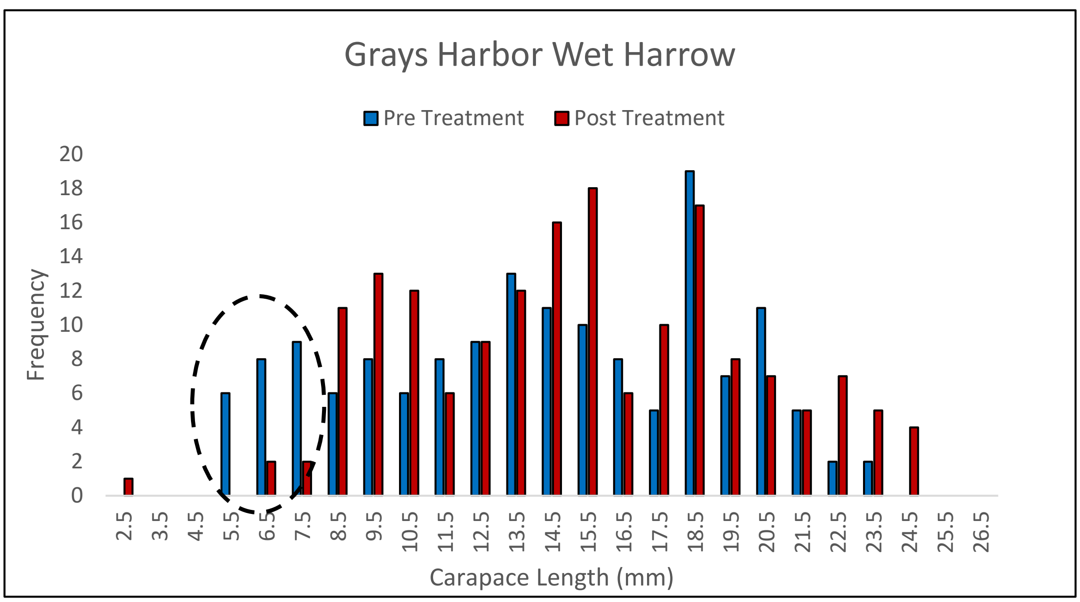


Figure 16. *Carapace Lengths (CL) from shrimp collected in nine cores at sites pre and post Wet Harrow treatment in Grays Harbor.*

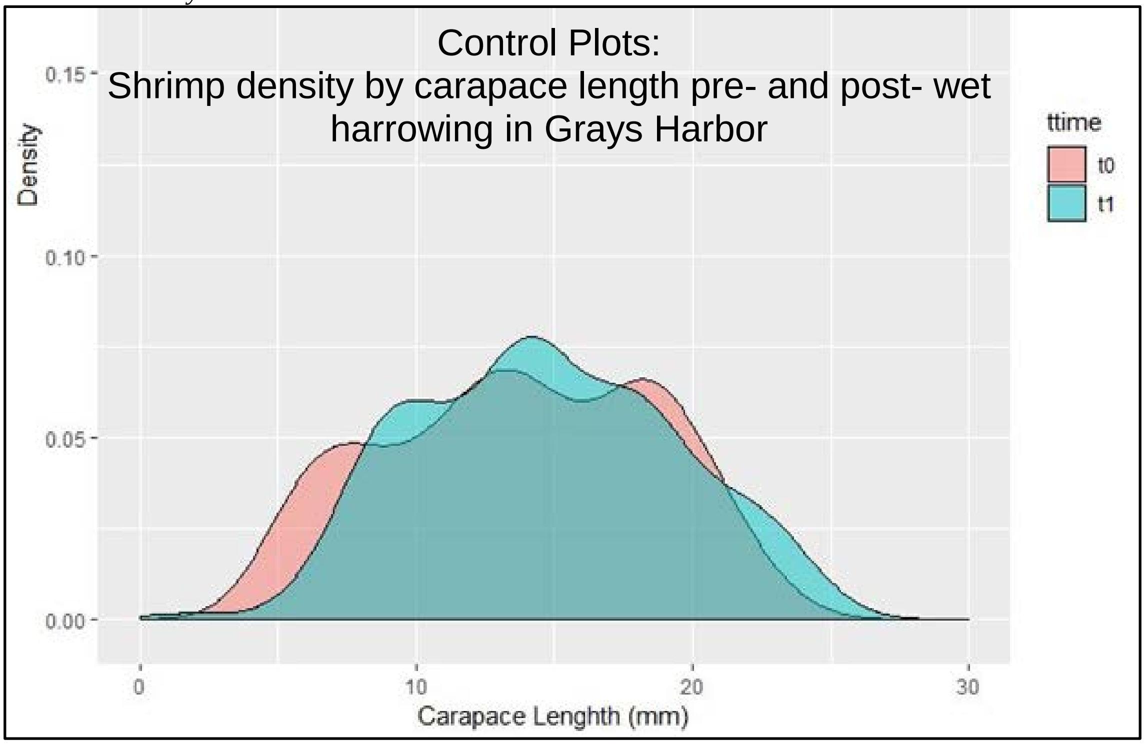


Figure 17. *Shrimp density for carapace lengths of all shrimp collected in control plots for Grays Harbor pre- and post- treatment*

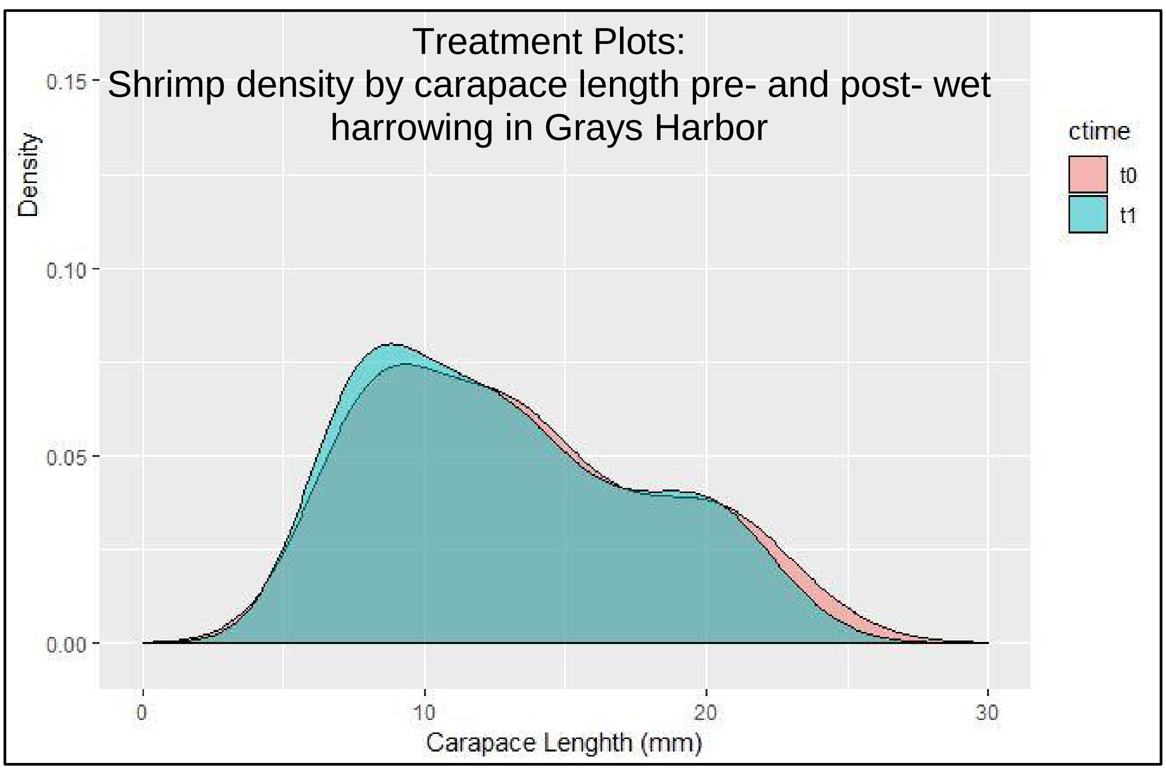


Figure 18. *Shrimp density for carapace length of all shrimp collected in treatment plots in Grays Harbor pre- and post- treatment.*

Grays Harbor - Treatment Effort

Treatment of Wet Harrow plots in Grays Harbor took a mean of 11.5 human hours per half acre (Figure 10 compares against other treatment methods). Plots were treated a total of 3 times each to evaluate effectiveness.

DISCUSSION

Results from this Proof of Concept study indicate that of three mechanical methods tested, Dry Harrowing was the only method that demonstrated a statistically significant reduction in burrowing shrimp density and biomass. In this study, plots treated by Dry Harrow experienced a 61% reduction in shrimp density, and a 67.5% reduction in biomass pre- and post-treatment. The Dry Harrow treatment was effective at reducing L, M, and S size classes. Further experimentation with timing with respect to shrimp recruitment is necessary to determine the effect on the extra small (XS) size class.

Wet Harrow treatments in Grays Harbor showed initial effectiveness in proportionally reducing the XS size class from 18% to 6% pre and post treatment (Figure 15). This reduction can also be seen in Figure 17, where plots treated with a Wet Harrow treatment experienced a population shift from left to right (a reduction in the number of XS shrimp 4.5 to 8.5 CL). At the same time, control plots maintained the same population structure from t0 to t1. New recruits, or "young of the year" (shrimp with CL equal to or less than 6mm in length), generally fit into age range of 0 to 2 years (Dumbauld 1996, Bosley and Dumbauld 2011). Their small body size limits their ability to burrow deeply into the sediment, so they generally inhabit the top 10 to 30 cm of sediment (Dumbauld 1996, Bosley and Dumbauld 2011). Further investigation of Wet Harrow treatment would be necessary to determine whether repeated passes of the 15 cm deep tines could effectively control the seasonal influx of juvenile recruits on plots. The large investment of human effort and time required however, limits the practicality of this approach.

CONCLUSION

Further investigation is necessary to evaluate whether *N. californiensis* populations can be successfully controlled by any mechanical technique. The most promising approach, Dry Harrowing, would need to be implemented at a larger-scale and on tidelands composed of sediment ranging in compaction, beaches of varying slopes and during different times of the year. Increased application intensity, and timing of treatment need to be examined for the duration of post-treatment effectiveness. While we experienced high levels of shrimp at Grassy Island relative to other locations in Willapa Bay, different sediment conditions could pose obstacles for using the Marsh-Master with roller-chopper in tow. To further investigate this method, we proposed a supplemental study designed to examine whether the reduction in shrimp density and biomass from Dry Harrowing persists over time, whether increased intensity of Dry Harrowing (more passes with the roller-chopper) has a greater influence on reducing shrimp population, and to evaluate recolonization of treated plots by shrimp from adjacent untreated mudflat.

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