

The Effect of Manila Clam Aquaculture on Invertebrate Diversity

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Marine Affairs

University of Washington

2017

Committee:

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Program Authorized to Offer Degree:

School of Marine and Environmental Affairs

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**Abstract**

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The success of commercial shellfish aquaculture is rooted in a healthy and productive nearshore marine ecosystem, making it essential to strike a balance between ecological and economic interests for farmers and regulators alike. Consequently, the removal of macroalgae from anti-predator nets and the development of new harvest techniques used in Manila clam (*Venerupis philippinarum*) aquaculture have raised important questions regarding their ecological impacts. In this study, I examine these impacts on the infaunal invertebrate communities of a Manila clam farm in Samish Bay, Washington. Using species data gathered from benthic and epibenthic sampling, I found that farmed sites support different invertebrate communities compared to reference sites, regardless of whether or not macroalgae is removed from anti-predator nets. Similarly, farmed sites support different invertebrate communities and lower taxon densities and richness values when compared to eelgrass habitats, regardless of

whether or not sites are harvested by hand or by machine. Within farmed sites, however, a significant decline in both density and richness was observed after hand harvesting events, but not after mechanical harvests. These results provide ecological evidence that supports the removal of macroalgae from anti-predator nets, and the continued use and development of mechanical forms of Manila clam harvest. Both technologies are responsible for increases in the economic returns from shellfish operations, but until now, their ecological effects have been less clear.

## **Introduction:**

Since the late 1800s, commercial shellfish aquaculture has been important to both the heritage and economy of the state of Washington. Over a 150-year period, the industry has grown to make Washington State the nation's leading producer of farmed oysters, clams, and mussels (Washington Sea Grant, 2015). Today, the commercial shellfish industry employs 3,200 people throughout the state's coastal communities, and provides a total contribution of \$270 million annually towards the state's economy (Washington Sea Grant, 2015). While Pacific Oysters (*Crassostrea gigas*) are the most profitable and common type of shellfish grown in Washington, Manila clams (*Venerupis philippinarum*) are the third most valuable shellfish resource in the state, contributing an estimated \$18 million towards the state's economy in 2013 (Washington Sea Grant, 2015). Given the success of the commercial shellfish industry and the location of its operations in nearshore habitats throughout the state, it is important to assess the impacts shellfish farms have on local fauna. In particular, two elements of Manila clam aquaculture bear scrutiny: the use of anti-predator netting, and the development of mechanical forms of harvest. Below, I describe each method before assessing the ecological impacts of each.

### *Anti-Predator Netting:*

In Washington State, Manila clams are grown primarily in the Hood Canal and the Southern Puget Sound, and increasingly in Willapa Bay and the Northern Puget Sound (Booth, 2010). Grown best in mid-intertidal habitats, Manila clams are generally raised in a mixture of sand and gravel and reside just below the surface of the sediment. Their shallow location makes them relatively easy to harvest, but also especially prone to predation by starfish, crabs, seabirds, and other organisms. In an effort to mitigate the risks of predation, growers commonly use mesh

netting to exclude predators and protect the clams as they grow to a harvestable size. This anti-predator netting is an essential tool used by farmers to reach profitable production levels and ensure successful aquaculture practices (Munroe et al., 2015). Proper maintenance of netting is an essential chore that accompanies its use: just one small hole or gap in a net is enough for an entire plot to be destroyed by predators like Dungeness crabs (D. Cheney, personal communication, March 27, 2017).

Placing the anti-predator nets directly on the sediment makes the nets especially prone to the bioaccumulation of macroalgae. High levels of such bioaccumulation can result in anoxic sediment conditions which negatively impact the growth of Manila clams (Toba, D., Dewey, W., 2005) by interfering with the exchange of nutrients and oxygen between the water and upper layers of sediment (Lavoie, McKindsey, Pearce, & Archambault, 2016). Accordingly, the removal of macroalgae from anti-predator nets is an essential component of Manila clam farming.

Despite its potential to harm clams, this macroalgae buildup can serve as habitat for other organisms, particularly invertebrates and juvenile fish. Spencer et al., 1997 reported that macroalgae buildup resulted in a greater density of infaunal species than comparable areas without such buildup (Spencer, Kaiser, & Edwards, 1997). Similarly, Powers et al. found the epibiota diversity within macroalgae buildup on anti-predator netting to be 20 times higher than that of adjacent, un-farmed areas (Powers, Peterson, Summerson, & Powers, 2007). These macroalgae habitats were found to support a wide variety of mobile invertebrates and juvenile fish at levels comparable to that of adjacent eelgrass beds, and three to seven times greater than that of adjacent un-farmed, sandy substrates (Powers et al., 2007).

Such juvenile fish habitat is an issue of increasing political and ecological importance in Washington State, particularly in regards to salmon recovery. Starting in the mid 1900s, salmonid populations throughout the Pacific Northwest rapidly declined, and by 1999, salmonids disappeared from 40% of their historic breeding ranges (Washington State Recreation and Conservation Office, 2016). Today, 15 populations of the state's salmonids are federally listed as either threatened or endangered under the Endangered Species Act. Accordingly, Washington state, in conjunction with local tribes and the federal government, has invested \$883 million towards increasing salmonid populations between 1997 and 2015 (Governor's Salmon Recovery Office, 2016). The majority of this money has gone to restore estuaries and eelgrass beds, two habitats that are essential for juvenile fish that have been degraded or lost due to high rates of coastal development (Bottom et al., 2011). One state agency has set a goal of increasing eelgrass coverage by 20% and restoring 7,380 acres of estuarine habitat by the year 2020 (Puget Sound Partnership, 2016). As previous research suggests (Powers et al., 2007; Spencer et al., 1997), the macroalgae buildup generated by commercial shellfish farms, may be an overlooked, but nonetheless important, contributor to improving salmon habitat.

*Mechanization:*

The advent of new, mechanized means of clam harvesting has raised important questions surrounding the impacts that Manila clam aquaculture has on surrounding ecosystems. Typically, Manila clams are harvested by hand, and studies have reported an immediate reduction in the diversity and abundance of infaunal invertebrates following such harvesting events (Spencer, Kaiser, & Edwards, 1998). After several months, however, the community composition returns

to its pre-harvest condition, but the rate of this return is greatly influenced by wave action and other environmental variables (Kaiser, Edwards, & Spencer, 1996; Spencer et al., 1997, 1998).

Hand-harvesting methods require a large labor force, resulting in labor to comprise a significant percentage of the costs associated with farming (Northern Economics Inc., 2013). Increasingly, growers are experimenting with mechanical forms of harvest as they have been shown to dramatically improve the efficiency and production potential of Manila clam farming (Saurel et al., 2014).

Taken together, the mechanization of Manila clam aquaculture holds promise for dramatically improving the cost efficiency of farming. Since implementing these mechanized processes, the grower on the farm used in this study has seen labor costs fall from between 15 – 25% of the total production cost to just 3 – 5% as the mechanical harvester is able to carry out the work typically performed by 8 individuals (D. Cheney, personal communication, March 27, 2017). The reduction in labor, results in the cost of harvesting to fall from \$0.45/pound in hand harvested clams to just \$0.06/pound when using a mechanical harvesting device (not accounting for equipment purchase and maintenance) (D. Cheney, personal communication, March 27, 2017).

These cost reductions have sparked interest in further developing the mechanization of Manila clam aquaculture. Shellfish aquaculture firms spend an estimated \$4,880 on each farmed acre, and 29% of these costs go towards employee payroll (Northern Economics Inc., 2013). With the reduced labor costs that accompany mechanized practices, firms have the potential to reduce the amount they spend on each acre farmed using mechanized growing and harvesting methods and increase the profit margins associated with Manila clam farming.

At present, the majority of Manila clam aquaculture is performed using traditional hand growing and harvesting methods, with only a handful of farms employing mechanized methods. At the farm used in this study, the grower has modified a 30-year old tulip bulb harvester to assist in harvesting the clams and maintaining the plots (Appendix 1). The machine has long metal “fingers” that insert into the sediment and move from side to side, shaking the sediment and loosening the clams. The tulip harvester has also been adapted to deploy and collect anti-predator nets and remove macroalgae buildup from them using a street sweeper brush.

Current mechanical methods are often tailored to a particular farm and its location. At Chuckanut Shellfish Farm (in Samish Bay, WA) for example, the Manila clams are grown in sand, which provides a substrate in which the tulip bulb harvester can easily operate. In farms where clams are grown in a combination of rocks and sand, this same machine would be difficult to move. Despite this, the gains achieved in the cost effectiveness from mechanical methods provide an incentive for farmers to invest in developing their own new technologies.

Previous research on Manila clam aquaculture has examined its influence on nutrient cycling (Lavoie et al., 2016; Nizzoli, Bartoli, & Viaroli, 2006), sediments (Gouletquer, Robert, & Trut, 1999; Sgro, Mistri, & Widdows, 2005), and the habitat quality provided by farms for other species (Toupoint, Godet, Fournier, Retière, & Olivier, 2008), while other research has looked at the impacts of mechanical harvesting of Manila clams on ecosystem function (Pranovi, Da Ponte, Raicevich, & Giovanardi, 2004; Spencer et al., 1998). However, little is known about the impacts that mechanical forms of Manila clam harvest have on invertebrate diversity. Studies on other forms of aquaculture have shown that the presence of aquaculture gear provides habitat for invertebrates (Dealteris, Kilpatrick, Rheault, & Dealteris, J.T., Kilpatrick, B.D., Rheault, 2004). For example, areas of oyster aquaculture have been shown to support a high diversity and

abundance of invertebrates (O'Beirn, Ross, & Luckenbach, 2004) and in turn, numerous fish species (Tallman & Forrester, 2007). Additionally, areas of oyster aquaculture have been reported to support a greater richness and abundance of economically and ecologically important species when compared to a natural oyster reef (Erbland & Ozbay, 2008).

Though non-harvested infaunal macroinvertebrates themselves hold no economic value for commercial shellfish farmers, they inherently hold ecological value for the greater ecosystem upon which the industry is reliant (Dumbauld, Ruesink, & Rumrill, 2009). Accordingly, shellfish growers throughout the Pacific Northwest have worked to advocate for both economic and environmental interests primarily through the creation and adoption of Best Management Practices (BMPs) and Environmental Codes of Practice (ECOP) (Dewey, Davis, & Cheney, 2011). These tools enable growers to come to a consensus on growing and harvesting practices that optimize both economic and environmental interests. Effective BMPs and ECOP provide guidelines to growers and regulators where more formal management frameworks are non-existent (Dewey et al., 2011).

The purpose of this study is to investigate how different methods and techniques of Manila clam farming impact the diversity of invertebrate communities found within a particular Manila clam farm in the North Puget Sound. In this study, I ask the following two questions: what is the effect of net-sweeping on epibenthic fauna and what is the effect of mechanized harvesting on benthic fauna?

## **Methods:**

### *Site Description:*

Chuckanut Shellfish Farm is a 6.5-acre Manila clam farm in Samish Bay, Washington (Figure 1). The farm's elevation ranges from -.2 m to .2 m with relatively even terrain. The areas surrounding the farm support eelgrass (*Zostera marina*) and macroalgae as well as sandy areas that lack vegetation. This farm is unique in that the grower has developed a suite of tools that have mechanized nearly the entire growing and harvesting process behind Manila clam aquaculture, principally among them:

- Clams are grown entirely in sand, for ease of mechanical harvest, as opposed to sand and gravel, which would limit the machines' mobility.
- Predator nets are mechanically installed and removed using a modified tractor and swept using a street sweeper brush.
- Clams are harvested using a tulip bulb harvester.

Unlike more common seeding methods of beach scattering, in which juvenile clams are scattered haphazardly across the sediment, this grower seeds the Manila clams (750 clams/m<sup>2</sup>) in meter-wide rows to facilitate mechanical harvesting. These rows comprise roughly 4.5 acres of the farm, the remainder serving as the aisles between them. The rows are covered with pieces of 1.2 meter-wide, reusable polypropylene anti-predator mesh netting (6-10mm holes) that range in length from 90 to 460 meters. The edges of netting are buried in the sediment and secured using large steel staples. The predators of most concern in this region are Dungeness crab

(*Metacarcinus magister*), Surf Scoters (*Melanitta perspicillata*), and White-winged Scoters (*Melanitta fusca*).

The clams are left to grow for two to three years, during which, anti-predator nets are occasionally swept, generally each month from the spring until fall to remove macroalgae buildup, mainly *Ulva sp.* and *Sargassum sp.*. Chuckanut Shellfish Farm is an active and working shellfish farm. As such, net sweeping occurred on an irregular basis during this study period, and the date on which macroalgae was removed was not recorded by the grower (D. Cheney, personal communication, March 27, 2017).

Clams are harvested over 3 hours during a low-tide period, generally in the early spring and late fall when the tides are most extreme. Anti-predator nets are removed the day before harvesting, after which the tulip-harvester is driven along the length of the harvest row.

Mechanical harvesting disturbs the first 8-to-10 cm of sediment, exposing a variety of invertebrates. After a tide cycle, however, the substrate returns to its original characteristics and is nearly indistinguishable from previously undisturbed areas. Though Chuckanut Shellfish Farms has transitioned to harvest clams exclusively by mechanical methods, a small portion of clams were harvested by hand for the purpose of comparing both harvesting techniques in this study. Hand-harvested clams are collected using a small rake that loosens the upper layer of the sediment. Clams are then sorted from the sediment and placed into collection buckets. Hand harvesting requires a much larger workforce to collect clams during a low tide period.

## Sample Collection:

### *Effect of Net Sweeping on Epibenthic Fauna:*

Epibenthic invertebrate samples were collected to assess the impacts of net sweeping on biological diversity. Samples from plots in which macroalgae buildup was recently removed from the anti-predator nets were classified as “swept”; those from other plots were classified as “unswept”. Additional samples were collected from un-farmed, adjacent eelgrass and sand habitats, which served as reference.

7 replicate samples were taken in each substrate type using a custom-built epibenthic pump: a bilge pump powered by a 12V motorcycle battery and constructed with a sampling cylinder 25 cm high, encompassing an area of 0.018 m<sup>2</sup> (Toft, Ogston, Heerhartz, Cordell, & Flemer, 2013; Washington Sea Grant, 2014). Once the cylinder was appropriately placed on the sediment, the pump was turned on and water was pumped for 20 seconds or until sediment was observed in the clear discharge tube. The organisms in the sample were passed through a 0.106µm sieve, and rinsed into a vial and immediately fixed in a buffered formalin solution (Toft et al., 2013; Washington Sea Grant, 2014). Samples were taken to the University of Washington in Seattle and identified to the species level for adult crustaceans and lower taxonomic levels for other groups.

Samples were collected in June and July of 2011 and May of 2012. These dates were selected as this is the period during which juvenile salmon feed on epibenthic crustaceans while they occupy nearshore habitats (Fresh, 2006).

### *Effect of Harvest Method on Benthic Fauna:*

Benthic invertebrate sampling was used to compare the impacts of hand harvesting and mechanical harvesting on invertebrate diversity, given that harvesting method disturbs the sediment (as opposed to net-sweeping, which just disturbs the mudflat surface). Samples were sequentially collected in July, and August of 2011 and May of 2012. For each sampling date, 7 benthic core replicates were collected immediately before and either one or two days after a sampling event from each of the following habitats: an area harvested by hand, a mechanically harvested area, and an unfarmed eelgrass reference site adjacent to the farm.

Cores were collected using a PVC benthic corer and measured 10cm deep and 5cm wide (0.0024m<sup>2</sup>). Immediately following collection, cores were fixed in 5% buffered formaldehyde solution and returned to labs at the University of Washington in Seattle for further analysis. The samples were then sieved through 500µm mesh and classified into four groups: mollusks, crustaceans, polychaetes, and other taxa, all of which were then preserved in 70% ethanol. Taxa in each of the groups were then sorted and identified to the species level for most adult specimens and higher taxonomic levels for other specimens.

### Data Analysis:

I used multivariate statistical methods to detect community-level changes in the invertebrate samples, using R version 3.2.2 and the vegan package (Oksanen et al., 2015; R Core Team, 2015). Unless otherwise specified, p-values less than 0.05 were considered to be significant.

### *Epibenthic and Benthic Data Analysis:*

The overall density (number of organisms/m<sup>2</sup>) and richness was calculated for both epibenthic (effect of net sweeping) and benthic data (effect of harvest type).

In order to visualize groupings of sites and treatments in multivariate space, a non-metric multidimensional scaling (NMDS) was conducted on both epibenthic and benthic samples using a Bray-Curtis dissimilarity coefficient so as to not make sites that lacked the same taxa appear to be more similar. Bray-Curtis values were calculated from log-transformed taxon count data for both epibenthic and benthic invertebrates. A statistical analysis on the species that most strongly correlated with one habitat type over the others was performed by analysis of variance (ANOVA).

PERMANOVA analyses were conducted to apportion variance in observed communities among sites, treatments, and sampling dates for both epibenthic and benthic invertebrates.

### **Results:**

#### *Effect of Net Sweeping on Epibenthic Fauna:*

A total of 125 unique taxa (Appendix 2) were observed over the 84 different sampling events (sites).

Taking all taxa together, there were no significant differences in the overall animal density and richness of epibenthic sites (Figure 2a and 2b). However, PERMANOVA analyses (Table 1) on species count data indicated significant differences in the ecological communities due to: 1) farmed (swept + unswept) and unfarmed (eelgrass + sand) site identity ( $p < 0.001$ ; Bray-Curtis); 2) the month in which samples were collected ( $p < 0.001$ ; Bray-Curtis); and 3) the

effect of each treatment ( $p < 0.001$ ; Bray-Curtis). The most influential of these differences (24.3% of the observed variation) was the month in which the samples were collected, indicating large changes in the epibenthic fauna independent of the measured treatments. By contrast, swept/unswept net treatments and the effect of farming (relative to unfarmed reference sites) accounted for a more modest 9.6% and 2.9% respectively (Table 1).

Multivariate analysis showed no overlap between eelgrass and sand sites in ordination space, indicating that these sites support distinct invertebrate communities (Figure 4a). Conversely, both swept and unswept sites showed overlap with eelgrass and sand sites, as well as with each other, indicating the farmed sites support epibenthic communities that share elements of both the sand and eelgrass unfarmed sites. Of the top ten taxa most strongly correlated with one habitat over the others, nine belonged to the phylum *Arthropoda* and class *Hexanauplia* (Figure 4b).

A pairwise PERMANOVA analysis of species count data between eelgrass, sand, swept, and unswept treatments revealed significant differences in the ecological communities of all treatments ( $p < 0.05$ ; Bray-Curtis) except for swept and unswept sites ( $p = 0.073$ ; Bray-Curtis). This means that swept and unswept sites support similar invertebrate communities, while the invertebrate communities are different between all other sites (Table 1).

#### *Effect of Harvest Method:*

A total of 147 unique taxa (Appendix 3) were identified amongst the 105 sampling events that took place.

Both taxon density and richness declined ( $p < 0.05$ ) after hand harvesting, while an insignificant decline in density and richness was observed after mechanical harvesting (Figure 3a

and 3b). However, all treatments were significantly less taxon-rich and diverse ( $p < 0.05$ ) than the reference eelgrass treatment.

PERMANOVA analyses (Table 1) on species count data indicated significant differences in the ecological communities for the month in which samples were collected ( $p < 0.001$ ; Bray-Curtis), the harvest treatment ( $p < 0.001$ ; Bray-Curtis), as well as the interaction between these factors ( $p < 0.001$ ; Bray-Curtis). Here, the treatment (hand/mechanical, before/after) explained twice as much of the observed variation (24.1%) as did the month in which the sample was collected (12.8%); the interaction of these two variables accounted for 23.2% of the variation.

Multivariate analysis revealed essentially no overlap in ecological communities between any of the harvest treatment sites and the eelgrass reference sites, indicating that the species supported by farmed sites and eelgrass sites differ (Figure 5a). Conversely, there is considerable overlap between the harvest treatment sites. There are similar degrees of variation between the mechanical post-harvest hull and the mechanical pre-harvest hull, while the hand pre-harvest exhibits much larger degrees of variation than the hand post-harvest hull. Of the top ten taxa most strongly correlated to one habitat over the others, all ten were more abundant in eelgrass habitats and belonged to the phyla *Arthropoda*, *Annelida*, and *Echinodermata* (Figure 5b; ANOVA on organism count data).

Pairwise PERMANOVA analyses on species count data revealed no significant differences in Bray-Curtis dissimilarities between hand pre- and post-harvest sites as well as mechanical pre- and post-harvest sites (Table 1). Although the density and richness experienced significant declines after hand harvesting, this result suggests the general composition of the invertebrate communities remains the same after both hand and mechanical harvest. Pairwise

PERMANOVA results also revealed significant differences in Bray-Curtis dissimilarities between both hand and mechanical post-reference sites with the reference eelgrass sites.

### **Discussion:**

The success of commercial shellfish aquaculture as an industry lies in the fact that the crops (shellfish) are left to grow naturally within their surrounding environment. Unlike large-scale, commercial terrestrial agriculture—which relies heavily on frequent treatments of fertilizers, supplemental watering, and pest removal—shellfish aquaculture is a relatively hands-free practice once the shellfish are planted in the environment, relying principally on the natural processes of the ecosystem in which it is being performed.

However, a tension is inherent within the industry: in order to grow and harvest shellfish, growers must alter the natural habitat upon which their crops are reliant. Hence the unique challenge with which the industry, and those tasked with regulating it are faced: how to grow shellfish in a simultaneously profitable and environmentally sustainable manner?

This study focused on the environmental impacts of two practices that have been shown to improve the economics of Manila clam aquaculture, but whose environmental impacts were less known. The first of which, mechanical harvest, is a relatively recent and increasingly relevant aspect to the industry, with the potential to save growers substantial amounts of money from reduced labor costs. The second, sweeping of macroalgae buildup from anti-predator nets, is an essential task that ensures clams are not lost to predation or suffocated from a lack of oxygen.

### *Effect of Net Sweeping:*

Overall, my results revealed no significant difference in the invertebrate communities between swept and unswept sites. Instead, these farmed sites were found to harbor invertebrate communities that fall somewhere between those found in eelgrass habitats and those found in sand habitats. That is, farming shifts the biota present, regardless of whether or not sites are swept or unswept, creating its own invertebrate community with species found in adjacent, unfarmed habitats.

This suggests that the effects associated with anti-predator nets are subtle compared to the benefits they provide to growers. With a timeline of three years from planting to harvest, Manila clams are a significant investment of time and resources making anti-predator nets essential in the growing process. These results provide evidence that the necessary task of removing macro algae build up from the anti-predator nets is no more detrimental to invertebrate communities than other aspects of Manila clam farming.

Both unswept and swept sites provide habitat that is indistinguishable when examined through invertebrate diversity. Here, we see that the act of sweeping, or not sweeping, has no influence over the invertebrate communities that are found within farmed sites, instead the presence of the anti-predator netting is what results in the observed differences in the invertebrate community structure between farmed sites and natural eelgrass or sandy sites.

That being said, the majority of the variation observed in the data was attributed to the month in which the samples were collected. Estuarine habitats are inherently dynamic: daily high and low tides, weather extremes, and seasonal changes lead to an ever-changing environment and community composition (Simenstad & Fresh, 1995). This, coupled with the repeated

disturbances that accompany Manila clam harvests result in a highly variable system that changes from month to month, depending on the intensity and timing of harvests.

Accordingly, natural variation will always have an impact on the results observed, but this study reveals that the impacts associated with farming, while significant, are a small piece of a much larger suite of influencing factors beyond those associated with natural variation.

#### *Effect of Harvest Method:*

Regardless of harvest technique, farmed sites had significantly lower taxon richness and density values when compared to reference eelgrass sites. However, these results suggest that mechanical harvesting is no worse than hand harvesting when it comes to the impacts of Manila clam harvest on infaunal invertebrate diversity.

The results of this study provide ecological support for the use of mechanized forms of Manila clam aquaculture. These results incentivize growers, whose livelihoods are dependent upon a healthy and productive ecosystem, to make investments towards developing mechanical harvesting technologies to reap the substantial economic and efficiency gains that accompany their use, without worsening the environmental impacts.

Farmers would be wise to invest in developing mechanical harvesting equipment to reap the economic benefits it provides in the form of reduced labor costs. Managers would be wise to encourage such investments that will serve to improve the environmental sustainability of commercial shellfish aquaculture and enable the industry to reach its profitability and production potential.

Limitations in the sampling design may have influenced these results. Principally among them being that samples were collected one to two days after harvesting occurred, resulting in

the plots being exposed to a full tide cycle. As mentioned earlier, both forms of harvesting disturbed the upper layer of sediment and numerous invertebrates were brought to the surface after harvesting events. Recordings by underwater cameras of the sites between the harvest and sampling events showed high rates of predation during the high tide immediately following a harvest by crabs and flatfish (D. Cheney, personal communication, March 27, 2017). Given the time constraints between harvesting and the rising tides, situations like this may be unavoidable, but should nonetheless be considered in future experiments.

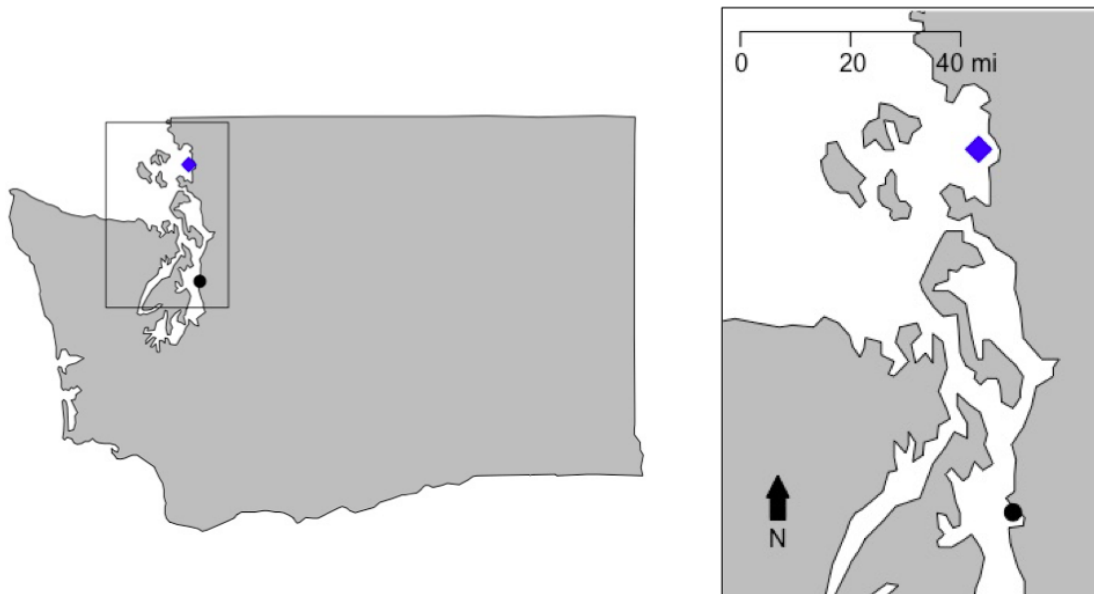
### **Conclusion:**

This study provides important insights regarding the ways in which the Manila clam aquaculture industry operates within the unique nearshore ecosystem of the Puget Sound. Since the industry began, commercial shellfish growers have been loyal stewards of environmental quality for the coastal habitats in which they operate, and that same activism is alive and well today. As our understanding of the importance of infaunal invertebrates has grown, so too has the support of the industry in working with researchers to create harvesting and growing practices that sustain both ecosystems and economies. Growers, like that of Chuckanut Shellfish Farms, are increasingly aware of the role in which scientific research can have on the success of their operations in the face of a rapidly changing marine environment. As the industry continues to expand and innovate with new tools and techniques, studies like this one will be essential in evaluating the environmental impacts of such innovations to ensure a productive ecosystem and a thriving industry for years to come.

**Acknowledgements:**

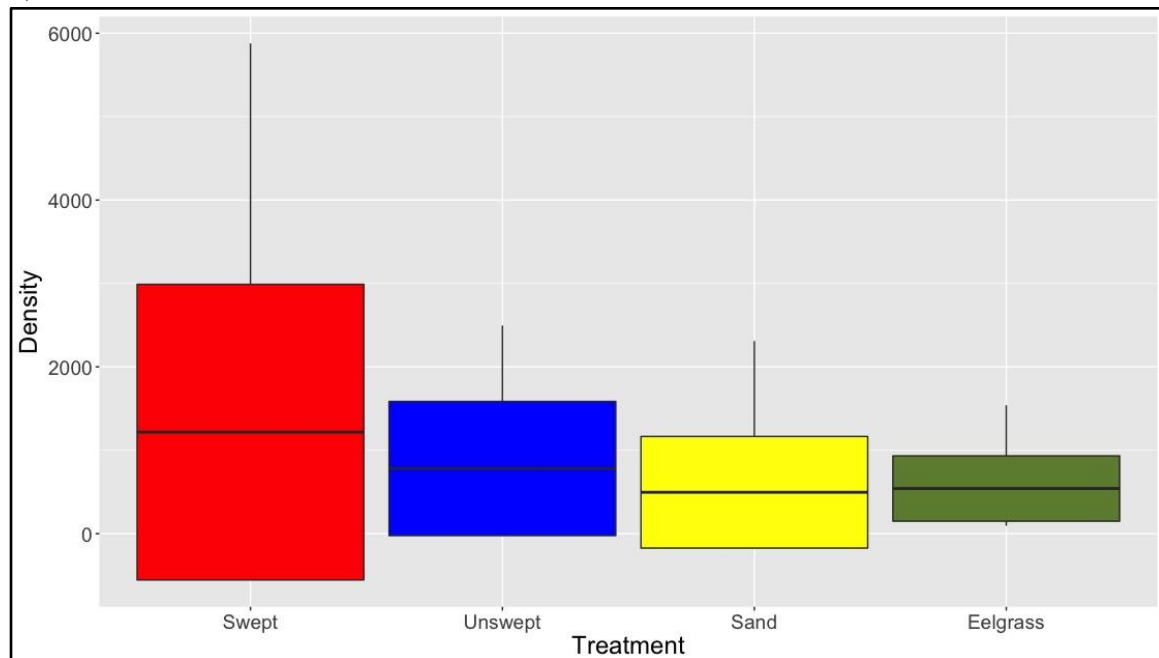
I would like to thank Dr. Ryan Kelly (University of Washington), Jeff Cordell M.S. (University of Washington), Jason Toft M.S. (University of Washington), Dr. Dan Cheney (Pacific Shellfish Institute) for their tremendous help and support towards producing this thesis. Jeff Cordell and Jason Toft conducted the fieldwork and processed the samples used in this study. Additionally, thanks go to Bill Dewey (Taylor Shellfish), the owner of Chuckanut Shellfish Farm, who made his farm available for this study.

Funding for this project was made possible by the The Saltonstall - Kenney Grant Program through NOAA Fisheries.

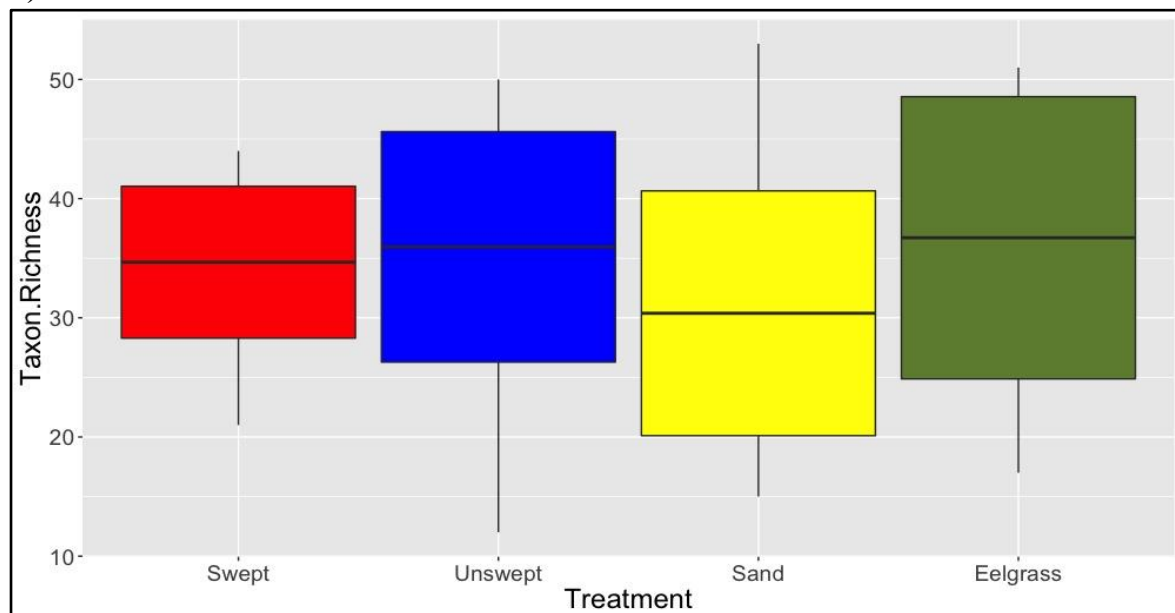


**Figure 1: Map of Washington State, USA focused on North Puget Sound.** The site location (Chuckanut Shellfish Farm) was located in Samish Bay (blue diamond). The farm is located ~73 miles North of Seattle, WA (black circle).

a)

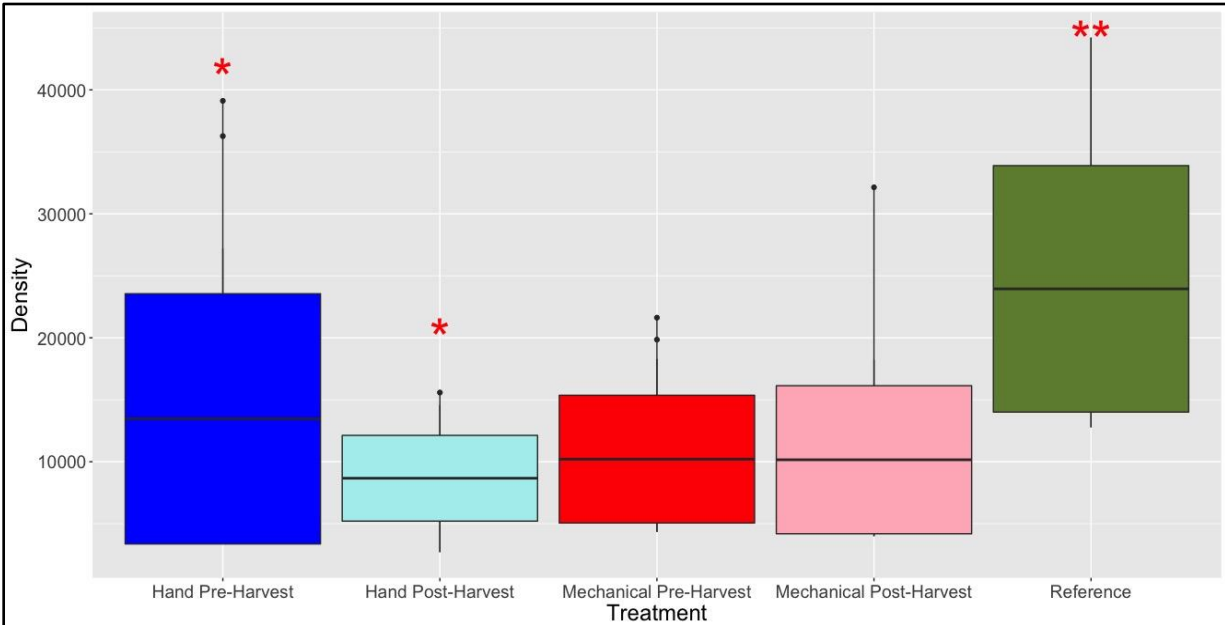


b)

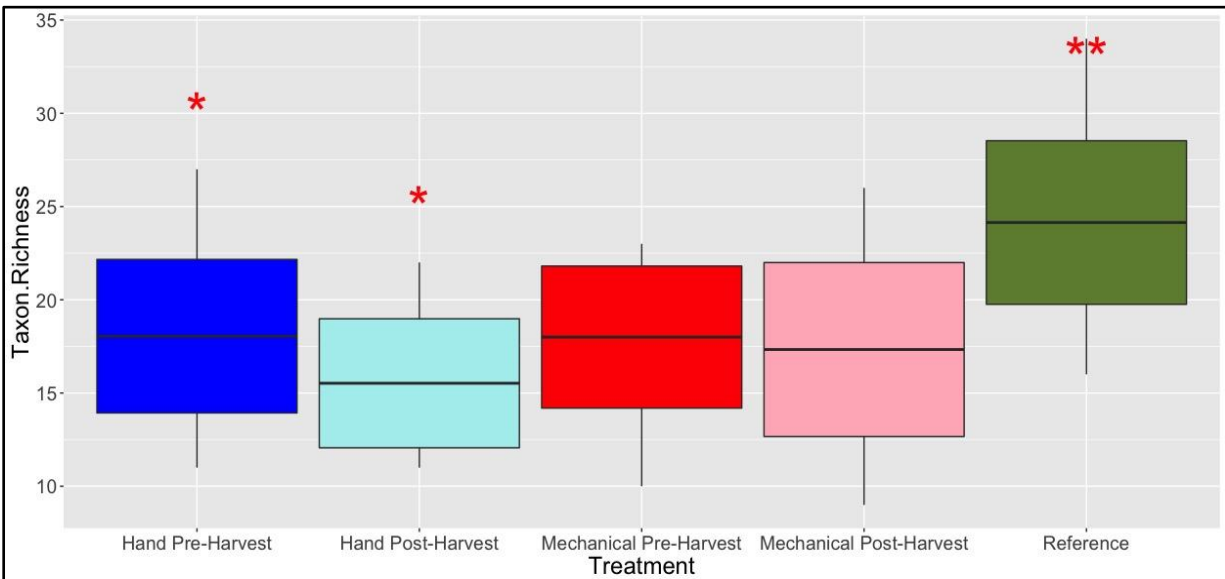


**Figure 2: Epibenthic taxon density and richness.** Taxon richness was calculated by aggregating the total number of taxa found in each of seven sampling replicates over three sampling days (total N = 21 for each of the four different treatments). Density was calculated in the same manner, by averaging the density (organisms/m<sup>2</sup>) of all organisms across each site, and then by treatment. There were no significant differences among any of the four different treatments in both density and richness.

a)

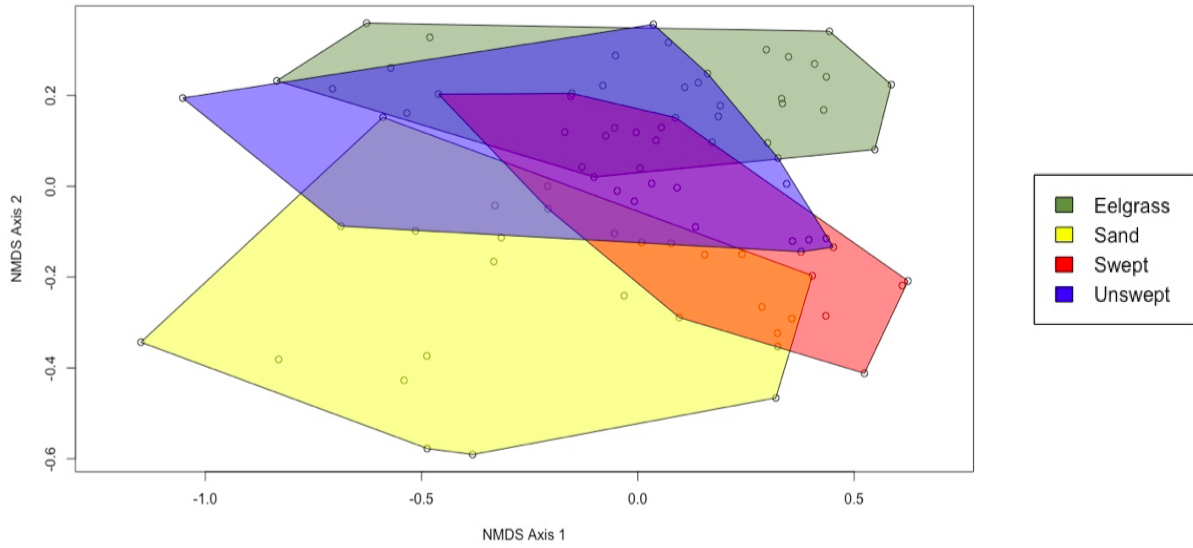


b)



**Figure 3: Benthic taxon density and richness.** Taxon richness was calculated by aggregating the total number of taxa found in each of seven sampling replicates over three sampling days (total N = 21 for each of the five different treatments). Density was calculated in the same manner, by averaging the density (organisms/m<sup>2</sup>) of all organisms across each site, and then by treatment. Asterisks indicate a significant ( $p < 0.05$ ) decline in richness and density after hand harvesting relative to before. Richness and density were significantly ( $p < 0.05$ ) lower in Hand Pre-Harvest, Hand Post-Harvest, Mechanical Pre-Harvest, and Mechanical Post-Harvest than in the Reference sites.

a)

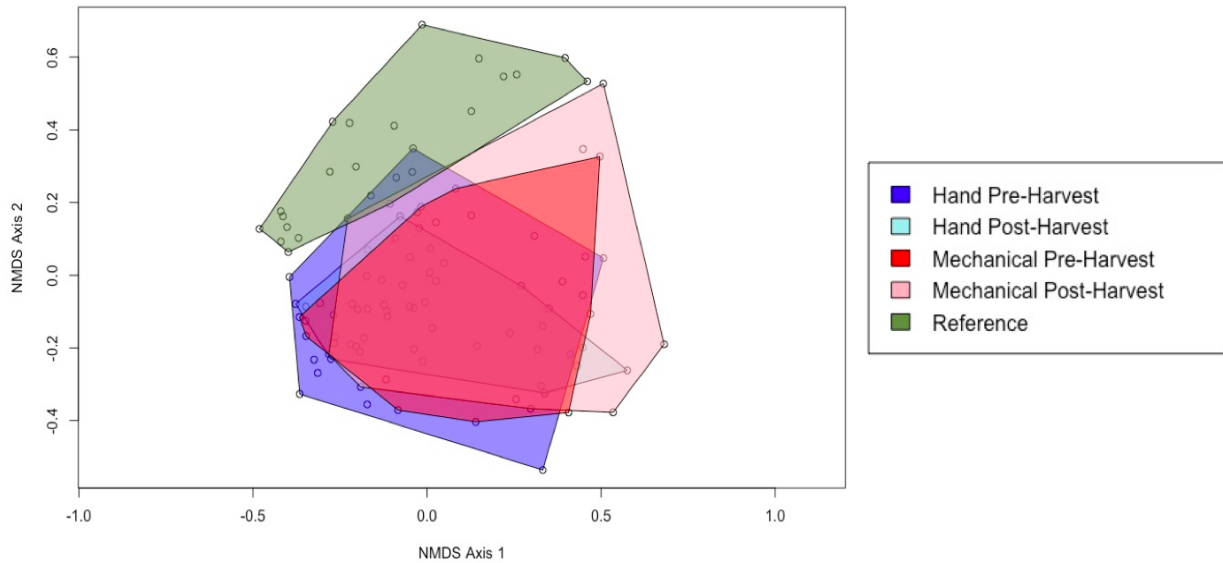


b)

Taxon	Phylum	Class	F	p	Number of Occurances	Number in Eelgrass	Number in Sand	Number in Swept	Number in Unswept
<i>Tegastidae</i>	Arthropoda	Hexanauplia	11.02	< 0.001	1136	532	12	129	463
<i>Dactylopusia paratisboides</i>	Arthropoda	Hexanauplia	9.73	< 0.001	53	43	0	4	6
<i>Tisbe spp.</i>	Arthropoda	Hexanauplia	7.96	< 0.001	4759	1999	301	560	1899
<i>Typhlamphiascus sp.</i>	Arthropoda	Hexanauplia	6.49	< 0.001	56	47	4	5	0
<i>Diosaccus spinatus</i>	Arthropoda	Hexanauplia	6.41	< 0.001	1322	1113	74	54	81
<i>Bulbamphiascus sp.</i>	Arthropoda	Hexanauplia	6.2	< 0.001	515	479	26	3	7
<i>Amphiascopsis cinctus</i>	Arthropoda	Hexanauplia	5.96	0.001	360	22	43	268	27
<i>Cyclopina sp.</i>	Arthropoda	Hexanauplia	5.69	0.001	15348	2429	1285	2775	8859
<i>Enhydrosoma hopkinsi</i>	Arthropoda	Hexanauplia	4.82	0.004	483	192	22	55	214
<i>Polychaeta larva juvenile</i>	Annelida	Polychaeta	4.73	0.004	489	309	140	19	21

**Figure 4: NMDS plot of sites and species studied with epibenthic sampling.** NMDS plots were created by tabulating the species abundance at each treatment across all sampling replicates and dates, producing 84 unique sampling events (circles). These were grouped according to treatment (swept, unswept, eelgrass, and sand). Species that were significantly associated with one habitat type over all others are listed in the table (ANOVA).

a)



b)

Taxon	Phylum	Class	F	p	Number of Occurances	Number in Mechanical Plots	Number in Hand Plots	Number in Eelgrass
<i>Leptochelia</i> sp.	Arthropoda	Malacostraca	96.73	< 0.001	4549	930	887	2732
<i>Exogone lourei</i>	Annelida	Polychaeta	40.65	< 0.001	3110	740	528	1842
<i>Cumella vulgaris</i>	Arthropoda	Malacostraca	36.68	< 0.001	48	5	8	35
<i>Rhynchospio glutaea</i>	Annelida	Polychaeta	33.23	< 0.001	1034	191	271	572
<i>Phyllodoce</i> sp. A	Annelida	Polychaeta	32.25	< 0.001	43	4	3	36
<i>Apodida</i>	Echinodermata	Holothuroidea	25.65	< 0.001	203	33	48	122
<i>Monocorophium acherusicum</i>	Arthropoda	Crustacea	24.52	< 0.001	344	56	73	215
<i>Euclymeninae</i>	Annelida	Polychaeta	18.69	< 0.001	52	6	3	43
<i>Anoplodactylus viridintestinalis</i>	Arthropoda	Pycnogonida	14.96	< 0.001	39	5	11	23
<i>Monocorophium</i> sp.	Arthropoda	Malacostraca	11.79	< 0.001	778	135	174	469

**Figure 5: NMDS plot of sites and species studied with benthic sampling.** NMDS plots were created by tabulating the species abundance at each treatment across all sampling replicates and dates, producing 105 unique sampling events (circles). These were grouped according to treatment (hand pre-harvest, hand post-harvest, mechanical pre-harvest, mechanical post-harvest, and reference). Species that were significantly associated with one habitat type over all others are listed in the table (ANOVA).

Study	Comparison	Factor	DF	SS	MS	F	R <sup>2</sup>	p
Epibenthic	All	Farm vs. Unfarmed	1	0.65	0.65	3.62	0.03	< 0.001
		Habitat	2	2.15	1.08	5.97	0.1	< 0.001
		Month	2	5.41	2.71	15.01	0.24	< 0.001
		Residuals	78	14.07	0.18		0.63	
		Total	83	22.29			1	
	Eelgrass vs. Sand	Habitat	1	1.66	1.66	6.85	0.15	< 0.001
		Residuals	40	9.71	0.24		0.85	
		Total	41	11.37			1	
	Eelgrass vs. Swept	Habitat	1	0.97	0.97	4.23	0.1	0.0039
		Residuals	40	9.2	0.23		0.9	
		Total	41	10.17			1	
	Eelgrass vs. Unswept	Habitat	1	0.69	0.69	3.12	0.07	< 0.001
		Residuals	40	8.83	0.22		0.93	
		Total	41	9.52			1	
	Sand vs. Swept	Habitat	1	0.68	0.68	2.56	0.06	0.021
		Residuals	40	10.66	0.27		0.94	
		Total	41	11.34			1	
	Sand vs. Unswept	Habitat	1	1.12	1.12	4.35	0.1	0.0019
		Residuals	40	10.29	0.26		0.9	
		Total	41	11.41			1	
	Swept vs. Unswept	Habitat	1	0.49	0.49	2.02	0.05	0.07493
Residuals		40	9.78	0.24		0.95		
Total		41	10.27			1		
Benthic	All	Month	2	1.74	0.87	14.49	0.13	< 0.001
		Treatment	4	3.27	0.82	13.64	0.24	< 0.001
		Month:Treatment	8	3.15	0.39	6.56	0.23	< 0.001
		Residuals	90	5.4	0.06		0.4	
		Total	104	13.55			1	
	Hand Pre- vs. Post-Harvest	Treatment	1	0.19	0.19	1.75	0.04	0.1059
		Residuals	40	4.31	0.11		0.96	
		Total	41	4.5			1	
	Mechanical Pre- vs. Post-Harvest	Treatment	1	0.15	0.15	1.64	0.04	0.1279
		Residuals	40	3.66	0.09		0.96	
		Total	41	3.81			1	
	Hand Post-Harvest vs. Reference	Treatment	1	2.06	2.06	20.85	0.34	< 0.001
		Residuals	40	3.95	0.1		0.66	
		Total	41	6.02			1	
	Mechanical Post-Harvest vs. Reference	Treatment	1	1.82	1.82	17.74	0.31	< 0.001
Residuals		40	4.1	0.1		0.69		
Total		41	5.92			1		

**Table 1: PERMANOVA analysis results.**

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## Appendix 1



The modified tulip bulb harvester used at Chuckanut Shellfish Farm to harvest Manila clams. Photo courtesy of Daniel Cheney, Pacific Shellfish Institute. 2012.

## Appendix 2: Epibenthic Taxa

Taxon	Total Count
Acrenhydrosoma sp.	34
Ameiridae	8175
Americhelidium sp.	1
Amonardia normani	20
Amonardia perturbata	80
Amphiascoides sp.	81
Amphiascoides sp. A	163
Amphiascopsis cinctus	360
Anoplodactylus viridintestinalis	1
Anthozoa	19
Aoroides sp.	2
Armandia brevis	7
Asciacea	1
Asteroidea Juvenile	9
Auchenorrhyncha nymph	1
Balanomorpha cyprid	27
Balanomorpha nauplius	21
Bivalvia Juvenile	175
Bulbamphiascus sp.	515
Caprella laeviuscula	53
Caprella sp. Juvenile	136
Caridea zoea	1
Cletodidae sp. 1	11
Corophiidae Juvenile	55
Cumacean Juvenile	16
Cumella vulgaris	10
Cyclopina sp.	15348
Cyclopoida	117
Cyclopoida copepodid	187
Cymothoidae	1
Dactylopusia crassipes	15
Dactylopusia paratisboides	53
Dactylopusia sp.	88
Dactylopusia vulgaris	168
Danielssenia typica	453
Diarthrodes sp.	4385
Diosaccus spinatus	1322
Dorvilleidae Juvenile	275
Echinodermata juvenile	2
Echinolaophonte hedgepethi	3
Ectinosomatidae other	6534
Enhydrosoma hopkinsi	483

Taxon	Total Count
Enhydrosoma sp.	1
Eobrolgus chumashi	45
Ephemeroptera nymph	1
Epicaridea	9
Euryte sp.	603
Foraminifera	5749
Gammaridea	1
Gastropoda Juvenile	71
Grandidierella japonica	152
Halacaridae	1351
Halectinosoma ornatum	783
Harpacticoida copepodid	12087
Harpacticoida nauplius	328
Harpacticoida unidentified	1
Harpacticus obscurus group	6666
Harpacticus uniremis	6
Harpacticus uniremis group	6
Hesionidae	9
Heterolaophonte longisetigera	15
Holothuroidea Juvenile	4
Ischyrocerus sp.	2
Isopoda Juvenile	1
Laophontidae copepodid	270
Laophontodes hedgepethi	211
Leptochelia dubia	173
Longipedia sp.	8068
Macrochiron sp.	5
Mesochra spp.	2514
Microarthridion littorale	2
Miraciidae unidentified	7
Monocorophium acherusicum	1
Monocorophium sp.	6
Nebalia sp.	1
Nematoda	12678
Nemertea	10
Nereididae Juvenile	3
Nippoleucon hinumensis	80
Normanella sp.	172
Oligochaeta	851
Ophiuroidea	24
Opisthobranchia	1
Orthopsyllus linearis	1092

<b>Taxon</b>	<b>Total Count</b>
Ostracoda_Podocopida	733
Paradactylopodia sp.	135
Paralaophonte_pacifica	6048
Paralaophonte_perplexa_group	87
Paralaophonte sp.	3
Parastenhelia_hornelli	1325
Parathalestris_californica	51
Parathalestris_sp.	1
Peltidiidae	3
Photis_sp.	2
Phyllodocidae_Juvenile	7
Polychaeta_larva/juvenile	489
Polynoidae_Juvenile	61
Pontogeneia_rostrata	201
Prionospio_elegans	6
Rhynchospio_glutaea	20
Rhynchothalestris_helgolandica	10
Robertsonia_sp.	1867
Rutiderma_lomae	2
Sarsamphiascus_minutus	131
Sarsamphiascus_sp.	120
Sarsamphiascus_sp._A	20631
Schizopera_sp.	1
Scutellidium_sp.	3
Serpulidae	1
Siphonostomatoida	1
Spionidae_larva/juvenile	4063
Stenhelia_peniculata	316
Stenhelia_sp._1	22
Stenhelia_sp._2	12
Syllidae	263
Tachidius_triangularis	13
Tanaidacea	5
Tegastidae	1136
Tisbe_spp.	4759
Turbellaria	454
Typhlamphiascus_sp.	56
Uromunna_ubiquita	611
Xouthous_purpurocinctus	8
Zaus_sp.	30
Zeuxo_sp.	1

### Appendix 3: Benthic Taxa

Taxon	Total Count
Alvania compacta	2
Americhelidium shoemakeri	2
Ampharetinae	1
Ampithoe sp. Juvenile	1
Anobothrus gracilis	1
Anoplodactylus viridintestinalis	39
Aoroides sp. Juvenile	1
Apodida	203
Armandia brevis	58
Bivalve	28
Bivalve A	1
Bivalve Juvenile	19
Cancer sp.	1
Capitellidae	90
Capitellidae Juvenile	1
Caprella drepanochir	14
Caprella laeviuscula	30
Caprella sp.	8
Caprella sp. Juvenile	28
Caprellidae Juvenile	90
Cardiidae	1
Chone minuta	2
Chone sp.	2
Cirratulidae	4
Cirratulus sp.	1
Clinocardium nuttallii	4
Columbellidae	4
Corophiidae Juvenile	2
Cumella vulgaris	48
Dipolydora socialis	1
Dipolydora sp.	1
Dorvillea longicornis	41
Dorvilleidae	5
Eobrolgus chumashi	830
Ephemeroptera Nymph	1
Eteone sp.	1
Eteone sp. Juvenile	1
Euclymene sp.	2
Euclymeninae	52
Eulalia californiensis	1
Eumida longicornuta	4
Eumidae sp. Juvenile	1

Taxon	Total Count
Eupolytmia heterobranchia	1
Exogone lourei	3110
Exogone sp.	12
Exogone sp. Juvenile	1
Fabriciinae	1
Foraminifera	2154
Gastropoda	19
Gastropoda Juvenile	61
Glyceriforma	1
Glycinde picta	15
Glycinde sp.	5
Glycinde sp. Juvenile	1
Goniadidae	5
Goniadidae Juvenile	1
Grandidierella japonica	49
Gyptis sp.	1
Halacaridae	3
Harmothoe imbricata	60
Harmothoe sp.	1
Harmothoinae	3
Harmothoinae Juvenile	97
Hemigrapsus oregonensis	4
Hemipodia simplex	1
Heptacarpus sitchensis	1
Hesionidae Juvenile	21
Leitoscoloplos pugettensis	2
Leptochelia sp.	4549
Leptoplanidae	1
Longipedia sp.	21
Macoma nasuta	15
Macoma sp.	10
Maldanidae	19
Micropodarke dubia	3
Monocorophium acherusicum	344
Monocorophium insidiosum	1
Monocorophium sp.	778
Monocorophium sp. Juvenile	13
Mya arenaria	1
Mytilidae Juvenile	1
Nematoda	19809
Neoamphitrite sp.	3
Nephtyidae	1

