# Biodiversity of Benthic Mobile Fauna in Geoduck

(Panopea generosa) Aquaculture Beds in

Southern Puget Sound, Washington.

by

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#### **ABSTRACT**

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Geoduck aquaculture has increased dramatically in the last decade in southern Puget Sound, Washington. Potential ecological impacts due to industry practices have been identified by the Washington State Legislature. This study's aim was to determine if aquaculture practices influenced the biodiversity of benthic mobile fauna in farm areas. Composition of species, relative abundances, and biodiversity status were measured. Surveys of geoduck farms in two different stages of aquaculture production were examined. Site one had predator protection tubes around the planted geoduck. Site two had no predator protection and was in grow-out phase. No significant differences were detected at either stage of aquaculture production. Geoduck aquaculture at both locations had no effect on species evenness. Species richness at both locations was not affected by the presence of geoduck or associated structures. Biodiversity was higher in the Nisqually Reach compared to Eld Inlet, but was not influenced by the occurrence of geoduck aquaculture activity at either study location. Species composition and abundance did not differ between treatment and control locations. Large differences in capture rates were noted between female and male Cancer gracilis crab in Eld Inlet and Nisqually Reach. With the exception of fall sampling at the Nisqually Reach locations, female crab were more abundant than male crab during both spring and fall, at both treatment and control locations. Further research, including baseline studies and long term monitoring are recommended.

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#### INTRODUCTION

Aquaculture production has sharply increased in recent decades as a method of supplementing global demand for seafood and to relieve pressures put on wild stocks of declining ocean products (Diana 2009). The environmental effects, including the effect on local biodiversity, of these various forms of aquaculture are often an area of contentious debate among scientists, stakeholders, and policy makers. Social benefits such as hunger relief, job creation, and local and global economic benefits are inherent in the aquaculture industry. For policy makers, balancing the social and economic benefits against potential and realized environmental impacts has become a challenging priority in recent decades (Pillay 1997, Burbridge et al. 2001, Tlusty et al. 2001). In some cases, the impacts of aquaculture have been found to have various deleterious effects (Ribeiro et al. 2007, McKindsey et al. 2007, Naylor et al. 2000). Other impacts are found to have positive or no measureable influences on the aquatic environment (Tlusty et al. 2001, Newell 2004, Newell and Koch 2007, Grant et al. 1994). Often, the results of such studies vary, depending upon the level of disturbance as described in Simenstad and Fresh (1995). They found that while small scale disturbances had little effect on benthic invertebrates, large scale impacts can produce a measurable shift in community dominants. In many instances, arguments can be made that both positive and negative effects are observed, so that no clear indication of environmental impact can be distinguished (Munroe and Mckinley 2007, Murray et al. 2007). In many cases, however, little is known about overall, long-term environmental impacts. Investigations into potential impacts of geoduck aquaculture have been minimal and have begun just recently within the last few years. A paucity of information exists in the scientific literature pertaining to the potential effects of geoduck aquaculture. Results of this research and that currently being conducted by University of Washington SeaGrant is expected to allow scientists and decision makers to determine the effects geoduck aquaculture industry practices are having on the local marine environment and ecological processes.

Intertidal geoduck culture is a relatively new aquaculture technology. Original methods of aquaculture by state hatcheries began out of an anticipated need by state managers to supplement wild stock harvest (WDNR 2010). These early attempts at geoduck culture were largely unsuccessful. Due to technologies developed by the private sector throughout the 1990's, planting stock is now readily available, and geoduck aquaculture has quickly become a very successful and lucrative business. Some estimate farm product value at one half million dollars an acre (Davis, 2004). Although prices fluctuate wildly, farmed geoduck often sell for an average of twelve to fifteen dollars per pound. This led to an explosion of small and large-scale geoduck farms throughout Puget Sound, and exports increased dramatically in the last decade (FAO 2010, Figure 2). The inland soft-sediment intertidal areas of Puget Sound provide favorable habitats for geoduck aquaculture. Intertidal areas in southern Puget Sound are especially productive and appealing to growers due to the wide expanse of softsediment beaches. To date, geoduck aquaculture occurs only on privately owned lands which are often leased by shellfish growers, or farmed by the landowners as a smallscale operation. This differs from the wild-stock commercial fishery which occurs in subtidal state managed aquatic lands throughout Puget Sound. Naturally occurring geoduck are found on intertidal areas, but not in abundances that would allow for a commercial fishery; therefore only recreational fishing occurs for intertidal geoduck on state tidelands.

Concerns over the potential ecological consequences of this aquaculture practice followed the rapid expansion of geoduck farming. Up until recently, there have been no restrictions or limitations placed on the geoduck aquaculture industry other than regular shellfish aquaculture permitting requirements (WAC 220-76 2006). Recent permitting requirements through Washington Department of Ecology and the United States Army Corps of Engineers have been instituted that involve more thorough biological assessments of new intertidal geoduck farm sites. Due to rising concerns over the practices involved with geoduck aquaculture, the 2007 Washington State Legislature passed SSHB 2220 (W.S.H.B. bill 2220-2007-08). This legislation had four primary directives: directing Washington Department of Ecology to develop plans within the Shoreline Master Program to include geoduck operations; mandating Washington Department of Fish and Wildlife to expand permit requirements regarding geoduck farms; creation of the Shellfish Aquaculture Regulatory Committee (SARC) to oversee all aspects of state regulation of geoduck aquaculture activities; and the commission of several scientific research projects directed at determining the various effects of geoduck aquaculture within Washington State. Research priorities include determining the environmental effects of predator protection structures, commercial harvesting

impacts, biogeochemical effects, impacts to species biodiversity, potential parasites and diseases, and genetic interactions with wild stocks. Much recent attention has been directed toward the effects of geoduck harvesting (Willner 2006, Fisher et al. 2008) both in literature, symposia, and informal forums. Research currently being conducted by the University of Washington Seagrant has focused primarily on potential effects on benthic in-faunal organisms. While this focus is valuable, there are many other possible effects to consider regarding the sustainability of intertidal geoduck aquaculture. Another area of particular interest to scientists and decision makers is the potential threat posed by wide-spread intensive aquaculture to local biodiversity of other organisms inhabiting these areas. In fact, SSHB 2220 specifically addresses this concern and mandates that research be done including investigations of "...impacts on species biodiversity and abundance of other benthic organisms".

Biodiversity is often used in ecological studies as an indicator of ecosystem health (Hooper et al. 2005, Worm et al. 2006) and effects of disturbance (Balata et al. 2007). Biodiversity loss and global extinction have become of increasing concern to conservation biologists worldwide. While ecologists have recognized the importance of maintaining biodiversity for many years, recently conservationists have come to realize the importance biodiversity conservation plays in maintaining important ecosystem functions (Covich et al. 2004, Solan et al. 2004, Folke et al. 2004). In order to investigate the potential effects of geoduck aquaculture on the biodiversity of marine benthos, studies were conducted in southern Puget Sound at two farm sites to determine if there were detectable differences in biodiversity parameters between aquaculture sites and

nearby undisturbed areas. These two farm sites were in two different stages of production throughout the sampling period. Site one was structured, with "geoduck tubes" and net toppers serving as predator protection. The second site had no structure and contained only geoduck in grow-out phase. These two stages of geoduck aquaculture were examined because they represent the two primary stages of geoduck production, those with and those without "geoduck tubes".

#### **METHODS**

Two geoduck farms were selected as test sites within southern Puget Sound (Figure 1). These areas were selected based primarily upon their stage of geoduck production at the time of sampling. Both sites were commercial scale geoduck farming operations and were representative of a typical geoduck farm in Puget Sound both in scale and design. The geoduck location at site one (Figure 3, treatment) was in the initial phase with geoduck tubes and predator protective singular netting. The aquaculture location of site two (Figure 4, treatment) was in grow out phase with all predator protection removed. Both sites were characterized by sandy to slightly muddy substrate, typical of geoduck farms, with geoduck being planted in the -0.7 m MLLW to +0.7 m MLLW intertidal zone. Both locations also were chosen because there were suitable control sites located near both experimental locations. It is important to select a control site that is most similar to the experimental site as habitat heterogeneity can influence species distributions, community composition and biodiversity (Hewitt et al. 2005, Dethier and Schoch 2004). Thus, selection of a nearby, similar beach type would

allow a proper comparison that lessened any sampling differences that could occur due to different habitat types. Specific site coordinates are not provided to protect the proprietary products of the participating shellfish growers.

Site one is located in Eld Inlet as shown in Figure 3. This location was sampled between May 8<sup>th</sup> 2009 – May 18<sup>th</sup>, 2009 and October 1<sup>st</sup> – October 17<sup>th</sup>, 2009. The treatment location at site one consisted of a large area of planted geoduck protected by approximately 10.2 cm diameter PVC predator protection tubes with single net tube toppers (Figure 5). The tubes had been in the ground planted with geoduck for approximately one year at time of experiment. The area is approximately 13,567 m² and nearly rectangular in shape. The geoduck tubes were planted approximately 0.3 m apart throughout the area. Geoduck in grow-out phase (no predator protection) was grown beside and below the geoduck plot in tubes. This method is typical of many geoduck farms as the crops are planted in a rotational manor, and in a given tract of land there will be several plantings of different stages of farmed geoduck as well as other species of aquaculture product. It is believed that the presence of the geoduck would have no effect on the trapping experiments as this area was over 45.7 m away from the sampling location, and there was no structure in this area. Oyster racks located above sampling location in the higher intertidal also would have had no effect on sampling as the structures attracted only fouling organisms such as barnacles, and would not likely provide habitat to larger mobile organisms subject to this study.

At site one, there was very little algal cover or presence during either the spring or fall. Water currents in the local area were low due to the location of the site in a small embayment. Traps were set in the middle of the rectangular geoduck plot approximately 10 m apart. The tidal height of this planted area ranged from +0.7 m MLLW to -0.7 m MLLW. Traps were set between the 0.0 m MLLW and -0.30 m MLLW mark. The paired control site for this location was situated approximately 0.40 km away from the geoduck testing area (Figure 6). The beach morphology and sediment type of the site one control location was very similar to the treatment location of site one. Current velocities were also low in this area, and algal cover was sparse. No naturally occurring geoduck were observed during low tides on this beach. Traps were set in the middle of this 4212 m<sup>2</sup> control area approximately 10 m apart. The beach was approximately rectangular, and boundaries were defined by property ownership on either side. There were no aquaculture activities directly proximal to either side of the control area during time of sampling. Derelict oyster racks were found on a nearby parcel at least one hundred meters away. These abandoned simple structures would not affect the presence or abundance of mobile organisms subject to this study. During sampling it was noted that geoduck were being planted on a parcel approximately one kilometer to the north of this control location. It is not suspected these activities had any bearing on the integrity of the beach to serve as a natural, undisturbed control site.

Site two is located in the Nisqually Reach area, near Dogfish Bight. The treatment area is shown in Figure 7 (treatment) and Figure 8 (control). This area was sampled June 12<sup>th</sup>, 2009 – June 19<sup>th</sup>, 2009 and October 24<sup>th</sup>, 2009 – October 30<sup>th</sup>, 2009.

This commercial geoduck farm had no predator protection and was in grow-out phase. Prior planting techniques involved broadcast planting of geoduck seed over large predator protection nets that were supported by PVC tubes planted every 36 inches along growing area. Protective netting was suspended by PVC tubes, and all structure was removed after approximately 2 years, meaning this site has been without structure for nearly two years. The geoduck was approximately four years old at the time of sampling. No structured aquaculture was occurring proximal to, or near the sampling location during the experimental timeframe. Geoduck planting (including tubes) was observed far from this site, likely a kilometer away. As suggested previously, it is highly unlikely these newly introduced structures, so far from the test site would influence the findings at the test location. This beach was characterized by firm sandy substrate, with a moderate current velocity. This location was less protected than site one, and therefore was subject to higher current velocities and wave action (Figure 4).

During spring sampling the geoduck bed at site two had high algal presence, primarily of the genera *Ulva* and *Enteromorpha*. The occurrence of algae varied on a daily basis and was transitory. A small amount of fouling of traps occurred, however this did not appear to influence the effectiveness of the traps. The sampling area consisted of a roughly rectangular area. The geoduck plot area was 24,048 m². Tidal height ranged from approximately -0.33 m MLLW to +1.0 m MLLW line. Traps were placed near the 0.0 m MLLW line in the linear center of the described bed. Traps were placed approximately 10 m apart from the center of the geoduck bed.

### SAMPLING DESIGN

Sampling was conducted during the spring and fall of 2009. Each site was monitored for seven days during both seasons. Most of the sampling was done on concurrent days; however during periods where the tide receded below sampling height, traps were set on the next day that would allow a soaking time of a full semi-diurnal tidal cycle.

Two types of traps were used in sampling. One was designed to catch crab and the other was designed to catch shrimp. Both types are used by recreational fishermen and are commercially available. Traps were modified in order to retain smaller organisms than the traps were originally intended. Crab and shrimp traps measured approximately 61 X 61 X 22.9 cm. All traps were fitted with 12.7 mm industrial polypropylene semi-rigid mesh originating from oyster grow-out bags used in aquaculture operations (Figure 9). Escape mechanisms of traps were covered in order to minimize trap escapement. Trap entries were fitted with mesh, in a manner still allowing one way movement of entry door. Using both crab and shrimp pots offered an advantage over using only one style of pot. By design, the crab trap allowed for catch and retention of larger organisms while the shrimp trap design allowed for capture and retention of smaller organisms. Another advantage to using both styles of traps was that they offered two different entrance methods. The crab traps had a one-way entry "door" and the shrimp pot offered a ramp-style, passive entry mechanism.

The four entry ports for the crab trap measured approximately 22.9 X 15.2 cm, while that of the shrimp trap measured approximately 10.2 cm in diameter. Side entry traps were chosen because certain species may more readily enter traps based on the orientation of trap door to the down-current flow of bait scent. Previous studies involving decapods crustaceans have shown side entry traps to be more effective than top entry styles (Miller 1979). Therefore, providing entry on each side may optimize catch of some species. Both crab and shrimp pots were effective trapping mechanisms for this research as they fished indiscriminately, catching and retaining a variety of benthic marine fauna.

Traps were baited with Friskies® Brand canned cat food type "Ocean Whitefish and Tuna Dinner Classic Paté". Criteria used to select bait were primarily based on personal communications with local crab and shrimp fisherman and personal experience. It has been found anecdotally that this particular bait fishes indiscriminately, attracting several different species from various groups of animals. Additionally, previous research has demonstrated that baits using mixed scent attractants have better catch efficiency (Miller 1990). For these reasons, this type of bait was used exclusively throughout the experiment.

Traps were allowed to "soak" for approximately 24 hours during each sampling event at each site location. This allowed for trap fishing during two ebb and two flow cycles. This was important because many species occupying this intertidal area move in coordination with the incoming and outgoing tide. Also, a single soaking time has been

found to be the best method in scientific trapping studies (Bennett, 1974, Miller 1990, Zhou and Shirley 1997), since trap soak time can affect catch rates. Generally, traps were not set on days where the tides would go near or below the tidal height at which the traps were set, which was near -1.0 to 0.0 MLLW. There were, however, a few days where the soak time was shortened by a few hours to prevent the trap from going dry, which could allow for escapement and/or desiccation of animals trapped. It is not believed that this abbreviated soak time influenced overall catch. Miller (1990) found in similar trapping studies involving the crab *Cancer magister* that entry into un-emptied traps did not differ significantly from emptied traps in catch after 5 hours. Therefore, the trap continues to fish on some scale, but the significant portion of catch occurs in the first five hours.

Trap placement was monitored and recorded using a GPS (Trimble® GeoExplorer XT<sup>TM</sup>). The two pots were placed approximately 10 m apart from center of the plot. Pots were set during low tide when possible to guarantee placement accuracy. The trap retrieval procedures were the same for all sampling days at all sampling locations. Traps were pulled and immediately emptied into two large counting bins that had been prefilled with saltwater. One was designated for the shrimp pot and the other for the crab pot. The traps were then inspected for functional integrity, re-baited, and reset in the appropriate location. Catch records were maintained for individual traps at all locations. The animals were then identified and enumerated. Sex data was recorded for the crab *Cancer gracilis*. Organisms that were smaller than 12.7 mm were not included in the catch record. This minimum size requirement was in place because

animals smaller that the mesh size would not be consistently retained as they could move in and out of traps at will. Presence was noted for animals not meeting the 12.7 mm size requirement, but no attempt was made to enumerate these individuals.

Animals were transported and released at an offshore location approximately 500 m from the test sites. This method was carried out to lower the chances of repeatedly catching the same individuals. Since many benthic mobile organisms using this habitat are relatively transient (Varnell et al. 1994, Lindegarth et al. 2000), it was important to lessen any influence or bias due to sampling activities.

### STATISTICAL ANALYSES

Descriptive statistics were utilized to identify differences in relative abundances of species present for all sampling locations. Differences in female and male *Cancer gracilis* crab were also analyzed using this type of statistical analysis. Percentages were charted in order to visually observe differences at each sampling location.

Species richness for all locations was calculated using MaoTau expected species accumulation and Coleman rarefaction curves using EstimateS (Colwell 2009). The species accumulation curve allows an examination of how many species are present in total, for all sampling events. A comparison can then be made between the two sites using a standardized function (i.e. number of samples). Rarefaction curves also allow a standardization of the data in order to make comparisons of species richness involving unequal sample sizes (Magurran 2004). This method, like species accumulation curves, also shows the number of total species, but as a function of the accumulated number of

individuals sampled (Gotelli and Colwell 2001). The comparison can then be made because it allows for comparison from samples of different abundances. The site with the higher abundance is "rarefied" down to that of the comparative site. Significant differences occur only if the curve with lower number of individuals falls outside the confidence bounds of the curve with higher number of individuals (Magurran 2004). This comparison occurs at the point of maximum number of individuals of the curve of fewer individuals.

In order to further investigate the differences between the aquaculture sites and non-planted sites Simpson's biodiversity indexes were calculated for sites one and two using Simpson's Index of Diversity (D) =  $\Sigma$  (n/N)², D-1 (Table 1). This index accounts for both species richness and species abundance, and is a common index used to compare species biodiversity.

### RESULTS

The results of this research have successfully demonstrated that the described geoduck aquaculture had no influence on biodiversity of mobile benthic fauna at these research locations. Methods such as these have been successful in other research aimed at enumerating mobile benthic species (Miller 1979, 1990). Results pertaining to *C. gracilis* crab are consistent with results of other research conducted in Puget Sound and the Pacific Northwest (Orensanz and Gallucci 1988, Miller 1990).

## **SPECIES EVENNESS**

Evenness is a measure of differences (or similarities) of species in their abundances. In Eld Inlet, overall low species evenness was observed. *Cancer gracilis* accounted for the majority of organisms caught at both the treatment and control location (Figures 10, 11, Table 4). At the geoduck farm site, *C. gracilis* constituted 94 % of all specimens. *Pandalas danae* was second and accounted for 2% (Figure 10). All other seven species constituted 1% or less of the total individuals captured at this location. Comparing these results to the control site, very little difference was found. At the control location, *C. gracilis* accounted for 97% of total specimens captured here. The other seven species encountered constituted 1% or less (Figure 11).

Site two had a much more even distribution of species for total individuals than site one (Figures 12, 13). Neither the treatment nor control site was drastically dominated by one species. At the geoduck grow-out site, *C. gracilis* constituted a majority at 43% of the individuals; however there were several other species present at relatively high numbers (Figure 12, Table 4). Only three of the seven species captured accounted for less than 1% of the sampled population. Similarly, the control area at site two showed moderate, albeit lower species evenness than the treatment area (Figure 13). *Leptocottus armatus* was encountered most often and represented 46% of the specimens (Figure 13). At this location, there were several species present at relatively high numbers (Figure 13). Four of the eight species observed accounted for less than 1% of the total individuals captured at the control location of site 2.

### SPECIES RICHNESS

Site one showed little difference in species richness between the treatment and control locations (Figures 14a, 15a). The treatment location was slightly richer, as shown in Figure 14a. Nine species were encountered at the treatment location, while eight species were found at the site with no aquaculture activity. This difference was not significant, as shown by the similarity of the curves. Also, the curves representing the 95% confidence interval bounds of both treatment and control areas overlapped, further indicating that there was no significant difference between the two locations. This similarity is also evident in the rarefaction curve representing site one (Figure 15a). When the treatment location was "rarified" to the control location (which had the lower sample size (n = 319)) a small difference emerged, which shows the treatment location of site one richer by just one species. This difference is not significant, as the curve falls within the 95% confidence intervals of the control curve at the point of maximum number of individuals of the treatment curve (Figure 15a). Nine different species were present out of 319 individual organisms captured at the aquaculture location versus eight species present out of the 421 individual specimens at the control location.

The treatment and control area of site two were very similar in species richness (Figure 14b). The control location of site two showed slightly higher richness with eight species present, compared to the seven species at the treatment location (Figure 14b). This difference however was not significant, and at low levels of sampling, the species accumulation curves overlap and are equal. The 95% confidence intervals of the

treatment and control areas overlap, which shows the similarity between the locations of site two. The rarefaction curves of site two showed little differences between the treatment and control areas (Figure 15b). Once the treatment location was "rarified" down to the control location (which had a lower sample size ((n = 180)) a small difference existed. This difference is not significant because the control location curve falls within the 95% confidence intervals of the treatment curve at the point of maximum number of individuals of the control curve (Figure 15b). The control location of site two had slightly higher species richness with eight species present, compared to the treatment location that had seven species.

#### BIODIVERSITY INDEX

This value that was calculated refers to the probability that two randomly selected individuals will belong to the same species. Simpson Index of Diversity values range between zero and one, with one being the most diverse value possible. Site one had low diversity values at the treatment (.115) and control (.0698) location. The treatment area containing geoduck had a higher level of diversity than the control area (Table 1).

Site two exhibited much higher biodiversity values than did site one (Table 1).

The treatment area containing geoduck had higher diversity values (.704) than the control location (.633).

## SEX RATIOS OF CANCER GRACILIS

Differences existed in the capture of female and male *Cancer gracilis* crabs (Figure 16a-c, Table 2). Female crabs accounted for 80% of total *C. gracilis* catch at site one (Figure 16b). Females were more abundant during spring sampling (Table 2). At site two, total male and female abundances were equal; however no females were captured during fall sampling (Figure 16c, Table 2).

The majority of *C. gracilis* crab (87% to 90%) was captured in the shrimp ramp style pot at all locations (Table 2). This may indicate that the shrimp style pot had a higher retention rate of smaller organisms, especially the sexually dimorphic female *C. gracilis* crab. The structures provided by the geoduck aquaculture of site one cannot explain the abundance of female crab in the treatment location as similar abundances were found in the control area where no structure was present. A total of 285 female crab was captured at the aquaculture area, and a total of 361 female crab was captured at the control area (Table 2).

## **OTHER SPECIES PRESENT**

Eleven other species were visually observed at low tide during spring and fall, but not counted as catch at any sampling site (Figure 3). Some organisms such as the amphipods and snails were too small to be retained in the traps. Others, such as the large pink sea star and starry flounder were too large to enter the traps. Others, like the Dungeness crab and Pacific sand lance were simply not captured in the traps for

unknown reasons. While many of the species occupying in the sampling areas were captured, it is clear through visual observations that not all species present were accounted for in this study. Sampling with traps effectively captured many benthic organisms, but by design excluded some of the species in these research areas. This was also expressed in the lack of asymptote of all species richness and rarefaction curves (Figures 14, 15).

#### DISCUSSION

Species evenness was low at both Eld Inlet sampling locations. Both the aquaculture area and the natural control area were dominated by one species *Cancer gracilis*. There are many possible explanations for variability of species richness and abundances in soft-sediment communities. These differences could be due to physical effects such as food availability, predator interactions, larval recruitment or habitat type. Indirect effects as such species competition may also explain the exceptional abundance of this species at site one. Posey (1987) suggests that a dominance of one species, or functionally similar group of species may exclude other species. In the Nisqually Reach site species were dispersed more evenly than at the Eld Inlet site. This site variability within Puget Sound has been observed in other studies (Dethier and Schoch 2004). While each location of site two contained one species that constituted a slight majority, not one species was extraordinarily dominant.

Species richness was not significantly different between the treatment and control locations of Eld Inlet or Nisqually Reach. No patterns in differences between or

among the sampling locations were detected. In Eld Inlet, the "tubed" geoduck bed had slightly more species than the nearby control area. The geoduck grow-out bed at Nisqually had slightly less species than the nearby control area. Sites one and two had similar species richness and were not directly compared due to the physical and spatial differences between the sites. Previous studies in Puget Sound have found that physical conditions tend to become increasingly different with distance among sampling sites (Dethier and Schoch 2004), which may make a direct comparison between site one and two invalid. In all sampling locations the line representing species richness did not approach the asymptote. This indicates that for all sampling locations, the population was not sampled enough to represent all species present. Sampling effort allowing an asymptote to be achieved is desirable in ecological studies. In research such as this, it is difficult and prohibitive to achieve an asymptote due to the level of sampling that would be required in order to reach this goal. It is clear that not all species present at these locations were accounted for in this research; however a clear trend in data can still be obtained from the results of this sampling.

There were differences in biodiversity between Eld Inlet and Nisqually Reach, but little distinction among the treatment and control areas of either site. Eld Inlet showed low biodiversity. The geoduck bed at site one showed a slightly higher biodiversity than the control location. Site two was much more diverse than site one. The treatment area of site two was slightly more diverse than the nearby geoduck area. No clear pattern in biodiversity existed between or among the sampling locations. While these results indicate that geoduck aquaculture activities have had no effect on species

biodiversity at these sites, it cannot be assumed that differences do not exist at any other area in Puget Sound.

The sex ratio differences of C. gracilis between site one and two may have been influenced by many is factors. Many more crab in totality were encountered in Eld inlet than in Nisqually Reach. This coincides with Orensanz and Gallucci (1988) who found C. gracilis to occupy more protected habitats such as Eld Inlet within Puget Sound. Predation by the staghorn sculpin (Leptocottus armatus) may also explain why fewer C. gracilis were found in the Nisqually Reach area. Research has suggested this fish preys upon C. gracilis (Orensanz and Gallucci 1988) and high numbers of this predator fish were encountered in these trapping studies. Previous research has suggested that female C. gracilis crab congregate for mating purposes in shallow intertidal areas (Orensanz et al. 2009). This may be another explanation for the high numbers of female crab found in Eld inlet. Small seasonal differences were noted in the abundance of female crab at both locations. Female crab were more abundant at both sites during spring sampling. In Orensanz and Gallucci (1988) C. gracilis in Puget Sound molted and subsequently mated between May and June. Additionally, Miller (1990) found that catchability in crab is highest after molt, which could explain why so many more female than male C. gracilis crab were encountered during spring sampling in June. No females were captured at site two during fall sampling. Results from other studies have also shown a difference in catch between males and females (Krouse 1980, Miller 1990). Migration, molting, mating, predation or other unknown factors, or combination of

factors may explain the predominance of female crab caught in Eld Inlet, as well as the season variability found at both locations.

The results presented here have shown that specific intertidal geoduck aquaculture techniques are having no measurable influence on mobile benthic fauna inhabiting the same areas included in this research. Similarly, Dumbauld et al. (2009) found that while there are localized short-term effects involved in bivalve aquaculture in West coast estuaries, no evidence of habitat loss or water quality decline can be detected. Variation in benthic fauna naturally exists both spatially and temporally (Dethier & Schoch 2004), so making broad basin-wide conclusions based on these data are not are not recommended. In southern Puget Sound there exists a broad scale of habitat types, and broad variability in community member assemblages, as evidenced by the differences between site one and two.

#### CONCLUSION

Intertidal geoduck aquaculture contributes largely to state and local economies.

Due to this lucrative form of aquaculture, increasingly more intertidal areas are being utilized for culture of this product. Until recently, this practice has gone unregulated in Washington State. Policy makers have realized the importance of determining what potential effects these practices may be having. One way to evaluate the possible impacts of geoduck aquaculture is to measure its effect on biodiversity of local habitats. Measuring and comparing biodiversity can be a useful tool in ecological assessments.

No measurable differences in biodiversity were detected between geoduck aquaculture sites and non-farmed natural areas during this research.

Future research in this area should involve similar studies examining the effect of large single predator netting, and post-harvest conditions to detect what, if any differences in biodiversity exist at these sites. Additional research to determine the effects of geoduck aquaculture should include long-term monitoring, including baseline studies of specific habitat types. While widespread effects may be difficult to discern in a temporally and spatially variable system such as the Puget Sound, long-term efforts are paramount in providing insight into potential effects of intertidal geoduck aquaculture.

# **FIGURES**

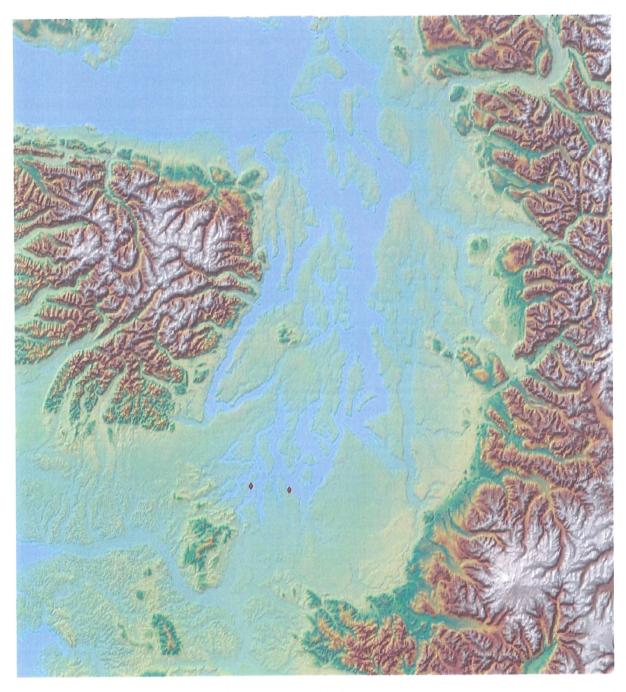


Figure 1. Puget Sound, WA. Study locations are illustrated by red diamonds in the far southern portion of Puget Sound.

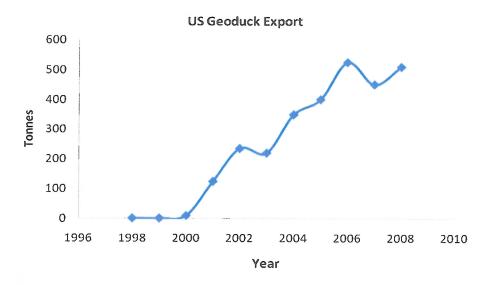


Figure 2. Exported geoduck aquaculture product from 1996 - 2009. Cultured geoduck did not become a major export product until near 2000. Raw Data Source: (FAO Fisheries and Aquaculture Department [online]).

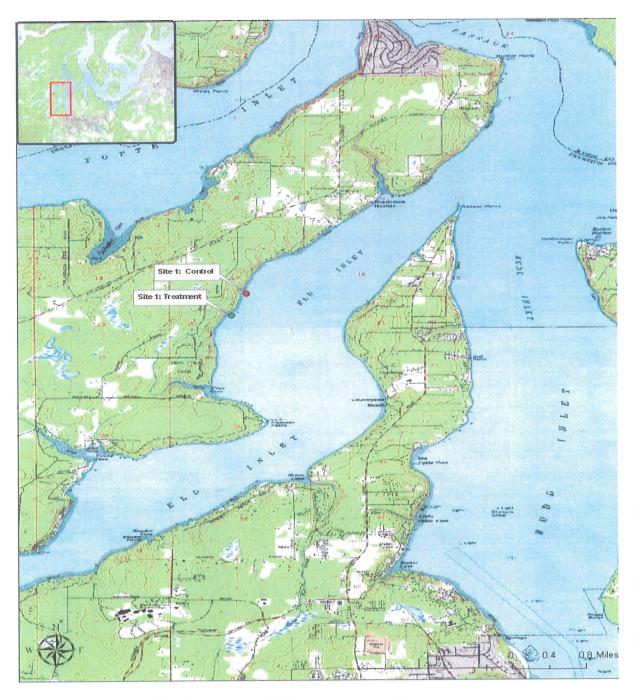


Figure 3. Location of site one treatment (geoduck tubes, green circle) and control (no aquaculture, red circle) locations are indicated in Eld Inlet, Puget Sound.

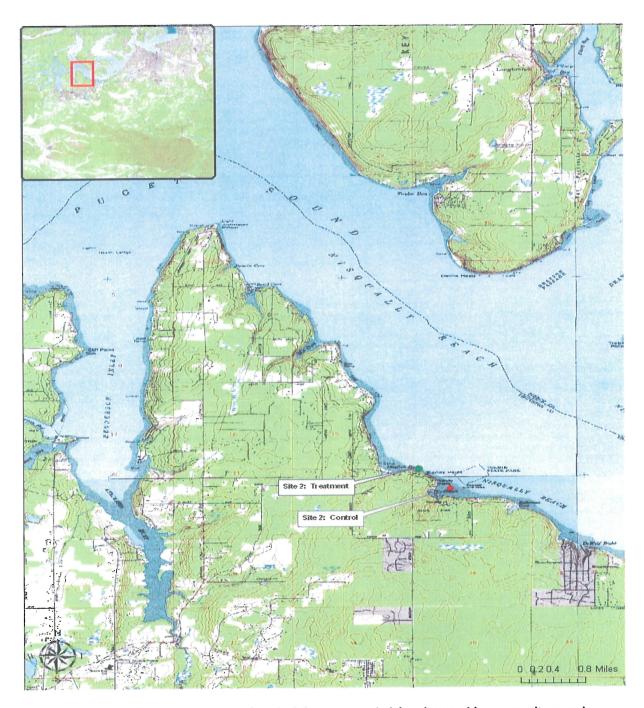


Figure 4. Location of site two treatment (geoduck farm, green circle) and control (no aquaculture, red circle) locations are indicated in the Nisqually Reach area of southern Puget Sound.



Figure 5. Site one at low tide during the spring of 2009. Geoduck tubes and net toppers are representative of the entire geoduck planted area.



Figure 6. Control area of site one at low tide during spring of 2009.

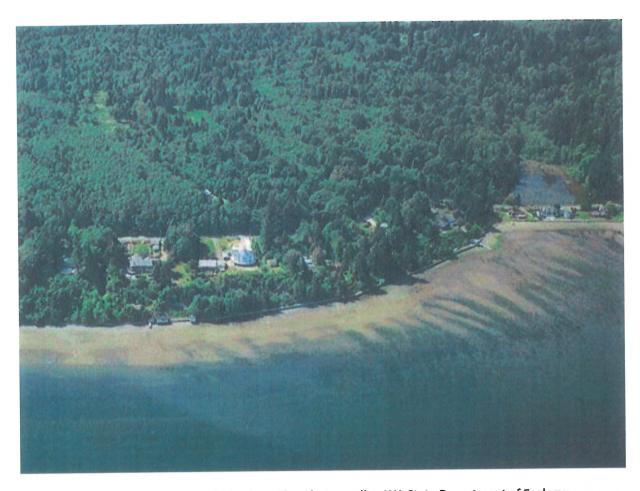


Figure 7. Site two treatment area during low tide. Photo credit: WA State Department of Ecology.

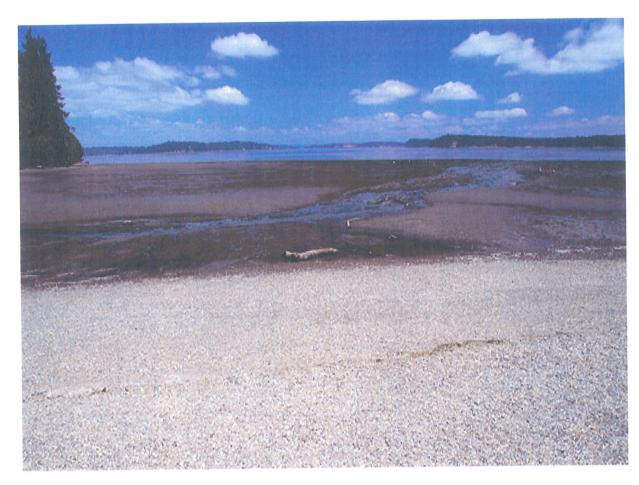


Figure 8. Site two control area during low tide. Photo credit: <a href="mailto:flickr.com/photos/10726866@N03/2712131158">flickr.com/photos/10726866@N03/2712131158</a>

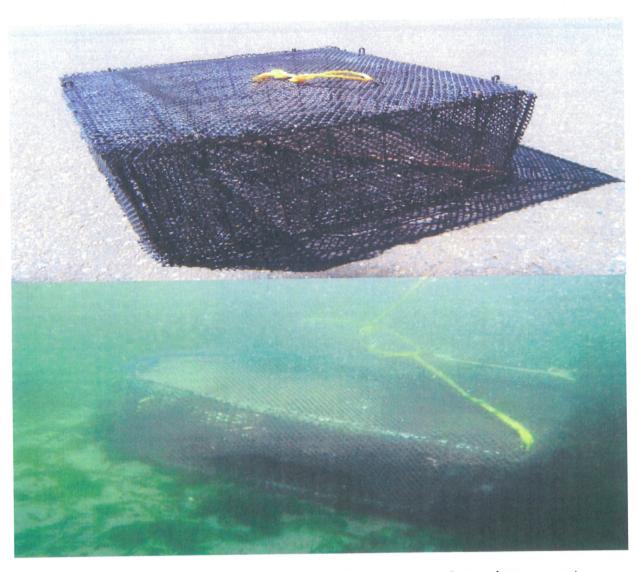


Figure 9. Modified crab and shrimp pots used to assess benthic populations. The top photo represents pre-launched crab pots and illustrates the manner in which all traps were modified with 12.7 mm polypropylene mesh material. The bottom photo shows an in-situ shrimp style pot after a 24-hour fishing period (test-launch not at treatment sites). During this test-launch traps were baited and set near 0 m MLLW. Captured organisms can be seen within the trap.

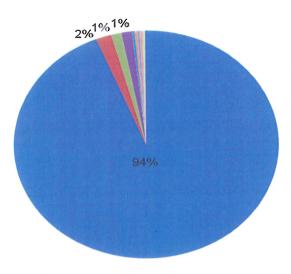


Figure 10. Species abundances of site one treatment area in Eld Inlet (n = 319). Nine different species were encountered, and five were encountered only once. One species, *C. gracilis* (cornflower) accounted for the majority of the catch at this location. *Pandalas danae* (red) represented two percent and *Leptocottus armatus* (green) and *Cancer productus* (purple) both represented one percent of the catch. The remaining two percent of species present were *Pholis laeta* (royal), *Pugettia product* (orange), *Cymatogaster aggregate* (periwinkle), *Pholis ornate* (pink), and an unknown sculpin species (mint). Species evenness was low at this location.

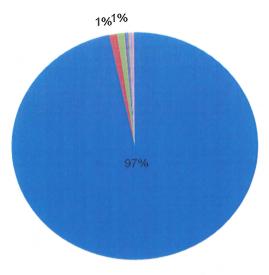


Figure 11. Species abundances of the control location at site one in Eld Inlet (n = 421). Eight different species were encountered, and five were encountered only once. One species, *C. gracilis* (cornflower), accounted for the majority of the catch at this location. *Pandalas danae* (red) and *Leptocottus armatus* (green) represented one percent each. *Cancer productus* (purple), *Pugettia producta* (royal), *Pycnopodia* helianthoides (orange), *Pagarus sp.* (periwinkle), and an unknown flatfish (pink) represented the remaining one percent of total catch. Species evenness was low at this location.

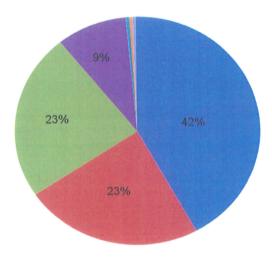


Figure 12. Species abundances of site two treatment area in Nisqually Reach (n = 226). Seven different species were encountered. Cancer gracilis (cornflower) accounted for the majority of the catch at this location.

Leptocottus armatus (red) and Cancer productus (green) both represented twenty three percent, and Pholis ornata (purple) accounted for nine percent. Pugettia product (royal), Hemigrapsus oregonensis (orange), and an unknown sculpin species (periwinkle) accounted for the remaining two percent of species present. This site exhibited moderate species evenness, and species were much more evenly distributed than both locations of site one.

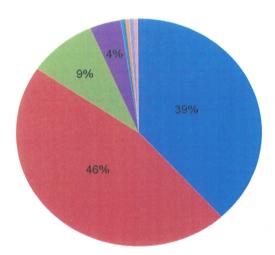
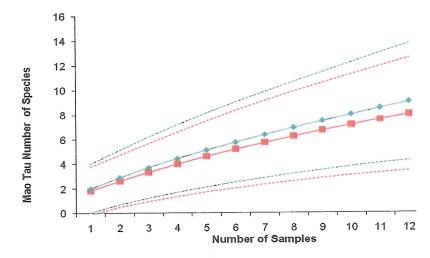


Figure 13. Species abundances of site two control area in Nisqually Reach (n = 180). Eight different species were encountered. Leptocottus armatus (red) represented the majority of the catch. Cancer gracilis (cornflower) accounted for thirty eight percent. Cancer productus (green) and Pholis ornata (purple) represented nine and four percent respectively. Cymatogaster aggregata (royal), Pycnopodia helianthoides (orange), Pandalas danae (periwinkle) and Crangon sp. (pink) accounted for the remaining two percent of species observed. This site exhibited moderate species evenness, although slightly lower than at the paired treatment location. Species were more evenly distributed than both locations of site one.

a)



b)

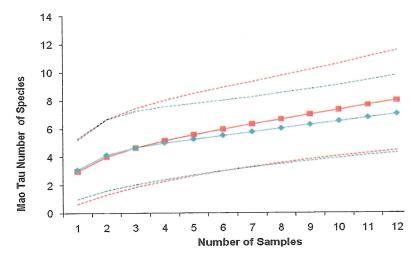
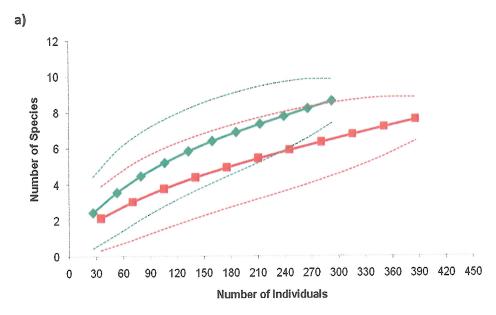


Figure 14. MaoTau Species accumulation curves represent species richness in the treatment and control areas of sites one (a) and two (b). The 95% upper and lower confidence intervals are represented by red (control) and green (treatment) dashed lines. Species abundances of the control sites (red square) and treatment sites (green diamond) do not differ significantly at either location. Site one (a) shows near identical species abundances between the aquaculture and control site at all levels of sampling intensity. A total of nine species were encountered at the treatment location and eight species at the control location during twelve sampling events. Site two (b) illustrates the similarity between species abundances at the aquaculture and control site at all levels of sampling intensity. A total of seven species were encountered at the treatment location of site two and six species were encountered at the control site at this location during twelve sampling events. Other species remain un-captured, as suggested by the lack of asymptote for both sites. Species accumulation curves were computed by *EstimateS* using the MaoTau method (Gotelli & Colwell 2001).



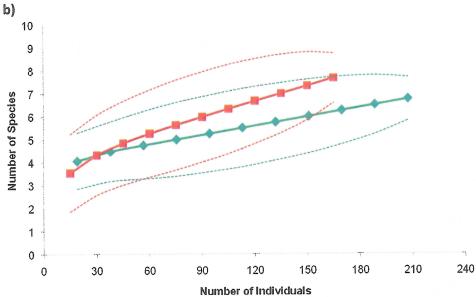
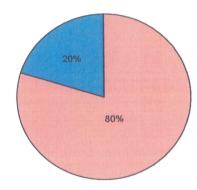
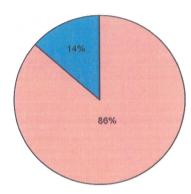


Figure 15. Rarefaction curves showing number of species as a function of number of individuals captured. The upper and lower 95 % confidence intervals are represented by color corresponding red dashed lines for control areas, and green dashed lines for treatment areas. This analysis allows us to compare species richness between the geoduck aquaculture area and the unplanted control. In site one (a) the structured aquaculture site (green diamond line) appears to be slightly richer than the control area (red square line) when accounting for all individuals (by 1 species). More individuals were captured in the control area. In site two (b) the control area (red square line) has a slightly higher richness than the grow-out phase aquaculture site (by less than 1 species), while the aquaculture area (green diamond line) has a much higher number of individuals. The sample based rarefaction curve was computed by *EstimateS* using the Coleman method and rescaled from samples to accumulated individuals (Gotelli & Colwell 2001).





b)



c)

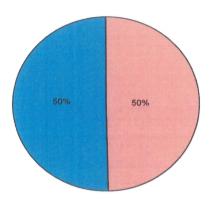


Figure 16. Sex ratio analyses for *Cancer gracilis* crab at all sites. Percentage of female crab is shown in pink and males in blue. Total abundance for all treatment and control sites combined is represented in (a). Overall catch resulted in a much higher proportion of females than males. In site one in Eld Inlet (b), females constitute the majority of the *C. gracilis* population in both treatment and control locales. Males and females were evenly distributed at site two in Nisqually Reach. Many more *C. gracilis* were

captured in site one (n = 747), than site two (n = 165), suggesting that higher numbers of female C. gracilis crab occur in the Eld Inlet area.

## **TABLES**

Table 1. Calculated Simpson's biodiversity values are shown for both site locations. Zero represents the lowest biodiversity while values approaching one represents the highest.

Site #1		Site #2		
Treatment	0.1152	Treatment	0.7040	
Control	0.0698	Control	0.6334	

Table 2. Seasonal and sex differences between sites and areas for *Cancer gracilis* captured in southern Puget Sound.

Site	Treatment		% shrimp	Control	0.000	% shrimp	Total	Total %
1			pot			pot		
	M	F		M	F			
Spring	15	185		29	258		380	0.51
Fall	21	100		36	103		299	0.4
total	36	285	0.88	65	361	0.90	747	0.82
SITE 2								
	M	F		M	F			
Spring	13	29		31	53		126	0.76
Fall	28	0		11	0		39	0.24
total	41	29	0.87	42	53	0.80	165	0.18

Table 3. Species that were observed, but not captured during the sampling period at site one and two. Some organisms were observed outside of the traps. Smaller organisms were found within or upon the traps, but were too small to be counted among the catch.

Species Name	Common Name		
Dendraster excentricus	Sand Dollar		
Polinices lewisii	Moon Snail		
Platichthys stellatus	Starry Flounder		
Heptacarpus sp.	Small Shore Shrimp		
Cancer magister	Dungeness Crab		
Ammodytes sp.	Pacific Sand Lance		
Pisaster brevispinus	Pink Sea Star		
Turbonilla sp.	Snail		
Unknown malacostracan	Grass Shrimp		
Unknown amphipod	Amphipod		
Unknown gastropod	Snail		

Table 4. Raw abundance data for sampling locations in Eld Inlet and Nisqually reach. Total abundance data for each species is represented by the total column on far right of the table.

		Geoduck Site	Control Site	Geoduck Site	Control	
Species	Common Name	#1	#1	#2	Site #2	Tota
Cancer gracilis	graceful crab	300	406	96	69	871
Pholis ornata	saddleback gunnel	1	0	21	7	29
Leptocottus armatus	staghorn sculpin	4	4	53	83	144
Cancer productus	red rock crab	4	1	53	17	75
Pugettia producta	kelp crab	1	1	1	0	3
Cymatogaster aggregata	shiner perch	1	0	0	1	2
pandalas danae	dock shrimp	6	6	0	1	13
Pholis laeta	crescent gunnel	1	0	0	0	1
unknown sculpin	sculpin	1	0	0	0	1
pycnopodia helianthoides	sunflower star	0	1	0	1	2
unknown sculpin	sculpin	0	0	1	0	1
Hemigrapsus oregonensis	shore crab	0	0	1	0	1
Crangon sp.	common shrimp	0	0	0	1	1
Pagarus sp.	hermit crab	0	1	0	0	1

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