FINAL REPORT

Geoduck Aquaculture Research Program

Report to the Washington State Legislature

Senate *Agriculture, Water & Rural Economic Development Committee*
Senate *Energy, Environment & Telecommunications Committee*
House *Agriculture & Natural Resources Committee*
House *Environment Committee*

November 2013

Washington Sea Grant has prepared this final progress report of the Geoduck Aquaculture Research Program to meet a requirement of Second Substitute House Bill 2220 (Chapter 216, Laws of 2007).

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November 2013 • WSG-TR 13-03

Acknowledgements

Washington Sea Grant expresses its appreciation to the many individuals who provided information and support for this report. In particular, we gratefully acknowledge research program funding provided by the Washington State Legislature, Washington State Department of Natural Resources, Washington State Department of Ecology, National Oceanic and Atmospheric Administration, and University of Washington. We also would like to thank shellfish growers who cooperated with program investigators to make this research possible. Finally, we would like to recognize the guidance provided by the Department of Ecology and the Shellfish Aquaculture Regulatory Committee.

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Recommended Citation

Washington Sea Grant (2013) Final Report: Geoduck aquaculture research program. Report to the Washington State Legislature. Washington Sea Grant Technical Report WSG-TR 13-03, 122 pp.

Contents

1 Overview

The geoduck (*Panopea generosa*) is North America's largest burrowing clam. It is found in soft intertidal and subtidal marine habitats in the northeast Pacific Ocean to depths of more than 200 feet. In Washington state this large clam has been cultured since 1991 and on a commercial scale since 1996. Today geoduck harvesting in Washington and British Columbia is an $80 million industry, with Washington supplying nearly half of the world's demand through wild and farmed operations. Aquaculture contributions to the annual state harvest have grown steadily and now total around 1.3 million pounds per year or 90% of global geoduck aquaculture production. While the clams are a valuable resource that can fetch $100 or more per pound overseas, until recently, little scientific information was available on the ecological impacts of common culture practices.

In 2007, the Washington Legislature enacted Second Substitute House Bill 2220 (Chapter 216, Laws of 2007) to commission studies assessing possible effects of geoduck aquaculture on the Puget Sound and Strait of Juan de Fuca environments. The bill called on Washington Sea Grant, based at the University of Washington (UW), to establish a six-year research program, reporting the results back to the Legislature by December 1, 2013. The following final report summarizes the results of the commissioned research studies, provides an overview of program activities and recommends future research and monitoring to support sustainable management of geoduck aquaculture in Washington state.

2 Background

The 2007 law directed Washington Sea Grant to review existing scientific information and examine key uncertainties related to geoduck aquaculture that could have implications for the health of the ecosystem and wild geoduck populations. The legislation established six priorities for measuring and assessing such implications:

1. the effects of structures commonly used in the aquaculture industry to protect juvenile geoducks from predation;
2. the effects of commercial harvesting of geoducks from intertidal geoduck beds, focusing on current prevalent harvesting techniques, including a review of the recovery rates for benthic communities after harvest;
3. the extent to which geoducks in standard aquaculture tracts alter the ecological characteristics of overlying waters while the tracts are submerged, including impacts on species diversity and the abundance of other organisms;
4. baseline information regarding naturally existing parasites and diseases in wild and cultured geoducks, including whether and to what extent commercial intertidal geoduck aquaculture practices impact the baseline;
5. genetic interactions between cultured and wild geoducks, including measurement of differences between cultured and wild geoducks in term of genetics and reproductive status; and
6. the impact of the use of sterile triploid geoducks and whether triploid animals diminish the genetic interactions between wild and cultured geoducks.

The Legislature assigned top priority to the assessment of the environmental effects of commercial harvesting and required that all research findings be peer-reviewed before reporting. The Shellfish Aquaculture Regulatory Committee (SARC), established by the 2007 law, and the Washington Department of Ecology (Ecology) were tasked with overseeing the research program.

Northwest Workshop on Bivalve Aquaculture and the Environment

To articulate a scientific baseline and encourage interest in the research program, Washington Sea Grant convened the Northwest Workshop on Bivalve Aquaculture and the Environment in Seattle in September 2007. Experts from the United States, Canada and Europe were invited to discuss recent findings and provide recommendations for research needed to support sustainable management of geoducks and other shellfish resources. The diverse range of attendees included state, federal and tribal resource managers, university researchers, shellfish farmers, conservation organizations and interested members of the public. All workshop materials are available on the Washington Sea Grant website at *wsg.washington.edu/research/geoduck/shellfish_workshop.html*.

Review of Current Scientific Knowledge

SSHB 2220 required a review of all available scientific research that examines the effect of prevalent geoduck aquaculture practices on the natural environment. Washington Sea Grant contracted with experts at the UW School of Aquatic and Fishery Sciences to conduct an extensive literature review of current research findings pertaining to shellfish aquaculture. The researchers evaluated 358 primarily peer-reviewed sources and prepared a draft document for public comment in September 2007. WSG received four formal comment submissions, which were considered by the authors while editing the final document and responded to in writing. The final literature review, "Effects of Geoduck Aquaculture on the Environment: A Synthesis of Current Knowledge," was completed in January 2008. It was revised and updated to include recent findings in October 2009; it was then significantly revised in April 2013[1] to include the evaluation of 62 additional publications. The literature review is available for download on the Washington Sea Grant website at *wsg.washington.edu/research/geoduck/literature_review.html*.

Commissioning of Research Studies

In October 2007, WSG issued a request for proposals and received responses from seven research teams. After rigorous scientific review, four projects were selected for funding, two of which were combined to develop a more integrated and comprehensive study. Selected projects addressed three of the six legislatively established priorities (1, 2, 4). Research on genetic interactions, priority (5), was already underway using funding from other sources. Funding for priority (6) and selection of a project to address the remaining priority (3) were deferred until later in the program, subject to the availability of additional resources.

The three selected projects together comprise the Geoduck Aquaculture Research Program (GARP). Project titles, principal investigators, research institutions and a brief description of selected studies are as follows:

A. **Geochemical and Ecological Consequences of Disturbances Associated with Geoduck Aquaculture Operations in Washington** (Glenn VanBlaricom, UW; Jeffrey Cornwell, University of Maryland). The project examined all phases of the aquaculture process — geoduck harvest and planting, presence and removal of predator exclusion structures, and ecosystem recovery. It assessed effects on plant and animal communities, including important fish and shellfish, in and on Puget Sound beaches, as well as the physical and chemical properties of those beaches.

B. **Cultured–Wild Interactions: Disease Prevalence in Wild Geoduck Populations** (Carolyn Friedman, UW). The study developed baseline information on pathogens to improve understanding of geoduck health and management of both wild and cultured stocks.

C. **Resilience of Soft-Sediment Communities after Geoduck Harvest in Samish Bay, Washington** (Jennifer Ruesink, UW). Capitalizing on eelgrass colonization of an existing commercial geoduck bed, this project examined the effect of geoduck aquaculture on soft-sediment tideflat and eelgrass meadow habitats.

Research Program Implementation

Funding for research and related program activities initially was provided through state appropriation to the geoduck aquaculture research account established under the 2007 law. This state funding of $750,000 supported the program through June 30, 2010 (Table 1). Although no additional monies were deposited in the account in fiscal year 2010–2011, the Department of Natural Resources (DNR) provided $300,827 through an interagency agreement with the UW. The largest project, the VanBlaricom-led disturbance study, also secured $39,972 from the UW's Royalty Research Fund and $22,207 from Ecology to supplement student and technical support that was not included in the DNR agreement.

Scientists adjusted their efforts to minimize research costs, and DNR, UW and Ecology funding ensured completion of the three research studies and program support. In October 2010, the National Sea Grant College Program awarded the VanBlaricom research team a competitive aquaculture grant to investigate the effects of aquaculture structures on related predator–prey interactions and food-web dynamics in geoduck aquaculture. While the goals of the new project differ somewhat from the priorities established in the 2007 law, the studies are complementary and permit resources to be leveraged as part of a shared program infrastructure.

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[1] Straus K. M., P. S. McDonald, L. M. Crosson, and B. Vadopalas. 2013. Effects of Pacific geoduck aquaculture on the environment: A synthesis of current knowledge. Washington Sea Grant, Seattle (Second Edition Edition). 83 p.

Ecology provided $39,742 through an interagency agreement with the UW to complete the final reporting tasks. No additional monies were secured to address deferred research priorities (3, 6) pertaining to the effects of geoduck aquaculture on overlying waters and the use of sterile triploid geoduck. Peer-reviewed and published research related to these priorities and priority (5), conducted outside the program, are addressed in the updated literature review.

Program Coordination and Communication

Washington Sea Grant staff and program researchers worked closely with staff from Ecology and DNR and provided regular presentations to members of the Shellfish Aquaculture Regulator Committee (*http://www.ecy.wa.gov/programs/sea/shellfishcommittee/*) until it was disbanded in March 2012. Program updates were provided in three interim progress reports to the Legislature (Dec 2009, Mar 2011 and Feb 2012), which are available on the Washington Sea Grant website (*http://wsg.washington.edu/geoduck*). In addition, research findings were communicated via media placements, publications and at more than 60 public presentations.

Table 1. Funding Source, Timing and Level

		WA State Geoduck in in Research Account	Ecology Agreement	DNR Agreement	UW Royalty Research Fund	National Sea Grant Strategic Investment in Aquaculture Research (competitive grant)	Ecology Agreement
Project Title	Study Duration	7/1/2007 – 6/30/10	4/1/2010 – 6/30/10	7/1/2010 – 6/30/11	7/1/2010 – 6/30/11	10/1/2010 – 9/30/13	1/1/2013 – 6/30/2013
Geochemical and Ecological Consequences of Disturbances Associated with Geoduck Aquaculture	Apr 2008 – June 2013	$459,935	$22,207	$210,390	$39,972	$397,672	
Cultured-Wild Interactions: Disease Prevalence in Wild Geoduck Populations	Apr 2008 – July 2011	$104,000		$65,688			
Resilience of Soft-Sediment Communities after Geoduck Harvestin Samish Bay, Washington	Apr 2008 – July 2011	$86,612		$11,000			
Program Administration	Jul 2007 – Dec 2013	$99,453		$13,749			$39,724
TOTAL		$750,000	$22,207	$300,827	$39,972	$397,672	$39,724

3 Summary of Research Projects

Each of the three GARP projects has produced research findings that generated at least one article for submission to a peer-reviewed scientific journal. While some of the articles are still in the process of being accepted for publication, all have been peer-reviewed and revised in response to the reviewer comments. Each article is summarized below, including authors and publication status. The full text of each manuscript is provided as an appendix to the final report.

Geochemical and Ecological Consequences of Disturbances Associated with Geoduck Aquaculture Operations in Washington

Glenn VanBlaricom, David Armstrong and Tim Essington, School of Aquatic and Fishery Sciences, University of Washington, and Jeffrey Cornwell and Roger Newell, Horn Point Marine Laboratory, University of Maryland

Ecological effects — harvest

Manuscript titled "Ecological effects of the harvest phase of geoduck clam (*Panopea generosa* Gould, 1850) aquaculture on infaunal communities in southern Puget Sound, Washington USA." Authored by Glenn R VanBlaricom, Jennifer L Price, Julian D Olden, and P Sean McDonald (Appendix I). Status: accepted, Journal of Shellfish Research.

The purpose of this study was to assess how harvesting cultured geoducks affects the structure of benthic macroinfaunal assemblages ("infauna") in intertidal sandy habitats of southern Puget Sound. Harvesting geoducks involves liquefaction of sediments surrounding individual clams to facilitate extraction from the sediment. The process produces many small-scale disturbances within a cultured plot, characterized by displaced sediments, changes in sediment water content and possible chemical modification of the sediments. Such disturbances were viewed at the outset as possibly significant to infaunal densities, population dynamics, productivity and biodiversity.

The investigators collected time-series data from large paired plots at three sites in southern Puget Sound. Each site involved a plot in active culture (cultured plot) and a nearby uncultured reference plot (separation distance ≥75 m). A primary goal of the study was to match the spatial and temporal scales of operation by commercial aquaculture companies to maximize the inferential value of the results in a management context. However, working within the timeline necessary to establish experimental farms was not feasible (outplanting to harvest requires a period of 5 to 7 years) and potential associated costs were prohibitive. Instead the investigators established collaborations with commercial geoduck growers to utilize cultured plots already established, and within 1 to 2 years of scheduled harvests dates, as the basis for the project. Collaborating growers made no effort to influence study design, sampling procedures, or data generation, analyses or interpretation.

The investigators sampled cultured plots approximately monthly, beginning no less than four months before scheduled initiation of harvest, continuing through the harvest period, and extending for a minimum of four months following conclusion of harvests. At each sampling event at the three study sites, randomly located samples were collected in the cultured plots and reference areas. Infauna densities were sampled with two methods: smaller infauna (e.g., small crustaceans, polychaete worms and juvenile bivalves) were assessed with sediment "cores"; larger infauna (e.g., adult bivalves, sand dollars and sea cucumbers) were assessed with larger "excavations." In addition, the investigators collected groups of core samples at varying pre-determined positions along transect lines extending away from cultured plot edges in a direction parallel to shore.

The study followed protocols of a "before-after-control-impact" (BACI) design. The investigators used multivariate data visualization and statistical methods, applied separately to data from cores and excavations. Analyses tested hypotheses that infaunal assemblages would be different — defined either by abundance data or the Shannon biodiversity index — during and after harvest of cultured clams compared with before harvest; that seasonal and within-site spatial variations would contribute significantly to patterns in the data; and that transect core data would reveal a "spillover" effect of harvest-associated disturbances on adjacent uncultured habitat.

Effects of harvest on resident macrofauna

Patterns in data from the three study sites were so different that consideration of the three sites as replicates was statistically inappropriate. As a consequence, analyses for the three sites were done separately, effectively increasing the sample size in a statistical context, but also reducing the statistical power of the analyses. Nevertheless, the approach provided sufficient power to produce several important insights:

- Effects of season and within-site location were significant. Thus, most of the variation in the data were linked to changes in infaunal abundance by season and in space, in the latter case often over relatively small distances.
- There was no support for a statistically significant effect of harvest disturbance on infaunal abundance data from the study sites, either for cores or excavation samples.
- Similarly, there was no support for a statistically significant effect of harvest disturbance on infaunal biodiversity data from the study sites, either for cores or excavation samples.
- With a single exception, there was no statistically significant variation of infaunal abundance data from cores with distance from the edges of cultured plots, which led the investigators to reject the hypothesis of a "spillover effect" of harvest on infaunal assemblages adjacent to but outside of cultured plots.

Conclusions

These data suggest that infauna at study sites in southern Puget Sound are characterized by a high level of variation by season and by location, even on small spatial scales. Natural spatial and temporal variation in the infaunal assemblages is far more significant than variations imposed by harvesting of cultured geoduck clams. Moreover, infauna at the study sites in southern Puget Sound may have generally become accommodated to natural disturbances such as storm events, and thereby have adapted to coping — either by physiological or physical resistance, or by appropriate post-disturbance population resilience — with disturbances associated with harvesting of cultured geoduck clams.

Ecological effects — outplanting

Manuscript titled "Effects of geoduck (*Panopea generosa*) outplanting and aquaculture gear on resident and transient macrofauna communities of Puget Sound, Washington, USA." Authored by P Sean McDonald, Aaron WE Galloway, Kate McPeek, and Glenn R VanBlaricom (Appendix II). Status: accepted, Journal of Shellfish Research.

The goal of this study was to examine the response of resident and transient macrofauna to geoduck aquaculture by comparing community attributes at cultured plots and nearby reference areas. Habitat complexity is known to enhance abundance and diversity by reducing interactions among competitors, by sustaining predator and prey populations, and by enhancing settlement processes and food deposition. Gear used in geoduck aquaculture enhances structural complexity on otherwise unstructured beaches.

The investigators collected data at geoduck aquaculture sites at three locations in southern Puget Sound prior to initiation of aquaculture operations (pre-gear); with protective PVC tubes and nets and outplanted juvenile geoducks (gear-present); and following removal of the structures during the grow-out period (post-gear). Regular surveys of resident benthic invertebrates were conducted using coring and excavation methods during low tide, while surveys of transient fish and macroinvertebrates were done at high tide via SCUBA. Shore surveys to quantify use of these habitats by juvenile salmonids were conducted during peak migration periods (March through July).

Species abundance, composition and diversity were examined because these characteristics are useful for understanding the ecological effects of aquaculture as a press (i.e., chronic) disturbance on intertidal beaches. Variability has been linked to the environmental stress of disturbance; thus, special consideration was given to variability of community composition in different phases of the culture cycle. By evaluating effects across phases of culture, the investigators were able to examine recovery following attenuation of the disturbance.

Effects of aquaculture gear and geoducks on resident macrofauna

Resident invertebrate communities were characterized by strong seasonal patterns of abundance and site-specific differences in composition. Highest densities typically occurred July to September, but patterns of higher density were inconsistent in either cultured plots or reference areas across months or sites. Dispersion in sample variation, which is commonly used to detect effects of disturbance, did not differ between cultured plots and reference areas when aquaculture gear was in place. Sampling methods were used to opportunistically examine forage fish spawning at study sites. Despite the presence of Pacific sand lance (*Ammodytes hexapterus*) in excavation samples (Rogers site, October 2010), no evidence of spawning (i.e., eggs) was observed in those or subsequent samples.

Effects of aquaculture gear and geoducks on transient macrofauna

Observations suggest a pronounced seasonal response of transient macrofauna at study sites, with most taxa conspicuously more abundant during spring and summer (April through September). Total abundance of fish and macroinvertebrates was more than two times higher at cultured plots than at reference areas during the structured phase of geoduck aquaculture (gear-present), indicating that geoduck aquaculture gear created favorable habitat for some types of Puget Sound macrofauna. In particular, habitat complexity associated with geoduck aquaculture attracted species observed infrequently in unstructured reference areas (e.g., bay pipefish, *Syngnathus leptorhynchus*), but displaced species that typically occur in these areas (e.g, starry flounder, *Platichthys stellatus*).

Analyses of community composition across phases of culture operations largely support descriptive observations. Composition was similar among cultured plots and reference areas prior to initiation of aquaculture operations; however, these communities diverged with placement of PVC tubes and nets and outplanting of juvenile geoducks. In general, functional groups such as crabs and seaperches showed higher affinity with cultured plots, while flatfishes were more often associated with reference areas. These differences did not persist once aquaculture gear was removed from cultured plots during the geoduck grow-out phase. Despite shifts in abundance and species composition, diversity, as calculated with the Shannon Diversity Index (H'), did not vary significantly between cultured plots and reference areas across phases of geoduck aquaculture operations.

Juvenile chum (*Oncorhynchus keta)* and pink salmon (*O. gorbuscha*) were observed in approximately 8% of shore surveys and in similar frequencies at cultured plots and reference areas. No discernable differences in behavior were observed. The investigators suggest that additional sampling using alternative methods (e.g., beach seine) is necessary to thoroughly evaluate habitat use by salmonids, given low encounter frequency in the present study.

Conclusions

Resident and transient macrofauna communities respond differently to changes in habitat complexity associated with geoduck aquaculture operations. Structures associated with geoduck aquaculture (i.e., PVC tubes and cover nets) appear to have little influence on resident benthic macroinvertebrates in this study. Differences among sites suggest location-specific habitat characteristics, including local patterns of natural disturbance, are more important than geoduck aquaculture practices in affecting community composition. These results are consistent with other ecological studies addressing effects of shellfish aquaculture on benthic invertebrate communities. The investigators postulate that effects may be more pronounced for geoduck aquaculture operations sited in low-energy embayments with weak flushing because accumulation of shellfish biodeposits has been linked to changes in invertebrate communities.

Geoduck aquaculture gear significantly alters abundance and composition, but not diversity, of transient macrofauna. In this study, the presence of PVC tubes and nets produced community shifts that favored species associated with complex habitats and excluded species that occur in unstructured areas, and behavioral observations suggested that aquaculture gear provides foraging habitat and refuge for a variety of taxa. Moreover, seasonal biofouling by macroalgae further enhanced habitat complexity within cultured plots. Despite these significant changes, effects of aquaculture operations only occurred when PVC tubes and nets were present; none of the changes carried over to the grow-out phase. Taken together, these results indicate that changes in habitat complexity associated with geoduck aquaculture produce short-term effects (1 to 2 years) on intertidal beaches, but the investigators caution that this study did not address spatial or temporal cumulative effects.

Geochemical effects

Manuscript titled "The influence of culture and harvest of geoduck clams (*Panopea generosa*) on sediment nutrient regeneration." Authored by Jeffrey C Cornwell, Michael S Owens, and Roger IE Newell (Appendix III). Status: submitted, Aquaculture.

The goals of this study were to examine the extent to which the culture and harvest of geoducks in Puget Sound affect the accumulation of inorganic nitrogen (N) and phosphorus (P) in sediments. The investigators measured nutrient concentrations within the pore water at various depths in the sediment where geoducks had been reared for 5 to 8 years (cultured plots) and compared these with nearby controls (reference areas) at five aquaculture farms in South Puget Sound and one in north Hood Canal. The investigators also measured the release of nutrients in the effluent water during commercial geoduck harvest and measured pore nutrient concentrations after harvest had occurred.

The investigators note that farming geoduck clams, like other bivalves, results in no net addition of nutrients to Puget Sound. Geoducks consume naturally occurring phytoplankton, sustained by a pool of nutrients comprising "new" nutrient inputs from anthropogenic sources, inputs from adjoining coastal waters and "old" nutrients regenerated via decomposition of organic material within the water body. Unlike fish aquaculture, no feed is added that would increase farm inputs.

Before harvest

Three different methods were used to determine pore-water inorganic nutrient concentrations. Pore-water equilibrators were placed in sediment, equilibrating water in the devices with the surrounding pore water. Standpipe piezometers were used to sample pore water at discrete depths and to measure the position of the water table relative to the sediment surface. Stainless steel microbore "sipper" tubes were inserted to depth within the sediments and small volumes of pore water withdrawn into a syringe. In addition to pore-water nutrient concentrations, rates of sediment-water exchange were measured by incubating stirred sediment cores.

A number of differences between cultured plots and reference areas were observed. Average soluble reactive phosphorus released from sediment to the water column during incubations in the absence of light was greater from cultured plots than from reference areas, though not statistically significant. This suggests the regeneration of sediment inorganic phosphorus, possibly via iron oxide-bound inorganic phosphorus attached to particles filtered by the geoducks and released in their particulate waste (biodeposits). Such bound phosphorus then becomes incorporated into sediments where oxygen is depleted and iron reduced, resulting in the release of soluble reactive phosphorus.

Rates of silica release from the sediment to the water column during dark incubations were also greater at cultured plots than at reference areas, although this was again not statistically significant. This suggests higher levels of remineralization of amorphous silica, likely from increased accumulation of diatom tests associated with geoduck biodeposits.

Average ammonium effluxes did not differ significantly between the cultured plots and reference areas in sediments incubated in darkness; with ambient light levels, fluxes (both efflux and influx) were lower than in darkness. This response of nutrient fluxes to light and dark is due to benthic microalgae actively taking up regenerated nutrients in the presence of light. High core-to-core variability, reflective of spatial variability in the amount of fecal material deposited to and ultimately incorporated into sediments, made statistical comparisons between cultured plots and reference areas difficult. At the Foss-Joemma and Chelsea-Wang sites, sipper-derived ammonium pore-water concentrations were significantly higher at cultured plots than reference areas.

During harvest

To establish background levels, the investigators collected and analyzed before and after samples of the water used to liquefy the sediments during geoduck harvest.

Mean ammonium concentrations in this effluent were slightly higher than the concentrations observed in the estuarine source water. At the Cooper site, effluent ammonium was significantly higher than both the cultured plot and reference area pore water levels, while at Thorndyke and Chelsea-Wang, the effluent ammonium concentrations were less than 10% of the mean porpore watere-water ammonium concentrations. The soluble reactive phosphorous concentrations in effluent water were quite low. The effluent silica concentrations were elevated relative to pore-water concentrations at Cooper, similar to pore-water concentrations at Thorndyke, and much lower than pore-water silica concentrations at Chelsea-Wang.

Conclusions

Compared to sediments in many other estuarine environments nationwide, the concentrations of pore-water solutes at all sites surveyed were generally low, leading to low sediment-water exchange rates and lower efflux rates during harvest.

The evidence for an effect of geoduck culture on pore-water nutrient concentrations was mixed. The study found that the cultivation of geoducks leads to generally low to moderate levels of accumulation of inorganic nutrients in the pore waters of the sediment.

The comparisons of pore water chemistry to harvest effluent suggest that harvest-related flushing of deep sediment releases a variable fraction of the pore water inorganic nitrogen and phosphorus. In general, the release of pore-water nutrients in the harvest effluent was low. To scale the size of effluent inputs to the waters of Puget Sound, the study estimated that nutrients flushed into adjacent waters during the harvest process comprise approximately 0.001% of the daily nutrient load from streams or wastewater plants. Geoduck harvesting is tied to market demand and tidal level, so nutrient inputs may be proportionately higher for short periods of time. Overall, however, the magnitude of nutrient release during harvest by current levels of geoduck aquaculture is an inconsequential fraction of anthropogenic nutrient inputs into Puget Sound. Moreover, it is prudent to note that effluxes from geoduck aquaculture are derived from a transformation of existing nutrients in the water column, not anthropogenic inputs associated with aquaculture practices.

Cultured-Wild Interactions: Disease Prevalence in Wild Geoduck Populations

Carolyn Friedman and Brent Vadopalas, School of Aquatic and Fishery Sciences, University of Washington

Manuscript titled "Characterizing trends of native geoduck (*Panopea generosa*) endosymbionts in the Pacific Northwest." Authored by Elene M Dorfmeier, Brent Vadopalas, Paul Frelier, and Caroline S Friedman (Appendix IV). Status: accepted, Journal of Shellfish Research.

The goals of the geoduck disease study were to (1) explore trends of parasite presence within wild geoduck populations and (2) characterize the influence of spatial distribution (site), collection depth and temporal distribution (season) on the diversity of parasite assemblages. This study provides an initial characterization of endoparasites in wild geoduck populations in Puget Sound and suggests that seasonal and geographic differences in distribution and intensity of infection of these organisms should be taken into account when moving geoducks among locales.

The parasite data set consisted of five tissue sections (ctenidia [gill], siphon [neck] muscle, siphon surface epithelium, intestine and ova) from each of 634 geoducks, containing information on three broad categories of taxa: rickettsia-like organisms (RLO), microsporidia-like organisms (MLO) and metazoans. Parasite **prevalence** describes the portion of a population observed to have a particular parasite. Parasite **intensity** describes the relative number of parasites in each tissue section. Each tissue section was assigned a semi-quantitative score of 0 to 4 where 0 = no parasites, 1 = few parasites (<10), 2 = small numbers of parasites (11 – 20), 3 = moderate numbers of parasites (21 – 30), 4 = large numbers of parasites (>30).

This study revealed five morphologically unique endosymbionts of wild Pacific geoducks in the Pacific Northwest: RLOs were observed in gill (ctenidia), an unidentified metazoan in the siphon, and two MLOs in siphon muscle and intestinal submucosa (connective tissue beneath a mucus membrane). A third MLO was observed in oocytes and is likely a *Steinhausia*-like organism (SLO).

Parasite prevalence

Spatial differences in parasite communities were evident. Freshwater Bay and Totten Inlet exhibited the greatest differences in parasite prevalence and intensity while Thorndyke Bay generally exhibited intermediate parasite prevalence and intensity. RLO prevalence was highest in Freshwater Bay (62%) relative to both Thorndyke Bay (35%) and Totten Inlet (19%). In contrast, prevalence of siphon metazoa was highest in Totten Inlet (57%) and Thorndyke Bay (46%) relative to only 9% in Freshwater Bay. Intestinal MLO and metazoan parasites were observed in highest prevalence at Totten Inlet and showed the lowest abundance at Freshwater Bay. Prevalence of the SLO, limited to reproductively active female geoducks, was similar among sites. Similarly, siphon MLOs were generally of low prevalence or absent at all sites.

Seasonal trends in metazoan prevalence were observed in geoducks from Freshwater and Thorndyke bays, where summer prevalence exceeded those of all other seasons. Both sites exhibited similar prevalence patterns of metazoan parasites. No trend was observed in Totten Inlet animals.

Collection depth influenced parasite prevalence. Higher RLO prevalences were observed in geoducks collected in shallow depths. Siphon MLOs were only observed in shallow collection depths. Both the intestinal MLO and metazoan parasites were more prevalent at the deeper collection depths.

Parasite intensity

Infection intensities differed by season and site among the endoparasites. RLO intensities did not vary among sites, but varied among seasons with the highest intensities observed in summer and winter. Metazoan intensities were temporally lowest in spring and spatially highest in Totten Inlet. The intensity of the intestinal MLO was significantly greater in fall than in winter, but similar among sites. In contrast, the intensity of the siphon MLO was similarly high among seasons and between Totten Inlet and Thorndyke Bay; it was not observed in Freshwater Bay. In contrast, the infection intensity of the SLO was similar among both seasons and sites.

Conclusions

The investigators revealed the presence of several previously unreported parasites in Puget Sound geoduck clams. Parasite presence in marine geoduck populations was significantly influenced by spatio-temporal differences in Puget Sound. The observed differences in parasite assemblages may be attributed to host physiology and density, seasonality of infective stages of parasites, temperature shifts or localized environmental factors. Parasite presence is ultimately dependent on both the environment of the host and the microenvironment of the parasite. Management of any future disease outbreaks in geoducks, whether in farmed or wild stocks, will benefit from the baseline knowledge gathered in this study.

Resilience of Soft-Sediment Communities after Geoduck Harvest in Samish Bay, Washington

Jennifer Ruesink and Micah Horwith, Department of Biology, University of Washington

Manuscript titled "Changes in seagrass (*Zostera marina*) and infauna through a five-year crop cycle of geoduck clams (*Panopea generosa*) in Samish Bay, WA." Authored by Micah J. Horwith and Jennifer Ruesink (Appendix V). Status: peer-reviewed and revised for submission to Pacific Science.

The goal of this study was to examine the response of native eelgrass, *Zostera marina*, to geoduck aquaculture in a single-site case study. This protected seagrass can recruit into geoduck farms during the culture cycle, and geoduck aquaculture may affect nearby eelgrass. The investigators studied the response of eelgrass and soft sediment communities at a site in Samish Bay, Washington, where *Z. marina* colonized the cultured plot after geoducks had been planted. The investigators measured eelgrass density, above- and below-ground biomass, sediment organic content, and infaunal abundance and diversity. These response variables were compared in and outside the cultured plot over the course of the aquaculture cycle, including during harvest of adult geoducks and subsequent replanting of new seed clams within PVC tubes under a protective blanket net. The response of eelgrass outside the plot may be relevant to discussions of buffer zones, given the implications of shoot density and biomass for habitat complexity and primary production. Infaunal abundance, taxa richness and diversity were measured annually in spring. The response of infauna may also be relevant to buffer zones considerations.

Effects of adult geoduck

Prior to harvest, adult geoducks were present at commercial densities within the cultured plot, and the density and above-ground biomass of *Z. marina* were not different between the cultured plot and reference area. Similarly, no differences were observed between the cultured plot and reference area in sediment organic content, infaunal abundance or taxa richness. However, *Z. marina* in the cultured plot had 102% higher below-ground biomass than in the reference area, and infaunal diversity was lower in the cultured plot than in the reference area.

Effects of geoduck harvest and replanting

Immediately after harvest, *Z. marina* was 44% less dense in the cultured plot than in the reference area. Above- and below-ground biomass were also lower in the cultured plot than in the reference area, and the cultured plot had lower sediment organic content.

Zostera marina was no longer present on the farm one year after harvest, following a period of heavy algal biofouling of the blanket nets after replanting. One year after the removal of nets and tubes, the farm was recolonized by *Z. marina*. Two years after the removal of nets and tubes, sediment organic content was higher in the cultured plot than in the reference area, suggesting that nets and tubes that were present earlier may reduce local sediment organic content. Sediment organic content was poorly predicted by quadrat-specific *Z. marina* biomass, suggesting that the effects of geoduck aquaculture on sediment organic content may be mediated by mechanisms other than eelgrass.

In the years following harvest and subsequent replanting, infaunal abundance and taxa richness in the cultured plot were lower than in the reference area. Diversity was lower in the cultured plot before harvest, and remained lower afterward. Infaunal abundance, richness and diversity were poorly predicted by quadrat-specific *Z. marina* biomass, suggesting that the effects of geoduck aquaculture on infauna are not mediated solely through eelgrass.

Conclusions

On the basis of the pre-harvest survey, the presence of adult geoducks at aquaculture densities appeared to have little influence on traits of *Z. marina* at the Samish Bay site. This result is consistent with findings from a previous study in South Puget Sound. Following harvest in this study, *Z. marina* density was 44% lower in the cultured plot than in the reference area. This difference is less than the 75% density reduction observed after harvest in South Puget Sound. The most dramatic effects of farming geoducks at this site were associated with biofouling of the blanket nets, which reduced light availability and resulted in the loss of *Z. marina* within the farm. The recovery of *Z. marina* began one year after the removal of tubes and nets during a subsequent culture cycle. It will likely take a number of years for eelgrass to recover to its pre-harvest density within this farm.

Following harvest, the cultured plot had lower infaunal abundance and richness, and temporarily reduced sediment organic content. Differences in eelgrass density did not explain these variations. More research is necessary to generalize the findings of this single-site study to geoduck aquaculture elsewhere.

4 Research Priorities & Monitoring Recommendations

The following research priorities and monitoring approaches are recommended to further assess possible ecological effects of geoduck aquaculture on the Puget Sound and Strait of Juan de Fuca environments. Needs were identified based on GARP project findings and the synthesis of current scientific knowledge provided in the updated literature review.

Research Priorities

Cumulative effects of geoduck culture

Bivalves in culture may alter nutrient cycling and affect ecological carrying capacity, but the scale of these changes is unknown. Models of nutrients, phytoplankton and zooplankton can be parameterized and targeted scenarios can be developed to predict these changes. Empirical data on the community structure and ecology in geoduck farms and reference plots should be integrated into predictive models (1) to evaluate direct and indirect ecosystem effects in scenarios involving future increases in the extent of geoduck aquaculture and (2) to identify appropriate indicator species that reflect the broader status of ecosystem health in response to geoduck aquaculture expansion. Such models can be used to broaden the context to basin-scale ecosystem function and multi-sector tradeoffs, and consider effects on species at higher trophic levels. Existing data sets could be leveraged to complete modeling tasks, and no new field programs would be necessary.

Water column effects

Performance indicators such as clearance efficiency or phytoplankton depletion footprints provide alternatives to ecological models for examining effects of geoduck culture on water quality. However, such approaches rely on accurate geoduck filtration rate data. Geoducks may locally reduce phytoplankton abundance and availability to other organisms. This localized feeding on phytoplankton (clearance) may reduce turbidity and, as a consequence, increase benthic macroalgae growth, resulting in shifts in primary productivity from pelagic to benthic sources. Additional information (e.g., accurate data on size- and age-specific clearance rates) is required to assess the impact of geoduck farms on water quality measurements, as well as the geoduck's ability to potentially compete with other suspension feeders and facilitate macrophyte growth. Although some data exist, new field and laboratory studies are likely necessary to develop accurate size- and age-specific clearance rate estimates.

Disease identification tools and prevalence in farmed populations

To fully assess the potential risks of geoduck diseases, continued exploration of the distribution, virulence and physiological tolerances of individual parasite species is needed. The recently found endosymbionts associated with wild geoduck populations may also affect cultured stocks. Conversely, the higher densities of farmed geoducks may exacerbate the possibility of amplifying parasite populations within farms or rapidly transmitting them to wild stocks. Gathering further information about geoduck endosymbiont life cycles, host–parasite interactions and prevalence in farmed stocks will assist in future fishery management decisions regarding geoduck aquaculture and stock movement. Extensive sample collection in the field and characterization of pathogens in the laboratory will be required to understand disease prevalence in farmed populations and potential transmission to wild geoducks.

Reproductive contribution from farms

The pelagic larval stages of geoducks provide genetic connectivity via migration among locales, yet little is known about the spatial and temporal distributions of geoduck larvae from farmed and wild populations. Almost nothing is known about settlement of juveniles. Understanding these pre-recruitment processes is important for sustainable shellfish aquaculture. The study of larval movement and settlement would enhance managers' ability to quantify the effects of farmed geoducks on wild populations, predict the synergistic effects of ocean acidification and declining water quality, and ensure self-sustaining wild populations. Field deployment of larval traps coupled with microchemical analyses of trapped larval shells and genetic analyses, or both, will be required to understand the dynamics of larval contributions from farms.

Sterile triploid reversion

Triploid geoducks may reduce risk of genetically perturbing wild stocks. Investigating triploid geoducks is critical for understanding the extent to which triploidy could help prevent genetic change to wild stocks. An analysis of the potential for triploid reversion at different sites is necessary, requiring a time series of flow cytometric analyses of certified triploid geoducks.

Local adaptation

Aquaculture of native shellfish can impact nearby ecological systems and wild conspecifics by creating opportunities for genetic impacts on native populations. Wild populations may be genetically adapted to local environmental conditions. Interbreeding with cultured geoducks from other locales may disrupt patterns of local adaptation, potentially jeopardizing wild populations by decreasing their adaptive potential. A significant impediment to sustainable aquaculture is the lack of information on adaptive differences between farmed and wild stocks. This information could be incorporated into a model to predict the genetic impacts of culturing native shellfish (see "Genetic risk model"). Transplant field experiments and new genomic information would be necessary to gain information on local adaptation.

Genetic risk model

The level of reproductive contribution from farmed stocks to wild systems that would result in low risk of genetic change depends on the effective population size in wild populations and the effective number of breeders used in hatcheries. This allowable genetic contribution from farmed stocks can be estimated using predictive models. A genetic risk model is needed that includes effects of environmental processes occurring on different scales as potential drivers of viability, allowable hatchery contributions and optimal yield for each region. Data are sufficient to complete initial modeling tasks and no new field programs are necessary; additional data (e.g., see preceding "Local adaptation") would refine model utility.

Site specificity of geoduck aquaculture's ecological effects

One important next step to understand the ecological effects of geoduck aquaculture and how farm siting may influence these effects is a carefully designed study of site characteristics focused on correlations among geoduck biodeposit accumulation, changes in community structure, and physical characteristics. Biodeposition by filter-feeding bivalves can alter benthic community structure, and the accumulation of biodeposits likely depends on specific physical site characteristics that affect flushing such as fetch, currents, exchange and freshwater inputs. Such a study would likely require extensive fieldwork across multiple sites to characterize physical and biological patterns over an extended period of time.

Innovations in aquaculture production

Research must be responsive to ongoing changes in practices and techniques used for geoduck aquaculture, including timing of outplants, predator protection, and density and tidal height. For example, novel methods for subtidal geoduck aquaculture may produce different effects than intertidal operations. The GARP results, as well as previous studies, suggest that patterns of natural disturbance are important criteria for predicting effects of shellfish aquaculture. Intertidal zones are typically more dynamic than subtidal zones and experience annual, extensive natural disturbance from storms, waves, boat wakes, flooding and so forth. Because of relatively frequent disturbance, community structure in intertidal zones is generally more resilient to disturbance than subtidal communities. Geoduck aquaculture disturbances in less variable subtidal zones may exert relatively stronger effects on the associated soft-bottom communities. Understanding effects in the subtidal environment would require extensive field data collection, which is complicated by water depth and would require a trained dive team.

Monitoring recommendations

Two new approaches for monitoring environmental effects of geoduck aquaculture are recommended. Ongoing monitoring should (1) be cost effective (2) use standard techniques and methods (3) be based on previous research findings and (4) accurately characterize the environment. The monitoring system should provide timely information as relevant environmental changes occur. The new approaches areas follows.

Benthic community structure monitoring

Results of GARP studies on resident macrofauna communities did not clearly identify indicator species (i.e., species that may act as an early warning of substantial effects) because no taxa showed strong, generalizable responses to aquaculture practices. Moreover, the traditional approach to monitor benthic communities, and thus indicator species, is sample collection for taxonomic identification and enumeration, which is labor intensive and costly. One potential proxy for identifying shifts in community structure is quantification of accumulated biodeposits (feces and pseudofeces). The literature review identified studies suggesting the balance of biodeposition and flushing may be the strongest determinants of community structure. Monitoring biodeposits (i.e., measuring sediment organic content) is relatively inexpensive and does not require highly technical methods, but it does hold promise as an indicator of changes associated with possible aquaculture effects. This approach would be informed by research on site specificity of geoduck aquaculture ecological effects, described previously as a priority.

Genetic monitoring of hatchery seed

It is important to monitor the genetic diversity and the number of seed produced by hatcheries to accurately estimate the allowable reproductive contribution from hatchery to wild populations. Hatcheries need to adopt breeding protocols to maximize genetic diversity and reduce the potential for genetic perturbation of wild stocks via interbreeding.

5

Program-Related Communications

Copies of representative presentations and publications are available on the WSG Geoduck Aquaculture Research Program website at *http://www.wsg.washington.edu/research/geoduck*.

Publications (Peer-Reviewed)

Vadopalas, B., T. W. Pietsch, and C. S. Friedman. 2010. The proper name for the geoduck: resurrection of *Panopea generosa* Gould, 1850, from the synonymy of *Panopea abrupta* (Conrad, 1849) (Bivalvia: Myoida: Hiatellidae). *Malacologia*, 52(1):169-173.

Publications (Not Peer-Reviewed)

Smith, R., and McDonald, P. S. 2010. Examining the effects of predator exclusion structures associated with geoduck aquaculture on mobile benthic macrofauna in South Puget Sound, Washington. *Northwestern Undergraduate Research Journal*, 5(2009-2010):11-16.

Theses and Dissertations

Price, J. 2011. Quantifying the ecological impacts of geoduck (*Panopea generosa*) aquaculture harvest practices on benthic infauna. M.S. thesis, University of Washington, Seattle.

Horwith, M. 2011. Plant behavior and patch-level resilience in the habitat-forming seagrass *Zostera marina*. Ph.D. dissertation, University of Washington, Seattle.

Media Placements

Wang, Deborah. 2008. Clam wars. *KUOW Puget Sound Public Radio News*, Seattle. Sept. 25.

Ma, Michelle. 2009. Skirmish continues over shellfish farming in Puget Sound. The *Seattle Times*, Seattle, Mar. 7.

Wang, Deborarh. 2009. University of Washington researchers say geoduck funding in jeopardy. *KUOW Puget Sound Public Radio News*, Seattle. Apr. 15.

Welch, Craig. 2009. Geoducks: Happy as clams. *Smithsonian*, Mar. Online: *http://www.smithsonianmag.com/science-nature/Happy-As-Clams.html*.

Stang, John. 2011. Economic benefits, ecological questions stall geoduck industry's growth. The *Kitsap Sun*, Kitsap County, Washington. Jul. 23.

Presentations

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McDonald, P. S. 2008. Effects of geoduck aquaculture on ecosystem structure and function: a progress report. Presentation to the National Shellfisheries — Pacific Coast Section/Pacific Coast Shellfish Growers Association Annual Meeting, Chelan, Washington, Oct. 3.

VanBlaricom, G. 2008. Guest class lecture for class, Ocean 506: Writing about science and technology for general audiences. University of Washington, Seattle, Oct. 8.

VanBlaricom, G. 2008. Geoduck clam aquaculture on the intertidal habitats of southern Puget Sound: Assessment of ecological impacts and mitigation of regional-scale cultural conflict. Presentation to the Water Center Seminar Series, University of Washington, Seattle, Oct. 28.

VanBlaricom, G. 2008. Ecological effects of geoduck aquaculture: The battle of southern Puget Sound. Presentation to a Workshop titled "Communicating Ocean and Marine Science." Centers for Ocean Sciences Education Excellence, University of Washington, Seattle, Nov. 22.

VanBlaricom, G. 2009. Geoduck aquaculture investigations in Puget Sound: Digging deep for answers. Presentation to the Sound Science Seminar Series, Washington Sea Grant, Union, Washington, Feb. 26.

VanBlaricom, G. 2009. Planting and harvest as disturbances in geoduck aquaculture: An overview and preliminary observations. Presentation to the 17th Conference for Shellfish Growers, Washington Sea Grant, Union, Washington, Mar. 3.

VanBlaricom, G. 2009. Another resource collision? Projecting interactions of sea otters with geoduck clam populations and fisheries in Washington and British Columbia. Presentation to Sea Otter Conservation Workshop VI, Seattle Aquarium, Seattle, Mar. 21.

Smith, R. 2009. Examining the effects of predator exclusion structures associated with geoduck aquaculture on mobile benthic macrofauna in South Puget Sound, Washington. Presentation to the 101st Annual meeting of the National Shellfisheries Association, Savannah, Georgia, Mar. 24.

VanBlaricom, G. 2009. Planting and harvest as disturbances in geoduck aquaculture: An overview and preliminary observations. Presentation in the State Capitol Fish & Wildlife Seminar Series, Washington Department of Fish and Wildlife, Olympia, Washington, Jun. 9.

Larson, K. 2009. Trophic implications of structure additions associated with intertidal geoduck aquaculture. Presentation to the National Shellfisheries — Pacific Coast Section/Pacific Coast Shellfish Growers Association Annual Meeting, Portland, Oregon. Sept. 30.

Price, P. 2009. Disturbance and recovery of a benthic community in response to geoduck aquaculture harvest. Presentation to the National Shellfisheries — Pacific Coast Section/Pacific Coast Shellfish Growers Association Annual Meeting, Portland, Oregon, Sept. 30.

VanBlaricom, G. 2009. Relative abundances of native (*Americorophium salmonis*) and invasive (*Monocorophium* spp.) gammaridean amphipods in geoduck aquaculture plots on intertidal habitats in southern Puget Sound. Presentation to the National Shellfisheries — Pacific Coast Section/Pacific Coast Shellfish Growers Association Annual Meeting, Portland, Oregon, Sept. 30.

Galloway, A. 2009. Effects of geoduck aquaculture planting practices on fish and macroinvertebrate communities in southern Puget Sound, Washington. Presentation to the National Shellfisheries — Pacific Coast Section/Pacific Coast Shellfish Growers Association Annual Meeting, Portland, Oregon, Sept. 30.

Larson, K. 2009. Trophic implications of structure additions associated with intertidal geoduck aquaculture. Presentation to the 63rd Joint Annual Meeting of the National Shellfisheries Association — Pacific Coast Section and the Pacific Coast Shellfish Growers Association. Portland, Oregon, Sept. 28-Oct. 1.

Price, J. 2009. Disturbance and recovery of a benthic community in response to geoduck aquaculture harvest. Presentation to the 63rd Joint Annual Meeting of the National Shellfisheries Association — Pacific Coast Section and the Pacific Coast Shellfish Growers Association. Portland, Oregon, Sept. 28-Oct. 1.

VanBlaricom, G. 2009. Relative abundances of native (*Americorophium salmonis*) and invasive (*Monocorophium* spp.) gammaridean amphipods in geoduck aquaculture plots on intertidal habitats in southern Puget Sound. Presentation to the 63rd Joint Annual Meeting of the National Shellfisheries Association — Pacific Coast Section and the Pacific Coast Shellfish Growers Association. Portland, Oregon, Sept. 28-Oct. 1.

Galloway, A. 2009. Effects of geoduck aquaculture planting practices on fish and macroinvertebrate communities in southern Puget Sound, Washington. Presentation to the 63rd Joint Annual Meeting of the National Shellfisheries Association — Pacific Coast Section and the Pacific Coast Shellfish Growers Association. Portland, Oregon, Sept. 28-Oct. 1.

Cornwell, J. C., R. I. E Newell, and M. Owens. 2009. The influence of geoduck clam culture and harvest in Puget Sound on sediment nutrient biogeochemistry. Presentation to the Coastal and Estuarine Research Federation 20th Biennial Conference, Portland, Oregon, Nov. 1-5.

Galloway, A. Culture practices and structure effects of intertidal geoduck aquaculture operations in Puget Sound: An evaluation of influence on mobile macrofauna. Presentation to the Coastal and Estuarine Research Federation 20th Biennial Conference, Portland, Oregon, Nov. 1-5.

McDonald, P. S. 2009. Trophic implications of complex littoral habitats: comparison of aquaculture structure, natural structure, and unstructured habitat, Washington. Presentation to the Coastal and Estuarine Research Federation 20th Biennial Conference, Portland, Oregon, Nov. 1-5.

Price, J. 2009. Assessing the impacts of geoduck aquaculture harvest practices on benthic infaunal communities. Presentation to the Coastal & Estuarine Research Federation 20th Biennial Meeting. Portland, Oregon, Nov. 5.

Cornwell, J. C., R. I. E Newell, and M. Owens. 2010. The influence of geoduck clam culture and harvest in Puget Sound on sediment nutrient biogeochemistry. Presentation to the 102nd Annual Meeting of the National Shellfisheries Association and World Aquaculture Society, Aquaculture 2010, San Diego, California, Mar. 1-5.

McDonald, P. S. 2010. Challenges to the evaluation of ecological effects of bivalve aquaculture: social and economic constraints, and contradictory incentives from ecological and statistical theory. Presentation to the 102nd Annual Meeting of the National Shellfisheries Association and World Aquaculture Society, Aquaculture 2010, San Diego, California, Mar. 1-5.

McDonald, P. S. 2010. A fisheye perspective on habitat complexity: Do structures associated with intertidal geoduck aquaculture affect trophic dynamics of nekton in unique ways? Presentation to the 102nd Annual Meeting of the National Shellfisheries Association and World Aquaculture Society, Aquaculture 2010, San Diego, California, Mar. 1-5.

Price, J. 2010. Difference in benthic community structure between geoduck (*Panopea generosa*) aquaculture sites and response to harvest events. Presentation to the 102nd Annual Meeting of the National Shellfisheries Association and World Aquaculture Society, Aquaculture 2010, San Diego, California, Mar. 1-5.

Price, J. 2010. Geoduck aquaculture harvest practices in southern Puget Sound, Washington: Assessing patterns of impact and recovery in benthic infaunal communities. Presentation to the 64th Joint Annual National Shellfisheries Association — Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Tacoma, Washington. Sept. 20-23, 2010.

McDonald P. S. 2010. Biotic communities associated with aquaculture structures: some aspects of recruitment, growth, and predation. Presentation to the 64th Joint Annual National Shellfisheries Association — Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Tacoma, Washington. Sept. 20-23, 2010.

VanBlaricom, G. R. 2011. Evaluation of ecological effects of geoduck aquaculture operations in intertidal communities of southern Puget Sound. Invited presentation at the Environmental Science Seminar Series, Environmental Program, Interdisciplinary Arts and Sciences Program, University of Washington, Tacoma, Washington, Feb. 7.

VanBlaricom, G. R. 2011. Ecological effects of geoduck aquaculture operations in southern Puget Sound. Invited presentation to the Panel on Aquaculture Research and Technical Support, Washington Sea Grant Program Site Review, Seattle, Mar. 3.

Price, J. L., P. S. McDonald, T. E. Essington, A. W. E. Galloway, M. N. Dethier, D. A. Armstrong, and G. R. VanBlaricom. 2011. Benthic community structure and response to harvest events at geoduck aquaculture sites in southern Puget Sound, Washington. Invited presentation to the Joint Annual Meeting, Society for Northwestern Vertebrate Biology and Washington Chapter of The Wildlife Society, Gig Harbor, Washington, Mar. 24.

Price, J. L., P. S. McDonald, G. R. VanBlaricom, J. R. Cordell, T. E. Essington, A. W. E. Galloway, M. N. Dethier, and D. A. Armstrong. 2011. Benthic community structure and response to harvest events at geoduck (*Panopea generosa*) aquaculture sites in southern Puget Sound, Washington. Oral presentation to the National Shellfisheries Association Annual Meeting. Baltimore, Maryland, Mar. 30.

Price, J. L. 2011. Geoduck harvest in Puget Sound: Is it an ecological problem? Invited presentation to the State Capital Seminar Series, Washington Department of Fish and Wildlife, Olympia, Washington, Jul. 13.

Price, J. L. 2011. Quantifying the ecological impact of geoduck (*Panopea generosa*) aquaculture harvest practices on benthic infauna. M.S. Thesis Defense, School of Aquatic and Fishery Sciences, University of Washington, Seattle. Aug. 8.

VanBlaricom, G. R. 2011. Ecological disturbances associated with harvests of cultured geoduck clams in southern Puget Sound, with implications for sustainability. Invited presentation to the Workshop on Washington State Environmental and Sustainability Learning Standards, Washington State Office of Public Instruction, Olympia, Washington, Aug. 24.

Hurn, H., J. Eggers, P. S. McDonald, and G. R. VanBlaricom. 2011. Effects of geoduck aquaculture on predation and growth of non-target clams. Oral presentation to the American Fisheries Society Annual Meeting, Seattle, Washington, Sept. 6.

McDonald, P. S., A. W. E. Galloway, J. L. Price, K. McPeek, D. A. Armstrong, G. R. VanBlaricom, and K. Armintrout. 2011. Effects of geoduck aquaculture practices on habitat and trophic dynamics of nekton and macroinvertebrates in Puget Sound. Oral presentation to the American Fisheries Society Annual Meeting, Seattle, Washington, Sept. 6.

McDonald, P. S., A. W. E. Galloway, J. L. Price, K. McPeek, D. A. Armstrong, and G. R. VanBlaricom. 2011. Patterns in abundance of fish and macroinvertebrates associated with geoduck aquaculture. Oral presentation to the 65nd Annual Meeting of the Pacific Coast Shellfish Growers Association and the National Shellfish Association – Pacific Coast Section, Salem, Oregon, Sept. 20.

Armintrout, K., P. S. McDonald, K. McPeek, D. Beauchamp, G. R. VanBlaricom. 2011. Trophic ecology within geoduck aquaculture habitat. Oral presentation to the 65th Annual Meeting of the Pacific Coast Shellfish Growers Association and the National Shellfish Association – Pacific Coast Section, Salem, Oregon, Sept. 20.

VanBlaricom, G. R., J. L. Price, P. S. McDonald, J. R. Cordell, T. E. Essington, A. W. E. Galloway, M. N. Dethier, and D. A. Armstrong. 2011. Geoduck aquaculture harvest impacts: The results. Oral presentation to the 65th Annual Meeting of the Pacific Coast Shellfish Growers Association and the National Shellfish Association – Pacific Coast Section, Salem, Oregon, Sept. 20.

McDonald, P. S. 2012. The ecological effects of geoduck aquaculture: effects on fish and mobile macroinvertebrates. Invited presentation at the Geoduck Research Symposium, Union, Washington, Mar. 6.

Newell, R. I. E., J. C. Cornwell, M. S. Owens, 2012. The influence of geoduck clam culture and harvest in Puget Sound on sediment nutrient biogeochemistry. Invited presentation at the Geoduck Research Symposium, Union, Washington. Mar. 6.

VanBlaricom, G. R., J. L. Price, P. S. McDonald, J. R. Cordell, M. N. Dethier, K. K. Holsman, K. C. McPeek, A. W. E. Galloway, T. E. Essington, and D. A. Armstrong. 2012. Ecological consequences of geoduck (*Panopea generosa*) aquaculture for infaunal assemblages in southern Puget Sound. Invited presentation at the Geoduck Research Symposium, Union, Washington, Mar. 6.

McDonald, P. S., P. F. Stevick, A. W. E. Galloway, K. McPeek, D. A. Armstrong, and G. R. VanBlaricom. 2012. Nekton, nets, and tubes: macrofauna response to intertidal geoduck aquaculture operations in Puget Sound, Washington USA. Oral presentation at the National Shellfisheries Association Annual Meeting, Seattle, Washington, Mar. 25-29.

VanBlaricom, G. R., A. W. E. Galloway, K. C. McPeek, J. L. Price, J. R. Cordell, M. N. Dethier, D. A. Armstrong, K. K. Holsman, and P. S. McDonald. 2012. Effects of predator exclusion structures as agents of ecological disturbance to infaunal communities in geoduck clam aquaculture plots in southern Puget Sound, Washington, USA. Oral presentation at the National Shellfisheries Association Annual Meeting, Seattle, , Mar. 25-29.

Price, J. L., G. R. VanBlaricom, and P. S. McDonald. 2012. Effects of harvest activity on infaunal communities in geoduck clam aquaculture plots in southern Puget Sound, Washington, USA. Oral presentation at the National Shellfisheries Association Annual Meeting, Seattle, Mar. 25-29.

McPeek, K. C., G. R. VanBlaricom, P. S. McDonald, and D. S. Beauchamp. 2012. Effects of geoduck aquaculture on the growth and stable isotope signatures of Pacific staghorn sculpin. Oral presentation at the National Shellfisheries Association Annual Meeting, Seattle, Washington, Mar. 25-29.

VanBlaricom, G. R. 2012. Beyond academia: Partnerships for success. Seminar and panel discussion with four other participants. Seminar series sponsored by Washington Sea Grant and the Centers for Ocean Sciences Education Excellence – Ocean Learning Communities: Beyond the Ivory Tower: Tools and techniques for reaching audiences and broadening the impacts of your research, University of Washington, Seattle, Mar. 26.

McPeek, K. C., G. R. VanBlaricom, D. A. Beauchamp, and P. S. McDonald. 2012. Patterns of utilization of geoduck aquaculture plots by Pacific staghorn sculpin in Puget Sound, Washington: results from mark-recapture and stable isotope studies. Oral presentation at the 66th Annual Joint Meeting, Pacific Coast Section, National Shellfisheries Association, and Pacific Coast Shellfish Growers Association, Tulalip, Washington, Sept. 23-27.

McDonald, P. S., Z. Oyafosu, A. W. E. Galloway, J. L. Price, K. C. McPeek, D. A. Armstrong, and G. R. VanBlaricom. 2012. Is a picture worth a thousand words? What results of photo analysis reveal about the effects of geoduck aquaculture practices. Oral presentation at the 66th Annual Joint Meeting, Pacific Coast Section, National Shellfisheries Association, and Pacific Coast Shellfish Growers Association, Tulalip, Washington, Sept. 23-27.

VanBlaricom, G. R., A. W. E. Galloway, K. C. McPeek, J. L. Price, J. R. Cordell, M. N. Dethier, D. A. Armstrong, and P. S. McDonald. 2012. Effects of predator exclusion structures as agents of ecological disturbance to infaunal communities in geoduck clam aquaculture plots in southern Puget Sound, Washington, USA. Oral presentation at the 66th Annual Joint Meeting, Pacific Coast Section, National Shellfisheries Association, and Pacific Coast Shellfish Growers Association, Tulalip, Washington, Sept. 23-27.

McPeek, K. C., G. R. VanBlaricom, D. A. Beauchamp, and P. S. McDonald. 2012. Patterns of utilization of geoduck aquaculture plots by Pacific staghorn sculpin in Puget Sound, Washington: Results from mark-recapture and stable isotope studies. Annual Cooperators Meeting, Washington Cooperative Fish and Wildlife Research Unit, US Geological Survey, Seattle, Sept. 27.

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Santacruz, A., B. Vadopalas, and C. S. Friedman. 2008. Endosymbiotic, commensal and parasitic organisms associated with wild geoduck clams (*Panopea abrupta*). Presentation to the Pacific Coast Shellfish Growers Association joint conference with the Pacific Coast Section of the National Shellfisheries Association in Chelan, Washington, Oct. 3.

Friedman, C. S., A. Santacruz, and B. Vadopalas. 2009. Endosymbiotic, commensal and parasitic organisms associated with wild geoduck clams. Presentation to the 17th Conference for Shellfish Growers, Washington Sea Grant Program, Alderbrook Resort, Union, Washington, Mar. 2-3.

Vadopalas, B., T. W. Pietsch, C. S. Friedman. 2009. Resurrection of *Panopea generosa* (Gould, 1850, from the synonymy of *P. abrupta* (Conrad, 1849). Presentation to the 63rd Joint Annual Meeting of the National National Shellfisheries Association — Pacific Coast Section and the Pacific Coast Shellfish Growers Association, Portland, Oregon, Sept. 28-Oct. 1.

Friedman, C. S., A. Santacruz, and B. Vadopalas. 2010. Endosymbiotic, commensal and parasitic organisms associated with wild geoduck clams (*Panopea generosa*). Presentation to the 102nd Annual Meeting of the National Shellfisheries Association and World Aquaculture Society, Aquaculture 2010, San Diego, California, Mar. 1-5.

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Friedman, C. S. 2012. Characterizing trends in endosymbionts of native geoduck, *Panopea generosa*. Invited presentation at the Geoduck Research Symposium, Union, Washington, Mar. 6.

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6 Appendices

Appendix I

Ecological effects of the harvest phase of geoduck clam (*Panopea generosa* Gould, 1850) aquaculture on infaunal communities in southern Puget Sound, Washington USA.

Revised submission draft: 11 October 2013

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Short title: Ecosystem effects of geoduck aquaculture harvest

Key words: *Aquaculture, benthic, disturbance, extralimital, geoduck, infauna, intertidal,* Panopea generosa*, Puget Sound, spillover.*

Abstract

Intertidal aquaculture for geoduck clams (*Panope generosa* Gould, 1850) is expanding in southern Puget Sound, Washington USA, where gently sloping sandy beaches are used for field culture. Geoduck aquaculture contributes significantly to the regional economy, but has become controversial because of a range of unresolved questions involving potential biological impacts on marine ecosystems. From 2008 through 2012 we used a "before-after-control-impact" experimental design, emphasizing spatial scales comparable to those used by geoduck culturists, to evaluate the effects of harvesting of market-ready geoduck clams on associated benthic infaunal communities. We sampled infauna at three different study locations in southern Puget Sound at monthly intervals before, during, and after harvests of clams, and along extralimital transects extending away from edges of cultured plots to assess effects of harvest activities in adjacent uncultured habitat. Using multivariate statistical approaches we found strong seasonal and spatial signals in patterns of abundance, but we found little evidence of effects on community structure associated with geoduck harvest disturbances within cultured plots. Likewise we found no indication of significant "spillover" effects of harvest on uncultured habitat adjacent to cultured plots. Complementary univariate approaches revealed little evidence of harvest effects on infaunal biodiversity and indications of modest effects on populations of individual infaunal taxa. Of ten common taxa analyzed only three showed evidence of reduced densities, although minor, following harvests, whereas the remaining seven taxa indicated either neutral responses to harvest disturbances or increased abundances, either during or in the months following harvest events. We suggest that a relatively active natural disturbance regime, including both small-scale and large-scale events that occur with comparable intensity but more frequently than geoduck harvest events in cultured plots, has facilitated assemblage-level infaunal resistance and resilience to harvest disturbances.

Introduction

Aquaculture operations are proliferating and diversifying in nearshore marine habitats across the globe (e.g., Naylor et al. 2000, Chopin et al. 2001, Goldburg & Naylor 2005, Buschman et al. 2009, Lorenzen et al. 2012, Samuel-Fitwi et al. 2012). Although frequently of positive societal benefit, aquaculture enterprises have raised concerns regarding possible negative ecological consequences among resource managers, scientists, conservation advocacy organizations, political leaders and legislators, and the interested lay public (e.g., Simenstad and Fresh 1995, Newell 2004, Sara 2007, Dumbauld et al. 2009, Forrest et al. 2009, Coen et al. 2011, Hedgecock 2011). Since the early 2000s localized but intensive political controversy has emerged in communities near southern Puget Sound, Washington USA, regarding development of geoduck clam (*Panopea generosa* Gould, 1850) aquaculture operations on gently-sloping intertidal sand habitats. Geoduck aquaculture activity is increasingly contributing to Puget Sound's total commercial geoduck production that also includes substantial wild harvests. In 2011 cultured geoducks comprised about 25% of the total commercial harvest in Washington and generated revenues of about US$20M. As a consequence of expanding geoduck aquaculture operations, many questions and concerns have emerged regarding ecological effects of harvesting activities.

Our focus is on evaluation of possible ecological changes to marine ecosystems as a result of habitat disturbances associated with geoduck aquaculture activity in southern Puget Sound. We regard ecological disturbance as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substratum availability, or the physical environment" (Pickett & White 1985). Disturbances generally may be natural or anthropogenic and may occur on a wide range of magnitudes and spatiotemporal scales. Natural disturbances are known to be important determinants of community dynamics in many marine benthic habitats (e.g., Connell 1978, VanBlaricom 1982, Sousa 1984, Dumbauld et al. 2009). However, frequent and intensive anthropogenic disruptions may overwhelm evolved natural resistance or resilience to habitat disturbance in benthic communities (Sousa 1984, Paine et al. 1998).

The geoduck aquaculture cycle includes the following phases, each constituting potential ecological disturbances to resident organisms. Young hatchery clams are outplanted at the initiation of the cycle. At the same time predator exclusion structures are placed to limit losses of young clams to mobile consumers such as crabs and shorebirds. Structures include arrays of vertically emplaced polyvinyl chloride (PVC) tubing extending above the sediment surface. Young clams are placed in sediments within the tubes (typically 3-4 individuals per tube), after which tubes are covered either with large nets that extend over the entire tube field, or individual "cap nets" that cover each tube but leave intervening spaces uncovered. Typical initial stocking density at outplanting is 20-30 clams/m^2, and the tubes and netting are removed 1-2 years after outplanting when clams are sufficiently large and deeply buried that risks of predation are minimal. Tube diameter, tube density, within-tube clam density at outplanting, netting type, and timing of removal of tubes and netting vary by grower preference. Clams are left in place for the grow-out phase until they reach optimal market size.

The culture cycle is terminated by harvest 5-7 years after outplanting. During low tides, individual clam siphons are located visually and marked with small wooden stakes pressed into the sediment. Individual clams so located are subsequently extracted by hand after liquefaction of sediments within a radius of 15-30 cm of the siphon, extending into the sediment the length of the clam siphon. Liquefaction is achieved with a handheld nozzle ("stinger") supplied with sea water pumped into an attached hose from a small barge offshore. The process is highly efficient in the hands of experienced harvesters, with extraction of each clam requiring 5 s or less under optimal conditions. Time required for complete harvest of a given cultured plot may range from a few days to many months. Duration of harvest varies with plot size, density of market-sized clams, weather and sea conditions, availability of skilled and experienced laborers, and grower preference. Harvests may be done during high tides by divers also using stingers if schedules for extreme low tides are unfavorable in the context of labor availability, market price, or shipping cost conditions.

Disturbance of sediments as a result of cultured geoduck harvests may have ecological consequences that extend beyond cultured plots to adjacent areas of un-harvested substrata, causing extralimital changes in benthic communities. There is significant management interest in potential "spillover" effects of geoduck harvest, particularly relating to regulation of spatial scope of cultured geoduck plots and potential requirements for uncultured buffer zones between cultured plot boundaries. Geoduck harvest activities produce disturbances confined to explicit spatial boundaries, and create a distinctive interface in physical processes between harvested and unharvested substrata. When harvest occurs, suspended sediments, biogenic detritus, and possibly benthic organisms could be carried onto adjacent sediments either by water pumped across intertidal habitats during harvest, or by along-shore currents during flood tides immediately following harvest. The export of benthic organisms, sediment, detritus and nutrient materials could affect resident infaunal populations at intensities varying with distances from edges of harvested plots.

Here we report results of a field study to determine if geoduck aquaculture harvest operations alter benthic infaunal invertebrate assemblages of intertidal sand flats in southern Puget Sound. We chose infaunal assemblages as response variables for three reasons: First, our opinion *a priori* was that selected organisms would likely be more sensitive to cultured geoduck harvest effects than other ecosystem components, given that the physical habitats of infauna are directly disturbed in harvest operations by design. Second, benthic infauna and epifauna in the Puget Sound region are known to be important as prey for mammals, birds, mobile invertebrates, and fish, including juvenile salmonid populations migrating from natal freshwater habitats seaward via Puget Sound. Minimization of detrimental disturbances to significant prey populations is viewed as crucial to restoration of imperiled salmonid populations in the region. Third, the known high densities of infauna in habitats used for geoduck aquaculture ensured that samples collected in our study would produce high counts of organisms, with zero values rare or absent, facilitating an effective and rigorous community-based investigation in a quantitative context.

We tested three related hypotheses (identified by number in subsequent text), using coupled multivariate and univariate statistical methods to evaluate the significance of relevant contrasts:

1. Within plots subject to harvests ("harvest plots"), infaunal assemblages will be similar to those in adjacent plots not designated for harvest ("reference plots") before harvest occurs;
2. Prior to harvest, infaunal assemblages over a range of distances away from the edge of harvest plots ("transect samples") will be similar to assemblages in harvest plots, and to adjacent reference plots. After harvest, data from transect samples will show a trend of increasing similarity to data from reference plots and decreasing similarity to data from within harvest plots, with increasing distances away from the edges of harvest plots;
3. Within harvest plots, benthic infaunal assemblages will be altered significantly following completion of harvests, as a consequence of harvest-related disturbances.

Methods

Study Areas

Our study was conducted at intertidal locations in the southern basin of Puget Sound, Washington, USA. Puget Sound is an estuarine fjord, with the southern basin defined as the interconnected marine waters south and west of Tacoma Narrows (47.27° N, 122.55° W). Surface area of the basin is 449 km^2 at mean high water, including 67.4 km^2 of intertidal habitat (Burns 1985). The area contains extensive gently sloping sandy and muddy intertidal habitats, many of which are biologically appropriate for bivalve aquaculture operations. Mean daily tidal fluctuation in the southern basin ranges from 2.7 to 3.2 m in a mixed semidiurnal pattern (Mofjeld et al. 2002), with a maximum range of 6.4 m for single tidal exchanges at the extreme southern limit of the basin (National Ocean Service, National Oceanic and Atmospheric Administration). Surface water temperatures range annually from ~8 to ~16° C, and salinities from 27 to 30‰ excepting periods of dilution from riverine flooding (Collias et al. 1974, Dethier & Schoch 2005).

Three study sites were chosen (Figure 1) based on three criteria. First, selected sites were involved in production-scale commercial aquaculture at the time of our anticipated field sampling. Our site selections had the purpose of fostering relevance of our study to the spatial and temporal scales typical of the geoduck aquaculture industry. Second, the culture cycle at selected sites was approaching the terminal harvest phase, allowing us to sample before, during, and after harvest at treatment and adjacent reference plots in time periods ≤30 months. Third, sediments, slope, and exposure to weather and sea were generally similar among the selected sites, and were in all cases similar to the typical physical attributes of sites customarily utilized by the geoduck aquaculture industry (gently sloping intertidal sediments that are primarily fine sands with silt/clay fractions <20% by mass, and at least moderately protected from exposure to wind and sea by local topography).

The three study sites were as follows. "Foss" (47.22˚ N, 122.82˚ W) was located on the eastern shore of Case Inlet near Joemma Beach State Park. "Manke" (47.20˚ N, 122.84˚ W) was near Pt. Wilson on the eastern shore of Harstene Island, which forms the western shore of Case Inlet. Cultured plots at Foss and Manke were operated by Taylor Shellfish, Inc. (Shelton, Washington USA) specifically for geoduck aquaculture at the time of our study. "Chelsea" (47.13˚ N, 122.96˚ W) was on the northwestern shore of Eld Inlet. At the time of our study the cultured plot at Chelsea was owned by Chelsea Farms LLC (Olympia, Washington USA), with nearby areas used for Manila clam (*Venerupis philippinarum* [Adams and Reeve, 1850]) and Pacific oyster (*Crassostrea gigas* [Thunberg, 1793]) aquaculture as well as for geoduck clams. Neither Taylor Shellfish, Inc., nor Chelsea Farms LLC made any effort whatsoever to influence study design, sampling procedures, generation and analyses of resulting data, or interpretations of results as provided herein or elsewhere.

Sampling Design and Methods

We utilized a "Before-After-Control-Impact" design (Green 1979), establishing a cultured (i.e. "impact") plot containing mature geoduck clams, and an unplanted reference (i.e. "control") plot, each measuring at least 2500 m^2, at each of our three sites. Cultured plots at each site were subject to geoduck harvest during the course of the study while reference plots experienced no harvest activity. None of our study plots had been used for geoduck aquaculture prior to our project. Within each site the cultured and reference plots were of equal size and shape, with similar sedimentary composition (based on qualitative assessments *a priori*), slope and elevation within the tidal zone. Cultured and reference plots were separated by a buffer zone of at least 75 m to minimize effects of intrinsic differences due to location, while simultaneously providing adequate separation distance to reduce potential extralimital effects of the harvest process on the reference plot (Figure 2a). Plots were marked with PVC stakes at the two shoreward corners. Cultured and reference plots were divided into 100 x 100 unit Cartesian grids and 10 sampling points were randomly selected within each plot for each sampling date, without replacement across sampling dates. One core sample was collected at each sampling point on each sampling date.

At each site at least one extralimital transect was established, extending away from each cultured plot and running parallel to shoreline for distances of 50-60 m. Each transect extended from an origin at the midpoint of one of the two edges of the cultured plot that ran perpendicular to the shoreline. The entire length of each transect was in an area free of planted geoduck clams or other types of aquaculture, except at Chelsea where the first ten meters of the transect crossed over a young cohort of planted geoducks. Areas spanned by transects experienced no harvest activity during the course of the study.

At each site three benthic core samples were taken on each sampling date at distances of 2, 5, 10, 20, and 50 m from the edge of the cultured plot along the transect (2, 5, 10, 12, 15, 20, 30, & 60 m at Chelsea). At each distance, one core sample was taken on the transect line, and one each approximately 30 cm to either side (in shoreward and seaward directions) of the transect line. Core sampling points along the transect lines were shifted slightly (≤ 1 m) to avoid re-sampling the same point during subsequent sampling events.

Benthic core samples were 5 cm in diameter with surface area 19.6 cm^2, depth 10 cm, and volume 196 cm^3. All contents of each core sample were placed unscreened in 500 ml jars and preserved in 10% buffered formalin solution immediately after collection. Following the laboratory processing methods of Simenstad et al. (1991) and Sobocinski et al. (2010), freshwater was added to each sample followed by mixing until sediments settled to the bottom and elutriated organisms floated to the surface. Fluid was decanted through a 500-micron screen and all organisms retained on the collection screen were removed and preserved in 70% isopropanol for eventual identification and enumeration. The process was repeated several times for each sample to ensure that all organisms had been separated from the sediments. Organisms were identified to the level of species or genus when feasible, but in all cases at least to family level. Family level identification of infaunal organisms has been found sufficient for many types of marine environmental studies (e.g., Ferraro & Cole 1990, Somerfield & Clarke 1995, Hernández Arana et al. 2005) including some in Puget Sound (e.g., Dethier 2010). Identified samples were subjected to quality assurance and control checks by specialists to ensure accurate identification. We did not estimate infaunal biomass densities in our study.

Each site was sampled as often as possible, but no more frequently than monthly, as allowed by low tide patterns and by competing sampling activities at other study sites. Our minimum goal for each site was four monthly sampling events prior to harvest, monthly sampling events during harvest activities for as long as they continued, and four monthly sampling events following completion of the harvest. Our study design did not include sampling targeted specifically to times immediately following harvest activity (i.e., within hours to a few days), possibly resulting in underestimation of short term ecological consequences of harvesting. The actual number of dates sampled was different from site to site due to variations in harvest timing and site accessibility. Harvest duration and sampling duration varied by site, and modest differences in sediment composition were detected among sites. As a result, data from each site were analyzed independently and the sites were not considered replicates.

For descriptive summaries, numbers of organisms in each core sample (hereinafter “sample”) were converted to estimated densities (individual organisms of all species per m^2). For each sampling date, all samples were averaged to single point estimates for each taxon in each plot by date, with certain exceptions as noted below. Standard errors were calculated for each point estimate.

For direct assessment of within-plot harvest effects, analyses were done for the following categories: “treatment” (samples collected on cultured versus reference plots), “date” (samples collected on each sampling date), and “harvest state” (samples collected during different periods of geoduck harvest). Harvest state subcategories were: before the geoduck harvest (“pre-harvest”), during harvest (“mid-harvest” or “harvest period”), and after harvest (“post-harvest”).

For assessment of extralimital effects of harvesting based on transect sampling, categories were “treatment” (samples collected in cultured and reference plots versus samples collected at various distances along transects from the cultured plot edges), “date” (samples collected on each sampling date), and “harvest state” (samples collected during different periods of geoduck harvest, subcategories as indicated above).

Patterns of abundance in a species of particular interest in a management context, the benthic gammaridean amphipod *Americorophium salmonis* (Stimpson, 1857), were evaluated along with organisms occurring frequently in samples. *A. salmonis* is known to be an important prey species for juvenile outmigrating salmonid fish populations in Puget Sound, particularly Chinook salmon (*Oncorhynchus tshawytscha* [Walbaum, 1792]).

Multivariate Analyses

Permutation based analyses of variance (perMANOVA; Anderson 2001) were used to test for differences by site, treatment, date, and harvest state according to square-root transformed abundance data and Bray-Curtis indices of community similarity (Bray & Curtis 1957). For extralimital transect data, perMANOVAs were used to evaluate differences by plot type and distance on transects (treatment), date, and harvest state. In addition, the interaction of data subsets representing treatment and harvest state was tested for data collected from treatment and reference plots. A significant result from a test of the [harvest state]*[treatment] interaction term indicated an effect of the harvest state on one of the treatments, specifically the effect of the mid-harvest state on the cultured plot or on locations along extralimital transect lines.

Distance based tests for homogeneity of multivariate dispersion (HMD; Anderson 2006) were conducted to contrast levels of variability in community structure between treatment and reference plots, and for contrasts among plots data and locations on extralimital transects. HMD uses a Bray-Curtis distance matrix of species data to calculate the average distance in multivariate space between individual samples and the calculated centroid of the sample group. The average distance and the associated variability are compared between groups and tested for significance with permutation tests. An increase in the multivariate dispersion of samples with increased disturbance was predicted by Caswell & Cohen (1991). In addition, a number of environmental impact studies have reported that the variability of species abundance in samples collected from disturbed areas was greater than the variability of samples collected from non-disturbed areas when evaluated with HMD (Warwick and Clarke 1993). For contrasts of data from treatment and reference plots using HMD analyses, data on infaunal abundance by individual sample were used since averaging samples could mask important inter-sample variability, given the large number of replicate samples collected. At each site, HMD analyses were used to test differences between the cultured and reference plots within each harvest state, within plots among harvest states, and among samples from plots and varying distances on extralimital transects.

Univariate Analyses

Individual sample diversity was calculated using the Shannon index (Shannon 1948; also known as Shannon's diversity index, Shannon-Wiener index, and Shannon-Weaver index) on log-transformed data (e.g., Warwick et al. 1990). Two-sample t-tests were used to assess differences in diversity indices between plots within sites for each sampling date. In addition, one-way univariate analyses of variance (ANOVA) were used to evaluate the significance of differences in diversity indices between plot types on each date, between plot types for each harvest state, and within plot types between harvest states.

Some components of our data failed to meet underlying assumptions on which ANOVA methods are based, including normality and homoscedasticity. The subject assumptions are often violated by ecological data, but ANOVA procedures are frequently robust to the discrepancies (e.g., Underwood 1981). ANOVA methods have been applied in a number of other studies with data characteristics similar to ours (e.g., Smith and Brumsickle 1989, Warwick et al. 1990, Thrush et al. 1996, Kaiser et al. 1996, 2006, Anderson and Underwood 1997).

We used generalized linear mixed models (GLMMs; McCullagh and Nelder 1989) assuming Poisson-distributed data to examine the factors contributing to abundance of selected individual infaunal taxa from our core samples. We applied these analyses to *Americorophium salmonis* and nine other individual taxa (species, genera, or families) identified from high frequencies of occurrence in core samples. In our univariate analyses data from all sites were considered together. The fixed effects of month, plot type, harvest phase, and their interaction were included, as well as random effects of site. Models were fitted by maximum likelihood assuming a Laplace approximation in the "lme4" package (Bates & Maechler 2010) of R software (R Development Core Team 2011). Likelihood ratio tests were utilized to formally compare models including the [harvest state]*[treatment] interaction term. Regression coefficients and their 95% confidence intervals were calculated for each model.

Results

Descriptive Patterns

Percentages of sand in benthic habitats were 99.1 at the Foss study site, 98.8 at Manke, and 86.0 at Chelsea (Price 2011). Overall we identified fifty discernible animal taxa in our samples. The numerically dominant taxa were generally small (<1 cm maximum length of individuals) and resided on, or within a few centimeters below the sediment surface. The sampled benthic communities at all three sites consisted primarily of small polychaete worms (annelida), crustaceans (arthropoda), and bivalves (mollusca; Tables 1 and 2). Polychaetes were numerical dominants at all sites followed by crustaceans (Figures 3a, b, and c). Taxonomic compositions of our samples generally resembled those reported previously for southern Puget Sound (Dethier et al. 2003, 2010, 2012, Dethier & Schoch 2005, Dethier 2005, 2010).

Multivariate Contrasts by Site and Plot Type

Infaunal abundances were significantly different among study sites (perMANOVA, Table 3). At Foss and Manke, the infaunal sample data from the cultured plots were significantly different from those of reference plots (perMANOVA, Table 3, Figures 4a & 4b). At Chelsea the core sample data from the two plots did not differ significantly (perMANOVA, Table 3, Figure 4c).

Our perMANOVA analyses identified a number of significant differences based on site, date, or treatment in contrasts within and between plots (Table 3). However, none of the three assessments of the interaction term [harvest state]*[treatment] were found to be significant (perMANOVA, Table 3). For within plot contrasts, there were several cases of significant effects of both date and harvest state on reference plot data, illustrating that harvest state is a proxy for date and emphasizing the premise that the [harvest state]*[treatment] interaction term is the uniquely informative metric for assessment of harvest effects within our study design. Analytical results were inconsistent with hypotheses 1 and 3 as defined above. Because the interaction term was not significant in any case, significant differences between plots at Foss and Manke were likely the result of factors other than harvest-related disturbances.

Results for homogeneity of multivariate dispersion (HMD) analyses for cultured and reference plots at the three study sites likewise did not fit expectations consistent with geoduck harvesting as a primary source of disturbance. Eight significant contrasts were identified for comparisons within plot type among harvest states, of which four were in reference plots and four in cultured plots (Table 4). These results are inconsistent with the hypothesis of greater compositional variation in cases of frequent disturbance as posited in the literature (e.g., Caswell & Cohen 1991, Warwick & Clarke

1993) if harvesting of cultured geoducks is the primary source of disturbance in cultured habitats. The results are also inconsistent with our hypotheses 1 and 3. Occurrence of significant contrasts for HMD values in reference plots is consistent with active sources of variability or disturbance other than geoduck harvesting in the study areas.

Multivariate Contrasts by Distance on Extralimital Transects

We found little indication of trends in summed infaunal densities with increased distance from the cultured plot in three of the four extralimital transects (Figure 5). On the Foss south transect, a significant trend was observed during the mid-harvest period. All other variations within transects were consistent with random distributions in space and time.

Significant effects of [harvest state]*[treatment] interaction terms were not detected for any combination of data from plots and transect distances at any of the study sites (perMANOVA, Tables 5, 6, & 7). By contrast, there were many cases of significant terms for contrasts of data from specific transect locations with treatment, date, and harvest state (Tables 6 and 7). Patterns in the results are inconsistent with an ecologically significant effect of harvest extending beyond the limits of the cultured plots. Conversely, the results are consistent with significant variation in transect and plot data based on processes independent of harvest activities. The results are also inconsistent with our hypotheses 2.

Within each site, the HMD values for community data from the pre-harvest state were similar across the cultured and reference plots and the various distances along transects (Tables 8 and 9). At Foss and Manke, the HMD values for cultured plots increased during the mid-harvest state while values in reference plots either remained relatively constant or decreased. For both sites HMD calculations for cultured plots during the mid-harvest state were significantly different from values at most transect distances and the reference plot (Table 9). During the post-harvest state at Foss, HMD values in the cultured plot remained high while values for most transect locations and the reference plot returned to near pre-harvest levels. At Manke post-harvest HMD values were similar to pre-harvest values at most transect distances and in cultured and reference plots. HMD values increased for most distances on the Chelsea transect during the mid-harvest state. However, permutation tests revealed that infaunal data from Chelsea were most similar among locations during mid-harvest (Table 9). In summary, HMD analyses for transect data generally were inconsistent with hypothesis 2.

Univariate Analyses

Values for the Shannon index for core samples at Foss and Chelsea were similar between the cultured and reference plots over time (Figures 6a and 6c). At Manke index values fluctuated more among dates on both plots but the cultured plot had consistently lower diversity indices (Figure 6b). When diversity values were averaged by harvest state, there was a mixture of significant and non-significant values in contrasts between plots for each harvest state and within plots among harvest states (Table 10).

Species-specific contrasts, using GLMMs, provided results in six categories for the ten taxa analyzed (Table 11). As noted the analyses were based on the protocol that a significant interaction result for [harvest state]*[treatment] was an indication of a significant effect of harvest activities on subject populations, manifested by density data either during or after the harvest events in the study areas. Three taxa, the gammaridean amphipod *Americorophium salmonis*, the cumacean *Cumella vulgaris*, and the polychaete family Capitellidae experienced increased abundances in harvest plots as compared to reference plots both during and following harvest activities. Conversely, two other taxa, the bivalve genus *Rochefortia* and the polychaete family Phyllodocidae experienced reductions in harvest plots as compared to reference plots during and after harvests. Two taxa in a third group, the nemertean genus *Micrura* and the polychaete family Spionidae were not affected positively or negatively by harvests either during or following harvest events. Data for the remaining three taxa indicated more complex population-level response patterns to harvests. The polychaete family Goniadidae showed increased abundance in harvested plots during harvest, as compared to reference plots, but the effect did not persist following completion of harvest. The polychaete family Polynoidae was not influenced numerically during harvests, but declined in harvest plots as compared to reference plots once harvests were completed. Finally, the polychaete family Hesionidae was negatively affected by harvest activities during the harvests as compared to reference plots, but the negative effect did not persist once the harvests were completed.

Discussion

Our study revealed only modest effects on infaunal communities from the harvest phase of geoduck aquaculture operations. Multivariate analyses indicated an absence of significant shifts in community composition (both means and variability) at any of the three study sites as a result of harvesting activities. Similarly, we found little evidence of a significant "spillover" effect of cultured geoduck harvest operations on resident infaunal communities. Univariate analyses of variance provided no evidence of significant impacts of cultured clam harvest on the biodiversity of resident infauna. Of the ten most frequently sampled infaunal taxa only three indicated evidence of reduction in abundance persisting as long as four months after conclusion of harvest activities. None of the proportionate changes in the three affected species approached local extinction.

Our results led us to reject our three hypotheses listed above. Some of our data suggested consistency with hypothesis 1, with significant differences between treatment category at the Foss and Manke sites. However, analyses of the [harvest state]*[treatment]* interaction term revealed that the subject differences were due to plot properties independent of harvest-related disturbance effects. Despite scattered temporary exceptions it is apparent that none of our hypotheses are generally applicable in our study sites.

Our results are similar to a recent experimental study of ecosystem-level effects of geoduck clam aquaculture done in British Columbia (BC), Canada (Department of Fisheries and Oceans 2012). Abundances of resident infauna showed temporary effects of clam harvest disturbance and a strong pattern of seasonal effects. There were observed effects of harvest on sediment chemistry and physical structure within but not beyond the planted area. All observed effects were temporary. Interpretation of results may have been compromised to some degree by the small plot size used in the BC study as compared to commercially operated geoduck farms.

The benthic community data we collected revealed variation in community compositions among sites. Sediment grain size distribution at our Chelsea study site was substantially different from the other two sites, which were similar to one another, and likely contributed to community differences (e.g., Gray 1981, Dethier & Schoch 2005). It has been shown that salinity decreases from north to south in Puget Sound (Collias et al. 1974, Dethier & Schoch 2005), and that variation in salinity can impact benthic community structure in a number of locations including Puget Sound (Tenore 1972, Bulger et al. 1993, Constable 1999, Smith & Witman 1999, Dethier & Schoch 2005). Differences among sites in resident benthic communities were consistent with previous studies that found substantial variation in benthic assemblages among intertidal sand flats in Puget Sound (Dethier et al. 2003, Dethier & Schoch 2005). Intertidal sand flats in Case Inlet, location of our Foss and Manke study sites, are particularly noteworthy for high beach-to-beach and year-to-year variation in resident benthos (Dethier 2005).

Because of habitat variations described above we determined that our three study sites could not be considered replicates. As a result we analyzed our data separately for each site. Such an approach had the unavoidable effect of reducing statistical power for detection of significant differences. Nevertheless, we found a number of significant differences in our data relating to date, a proxy for both season and harvest state, and between study plots within our study sites. Our resulting contention is that our study had the ability to detect major patterns of variation in the system, and that natural spatial and temporal variability in the subject assemblages were substantially more important than effects of harvest disturbances. When we found differences in abundance patterns between plots within study sites associated with harvest state, we invariably also found that harvest state was effectively a proxy for seasonal variation in harvested plots. Thus, harvest state unavoidably co-varied with date and associated seasonal effects and was not an informative stand-alone treatment factor for understanding harvest effects. Consistently, our most informative metric for an unambiguous harvest impact, the [harvest state]*[treatment] interaction term, was not significant in our analyses. Interaction term R^2 values were consistently low, typically explaining less than five percent of variation in the data. When date was used as the explanatory variable, significant values resulted in nearly all cases. Date as a factor had high R^2 values, usually accounting for more than 50% of the variation in the community dataset.

With regard to multivariate assemblage contrasts and univariate biodiversity analyses used in our study, our decision to analyze data from different study sites independently raises questions regarding the propriety of applying analyses of variance to our data (e.g., Hurlbert 1984). The dilemma in design of our study was the large size and relative scarcity of potential study plots that fit our selection criteria. Hurlbert's design rubrics to the contrary notwithstanding, Oksanen (2001) has argued that large-scale field studies with attributes such as ours are fully appropriate for application of analyses of variance. We note that Hurlbert's dogmatic perspective on design and analysis in field ecology has become increasingly questioned (e.g., Oksanen 2001, Schank and Koehnle 2009). Oksanen asserts that reflexive application of Hurlbert's dogma, to cases of design dilemmas such as in our study, amounts to "entirely unwarranted stigmatization of a reasonable way to test predictions referring to large scale systems."

In contrast to our results, other investigations of effects of shellfish harvesting have reported detectable impacts and variable durations of community recovery ranging from a few months to a year (Kaiser et al. 1996, Hall & Harding 1997, Spencer et al. 1998, Mistri et al. 2004, Morello et al. 2006). Results of our study are also different from many other experimental studies that found significant effects of various types of disturbance on benthic infauna, with recovery times ranging from several weeks up to 9 months in duration (e.g. VanBlaricom 1982, Smith & Brumsickle 1989, Thrush et al. 1996, Dernie et al. 2003, Zajac & Whitlatch 2003, Kaiser et al. 2006). There are several possible reasons for the strikingly different results in our study. First, physical habitat modifications associated with geoduck harvest may be unlike other types of harvest-associated disturbances of benthic infauna. Bottom trawling, suction dredge harvesting and clam raking, as examples, are substantially different methods with associated disturbances qualitatively distinctive from one another as well as from geoduck harvest. Second, experimental studies on benthic community disturbance have used methods such as sediment removal, sterilization, and defaunation, setting the point of initiation of observed recovery sequences at zero abundance by definition. The method by which geoduck clams are harvested has the potential to displace benthic organisms without injury or death, allowing recolonization of disturbed patches immediately after harvest. Third, the scales of disturbances evaluated in other published studies are different from the scale of disturbances occurring at harvest of cultured geoducks. Most experimental studies reported in the peer-reviewed literature utilized small patches (< 5 m^2 surface area) to quantify disturbance effects and implemented a spatially uniform disturbance regime. Geoduck harvest occurs on large spatial scales (plots that are typically 2500 m^2 or more in surface area) and creates a non-uniform disturbance regime within harvested plots. Survival of outplanted geoducks, typically placed in uniform distributional arrays, is generally less than 100% over time. Spatial variability of clam mortality is normal within a cultured plot during the multi-year production cycle, often resulting in non-uniform spatial distributions of clams within cultured plots at the time of harvest. It follows that disturbances associated with harvest of a cultured plot will be patchy in space. Another level of patchiness is associated with likely variation among individual cultured clams in detection probability of siphons on the sediment surface at harvest. If the visibility of individual geoducks to a harvester is patchy in space, then clam-by-clam harvest disturbances will also be patchy in space. The scale and patchiness involved in geoduck harvest as compared to the uniform disturbance and small scale of other experimental disturbance studies could diffuse any impacts over such a large area that the effect of harvest is undetectable and possibly trivial from the ecosystem perspective.

Our univariate analyses of selected individual taxa involved inclusion of site as a random effect and are not subject to the criticisms of design as emphasized by Hurlbert (1984). We identified three taxa with abundances that increased during the harvest phase in cultured plots and remained elevated in the months following completion of harvest. Such patterns suggest the possibility that the presence of adult geoduck clams at high densities near the termination of the culture cycle had a negative effect on the subject populations, and that the effect was removed at the time of harvest. The putative mechanisms for such an impact are unclear, but potentially could include modification of chemical or physical attributes of the sediments. Another plausible mechanism is subtle modification of micro-scale patterns of water movement as a consequence of the high living biomass density of geoduck clams in cultured plots. Cummings et al. (2001) identified variations in abundances of some species of an infaunal assemblage that were linked inversely to variations in densities in adult populations of a large filter-feeding bivalve. Elucidation of causal linkages between reduced densities of geoduck clams at harvest, and subsequent infaunal abundance patterns, was beyond the scope of our study. The matter would be an informative topic for future study.

We suggest that a principal reason for the apparent insensitivity of resident infauna to cultured geoduck harvest disturbances in southern Puget Sound is accommodation of the infaunal assemblage to a significant natural disturbance regime. It has been hypothesized that rates of ecosystem recovery from disturbances correlate with the extent to which species in the subject ecosystem have adapted to past disturbances (e.g., Connell 1978, Connell & Keough 1985), and that benthic ecosystems in sandy sediments show rapid resilience to disturbances (Collie et al. 2000). The intertidal zone of Puget Sound is affected by an array of disturbance processes that vary by frequency, intensity, physical and chemical attributes, and spatial scale. Disturbances with a high potential for ecological significance in the region include: a) small waves resulting from normal wind shear (e.g., Maunder 1968, Anderson 1972, Clarke et al. 1982, Gabrielson and Lukatelich 1985); b) wakes from vessel passage (e.g., Crawford 1984, Garrad & Hay 1987, Osborne & Boak 1999, Bishop 2007); c) thermal stress associated with daytime low tides in summer months (e.g., Dethier 2010, Dethier et al. 2010 & 2012); d) large waves caused by wind storms (e.g., Lynott and Cramer 1966, Reed 1980, Steenburgh and Mass 1996, Mass and Dotson 2010); e) flooding events caused by maxima in rainfall or snowmelt in watersheds draining to Puget Sound (e.g., Ferber et al. 1993, Zhu and Newell 1998, Colle and Mass 2000, Frascari et al. 2006, Lohrer et al. 2006, Forrest et al. 2007, Warner et al. 2012); and f) sediment liquefaction and small tsunami generation by seismic activity and associated subaerial and possibly submarine landslides (e.g., Atwater 1987, Hampton et al. 1996, Atwater 1999, Sherrod 2001, Williams and Hutchinson 2000, Gonzáles 2002, Ichinose et al. 2004, Wiest et al. 2007, Kao et al. 2008, Arcos 2012). Tidally-driven along-shore currents may intensify disturbance effects by transporting suspended or epibenthic materials away from disrupted locations (e.g., Adams et al. 2007, Bourrin et al. 2008, Denny et al. 2013). Benthic communities of Puget Sound have likely adapted to the array of natural disturbances and could therefore be resilient to other similar types of physical disturbances, including those of anthropogenic origin. The small-scale and large-scale natural disturbances typical of the area provide a rate of physical intervention to intertidal sedimentary environments substantially higher than rates of significant disturbances caused by geoduck aquaculture operations in a given plot. In addition, we note that Puget Sound is quite young in geological and oceanographic contexts, being only 5,000 years of age in current configuration following glacial recession, resultant isostatic rebound, and eustatic sea level rise (Armstrong et al. 1965, Easterbrook 1969, Burns 1985, Thorson 1989, Bucknam et al. 1992, Finlayson 2006). As a consequence, resident marine assemblages may be dominated by relatively opportunistic species arguably accommodated to, and relatively unaffected by, physical disturbance of various types. Thus, we argue that the prevailing natural disturbance climate in the region effectively has selected the infaunal assemblage toward tolerance of, and resilience to, the types of disturbances associated with geoduck aquaculture operations. Naturally-evolved characteristics pre-adaptive to effects of anthropogenic disturbances are known for a number of marine and fresh-water benthic species across many habitat types (e.g., Pearson & Rosenberg 1978, Tomassetti and Porrello 2005, Melzner et al. 2009, Gabel et al. 2011).

As also noted in McDonald et al. (in press), we caution that projection of our results to larger temporal or spatial scales may be inappropriate in the absence of additional studies. Our study sites were relatively isolated from other geoduck aquaculture plots, and were being utilized for aquaculture of geoducks for the first time. Our data may not provide a sufficient basis for unequivocal extrapolation to cases wherein a given plot is exposed to a long series of successive geoduck aquaculture cycles. Likewise, it may not be appropriate to extend our findings to cases where a number of separate plots are adjacent to one another and encompass significantly larger surface areas than any single plot. Resolution of the questions of larger spatial and temporal scales will be a major challenge for geoduck farmers as they continue production on existing plots and expand into new areas, and an important research goal in the interests of informed management policies by natural resource agencies.

Acknowledgments

Funding for our work was provided by the Washington State Legislature, the National Aquaculture Research Program of the National Oceanic and Atmospheric Administration, the Washington State Departments of Ecology and Natural Resources, the Royalty Research Fund of the University of Washington, the Shellfish Management Department of the Point No Point Treaty Council, and the Ecosystems Branch of the U.S. Geological Survey. Access to study sites was facilitated by Taylor Shellfish Inc., Chelsea Farms LLC, D. Adams, M. Adams, T. Bloomfield, S. Bloomfield, B. Foss, L. Foss, and B. Phipps. J. Cordell, M. Dethier, and J. Toft provided guidance and expertise on laboratory procedures and infaunal taxonomy crucial to completion of our study. K. Armintrout, K. Connelly, B. Cummings, J. Eggers, A. Fuller, A. Galloway, M. Langness, K. McPeek, P.F. Stevick and many volunteers provided vital laboratory and field support for the project. The staff of the Washington Sea Grant Program, particularly P. Dalton and R. Waters, provided generous and valuable administrative support. C. Schwartz provided expert and patient guidance and artistic skill in improving our graphics. Constructively critical comments were provided on draft versions of the manuscript by D. Cheney, S. Shumway, and two anonymous reviewers. J. Davis and B. Vadopalas provided additional editorial guidance and assistance. We offer sincere thanks to all. Any use of trade product or firm name herein is for descriptive purposes only and does not imply endorsement by the US Government.

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Tables and Figures

Table 1. Dominant infaunal taxa in core sample data, selected on the basis of frequencies of occurrence or (for A. salmonis) ecological significance. Frequency calculations are based on all core samples taken on all sampling events within cultured and reference plots at all three study sites during the study. Codes for "ecological notes" are: BD: Burrow-dweller; CTD: Commensal dweller in tubes of other invertebrates; DF: Deposit feeder; EFDF: Epistrate feeder (scrapes attached detrital or living plant or bacterial cells from individual sand grains) when living in sandy habitats, deposit feeder when living in muddy or silty habitats (Weiser 1956); M: Mobile; MCOS: Mobile carnivore, omnivore, or scavenger (varies by species within the family); SDSS: Selective detritivore on sediment surface; SF: Suspension feeder; TD: Tube-dweller. In the Spionidae, mode of habit (tube-dweller or mobile) varies by species.

Taxon	Category	Frequency	Ecological notes
Americorophium salmonis (Stimpson, 1857)	Amphipod crustacean	0.71	TD, SDSS
Cumella vulgaris (Hart, 1930)	Cumacean crustacean	0.92	EFDF
Rochefortia spp. Vélain, 1877	Bivalve mollusk	0.98	CTD, SF
Micrura spp. Ehrenberg, 1871	Nemertean	0.94	M, DF
Capitellidae Grube, 1862	Polychaete annelid	0.94	BD, DF
Goniadidae Kinberg, 1866	Polychaete annelid	0.94	MCOS
Spionidae Grube, 1850	Polychaete annelid	0.98	TD or M, SDSS
Hesionidae Grube, 1850	Polychaete annelid	0.94	MCOS
Phyllodocidae Örsted, 1843	Polychaete annelid	0.81	MCOS
Polynoidae Malmgren, 1867	Polychaete annelid	0.81	MCOS

Table 2. Mean densities (individuals/m2 (se)) rounded to nearest integer, by site and plot type for all sampling dates during the study as determined from core samples. Listed taxa are those identified and described in Table 1.

Taxon	Foss		Manke		Chelsea		Culture Mean	Reference Mean	Overall Mean
	Culture	Reference	Culture	Reference	Culture	Reference			
Americorophium salmonis	3,529 (882)	11,936 (710)	1,579 (796)	2.498 (952)	15 (8)	7 (5)	1,568 (441)	4,140 (1,080)	2,854 (597)
Cumella vulgaris	567 (194)	490 (127)	435 (80)	1,531 (307)	1,611 (540)	1,630 (637)	862 (203)	1,291 (254)	1,077 (163)
Rochefortia spp.	287 (92)	367 (113)	1,462 (419)	3,395 (743)	1,181 (190)	2,584 (497)	1,061 (194)	2,332 (388)	1,696 (227)
Micrura spp.	188 (52)	520 (94)	268 (38)	347 (46)	192 (35)	211 (60)	222 (24)	347 (40)	284 (24)
Capitellidae	718 (596)	310 (185)	979 (434)	772 (404)	4,368 (2501)	1,241 (258)	2,040 (883)	807 (195)	1,424 (454)
Goniadidae	1,217 (450)	1,700 (636)	900 (234)	1,436 (452)	1,369 (366)	1,125 (268)	1,139 (182)	1,401 (261)	1,270 (162)
Spionidae	766 (154)	602 (159)	406 (101)	833 (150)	1,567 (446)	1,499 (367)	887 (174)	995 (151)	941 (115)
Hesionidae	2,728 (449)	9,495 (3,304)	4,288 (2,110)	5,547 (598)	552 (286)	848 (280)	2,634 (920)	5,014 (1,175)	3,824 (755)
Phyllodocidae	252 (80)	126 (47)	505 (113)	538 (80)	124 (47)	269 (105)	312 (58)	341 (55)	326 (40)
Polynoidae	97 (33)	146 (58)	123 (26)	332 (56)	187 (51)	207 (88)	137 (22)	242 (41)	190 (24)

Table 3. Summary of perMANOVA results for contrasts at scales of study sites and plots.

*•••: p<0.001; ••: 0.001≤p<0.01; •: 0.01≤p<0.05; NS: p≥0.05.

Scale	Contrast	R^2	df	p*
Among sites	All sites	0.37	2	•••
	Foss vs. Manke	0.19	1	•••
	Foss vs. Chelsea	0.44	1	•••
	Manke vs. Chelsea	0.27	1	•••
Among sites within plot type, cultured plots	Foss vs. Manke	0.19	1	•••
	Foss vs. Chelsea	0.41	1	•••
	Manke vs. Chelsea	0.24	1	•••
Among sites within plot type, reference plots	Foss vs. Manke	0.39	1	•••
	Foss vs. Chelsea	0.56	1	•••
	Manke vs. Chelsea	0.38	1	•••
Within site between plot type, by treatment	Foss	0.41	1	•••
	Manke	0.45	1	•••
	Chelsea	0.09	1	NS
Within site between plot type, by date	Foss	0.60	10	•
	Manke	0.62	16	•••
	Chelsea	0.75	13	•••
Within site between plot type, by harvest state	Foss	0.18	2	•
	Manke	0.17	2	•••
	Chelsea	0.08	2	NS
Within site between plot type, [harvest state]*[treatment] interaction	Foss	0.02	2	NS
	Manke	0.03	2	NS
	Chelsea	0.03	2	NS
Within site within plot type, by date, cultured plots	Foss	1.00	10	•••
	Manke	1.00	16	•••
	Chelsea	1.00	13	•••
Within site within plot type, by harvest state, cultured plots	Foss	0.25	2	NS
	Manke	0.25	2	•••
	Chelsea	0.13	2	NS
Within site within plot type, by date, reference plots	Foss	1.00	10	•••
	Manke	1.00	16	•••
	Chelsea	1.00	13	•••
Within site within plot type, by harvest state, reference plots	Foss	0.32	2	•
	Manke	0.25	2	••
	Chelsea	0.11	2	NS

Table 4. Summary of Homogeneity of Multivariate Dispersion (HMD) analytical results for contrasts at scales of study sites and plots. Probability codes are defined in Table 3.

Scale	Contrast	df	p
Among harvest states within plot type, Foss cultured plots	Pre-harvest vs. mid-harvest	1	••
	Pre-harvest vs. post-harvest	1	NS
	Mid-harvest vs. post-harvest	1	••
Among harvest states within plot type, Manke cultured plots	Pre-harvest vs. mid-harvest	1	NS
	Pre-harvest vs. post-harvest	1	NS
	Mid-harvest vs. post-harvest	1	NS
Among harvest states within plot type, Chelsea cultured plots	Pre-harvest vs. mid-harvest	1	•
	Pre-harvest vs. post-harvest	1	•
	Mid-harvest vs. post-harvest	1	NS
Among harvest states within plot type, Foss reference plots	Pre-harvest vs. mid-harvest	1	NS
	Pre-harvest vs. post-harvest	1	••
	Mid-harvest vs. post-harvest	1	NS
Among harvest states within plot type, Manke reference plots	Pre-harvest vs. mid-harvest	1	••
	Pre-harvest vs. post-harvest	1	NS
	Mid-harvest vs. post-harvest	1	•
Among harvest states within plot type, Chelsea reference plots	Pre-harvest vs. mid-harvest	1	NS
	Pre-harvest vs. post-harvest	1	•
	Mid-harvest vs. post-harvest	1	NS
Within sites within plot type, among harvest states	All states, Foss culture plot	2	••
	All states, Foss reference plot	2	••
	All states, Manke culture plot	2	NS
	All states, Manke reference plot	2	•
	All states, Chelsea culture plot	2	NS
	All states, Chelsea reference plot	2	•
Within sites between plot type, within harvest states	Foss, pre-harvest	1	NS
	Foss, mid-harvest	1	••
	Foss, post-harvest	1	•
	Manke, pre-harvest	1	••
	Manke mid-harvest	1	•••
	Manke post-harvest	1	NS
	Chelsea pre-harvest	1	NS
	Chelsea mid-harvest	1	NS
	Chelsea post-harvest	1	NS

Table 5. Summary of perMANOVA results for contrasts within plots and transect locations within study sites, by date and harvest state. Locations include cultured plot, reference plot, and each sampled distance on transect lines. Probability codes are defined in Table 3.

Transect and contrast	Location on transect (m)	R^2	df	p
Foss North, date	2	1.00	10	•••
	5	1.00	10	•••
	10	1.00	10	•••
	20	1.00	10	•••
	50	1.00	10	•••
Foss North, harvest state	2	0.38	2	•••
	5	0.33	2	•
	10	0.26	2	NS
	20	0.27	2	NS
	50	0.25	2	NS
Foss South, date	2	1.00	10	•••
	5	1.00	10	•••
	10	1.00	10	•••
	20	1.00	10	•••
	50	1.00	10	•••
Foss South, harvest state	2	0.27	2	NS
	5	0.29	2	NS
	10	0.27	2	NS
	20	0.27	2	NS
	50	0.37	2	•
Manke North, date	2	1.00	16	•••
	5	1.00	16	•••
	10	1.00	16	•••
	20	1.00	16	•••
	50	1.00	16	•••
Manke North, harvest state	2	0.23	2	••
	5	0.16	2	••
	10	0.27	2	•••
	20	0.24	2	•••
	50	0.12	2	••
Chelsea North, date	2	1.00	13	•••
	5	1.00	13	•••
	10	1.00	13	•••
	12	1.00	13	•••
	15	1.00	13	•••
	20	1.00	13	•••
	30	1.00	13	•••
	60	1.00	13	•••
Chelsea North, harvest state	2	0.12	2	NS
	5	0.18	2	NS
	10	0.15	2	NS
	12	0.12	2	NS
	15	0.16	2	NS
	20	0.16	2	NS
	30	0.16	2	NS
	60	0.26	2	NS

Table 6. Summary of perMANOVA results for contrasts within plots within study sites and within transect locations, by treatment, date, and harvest state (part 1). Analyses were done for all transect locations (cultured plot and reference plot as well as each transect location), but only statistically significant results are shown. Probability codes are defined in Table 3.

Transect and contrast	Location on transect (m)	R^2	df	p
Foss North, cultured plot, treatment	2	0.10	1	•
	5	0.17	1	•••
Foss North, cultured plot, date	2	0.62	10	••
	5	0.59	10	•
	10	0.67	10	•••
	20	0.68	10	•••
	50	0.68	10	•••
Foss North, cultured plot, harvest state	2	0.21	2	•••
	5	0.18	2	••
	10	0.19	2	••
	20	0.18	2	•
	50	0.17	2	•
Foss North, reference plot, treatment	2	0.23	1	•••
	5	0.28	1	•••
	10	0.17	1	••
	20	0.17	1	•••
	50	0.11	1	•
Foss North, reference plot, date	10	0.64	10	••
	20	0.59	10	•
	50	0.66	10	•••
Foss North, reference plot, harvest state	2	0.18	2	•
	10	0.16	2	•
	20	0.16	2	•
	50	0.18	2	•
Foss South, cultured plot, treatment	2	0.15	1	•••
	5	0.14	1	•••
	10	0.11	1	•
	20	0.13	1	•••
	50	0.19	1	•••
Foss South, cultured plot, date	2	0.58	10	•
	5	0.62	10	••
	10	0.64	10	•••
	20	0.60	10	••
Foss South, cultured plot, harvest state	2	0.16	2	•
	5	0.17	2	•
	10	0.18	2	•
	20	0.16	2	•
Foss South, reference plot, treatment	2	0.19	1	•••
	5	0.21	1	•••
	10	0.16	1	•••
	50	0.18	1	••
Foss South, cultured plot, date	10	0.58	10	•
	20	0.70	10	•••
	50	0.64	10	•
Foss South, cultured plot, harvest state	2	0.16	2	•
	5	0.17	2	•
	10	0.17	2	•
	20	0.18	2	••
	50	0.19	2	•

Table 7. Summary of perMANOVA results for contrasts within plots within study sites and within transect locations, by treatment, date, and harvest state (part 2). Analyses were done and are presented as described in Table 6. Probability codes are defined in Table 3.

Transect and contrast	Location on transect (m)	R2	df	p
Manke North, cultured plot, treatment	5	0.05	1	•
	20	0.10	1	•••
Manke North, cultured plot, date	2	0.66	16	•••
	5	0.62	16	•••
	10	0.65	16	•••
	20	0.57	16	••
	50	0.63	16	•••
Manke North, cultured plot, harvest state	2	0.16	2	•••
	5	0.16	2	•••
	10	0.18	2	•••
	20	0.14	2	•••
	50	0.17	2	•••
Manke North, reference plot, treatment	2	0.09	1	•••
	5	0.05	1	•
	10	0.06	1	••
	20	0.06	1	•
Manke North, reference plot, date	2	0.57	16	••
	5	0.67	16	•••
	10	0.64	16	•••
	20	0.66	16	•••
	50	0.64	16	•••
Manke North, reference plot, harvest state	2	0.16	2	•••
	5	0.19	2	•••
	10	0.17	2	•••
	20	0.16	2	•••
	50	0.14	2	•••
Chelsea North, cultured plot, treatment	60	0.07	1	•
Chelsea North, cultured plot, date	2	0.72	13	•••
	5	0.69	13	•••
	10	0.75	13	•••
	12	0.68	13	•••
	15	0.66	13	•••
	20	0.67	13	•••
	30	0.69	13	•••
	60	0.66	13	•••
Chelsea North, cultured plot, harvest state	5	0.11	2	•
	20	0.11	2	•
	60	0.12	2	•
Chelsea North, reference plot, treatment	30	0.07	1	•
	60	0.12	1	•••
Chelsea North, reference plot, date	2	0.69	13	•••
	5	0.68	13	•••
	10	0.70	13	•••
	12	0.66	13	•••
	15	0.64	13	•••
	20	0.67	13	•••
	30	0.67	13	•••
	60	0.58	13	••
Chelsea North, reference plot, harvest state	60	0.11	2	•

Table 8. Summary of Homogeneity of Multivariate Dispersion (HMD) analytical results within study sites and plots, among transect locations. Transect locations include cultured plot and reference plot as well as each sampled location on transects. All indicated contrasts had six degrees of freedom. Probability codes are defined in Table 3.

Site	Harvest State	p
Within site within harvest state, among transect locations, Foss North	Pre-harvest	NS
	Mid-harvest	••
	Post-harvest	••
Within site within harvest state, among transect locations, Foss South	Pre-harvest	•
	Mid-harvest	••
	Post-harvest	••
Within site within harvest state, among transect locations, Manke North	Pre-harvest	•
	Mid-harvest	•••
	Post-harvest	•
Within site within harvest state, among transect locations, Chelsea North	Pre-harvest	••
	Mid-harvest	•
	Post-harvest	NS

Table 9. Summary of Homogeneity of Multivariate Dispersion (HMD) analytical results within study sites between cultured plots and transect locations (the latter include reference plot as well as each sampled location on transects), for each study site. Probability codes are defined in Table 3.

Contrast and location (m)	Harvest State	p, Foss North	p, Foss South	p, Manke North	p, Chelsea North
Cultured plot vs. Reference plot	Pre-harvest	NS	NS	•••	NS
	Mid-Harvest	•••	•••	•••	NS
	Post-Harvest	•	•	NS	NS
Cultured plot vs. 2 m	Pre-harvest	NS	NS	NS	NS
	Mid-Harvest	NS	•••	•••	NS
	Post-Harvest	•••	•	NS	•••
Cultured plot vs. 5 m	Pre-harvest	NS	NS	NS	NS
	Mid-Harvest	•••	••	•••	NS
	Post-Harvest	•••	•	••	NS
Cultured plot vs. 10 m	Pre-harvest	NS	•	••	••
	Mid-Harvest	•	NS	•••	NS
	Post-Harvest	NS	•	NS	NS
Cultured plot vs. 12 m	Pre-harvest	-	-	-	NS
	Mid-Harvest	-	-	-	NS
	Post-Harvest	-	-	-	NS
Cultured plot vs. 15 m	Pre-harvest	-	-	-	NS
	Mid-Harvest	-	-	-	NS
	Post-Harvest	-	-	-	NS
Cultured plot vs. 20 m	Pre-harvest	NS	NS	••	NS
	Mid-Harvest	••	•	•••	NS
	Post-Harvest	NS	••	•	NS
Cultured plot vs. 30 m	Pre-harvest	-	-	-	•••
	Mid-Harvest	-	-	-	NS
	Post-Harvest	-	-	-	•
Cultured plot vs. 50 m	Pre-harvest	NS	NS	NS	-
	Mid-Harvest	•••	•••	••	-
	Post-Harvest	•	•••	NS	-
Cultured plot vs. 60 m	Pre-harvest	-	-	-	••
	Mid-Harvest	-	-	-	••
	Post-Harvest	-	-	-	NS

Table 10. One-way ANOVA results for Shannon indices of diversity for samples at all sites. Analyzed contrasts include differences between reference and cultured plots for each state as well as differences between states within each plot. All indicated contrasts had one degree of freedom. Probability codes are defined in Table 3.

Study site & scale	Contrast	F	p
Foss, between treatments	Pre-harvest	0.68	NS
	Mid-Harvest	0.24	NS
	Post-Harvest	3.49	NS
Manke, between treatments	Pre-harvest	19.24	•••
	Mid-Harvest	30.12	•••
	Post-Harvest	12.92	•••
Chelsea, between treatments	Pre-harvest	5.35	•
	Mid-Harvest	0.001	NS
	Post-Harvest	1.60	NS
Foss, within cultured plot, between harvest states	Pre-harvest vs. mid-harvest	0.17	NS
	Pre-harvest vs. post-harvest	17.74	•••
	Mid-harvest vs. post-harvest	13.59	•••
Manke, within cultured plot, between harvest states	Pre-harvest vs. mid-harvest	15.36	•••
	Pre-harvest vs. post-harvest	4.97	•
	Mid-harvest vs. post-harvest	2.41	NS
Chelsea, within cultured plot, between harvest states	Pre-harvest vs. mid-harvest	0.04	NS
	Pre-harvest vs. post-harvest	4.79	•
	Mid-harvest vs. post-harvest	3.04	NS
Foss, within reference plot, between harvest states	Pre-harvest vs. mid-harvest	0.56	NS
	Pre-harvest vs. post-harvest	3.70	NS
	Mid-harvest vs. post-harvest	0.67	NS
Manke, within reference plot, between harvest states	Pre-harvest vs. mid-harvest	0.37	NS
	Pre-harvest vs. post-harvest	4.08	•
	Mid-harvest vs. post-harvest	4.84	•
Chelsea, within reference plot, between harvest states	Pre-harvest vs. mid-harvest	10.38	•••
	Pre-harvest vs. post-harvest	3.58	NS
	Mid-harvest vs. post-harvest	0.14	NS

Table 11. Results of univariate assessments of harvest impacts with Generalized Linear Mixed Models for abundant or ecologically significant individual infaunal taxa as sampled by coring. The test statistic is the likelihood ratio test for the interaction term [harvest state]*treatment]. The metric represented is the sign of the coefficient of the interaction term, where harvest phase is before-harvest, mid-harvest, or post-harvest, and treatment is either cultured plot or reference plot. All indicated contrasts had two degrees of freedom. Taxa are those described in Tables 1 and 2. Probability codes are defined in Table 3

Taxon	Results of likelihood ratio tests		Apparent effect of harvest on populations	
	X^2	p	During harvest	Following harvest
Americorophium salmonis	108.54	•••	Positive	Positive
Cumella vulgaris	82.13	•••	Positive	Positive
Rochefortia spp.	38.19	•••	Negative	Negative
Micrura spp.	0.82	NS	Neutral	Neutral
Capitellidae	271.51	•••	Positive	Positive
Goniadidae	15.89	•••	Positive	Neutral
Spionidae	1.41	NS	Neutral	Neutral
Hesionidae	362.82	•••	Negative	Neutral
Phyllodocidae	24.32	•••	Negative	Negative
Polynoidae	8.07	•	Neutral	Negative

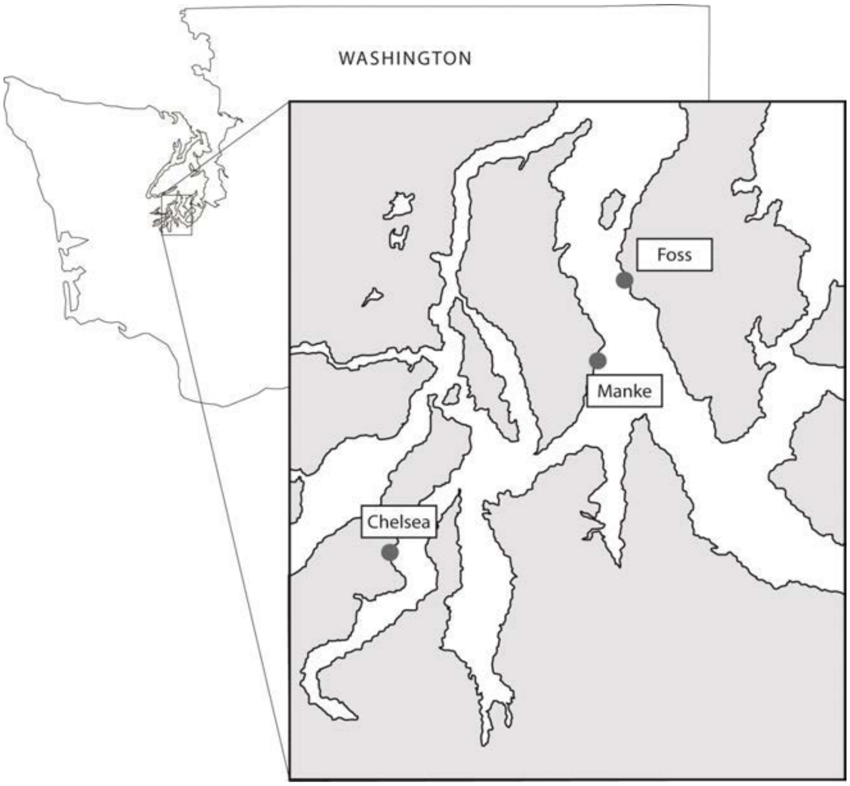

Figure 1. Locations of study sites in southern Puget Sound, Washington USA. Coordinates (latitude and longitude) for each site are provided in text. Shaded areas are land, white areas are water.

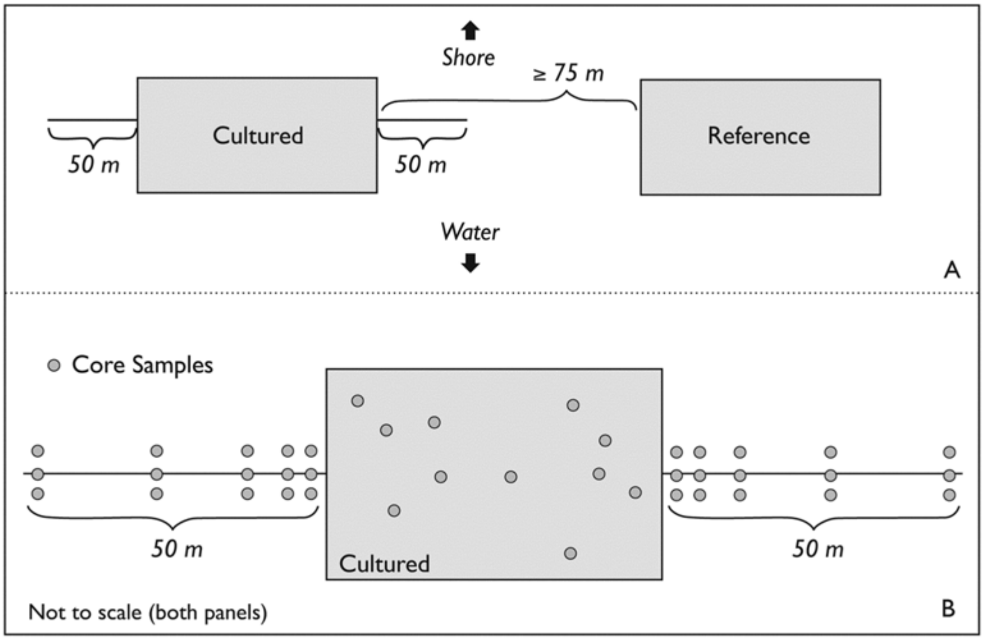

Figure 2. Diagram of physical layout (plan view) used for each of the three study areas. A: Relative positions of cultured and reference plots at each site, and placement of extralimital transects at Foss (only one transect was established at Manke and Chelsea, respectively). B: Example random placement of core sample sites for cultured plot at each site on each sampling date, and layout of transect core sample placement at Foss. Similar core sample placement protocols were used on the single transects at Manke and Chelsea. Diagrams are not to scale. Additional details are provided in text.

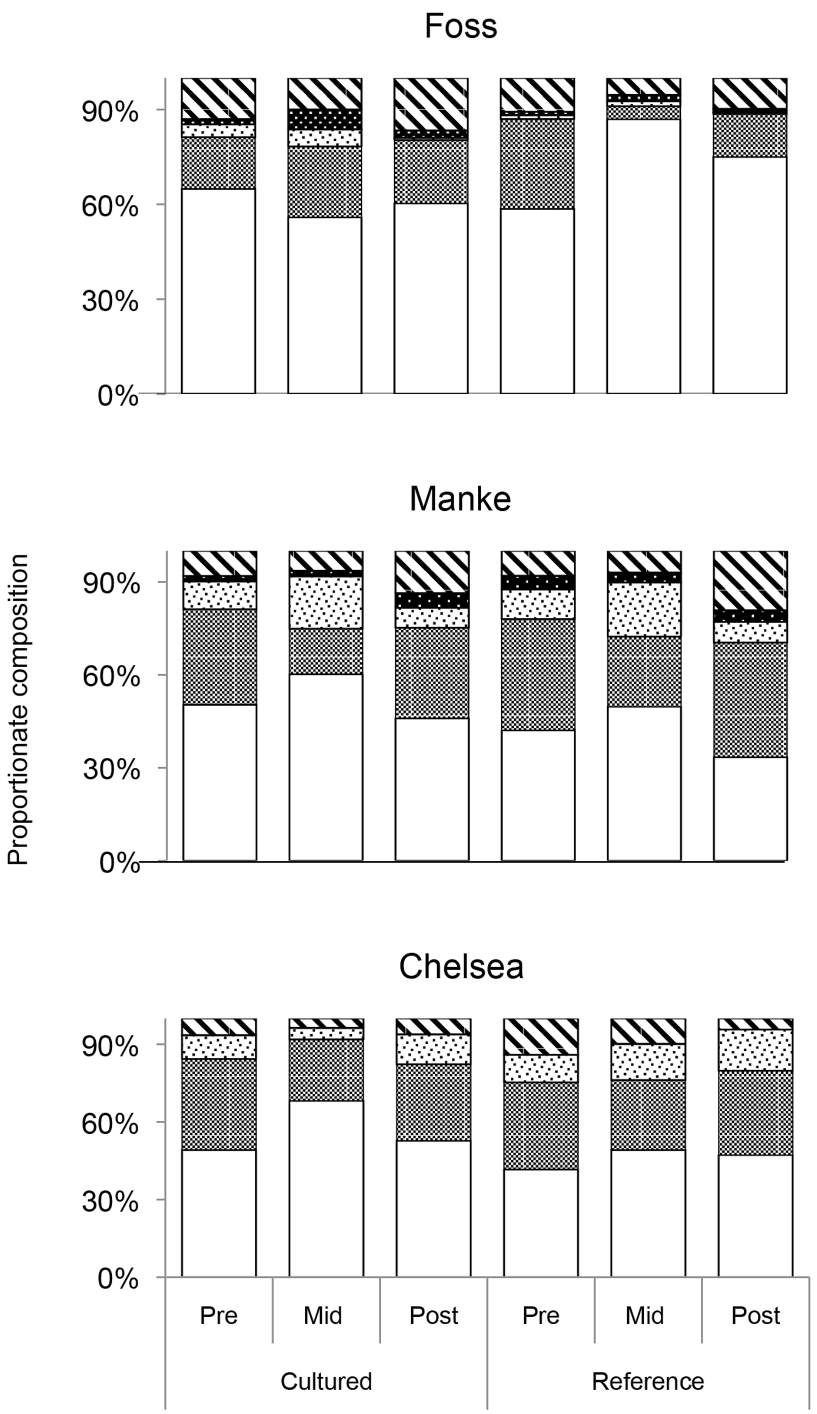

Figure 3. Taxonomic composition of all infauna summed, as proportions of numbers of individuals in samples, in cultured and reference plots during pre-harvest, mid-harvest, and post-harvest states at each study site. In each plot taxonomic categories are, from bottom to top, polychaetes, crustaceans, bivalves, echinoderms, and all other taxa combined. The echinoderm category does not appear in the Chelsea plot because numbers in samples were zero or near zero.

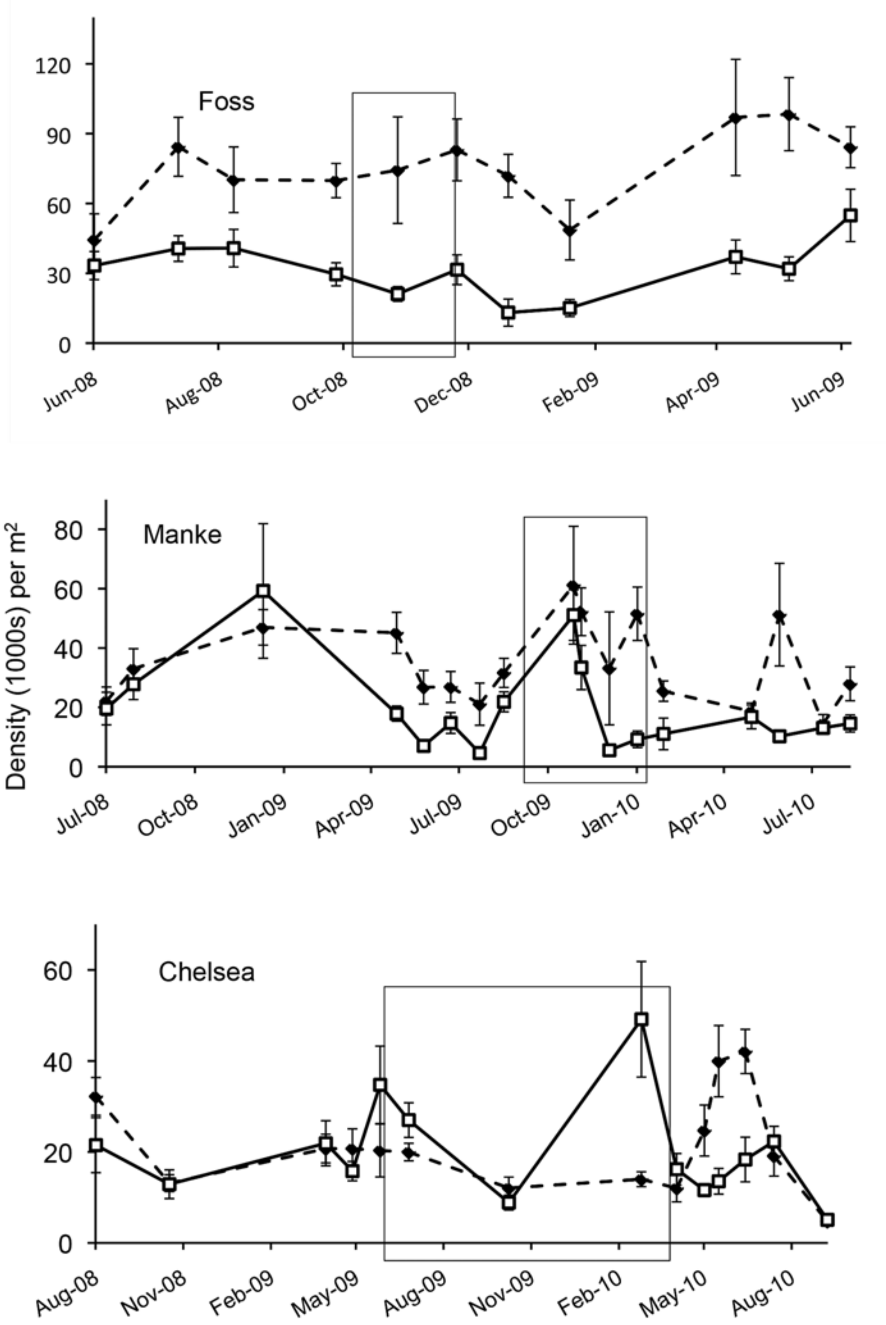

Figure 4. Mean densities of all infauna summed, as thousands of individuals per m^2 (± one standard error) from samples on each plot for each sampling date at each study site. Data from cultured plots are shown with white boxes and solid lines, and from reference plots with black diamonds and dashed lines. Vertically-oriented rectangles represent mid-harvest periods on cultured plots. Note that scales on both horizontal and vertical axes differ among study sites.

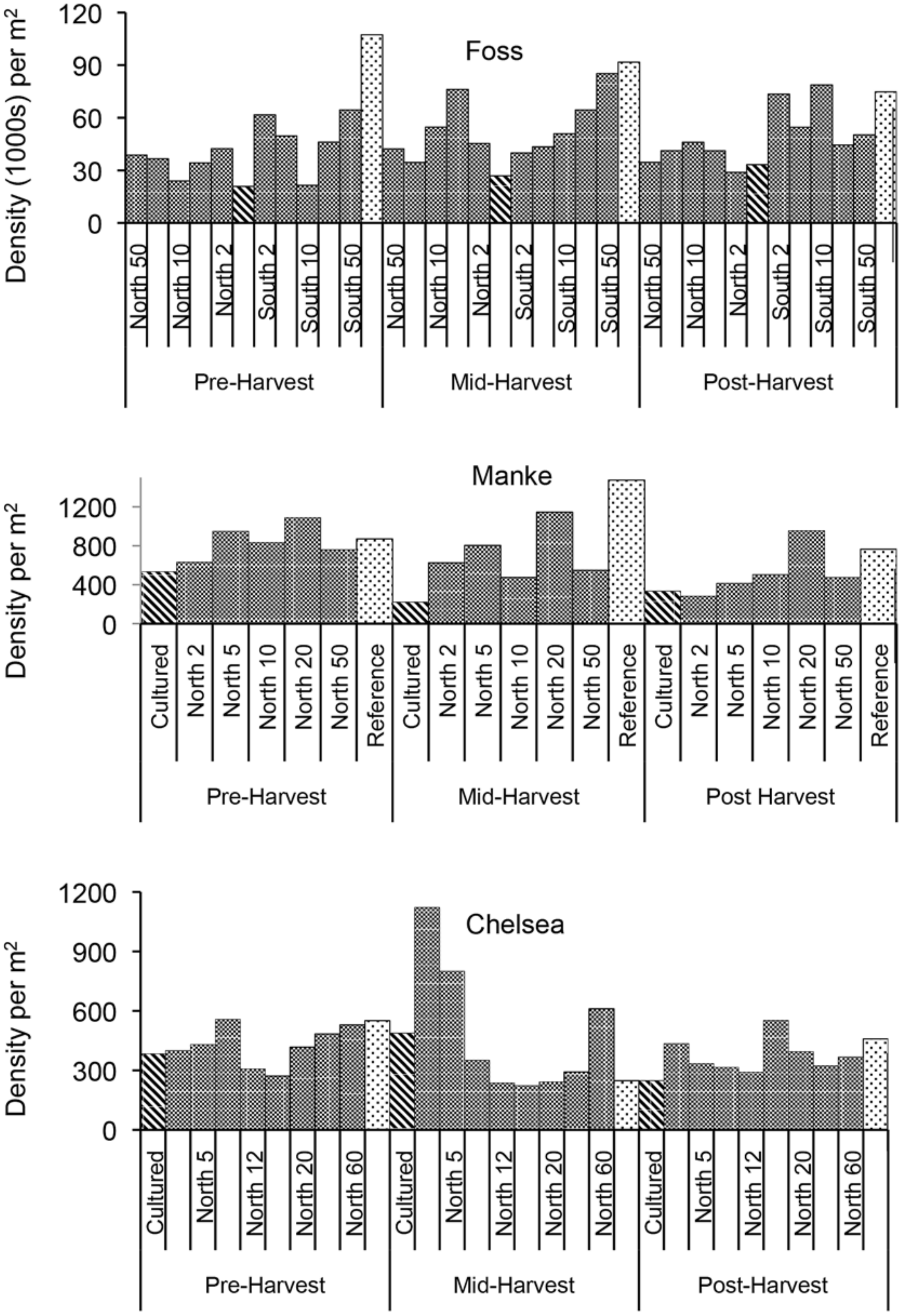

Figure 5. Mean densities of all infaunal organisms summed, as individuals per m^2, from samples in cultured and reference plots and on extra-limital transects at each distance, within harvest states. Diagonally-hatched bars represent densities within cultured plots, coarsely-stippled white bars reference plots. Finely-stippled gray bars indicate densities at specific distances (in m) from cultured plot edges on transects. Note that scales on both horizontal and vertical axes differ among study sites.

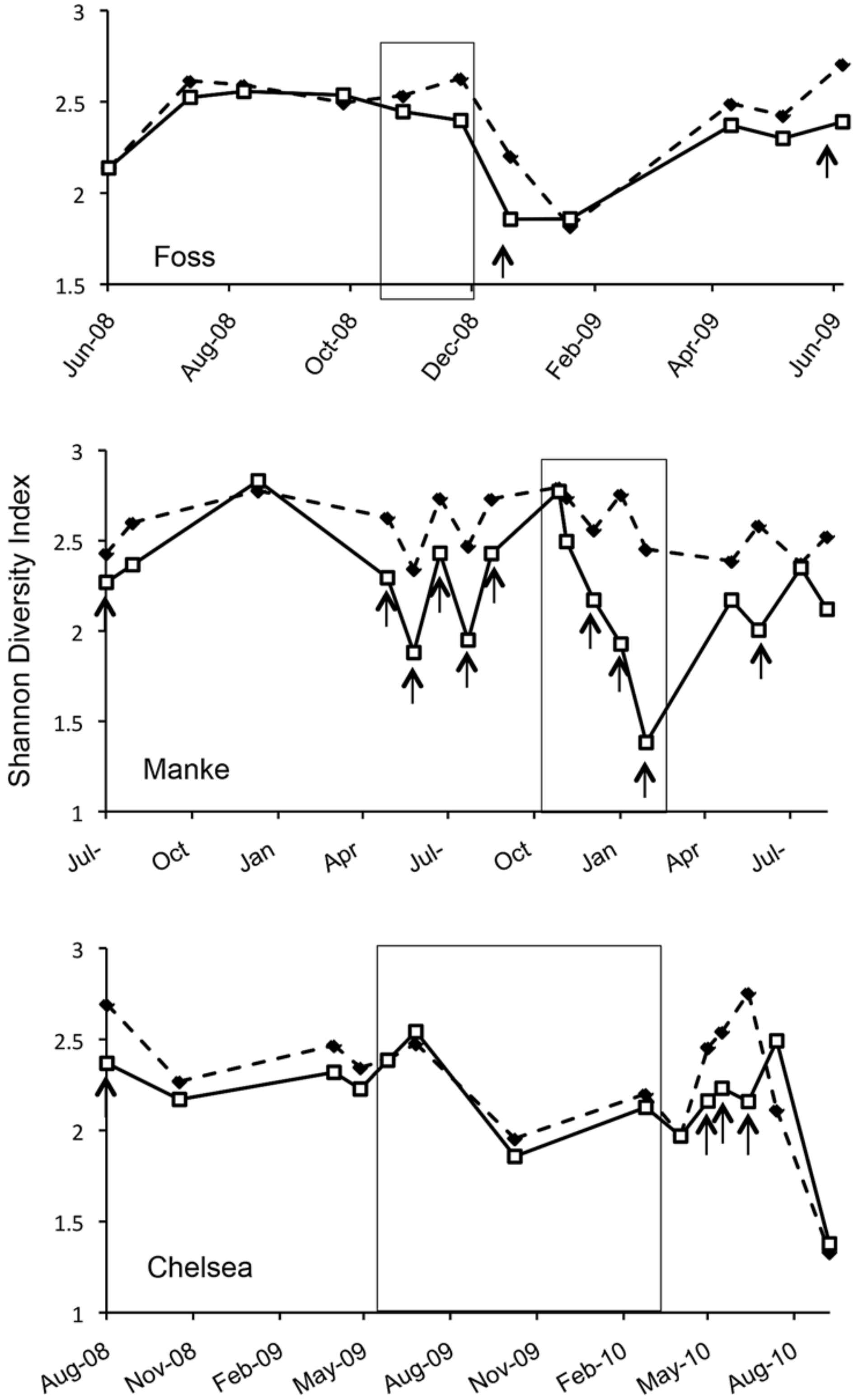

Figure 6. Shannon diversity index values from samples on each plot for each sampling date at each study site. Data from cultured plots are shown with white boxes and solid lines, and from reference plots with black diamonds and dashed lines. Arrows indicate sample dates with significant differences between reference and cultured plots ($p < 0.05$). Vertically-oriented rectangles represent mid-harvest periods on the cultured plots. Note that scales on both horizontal and vertical axes differ among study sites.

Appendix II

Effects of geoduck (*Panopea generosa* Gould, 1850) aquaculture gear on resident and transient macrofauna communities of Puget Sound, Washington, USA

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Keywords: *aquaculture effects, benthic community, geoduck, habitat provision, macrofauna, press disturbance, structural complexity*

Abstract

In Washington State, commercial culture of geoduck clams (*Panopea generosa*) involves large-scale out-planting of juveniles to intertidal habitats and installation of PVC tubes and netting to exclude predators and increase early survival. Here we examine whether structures associated with this nascent aquaculture method affect patterns of use by resident and transient macrofauna. We summarize results of regular surveys of aquaculture operations and reference beaches in 2009-2011 at three sites during three phases of culture: 1) pre-gear [- geoducks, -structure]; 2) gear-present [+geoducks, +structures]; and 3) post-gear [+geoducks, -structures]. Resident macroinvertebrates (infauna and epifauna) were sampled monthly (in most cases) using coring methods at low tide during all three phases. Differences in community composition between culture plots and reference areas were examined with Permutational Analysis of Variance (PerMANOVA) and homogeneity of Multivariate Dispersion (HMD) tests. SCUBA and shoreline transect surveys were used to examine habitat use by transient fish and macroinvertebrates. Analysis of Similarity (ANOSIM) and complementary non-metric Multidimensional Scaling (NMDS) were used to compare differences between species functional groups and habitat type at different aquaculture phases. Results suggest that resident and transient macrofauna respond differently to structures associated with geoduck aquaculture. No consistent differences in the community of resident macrofauna were observed at culture plots or reference areas at the three sites during any year. Conversely, total abundance of transient fish and macroinvertebrates were more than two times higher at culture plots than reference areas when aquaculture structures were in place. Community composition differed (ANOSIM) between culture and reference plots during the gear-present phase, but did not persist to the next farming stage (post-gear). Habitat complexity associated with shellfish aquaculture may attract some structure-associated transient species observed infrequently on reference beaches, while displacing other species that typically occur in areas lacking epibenthic structure. This study provides the first look at the effects of multiple phases of geoduck farming on macrofauna and has important implications for management of a rapidly expanding sector of the aquaculture industry.

Introduction

Habitat complexity influences diversity and abundance of species through strong effects on predation (Crowder & Cooper 1982) and competition (Grabowski & Powers 2004), as well as processes such as recruitment, food delivery, and biodeposition driven by flow and turbulence (e.g., Spencer et al. 1997, Lapointe & Bourget 1999, Lenihan 1999). Placement of structures on soft-sediment substrata is known to initiate a number of physical, geochemical, and ecological processes within the disturbed area (e.g., Wolfson et al. 1979, Davis et al. 1982). Within the conceptual framework of ecological disturbance (*sensu* Pickett & White 1985), placement of structures constitutes a longer-lasting or chronic event (i.e., "press" disturbance; Glasby & Underwood 1996) that may affect a number of ecological functions and processes over long time periods. Organisms that are absent from adjacent unstructured areas may colonize newly available surfaces and interstices, dramatically altering species diversity. Moreover, macroalgae growing on aquaculture structures can further enhance emergent structure and provide additional biogenic habitat (Powers et al. 2007). These changes may attract mobile consumers, such as transient fish and macroinvertebrates (e.g., Davis et al. 1982), a pattern attributed to enhanced resource supplies for detritivores (e.g., sea cucumbers), herbivores (e.g., urchins and some crab species) and predators (e.g., sea stars and other crab species; Inglis & Gust 2003, Dubois et al. 2007). Moreover, these structures may serve as refugia that reduce individuals' predation risk (e.g., Dealteris et al. 2004). Conversely, species that require soft-sediment habitat or prey therein may be excluded when structure additions occur (e.g., Woodin et al. 1981). These disturbances may modify predation pressure and alter patterns of primary production (indirect mediation of top-down control; Genkai-Kato 2007), and trophic dynamics (Grabowski 2004, Grabowski & Powers 2004).

Projections of future aquaculture production to meet human food demands (Costa-Pierce 2002; Dumbauld et al. 2009) imply an expanding ecological footprint for these activities in nearshore environments. Addition of cultured shellfish (e.g., live animals, shell) and aquaculture gear including bags, racks, and ropes, may substantially increase structural complexity in soft-sediment habitats where these activities frequently occur, and this can affect resident and transient fish and macroinvertebrates. For example, netting used to reduce predation of Manila clams (*Venerupis philippinarum*) in aquaculture operations in the United Kingdom alters patterns of biodeposition leading to changes in community composition of resident macroinvertebrates, including deposit-feeding polychaetes, consistent with organic enrichment (Spencer et al. 1997). Similarly, Inglis & Gust (2003) observed significantly higher densities of predatory sea stars (*Coscinasterias muricata*) associated with long-line mussel farms in New Zealand compared to adjacent reference sites, and scup (*Stenotomus chrysops*) in Narragansett Bay experienced lower disappearance rates (emigration + mortality) at an oyster grow-out site than adjacent areas (Tallman & Forrester 2007). Regardless of the processes involved (e.g., biodeposition or the provision of prey and/or habitat), published literature suggests differences in abundance and diversity at shellfish aquaculture sites relative to unstructured areas (Erbland & Ozbay 2008, see review by Dumbauld et al. 2009).

Pacific geoduck clams (*Panopea generosa* Gould 1850; hereinafter geoducks) are the largest burrowing bivalve known (Goodwin & Pease 1987) and range from Baja, California north to Alaska (Bernard 1983). Aquaculture of geoducks has occurred on a commercial scale since 1996 (Jonathan P. Davis, Taylor Resources Inc., personal communication) and has rapidly developed into an important industry in Washington State and British Columbia, with estimated annual production valued at $21.4 million USD (FAO 2012). Culture practices involve large-scale out-planting of hatchery-reared juvenile clams to intertidal habitats and installation of polyvinyl chloride (PVC) tubes and netting to exclude predators and increase early survival. Juvenile clams (10-20 mm shell length; SL) are placed within tubes (10-15 cm diameter) set vertically in the sediment. Nets typically consist of either small plastic mesh caps stretched over the opening of individual tubes or large continuous covers over entire plots. Predator-exclusion structures are removed once clams reach a size refuge from predators, generally 1-2 years after planting. Clams are harvested after an additional 3-5 year grow-out period (see VanBlaricom et al. in press for details).

While commercial geoduck aquaculture operations boost local economies and increase employment and international trade opportunities, there is a dearth of information regarding potential impacts to nearshore ecosystems. Thus, rapid expansion of geoduck aquaculture operations in intertidal habitats of Puget Sound in Washington State, USA, has raised concern among managers, conservation organizations, and the public regarding industry practices that may alter resident ecological communities. In response, the 2007 Washington State Legislature passed Second Substitute House Bill 2220, which commissioned a series of scientific studies to "measure and assess" the possible ecological impacts of current practices, including use of predator-exclusion structures.

The objectives of the present study were to assess differences in the abundance and diversity of resident and transient macrofauna at sites with (culture) and without (reference) geoduck aquaculture at distinct phases of the aquaculture sequence (prior to gear addition, gear-present and after gear removal). Here "resident" describes macrofauna species that occupy intertidal beaches throughout their entire benthic life history and demonstrate limited post-larval dispersal, whereas "transient" macrofauna make frequent (often daily, linked to tidal fluctuations in water level) migrations between intertidal and subtidal habitats. The following questions were posed: do the abundance and diversity of resident and transient macrofauna differ between culture plots and reference areas? What is the response of the macrofauna community to the addition and subsequent removal of aquaculture gear? The culture plots and reference areas at each site were located close enough together (75-150 m) to be considered functionally similar habitats. Evidence of an effect would consist of little or no difference prior to aquaculture, but a distinction between culture plots and reference areas once structure was added. If any differences in resident or transient macrofauna communities were detected when habitat complexity was increased (i.e., while aquaculture gear was present), we hypothesized that these changes would not persist once gear was removed and the disturbance associated with structure addition was ameliorated.

Methods

Study Sites

Work described here was done in south Puget Sound, Washington, USA, a sub basin of Puget Sound composed of those marine waters south and west of Tacoma Narrows (47°16'7.97"N, 122°33'2.76"W; Fig. 1 inset). The sub basin is shallow (mean depth 37 m) and characterized by extensive littoral mud and sandflats (674 km^2) that constitute more than 15% of the total area. Because of abundant suitable habitat, South Puget Sound supports substantial commercial culture of bivalves, predominately Pacific oyster (*Crassostrea gigas*), mussel (*Mytilus* spp.), Manila clams (*Venerupis philippinarum*) and most recently geoduck. Three study sites with similar habitat characteristics (Table 1) were selected for this study; Stratford (47°19'10.86"N, 122°47'38.56"W) and Rogers (47°14'53.13"N, 122°49'37.38"W) are located on the east shore of Case inlet, and Fisher (47°10'32.28"N, 122°56'33.79"W) is located on south shore of the northeastern portion of Totten Inlet (Fig 2). None of these sites had previously been used for geoduck aquaculture, which afforded the opportunity to examine the resident and transient macrofauna community prior to the initiation of aquaculture operations (pre-gear) and the early phases of culture, including the addition of aquaculture structure (gear-present) and subsequent removal approximately two years later (post-gear).

Surveys of resident macroinvertebrates (infauna and epifauna)

To investigate the resident benthic macroinvertebrate assemblage at the three study sites, surveys were conducted during low tides (0.5 to -1 m MLLW) from 2009-2011 at culture plots and adjacent reference areas. Ten randomly distributed core samples (5 cm in diameter, depth 10 cm, 196 cm^3) were collected in culture plots and adjacent reference areas. In addition, ten larger excavation samples (29 cm in diameter, depth 20 cm, surface area 660.5 cm^2, volume 13.2 liters) were taken on each sampling date occurring prior to deployment of protective PVC tubes and nets (pre-gear) and following removal of the structures (post-gear). The small core size was chosen as a cost-effective method for sampling the study plots, and analysis of preliminary samples demonstrated that most benthic infauna were adequately sampled (see VanBlaricom et al, in press). Moreover, small cores are frequently used to assess benthic infauna (Simenstad et al. 1991). The excavation samples were used to assess the abundance of larger invertebrates (e.g., sand dollars) that appear infrequently in the smaller cores. Core samples were preserved in 10% buffered formalin solution immediately after collection. Excavation samples were sieved (0.5 mm mesh) and enumerated in the field, with retained organisms similarly preserved for laboratory identification when necessary.

Core samples were processed in the laboratory using a standard method of winnowing to extract infaunal organisms (Simenstad et al. 1991, Sobocinski et al. 2010). Freshwater was added to a sample, the sample was mixed so that sediments settled to the bottom and the elutriated organisms floated to the surface. Water was decanted through a 500-micron sieve and organisms were retained on the collection screen. This process was repeated several times for each sample to ensure that all organisms had been separated from sediments. Organisms were identified to species or genus when practical, but in all cases at least to family. Family level identification has been sufficient to support meaningful quantitative analyses in previous studies (Ferraro & Cole 1990, Dethier 2005). In addition, we used the processing method above to opportunistically examine beach spawning by Pacific sand lance (*Ammodytes hexapterus*) at study sites during the peak spawning period (November-April). While our methods did not specifically target spawning (e.g., Moulton & Penttila 2000), winnowing or elutriation has previously been used to assess sand lance spawning because the process of agitating the sample loosens the adhesive eggs from sand grains (Thuringer 2003).

Permutation based multivariate analysis of variance (PerMANOVA; Anderson 2001) was used to test for differences in the community data within core samples among plot type (culture plots and reference areas within each site) and phases of culture (pre-gear, gear-present, and post-gear) separately for each site (Fisher, Rogers, Stratford). In addition to the main effects, we tested the interaction of plot type and culture phase; a significant interaction term was interpreted as evidence that gear addition or removal influenced the community of macroinvertebrate infauna. Thus, evaluation of the interaction term was our principal metric for determining the effect of culture practices. Analyses were conducted in R software (R Development Core Team 2011); significance was set at alpha (α)=0.05.

Distance based tests for the Homogeneity of Multivariate Dispersion (HMD; Anderson 2006) were also conducted for further characterization of contrasts of core data between culture plots and reference areas. HMD uses a Bray-Curtis distance matrix of species data to calculate the average distance in multivariate space between individual samples and the calculated centroid of the sample's group. The average distance and the associated variability are compared between groups and tested for significance with permutation tests. Caswell & Cohen (1991) hypothesized a positive relationship between multivariate dispersion of samples and disturbance, and previous assessments of disturbance effects have pointed to higher variability of species abundance in samples collected from disturbed areas relative to non-disturbed areas when evaluated with HMD (Warwick

& Clarke 1993). Because variability is the response of interest in HMD analyses, tests were performed on individual core and excavation samples as the replicated unit; sample averaging would have masked important inter-sample variability. At each site, HMD analyses were used to test differences between the culture plots and reference areas within each culture phase and within plots across culture phases. Analyses were conducted in R software (R Development Core Team 2011); significance was set at alpha (α)=0.05.

In addition to the community analyses above, we used generalized linear mixed models (GLMMs; McCullagh & Nelder 1989) assuming Poisson distributed data to examine the factors contributing to abundance of selected individual macroinfaunal taxa. In univariate analyses, data from all sites were considered together. The effects of plot type, phase, and their interaction were included, as well as random effects of site and month of sampling. Models were fitted by maximum likelihood assuming a Laplace approximation in the "lme4" package (Bates & Maechler 2010) of R software (R Development Core Team 2011). Likelihood ratio tests were utilized to formally compare models including the interaction term as part of a 'frequentist' hypothesis testing approach. Regression coefficients and their 95% confidence intervals were calculated for each model.

Surveys of transient fish and macroinvertebrates

In order to investigate transient fish and macroinvertebrate assemblages at the three study sites, SCUBA surveys were conducted during daytime high tides (3 to 4.25 m above MLLW) from 2009-2011. A pair of divers used a metric underwater transect tool adapted from Bradbury et al. (2000) to conduct line transects at each site; each diver surveyed a 1 m swath. Sites were comprised of two 2500 m^2 habitat spaces: a culture plot with active geoduck farming and a nearby reference area (the same reference area as utilized in the core sampling) with no aquaculture activity. Two 45 m transects were done on each habitat, although there was some variation in transect length depending on weather conditions and dimensions of the culture plots. Successful surveys were dependent on sufficient water clarity for underwater visibility, coinciding to horizontal Secchi-disk measurements of at least 2.5 m. SCUBA surveys were conducted monthly from March through August and bimonthly from September through February.

We identified and enumerated all observed fish and macroinvertebrates >60 mm to species or genus and recorded observations of size (estimated total length [TL] for fish, and diameter, carapace width [CW], or length for sea stars, crabs, other benthic invertebrates, respectively), water column position, behavior, and associated substrate type (sand, gravel, tubes + netting, tubes – netting). Observed species were assembled into ten functional groups: sea stars, moon snails, hermit crabs, crabs (Brachyura), other benthic invertebrates, flatfishes, sculpins, other demersal fishes, other nearshore fishes, and seaperches (Table 1). Numbers of organisms were converted to raw density values to offset the different transect lengths. Species that occurred in less than five percent of surveys were not included in the data analysis.

Based on observations during SCUBA surveys, it was apparent that many of the transient fish and macroinvertebrates do not occupy intertidal habitats during the winter months (Fig. 2). To reduce the effect of seasonal variability on the abundance of many functional groups, data analysis focused only on the April-September period. Three phases of the aquaculture cycle were represented in the dataset: pre-gear (in 2009, prior to any aquaculture operations [-geoducks, -structure]), gear-present (in 2010, during active geoduck aquaculture operation, aquaculture gear in place at culture plots [+geoducks, +structure]), and post-gear (in 2011, protective tubes and nets were removed but geoducks remained during grow-out [+geoducks, -gear]). While the 2010-2011 data represent periods in which aquaculture was active, farming only occurred at culture plots; thus there was no change in epibenthic structure at reference areas.

Data from the three survey sites were not analyzed individually as all sites were considered to have functionally similar habitat for mobile macrofauna. Additionally, in some cases the sample sizes would have been smaller than practical for the methods applied if the data were separated by site. Data were (log x+1)-transformed in R software with the vegan package (R Development Core Team 2011); with α=0.05 for statistical tests of significance.

We conducted Analysis of Similarity (ANOSIM; Clark 1993) to assess differences in functional groups between culture plots and reference areas across aquaculture phases. A Bray-Curtis dissimilarity matrix (Bray & Curtis 1957) was used in ranking pairwise combinations of the absolute densities for all functional groups and survey events. Test statistics (R) and p-values were generated using Monte Carlo permutation tests with 999 iterations. Values of the R statistic ranged from -1 to 1, with negative values suggesting larger differences within groups (Clarke & Gorley 2001) and positive values indicating larger differences among groups (McCune et al. 2002). A R-value of zero indicates no differences (McCune et al. 2002).

We explored visual representations of species abundance in different habitat types and aquaculture phases using non-metric Multidimensional Scaling (NMDS: Kruskal & Wish 1978). Because NMDS has no assumptions of linearity, it is suitable for any dissimilarity matrix (McGarigal et al. 2000), which makes the procedure useful for visualizing relationships in non-normal datasets of species abundance (McCune et al. 2002). We conducted NMDS on a Bray-Curtis dissimilarity matrix of the untransformed, raw density data and 1000 iterations were performed to ensure convergence with minimal stress. Stress significance was tested using a Monte Carlo randomization approach. We used linear correlation of the functional groups and NMDS axis scores to calculate variable weights. Significant functional groups were determined with permutation tests and overlaid as vectors on the NMDS plots, which facilitated interpretation of the position of each survey event in ordination space.

Addition of aquaculture gear is a press disturbance (see review by Dumbauld et al. 2009), and disturbance is generally considered one of the main factors influencing variations in species diversity (e.g., Connell 1978; but see Mackey & Currie 2001). The Shannon index was utilized to compare differences in diversity between plots for each aquaculture phase. This measure is commonly used in ecological studies and combines aspects of species richness and relative abundance to produce a value typically from 0 to 3.5 (Shannon 1948, Shannon & Weaver 1949). A higher index value indicates higher diversity. Two-sample Welch's t-tests (Zar 2010) were used to assess differences in diversity between plots at each stage of geoduck farming.

Supplementary observations of salmon smolts

In addition to the fish sampling described above, observations were made of salmon smolts in the vicinity of aquaculture operations. Pilot observations by divers and snorkelers indicated that smolts at our sites were not effectively sampled by those methods, possibly because observers altered fish behavior. Moreover, salmon smolts, particularly chum (*Oncorhynchus keta)*, typically move along shorelines in shallow water (<2 m; Healey 1979, Simenstad et al. 1982). Shore-based surveys have been developed as a method of monitoring fine scale use of shallow nearshore areas by juvenile salmonids (e.g., Young 2009). Concurrent with SCUBA surveys, shore-based visual surveys were conducted monthly during the spring and summer (March-July) to coincide with outmigration of chum, Pink (*O. gorbuscha*), and coho (*O. kisutch*) salmon smolts (Simenstad et al. 1982). An observer at the water's edge slowly walked along a 50 m transect line parallel to shore spending 1 min within each 10 m section. Observations were made of all fish encountered up to 5 m offshore. Polarized sunglasses were used when necessary to improve observations. Salmonids were identified to species when possible and enumerated. Additional observations of fish length (TL) and behavior were recorded. On each sampling date, one survey each was completed adjacent to the culture plot and reference area. Successful surveys were dependent on surface conditions, coinciding to Beaufort scale 0-1 (calm or light air).

Results

Surveys of resident macroinvertebrates (infauna and epifauna)

At all three sites, the community of resident macrofauna consisted primarily of polychaete worms (Annelida), small crustaceans (Arthropoda), and small bivalves (Mollusca). In some locations echinoids (Echinodermata), larger bivalves, burrowing sea anemones (Cnidaria) and sea cucumbers (Echinodermata) were important community components. All sites were characterized by substantial seasonal variation, and highest densities typically occurred July-September (Fig. 3). Total taxa density in core samples showed substantial site-specific variation, with no consistent pattern of higher density in either culture plots or reference areas across months or sites (Fig. 3). Similar taxa were recorded in cores and excavation samples in most cases. In October 2010, adult sand lance were captured in excavation samples collected at the culture plot and reference area at the Rogers site; densities were 24.2 ± 11.9 m^{-2} and 278.6 ± 115.7 m^{-2}, respectively. However, subsequent evaluation of core samples revealed no evidence of spawning. No adult sand lance, other forage fish, or fish eggs of any type, were observed at the other sites.

We collected and identified 68 taxa in 63 sampling events. Results of the PerMANOVA analyses illustrate differences in community structure across months of sampling, plot types, and phases at each site (Table 3); however, there were no community-level effects of aquaculture operations as indicated by non-significant plot type × phase interaction terms (Fisher site Pseudo-F=0.049, p=0.116; Rogers site Pseudo-F=0.023, p=0.643; Stratford site Pseudo-F=0.029, p=0.529).

Within each site, Homogeneity of Multivariate Dispersion (HMD) values for the community data from the pre-gear phase were similar at culture and reference plots (Table 4). Similarly, there were no significant differences in HMD values for culture and reference plots at any site when aquaculture structures were in place (gear-present), although the values were somewhat higher at Rogers and Fisher sites (Table 4). During the post-gear phase, values for culture plots and reference areas were lower (relative to the previous phase) and not significantly different at Rogers and Fisher (p=0.335 and p=0.436, respectively). At Stratford, the post-gear HMD values for the benthic community were similar to values when aquaculture gear was in place (gear-present); however, there was a significant difference in values between the culture plot and reference area (p=0.003; Table 4).

Twelve taxa were selected for univariate analyses using GLMMs based on their frequency in samples (>90%) and presumed ecological importance. Abundance of individual taxa showed marked differences across months, plot type, phases, and the interaction of plot type and phase. Taxa showed no consistent response to geoduck aquaculture. Regression parameter estimates and 95% confidence intervals for GLMMs are included in Figure 4. The abundances of six taxa were negatively affected by geoducks and aquaculture gear, as indicated by a significant plot type × phase interaction (GLMM χ^2, $p<0.05$) and negative parameter estimates for the gear-present phase (Fig. 4). However, only two taxa experienced persistent negative effects: the polychaete Families Spionidae ($\chi^2=22.89$, df=2, $p<0.001$) and Orbiniidae ($\chi^2=109.17$, df=2, $p<0.001$). Abundance of the amphipod Americorphium salmonis ($\chi^2=174.23$, df=2, $p<0.001$) and polychaete Family Hesionidae ($\chi^2= 341.18$, df=2, $p<0.001$) were reduced by the presence of aquaculture gear but recovered once gear was removed, and the cumacean Cumella vulgaris ($\chi^2=199.16$, df=2, $p<0.001$) and polychaete Families Glyceridae ($\chi^2=94.75$, df=2, $p<0.001$) and Ophellidae ($\chi^2=105.31$, df=2, $p<0.001$) increased in the post-gear phase in culture plots relative to reference areas. Additionally, the abundance of the polychaete Family Goniadidae ($\chi^2=10.94$, df=2, $p=0.004$) and anemone Family Edwardsiidae ($\chi^2=20.505$, df=2, $p<0.001$) increased when gear was present and recovered to pre-gear levels once gear was removed. The bivalve genus Rochefortia ($\chi^2=6.99$, df=2, $p=0.030$), nemertean genus Micrura ($\chi^2=0.52$, df=2, $p=0.772$), and polychaete Family Capitellidae ($\chi^2=4.83$, df=2, $p=0.089$) showed no response to geoduck aquaculture activities.

Surveys of transient fish and macroinvertebrates

The presence of aquaculture gear affects composition of transient fish and macroinvertebrate communities (Fig. 5). No significant differences between culture plots and reference areas were detected by ANOSIM when PVC tubes and nets were absent, either pre-gear or post-gear (Table 5). However, a significant difference was detected between culture plots and reference areas when aquaculture gear was present ($R=0.081$, $p=0.035$). ANOSIM tests between aquaculture phases (Table 5) resulted in a statistically significant difference when comparing the pre-gear vs. gear-present phases and gear-present vs. post-gear phases for culture plots ($R=0.156$, $p=0.040$ and $R=0.164$, $p=0.003$, respectively). There was also a significant difference between gear-present and post-gear reference plots ($R=0.090$, $p=0.029$). Low R-values of these tests indicate minimal separation in contrasts between the habitats.

Several two dimensional NMDS plots were employed to aid in visualization of differences between habitats within sites and across phases of aquaculture operations. NMDS plots also confirmed our assumption that the three sites were functionally similar for purposes of analyzing transient macrofauna communities during April-September. NMDS ordination of the reference plot data shows some inter-mixing of sites and clustering of the three sites in multivariate space (Fig. 6). Information on stress, Monte Carlo randomization and goodness of fit testing is included in the caption for each plot (Figs. 6-9).

During 2010, when nets and tubes were used in aquaculture operations (gear-present phase), surveys of culture plots and reference areas were generally separated in ordination space (Fig. 7). Neither habitat type was consistently associated with unique functional groups. However, differences in assemblages between culture plots and reference areas were illustrated by significant vector loadings associated with flatfish, hermit crab, sculpin, sea star, snail and true crab (Brachyura). True crab showed weak associations with reference areas overall, while sculpin and flatfish were highly correlated and more often associated with reference areas. Two additional NMDS ordination plots represent comparisons of the pre-gear and gear-present phases (Fig. 8) and the gear-present and post-gear phases (Fig. 9).

Survey data for the culture plots when PVC tubes and nets were present were more widely dispersed in ordination space compared to the pre-gear phase (Fig. 8). Differences in assemblages between pre-gear and gear-present phases were illustrated by significant vector loadings associated with flatfish, hermit crab, sculpin, sea star, and true crab (Brachyura). Prior to gear deployment, culture plots and reference areas were characterized by flatfish and sea star. Conversely, while communities associated with culture plots were represented by a variety of functional groups when nets and tubes were in place (gear-present), flatfish were conspicuously underrepresented. At the same time, reference areas were characterized by flatfish and hermit crab, and less so by true crab and sea star.

In comparisons of gear-present and post-gear phases, data from culture plots appear mostly separated in multivariate space but reference area data overlap and appear more homogenous (Fig. 9). Differences in assemblages between gear-present and post-gear phases were illustrated by significant vector loadings associated with clam, flatfish, hermit crab, other nearshore fish, sculpin, and true crab (Brachyura). Of the significant functional groups in Figure 9, true crab and other nearshore fish show strongest associations with culture plots during the gear-present phase when PVC tubes and nets were in place.

Species diversity, as calculated by the Shannon diversity Index (H'), was unaffected by geoduck aquaculture operations (Table 5). There was no significant difference in diversity between culture plots and reference areas in the phases of culture examined in this study: prior to gear deployment ($t=0.703$, df=11, $p=0.496$); gear-present ($t=0.727$, df=18, $p=0.476$), or after gear had been removed ($t=0.309$, df=25, $p=0.760$). Total numbers of organisms observed at culture and reference plots were similar prior to gear deployment (pre-gear, 2009) and after gear removal (post-gear, 2011). However, there was an overall increase in total abundance while aquaculture gear was present, and macrofauna counts were more than two times higher at culture plots compared to the reference areas (Table 5).

Supplementary observations of salmon smolts

Salmon smolts, chum (*O. keta)* and pink (*O. gorbuscha*), were rarely observed during shore-based visual surveys (8% total). When present, schools of salmon traveled parallel to the shoreline in < 2 m of water. We observed no difference in the occurrence of salmon smolts adjacent to culture plots and reference areas, although evidence is anecdotal given the low encounter rate. No discernable differences in behavior were observed.

Discussion

Resident and transient macrofauna communities respond differently to changes in habitat complexity associated with geoduck aquaculture operations. Although results of the present study suggest that structures associated with geoduck aquaculture have little influence on community composition of resident benthic macroinvertebrates (i.e., non-significant plot type × phase interaction in PerMANOVA), overall densities of resident epifauna and infauna tended to be lower on culture plots relative to reference areas at two of the three study sites. Resident invertebrate communities were characterized by strong seasonal patterns of abundance and site-specific differences in composition. Dispersion in sample variation, which is commonly used to detect effects of disturbance, did not differ between culture plots and reference areas when aquaculture gear was in place. Some individual taxa responded negatively to the presence of geoducks and aquaculture gear (e.g., polychaete Families Spionidae and Orbiniidae), while others responded positively (e.g., polychaete Family Goniadidae and anemone Family Edwardsiidae) and still others were unaffected (e.g., bivalve genus *Rochefortia* and polychaete Family Capitellidae).

The paucity of strong effects on the resident macrofauna community (epifauna and infauna) may not be unexpected. Previous studies have suggested that aquaculture effects on benthic infauna are most pronounced in soft sediment habitats directly below, or immediately adjacent to, shellfish aquaculture operations as a function of organic enrichment via biodeposition (see review by Dumbauld et al., 2009). Interestingly, the two taxa experiencing persistent negative effects of geoduck aquaculture activities, Families Spionidae and Orbiniidae, are selective detritivores and deposit-feeders, respectively (see Table 1 of VanBlaricom et al. in press). In off-bottom aquaculture (e.g., suspended culture), the balance of biodeposition and water flow, which removes deposits, tend to be the strongest determinants of community structure (Mattsson & Linden. 1983). In on-bottom aquaculture operations, effects of structural complexity and space competition are difficult to separate from changes in biodeposition (Dumbauld et al. 2009). Quintino et al. (2012) specifically investigated the relative contribution of biodeposition and aquaculture gear (i.e., oyster trestles) and found that structures alone had no effect, whereas biodeposition from sedimentation and organic waste did alter the benthic community. However, Spencer et al. (1997) found that the netting used to reduce Manila clam predation reduced flow and led to changes in benthic community composition consistent with organic enrichment. In the present study, several infaunal taxa recovered to pre-gear abundance, or increased in abundance, once aquaculture gear was removed. Effects on resident macrofauna, particularly infauna and epifauna, may be site-specific and likely driven by inherent levels of natural disturbance (Simenstad & Fresh 1995) or flushing (Dumbauld et al. 2009), which may be mediated by aquaculture gear. Physical and chemical variables (e.g., sediment grain size, pore water nutrients) that may contribute to site-specific differences were not examined in the present study. Thus, elucidating potential mechanisms responsible for differences in the response of infauna will require additional study. Additional data and analytical inference would also permit more direct comparison to previous studies done by Spencer et al. (1997), Quintino et al. (2012), and others.

Unlike resident macrofauna, the transient fish and macroinvertebrate community was clearly affected by aquaculture activities. Presence of PVC tubes and nets significantly altered abundance and composition, but not diversity, of transient macrofauna. Over two times more organisms were observed during surveys at the culture plots than at reference areas during the structured phase of geoduck aquaculture, indicating that geoduck aquaculture gear created favorable habitat for some types of Puget Sound macrofauna. ANOSIM results demonstrated a statistically significant difference between the transient macrofaunal communities in culture plots and reference areas when aquaculture gear was present (Table 5; R=0.081, p=0.035). Yet the low R-value of the test suggests minimal ecological difference between the habitats. NMDS plots provide insight into functional groups that may show preference for culture plots (structured habitat) or reference areas (unstructured habitat) when aquaculture gear is present. In general, true crabs, sea stars, and seaperches were more associated with culture plots and flatfishes and snails were often associated with reference areas.

The large increase in total abundance of transient macrofauna when aquaculture gear was present suggests that increased complexity afforded by PVC tubes and nets attracted some fish and macroinvertebrates to the habitat. Aggregation of macrofauna to structured habitat, and aquaculture gear in particular, has been well documented (Dealteris et al. 2004, Dubois et al. 2007, Dumbauld et al. 2009). Our data suggest that provision of foraging and refuge habitat is the primary mechanism for the attraction; crabs and sea stars were frequently observed feeding within culture plots, and we observed smaller fish and crabs retreating under netting when larger animals or divers approached. Similarly, Inglis & Gust (2003) observed increased predation by sea stars within New Zealand long-line mussel farms, while Tallman & Forrester (2007) identified refuge value as a major factor leading to higher site fidelity of juvenile scup (*Stenotomus chrysops*) to aquaculture structures in Rhode Island. Increased foraging pressure by transient macrofauna may also provide an additional mechanism to explain slightly depressed densities of resident macrofauna in culture plots relative to reference areas.

In the present study, some taxa, particularly flatfish and the snail, *Lunatia lewisii*, were rare in culture plots when gear was present. These organisms may actively avoid habitat complexity created by aquaculture gear. Holsman et al. (2006) found that subadult Dungeness crab (*Metacarcinus magister*, formerly *Cancer magister*) similarly avoid complex habitats, including on-bottom oyster culture, and preferentially use unstructured habitats during intertidal forays. For taxa adapted to unstructured habitat, complexity may hinder movement and reduce foraging efficiency (e.g., Holsman et al. 2010). The habitat value of unstructured areas to these taxa is substantial and should be considered along with any perceived positive habitat value of aquaculture gear to structure-oriented or crevice-dwelling fish and macroinvertebrates.

Effects of aquaculture on transient macrofauna did not persist once PVC tubes and nets were removed during grow-out. There was a significant difference between the culture plots for the last two aquaculture phases: gear-present vs. post-gear (R=0.160, p=0.003), and the ANOSIM R-value for this test was the highest of all tests conducted, suggesting moderate ecological significance that is corroborated by the NMDS plot in Figure 8. Moreover, when PVC tubes and nets were removed, the transient macrofauna community was no different from the pre-gear condition (ANOSIM R=-0.085, p=0.842). These data suggest transient macrofauna communities associated with these intertidal beaches begin to recover to pre-aquaculture conditions within a few months of removal of the PVC tubes and nets.

Transient macrofaunal communities in reference areas were also significantly different between gear-present and post-gear phases. The similar pattern observed in both culture plots and reference areas may be attributed at least in part to annual variation in species abundance and composition. Spatial and temporal variability can strongly influence transient macrofauna communities on a variety of scales (Jackson & Jones 1999, Hurst et al. 2004), and these changes can produce effects across trophic levels (Reum & Essington 2008). Reference areas in our study may also be somewhat affected by removal of aquaculture structures between gear-present and post-gear phases through spillover effects (e.g., Ries & Sisk 2004). Culture plots and reference areas were 75-150 m apart. Previous work has demonstrated spillover effects on transient macrofauna from both natural (Almany 2004) and artificial structures (Helvey 2002).

Geoduck aquaculture practices did not affect diversity of macrofauna. No consistent differences in diversity of resident macrofauna were observed in the present study. Average diversity of transient macrofauna at culture plots when gear was present was slightly higher than at reference areas (but not significant), and diversity measures for the pre-gear and post-gear data were almost identical between habitat types. It is important to note that the Shannon index is based on relative instead of absolute abundance. This distinction is a potential limitation for a study such as ours, which focuses on distinguishing between the raw abundances of species groups in different areas. Nevertheless, our results clearly contrast with previous work linking aquaculture disturbance with changes in diversity (Erbland & Ozbay 2008, see review by Dumbauld et al. 2009). Brown & Thuesen (2011) observed higher diversity of transient macrofauna associated with geoduck aquaculture gear in trapping surveys. However, taxa richness was low in that study and results were driven by a large number of graceful crab, *Metacarcinus gracilis* (formerly *Cancer gracilis*) captured in the reference area. Overall, more organisms were captured in traps set in the reference area than within geoduck aquaculture plots (Brown & Thuesen 2011).

Managers and stakeholders have raised concerns about potential effects of geoduck aquaculture practices on forage fish spawning habitat, particularly Pacific sand lance (*Ammodytes hexapterus*), which spawn on littoral beaches at high tidal levels (November-April; Penttila, 2007). Despite the presence of adult fish in excavation samples (Rogers site, October 2010), no evidence of spawning (i.e., eggs) was observed. It is possible that adult sand lance do not form winter aggregations in the same littoral habitats where spawning occurs (Quinn 1999). Moulton & Penttila (2000) suggest that spawning typically occurs at 2-2.75 m above MLLW, which is well above geoduck aquaculture operations and sampling in this study (Table 1). No other adult forage fish (e.g., surf smelt (*Hypomesus pretiosus*), herring (*Clupea pallasi*)) or evidence of spawning activities were observed in our study. While these results suggest negligible effects, our opportunistic sampling may be inadequate given spatio-temporal variability in spawning behavior, and further targeted investigation is warranted to elucidate potential broader impacts on forage fish populations.

The present study provides insight into the response of resident and transient macrofauna to geoduck aquaculture practices. Taken together, these results indicate that changes in habitat complexity associated with geoduck aquaculture produce short-term effects (1-2 years) on intertidal beaches. However, we caution that the present study focused exclusively on diversity and abundance of fish and macroinvertebrate communities. Additional impacts might be demonstrated by considering different metrics, including growth. For example, Tallman and Forrester (2007) found that scup were 40% smaller in oyster cages relative to natural rocky areas, despite higher abundance of the species at aquaculture sites. Our work also focused on three isolated aquaculture operations over a single culture cycle. Thus it is not possible to extrapolate results to consider the cumulative effects of multiple culture cycles in a single location through repeated disturbance or the landscape effects of a mosaic of adjacent aquaculture areas interspersed with other habitat types (see Dumbauld et al. 2009). Additional monitoring effort and spatially-explicit modeling work will be required to develop an understanding of these phenomena, which will be critical if this method of aquaculture continues to expand in the region. Moreover, our sampling was not adequate to assess rare or patchy species, particularly salmonids. SCUBA surveys and shoreline transects provide only a cursory appraisal of salmonid habitat use in this context, and given the contentious nature of salmon management in the region, rigorous assessment is critical. We recommend using alternative sampling methods such as beach seining to evaluate use of geoduck aquaculture by outmigrating smolts.

Future research should focus on the issues described above, as well as ecosystem effects on higher trophic levels. Nevertheless, our results provide valuable insight into the ecological effects of geoduck aquaculture practices and add to a growing body of work describing the effects of anthropogenic disturbance on nearshore marine ecosystems. Most importantly, these data will aid regulatory authorities and resource managers in placing aquaculture-related disturbance in appropriate context for decision-making so as to balance the needs of stakeholders and environmental protection.

Acknowledgements

We thank J. Eggers, N. Grose, M. Langness, F. Stevick, D. Todd, J. Toft, and others who assisted with SCUBA and collected field data in southern Puget Sound. J. Olden consulted on aspects of the analyses. Helpful comments and assistance from K. Holsman greatly improved the manuscript. This research was funded in part by the Washington State Legislature, Washington Department of Ecology, and Washington Department of Natural Resources. Additional support to A.W.E.G. was from the Castagna Student Grant for Applied Research (2009; National Shellfisheries Association). The views expressed herein are those of the authors and do not necessarily reflect the views of the funding agencies. Any use of trade product or firm name is for descriptive purposes only and does not imply endorsement by the US Government.

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Tables and Figures

Table 1. Description of local conditions and biota at geoduck aquaculture sites in Puget Sound (see also Figure 1).

Site/Status	Description	Biota
Stratford site – gear placement June 2009 (gear removed April 2011)		
5,100 m^2 farm; 2,500 m^2 plots	The site is on the east shore of Case Inlet (47°19’10.86”N, 122°47’38.56”W). Sandy substrate. (~ 500 μm grain size); slope moderate from +0.61 m to -0.61 m MLLW; Reference area is 150 m to the south on private property.	Horse clams and cockles present; Sand dollars patchy.
Rogers site – gear placement November 2008 (gear removed April 2011)		
5,100 m^2 farm; 2,500 m^2 plots	The site is on the east shore of Case Inlet (47°14’53.13”N, 122°49’37.38”W). Substrate is sandy to muddy sand. (~ 250-500 μm grain size). Beach is steeper and narrower than other sites. Green algae is abundant and freshwater seepage occurs. Reference area is 150 m to the south on private property.	Horse clams and cockles present; graceful crab abundant. Sand dollars patchy.
Fisher site – gear placement June 2009 – July 2009 (90% gear removed April 2011)		
2,500 m^2 farm; 2,500 m^2 plots	The site is in the northeast portion of Totten Inlet on the south shore, in the Carlyon Beach area (47°10’32.28”N, 122°56’33.79”W). Substrate is muddy sand (~ 250 μm grain size). Reference area is 75 m to the east on private property.	Horse clams present; crabs, sea stars and moon snails abundant.

Table 2. Functional groups for commonly observed taxa in SCUBA surveys of three geoduck aquaculture sites in Puget Sound, Washington, 2009-2011.

Functional group	Common name	Scientific name	Frequency in surveys (%)
cockle	heart cockle	*Clinocardium nuttallii*	29.6
crab (true crab)	graceful crab	*Metacarcinus gracilis*	89.4
	kelp crab	*Pugettia product*	47.0
	red rock crab	*Cancer productus*	29.6
	graceful decorator crab	*Oregonia gracilis*	7.6
hermit crab	black-eyed hermit crab	*Pagurus armatus*	65.2
	Bering hermit crab	*Pagurus beringanus*	15.9
moon snail	Pacific moon snail	*Lunatia lewisii*	55.3
other benthic invertebrate	dendronotid nudibranch	*Dendronotus* spp.	10.6
	black-tailed crangon	*Crangon nigricauda*	4.6
	gian sea cucumber	*Parastichopus californicus*	0.8
sea star	sunflower star	*Pycnopodia helianthoides*	53.0
	pink sea star	*Pisaster brevispinus*	38.6
	mottled sea star	*Evasterias troschelli*	22.7
	ochre sea star	*Pisaster ochraceus*	15.9
flatfish	speckled sanddab	*Citharichthys stigmaeus*	42.4
	starry flounder	*Platichthys stellatus*	18.9
	sand sole	*Psettichthys melanostictus*	6.8
gunnel	saddleback gunnel	*Pholis ornata*	6.1
	pinpoint gunnel crescent	*Apodichthys flavidus*	1.5
	gunnel	*Pholis laeta*	0.8
other demersal fish	plainfin midshipman	*Porichthys notatus*	4.6
	sturgeon poacher	*Podothecus accipenserinus*	5.3
other nearshore fish	bay pipefish	*Syngnathus leptorhynchus*	18.9
	snake prickelback	*Lumpenus sagitta*	8.3
	tubesnout	*Aulorhynchus flavidus*	0.8
sculpin	staghorn sculpin	*Leptocottus armatus*	37.1
	roughback sculpin	*Chitonotus pugetensis*	3.0
seaperch	shiner surf perch	*Cymatogaster aggregate*	6.1
	striped surf perch	*Embiotoca lateralis*	0.8

Table 3. PerMANOVA results for multivariate abundance data for all resident macroinfaunal taxa in core samples. Models included month of sampling (MONTH), plot type (culture plot or reference area; PLOT), phase of culture (pre-gear, gear-present, post-gear; PHASE), and the interaction of plot type and phase. Significance alpha (α)=0.05; significant results are bold italics.

Site	Factor	df	SS	MS	R^2	F	P
FISHER	MONTH	9	1.269	0.141	0.266	2.252	0.001
	PLOT	1	0.496	0.496	0.253	7.927	0.001
	PHASE	2	0.301	0.151	0.047	2.406	0.008
	PLOT:PHASE	2	0.195	0.098	0.023	1.558	0.116
	Error	27	1.691	0.063	0.411		
	Total	41	3.952				
ROGERS	MONTH	9	1.335	0.148	0.266	2.229	0.001
	PLOT	1	1.269	1.269	0.253	19.077	0.001
	PHASE	2	0.236	0.118	0.047	1.770	0.039
	PLOT:PHASE	2	0.113	0.057	0.023	0.848	0.643
	Error	31	2.063	0.067	0.411		
	Total	45	5.016				
STRATFORD	MONTH	9	2.278	0.253	0.398	2.757	0.001
	PLOT	1	0.792	0.792	0.138	8.623	0.001
	PHASE	2	0.380	0.190	0.066	2.072	0.020
	PLOT:PHASE	2	0.168	0.084	0.029	0.916	0.529
	Error	23	2.111	0.092	0.369		
	Total	37	5.729				

Table 4. Results of the test of multivariate homogeneity comparing multivariate dispersion (HMD test) of resident macroinvertebrate communities of culture plots and reference areas. Multivariate dispersion, a measure of beta-diversity, is associated with environmental stress and disturbance; the measure is calculated as the mean distance of all culture phase/habitat community samples to their group centroid in principal coordinate space defined by Bray–Curtis compositional dissimilarity. Significance alpha (α)=0.05; significant results are bold italics.

		Multivariate dispersion			
site	**phase**	**culture**	**reference**	**F**	**P**
Stratford	pre-gear	0.34	0.33	0.007	0.93
	gear-present	0.32	0.35	0.178	0.68
	post-gear	0.35	0.25	**14.608**	**<0.01**
Rogers	pre-gear	0.18	0.19	0.162	0.70
	gear-present	0.28	0.31	0.480	0.69
	post-gear	0.21	0.23	1.026	0.34
Fisher	pre-gear	0.20	0.22	0.355	0.57
	gear-present	0.27	0.28	0.261	0.64
	post-gear	0.25	0.22	0.790	0.44

Table 5. Results of two-way crossed ANOSIM tests comparing the transient fish and macroinvertebrate community assemblage in geoduck culture plots and reference areas across three phases of aquaculture operations: pre-gear, gear-present, and post-gear. A Monte Carlo permutation test with 999 iterations generated the test statistics (R). Significance alpha (α)=0.05; significant results are bold italics.

Test Groups	ANOSIM R	P
(Pre-gear) reference area vs. culture plot	-0.0501	0.761
(Gear-present) reference area vs. culture plot	***0.0808***	***0.035***
(Post-gear) reference area vs. culture plot	-0.0254	0.789
(Pre-gear) vs. (Gear-present) reference area	0.1176	0.093
(Pre-gear) vs. (Gear-present) culture plot	***0.1557***	***0.040***
(Pre-gear) vs. (Post-gear) reference area	-0.0268	0.600
(Pre-gear) vs. (Post-gear) culture plot	-0.0851	0.842
(Gear-present) vs. (Post-gear) reference area	***0.0900***	***0.029***
(Gear-present) vs. (Post-gear) culture plot	***0.1604***	***0.003***

Table 6. Results of Shannon diversity index (H') calculations for transient fish and macroinvertebrates at geoduck culture plots and reference areas across three phases of aquaculture operations: pre-gear, gear-present, and post-gear. Differences among culture plots and reference areas were examined with Welch's t-test with alpha (α)=0.05; significant results are bold italics. Total abundance of all observed organisms is included.

Phase	Plot type	Shannon diversity index (H')	t-test results for diversity values	Total # of organisms observed
pre-gear	reference	1.111	t=0.703, df=11, p=0.496	530
	culture	1.188		628
gear-present	reference	0.923	t=0.727, df=18, p=0.476	795
	culture	1.021		1692
post-gear	reference	1.163	t=0.309, df=25, p=0.760	621
	culture	1.207		694

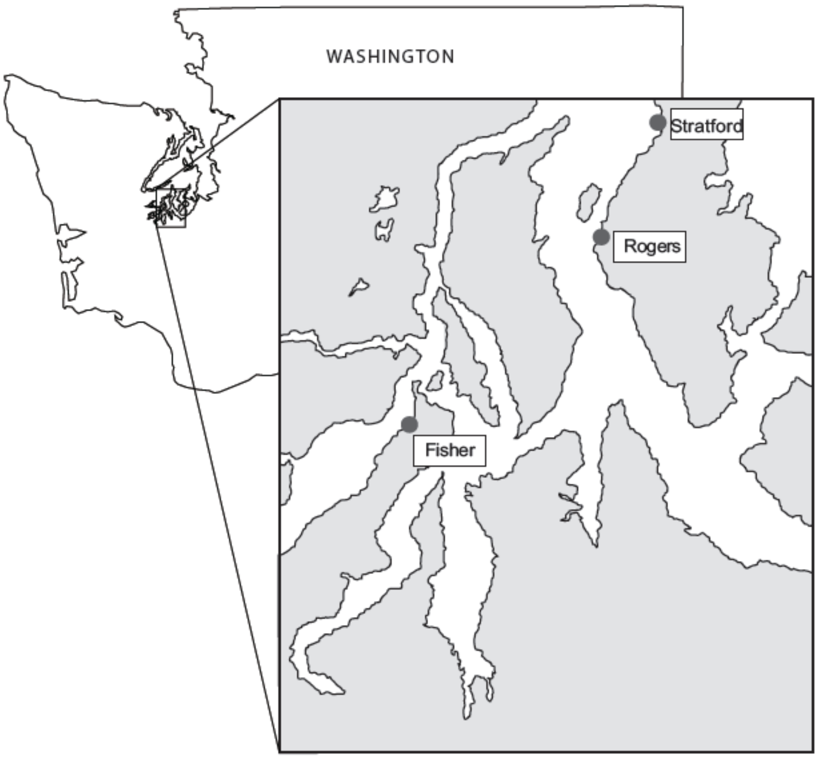

Figure 1. Locations of study sites in south Puget Sound, Washington, USA. Inset map shows the region of interest; most geoduck aquaculture in Washington State occurs within the area demarcated by the box.

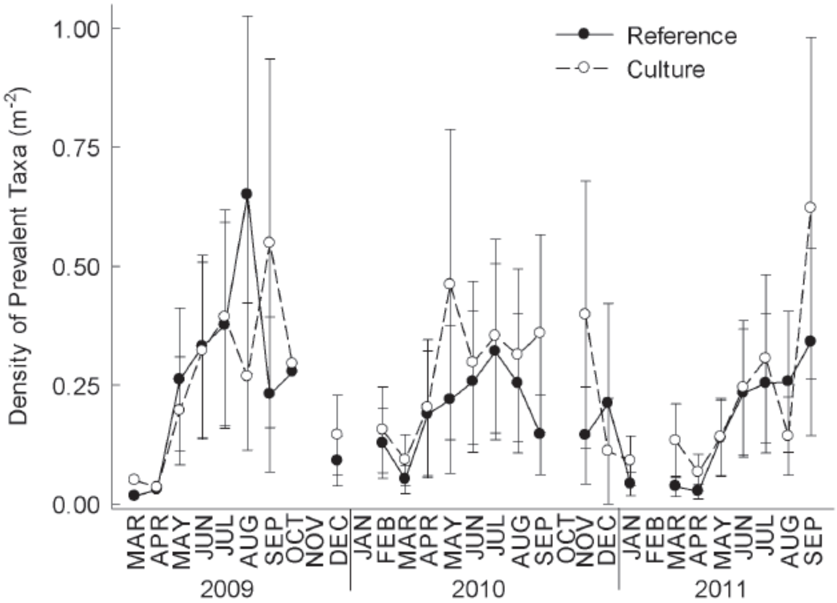

Figure 2. Density of prevalent taxa in SCUBA surveys of transient macrofauna (fish and invertebrates) defined as species present in at least 10% of surveys. Data were collected on culture plots (culture) and adjacent reference areas (reference) at three sites in southern Puget Sound during SCUBA surveys 2009-2011. Note: northern kelp crab (*Pugettia producta*) are excluded. Error bars are ±SE.

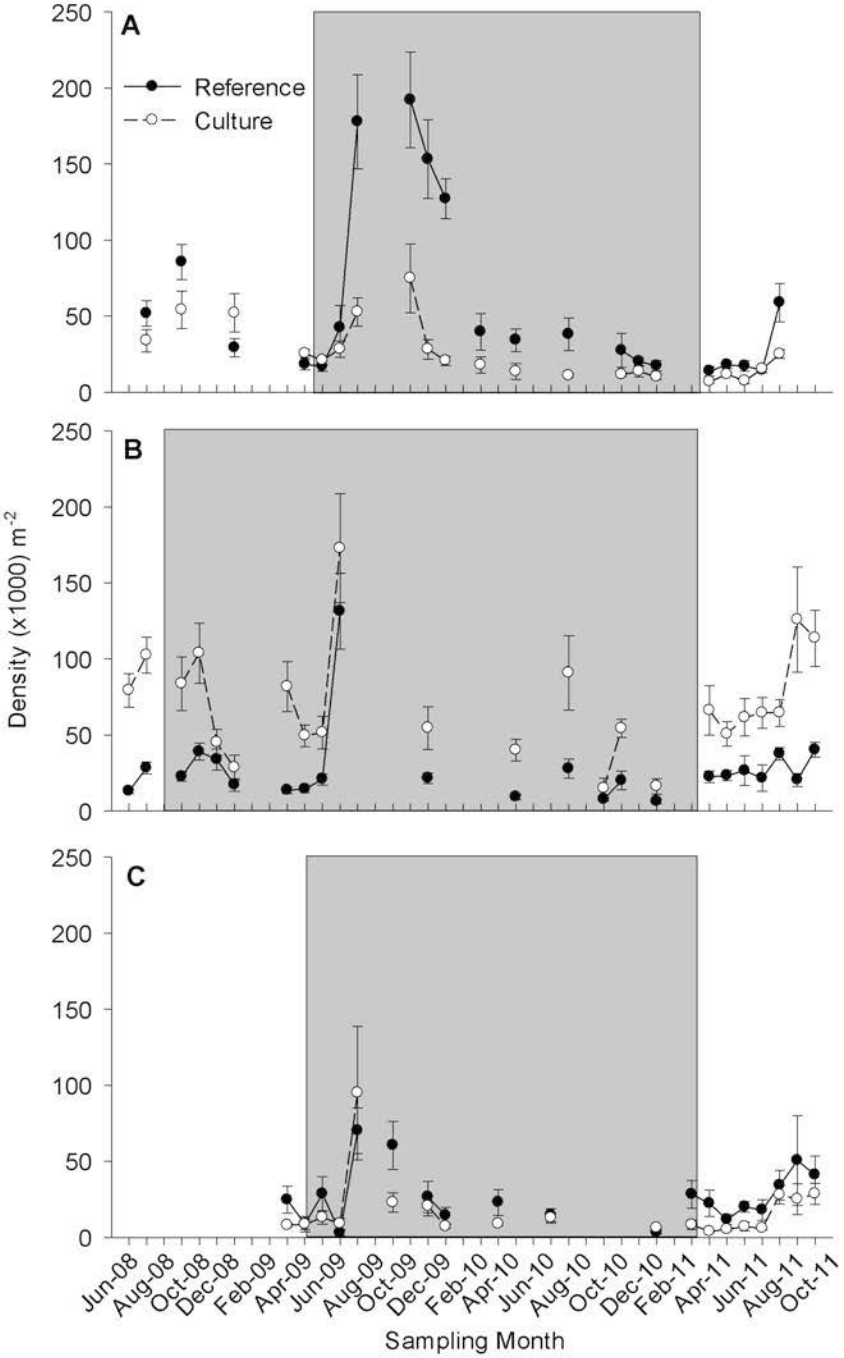

Figure 3. Density (in 1000s per m^2) of total taxa in surveys of resident macrofauna (infauna and epifauna). Data were collected on culture plots (culture) and adjacent reference areas (reference) at three sites in southern Puget Sound: Fisher (A), Rogers (B), and Stratford (C). Shaded areas illustrate the aquaculture phase when PVC tubes and nets were in place to protect juvenile geoducks (gear-present). Error bars are ±SE.

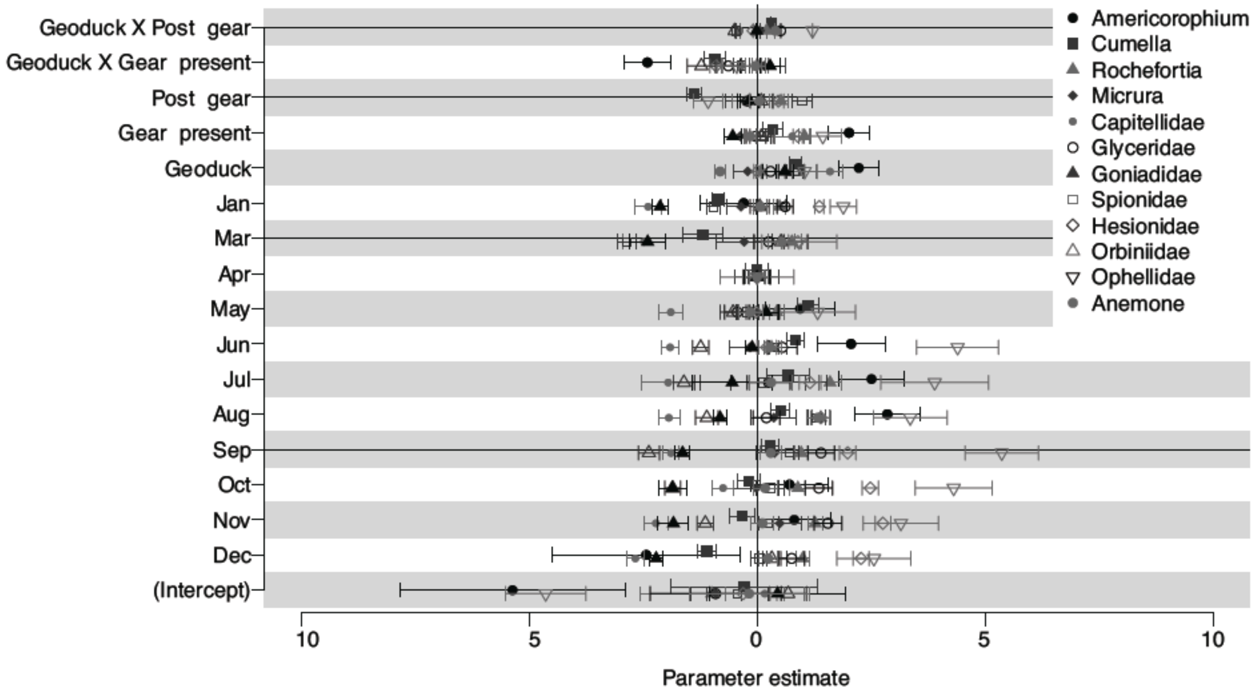

Figure 4. Parameter estimates and 95% confidence intervals for generalized linear mixed models of selected macroinfauna. The models included main effects of month of sampling, plot type (geoduck culture or reference area), phase (pre-gear, gear-present, post-gear), and their interaction, as well as random effects of site (Fisher, Rogers, and Stratford). As noted in the text, a significant interaction term provides evidence of an effect of aquaculture operations on abundance.

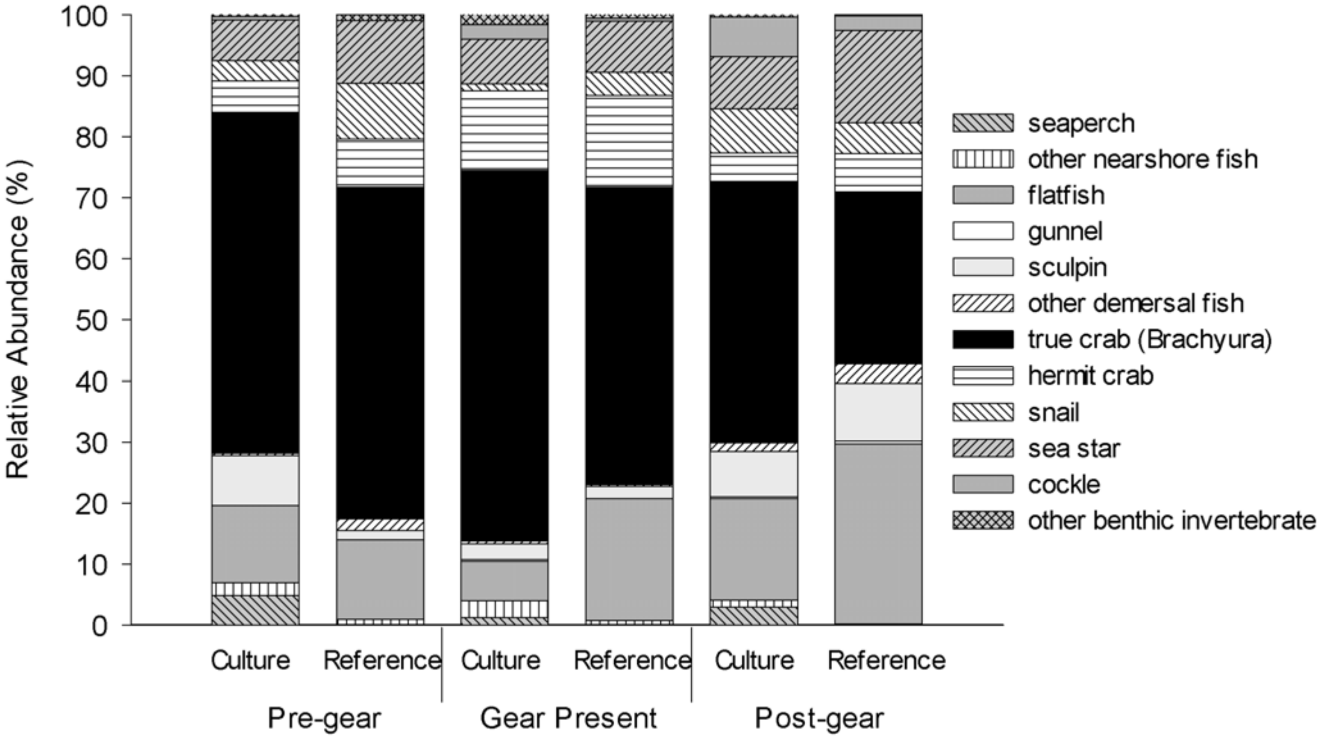

Figure 5. Relative abundance of 10 functional groups of transient fish and macroinvertebrates on geoduck culture plots (culture) and adjacent reference beaches (reference) during SCUBA surveys at three sites in southern Puget Sound (2009-2011). Data are presented in three April-October periods comprising three phases: 1) "Pre-gear", prior to placement of geoducks or aquaculture gear; 2) "Gear-present", when tubes and nets are in place; and 3) post-gear, after nets and tubes have been removed and geoducks are in place.

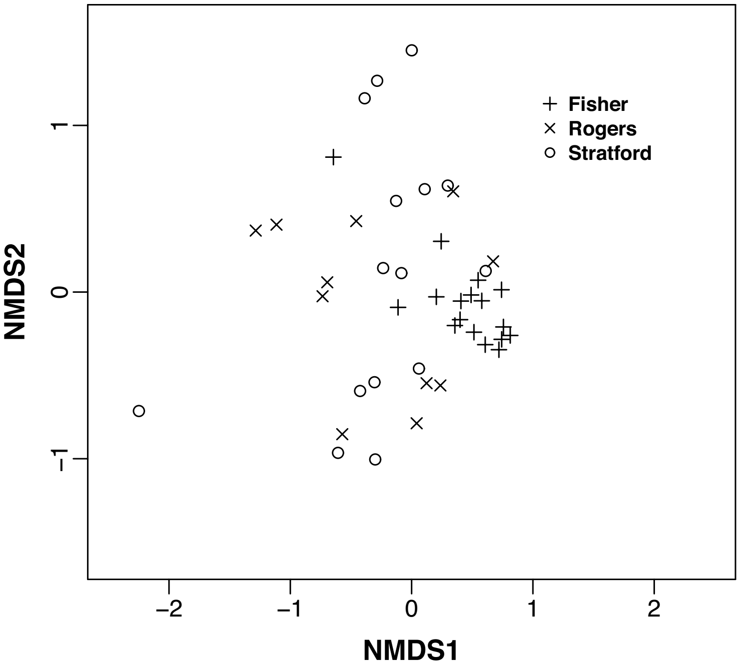

Figure 6. Two-dimensional NMDS ordination of SCUBA surveys at reference areas during 2010, which corresponds to when aquaculture gear was in place (gear-present) on the culture sites. Stress=17.24. Stress tested statistically significant under the Monte Carlo randomization approach ($p<0.01$). A goodness of fit Shepard plot showed good correlation between the ordination distances and the Bray-Curtis dissimilarities (linear fit $R^2=0.882$).

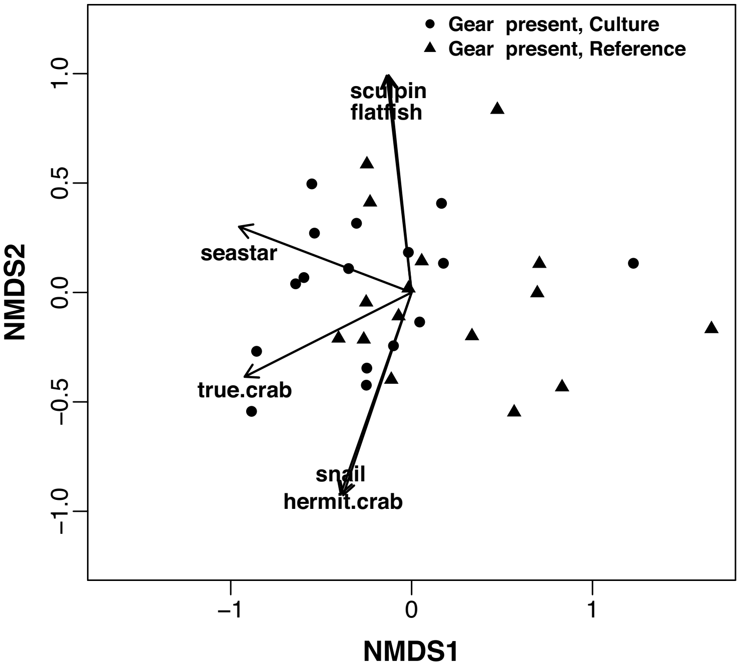

Figure 7. Two-dimensional NMDS plot of SCUBA surveys at culture plots (green circles) and reference areas (orange triangles) when aquaculture gear was in place (gear-present). Functional group vectors shown are those with $p<0.05$. Stress=13.87. Stress value tested statistically significant under the Monte Carlo randomization approach ($p=0.02$). A Shepard plot showed good correlation between the ordination distances and the Bray-Curtis dissimilarities (linear fit $R^2=0.925$). Vector loadings are shown for significant functional groups ($p<0.05$).

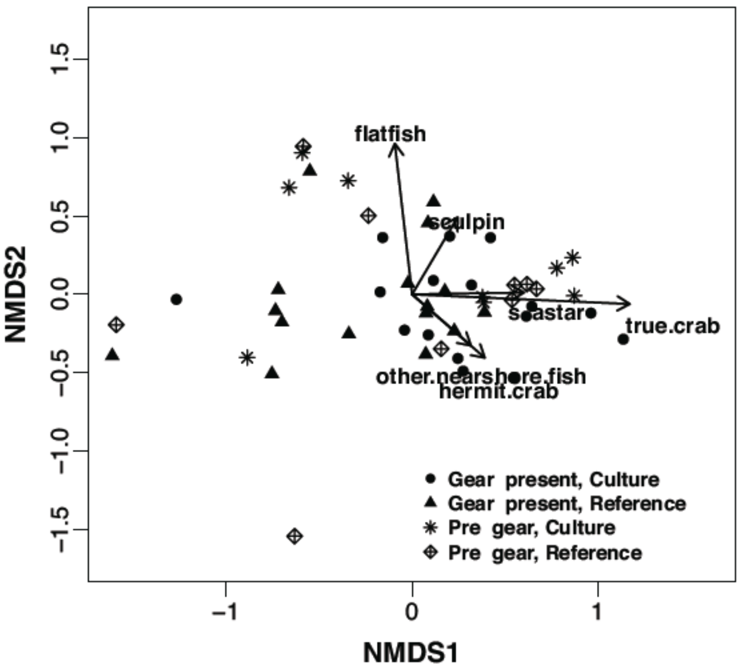

Figure 8. Two-dimensional NMDS plot of SCUBA surveys at culture plots and reference areas prior to deployment of aquaculture gear (pre-gear) and when aquaculture gear was in place (gear-present). Functional group vectors shown are those with $p<0.05$. Stress=14.498. Stress value tested statistically significant under the Monte Carlo randomization approach ($p<0.01$). A goodness of fit Shepard plot showed good correlation between the ordination distances and the Bray-Curtis dissimilarities (linear fit $R^2=0.918$). Vector loadings are shown for significant functional groups ($p<0.05$).

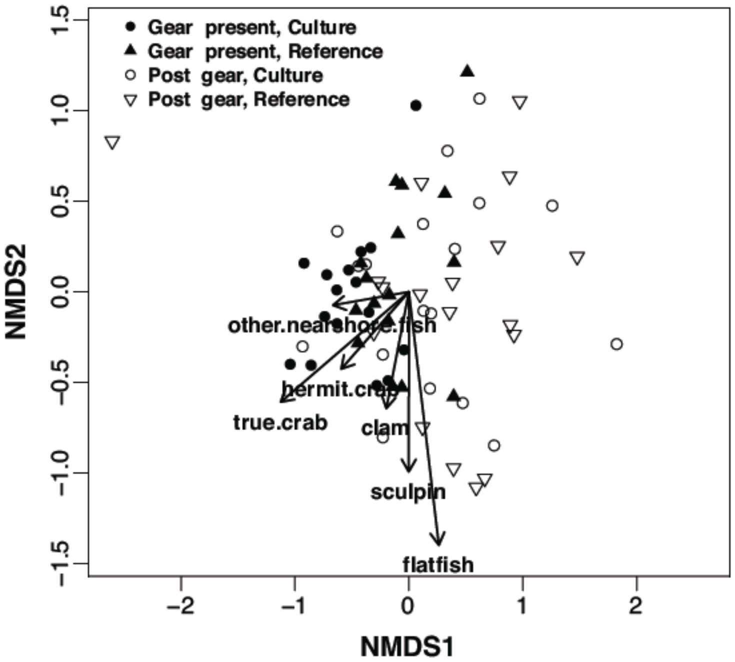

Figure 9. Two-dimensional NMDS plot of SCUBA surveys at culture plots and reference areas when aquaculture gear was in place (gear-present) and after gear was removed (post gear). Functional group vectors shown are those with $p<0.05$. Stress=18.08. Stress value tested statistically significant under the Monte Carlo randomization approach ($p=0.03$). A goodness of fit Shepard plot showed good correlation between the ordination distances and the Bray-Curtis dissimilarities (linear fit $R^2=0.877$). Vector loadings are shown for significant functional groups ($p<0.05$).

Appendix III

The influence of culture and harvest of geoduck clams (*Panopea generosa*) on sediment nutrient regeneration

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Abstract

The effects of geoduck (*Panopea generosa*) cultivation and harvest on local and whole ecosystem nutrient balances has been evaluated using biogeochemical measurements. Geoducks are cultivated at high densities (15 m^{-2}) in the low intertidal zone of Puget Sound and some of the public opposition to such cultivation has centered around concerns about nutrient releases during harvest. Geoducks are located at depths to 0.6 m in the sediment and commercial harvest utilizes a water jet to liquefy the sediment to aid manual removal of the clam. We examined nutrient pore water chemistry of geoduck and reference sediments, sediment-water exchange rates of nutrients using incubation techniques, and the release of nutrients during harvest. Pore water nutrient concentrations (N, P species) were higher in geoduck cultivation areas than in reference areas in several of the cultivation areas we examined, with elevated soluble reactive P concentrations suggesting P biogeochemical processes included desorption from the particulate phase. Similarly, higher effluxes of soluble reactive P were observed at the one site we examined. The release of N and P species during geoduck harvest resulted in a modest elevation of nutrient concentrations of the water used for harvest, suggesting that during harvest the liquefaction does not quantitatively remove the pore water and that effluxes are generally low. When extrapolated to all Puget Sound cultivated geoduck harvest on a daily basis, the harvest release of N primarily as NH_4^+ represents ~0.001% of South Puget Sound wastewater or riverine inputs. This means that the major concern that this harvest may release sufficient DIN and DIP to stimulate an algal bloom is not supported. Overall, the magnitude of nutrients released by current levels of geoduck aquaculture is small compared to new anthropogenic nutrient inputs into Puget Sound.

1. Introduction

Verwey (1952) first identified the pivotal role that suspension-feeding bivalves serve in benthic-pelagic coupling by filtering seston from the water column and transferring undigested particulate material in their feces and pseudofeces (= biodeposits) to the sediment surface. Subsequent research (Dame, 2012; Newell, 2004) has shown that inorganic nitrogen and phosphorus are released back to the water column both by direct excretion from the bivalves and through regeneration of organic biodeposits by infaunal microbes and bioturbating metazoans. It is the balance between the removal of nutrients in particulate material from the water column and the subsequent burial or regeneration of inorganic nutrients from their biodeposits that make bivalve suspension feeders an important component of nutrient cycling in shallow coastal systems (Cerco and Noel, 2007; Newell et al., 2005).

Although the ecosystem benefits of natural stocks of suspension feeding bivalves in the mediation of benthic-pelagic coupling and other ecosystem services are recognized (Coen et al. 2007; Shumway, 2011) there is generally less information available for the higher densities of bivalves typically maintained on commercial aquaculture farms (Newell, 2004). However, in recent years several examples of detailed sediment nutrient balances with clam aquaculture have been developed (Nizzoli et al. 2006; 2007). For example, geoducks, *Panope generosa* Gould 1850 (Vadopalas et al., 2010) are commercially valuable and have been harvested for many years from natural sub-tidal natural stocks in Puget Sound Washington, USA. Geoducks are long-lived and extremely large infaunal clams, with average adult shell lengths of 13.5 cm (Straus et al., 2008). Most notably, this species is very deep burrowing, with large adult clams being generally found at depths of 0.5 to 0. 6 m, but also to depths of 1 m (Straus et al., 2008). Geoduck cultivation began alongside shellfish hatchery technology in the mid-1990's with densities of ~15 m^2 in muddy-sand sediment in the low intertidal zone of Puget Sound, and in 2008 through 2010 production of farmed geoducks averaged 637,302 kg live whole weight (Pers. Comm. Bobbi Hudson, Pacific Shellfish Institute), equivalent to ~ 936,675 individual clams (Table 1). Although the amount of low intertidal zone devoted to geoduck cultivation is not precisely known, growers are currently harvesting ~51 acres (21 ha) per year, so given a seven year grow-out cycle, it is estimated that in 2010 there were ~ 355 acres (144 ha) populated with various age classes of geoducks (adapted from Anchor QEA 2011; other data suggest a lower harvest area, see Table 1). Our best estimate for the average harvest time per day suggested for Puget Sound is ~21.4 h d^{-1}; with most harvest at very low tidal stages, the effective amount of harvest effort within this ecosystem could be 10 fold higher (i.e. ~200 h d^{-1}) during harvest periods.

It takes about five to seven years for cultivated geoducks to reach market size and during this period the animals may provide some of the same valuable ecosystem services as natural clam populations. It is possible, however, that some of the benefits from geoduck aquaculture may be reduced or even negated due to the harvest process. Unlike other commercially cultivated epibenthic bivalves, such as oysters and mussels, geoducks live buried deeply in the sediment. Consequently they have to be harvested with a water jet that liquefies the sediments, thereby allowing the harvester to reach into the sediment and retrieve the buried clam (Strauss et al. 2008). This harvest process is potentially disruptive of the benthic plant, animal and microbial communities that are essential to maintain normal sedimentary processes; plant and community effects of culture and harvest suggest such communities are resilient (Price 2011, Ruesink and Rowell 2012). Sediment bioturbation by benthic animals can be a major control of biogeochemical processes through the movement of oxygen and water into the sediment, enhancing organic matter decomposition, and stimulating nitrification (Aller, 1982). The microbial communities that are major agents of organic mineralization, including coupled nitrification-denitrification, only flourish under particular oxygen concentrations and these gradients will altered by the harvesting process. These communities can take a prolonged period to recolonize sediments and reestablish biogeochemical gradients (Porter et al. 2006). Finally, N and P that is either in deep pore water or bound to sediments may be flushed out in a large pulse to become part of the water column nutrient pool, that supports phytoplankton growth, and potentially stimulate harmful algal blooms.

We examined if, and to what extent, culturing and harvesting geoducks in Puget Sound, Washington USA, increased inorganic N and P nutrient accumulation in sediments. Most sites were chosen to correspond to locations in which geoduck culture and harvest effects on benthic infauna were assessed (VanBlaicom et al., *In Press*). We worked at several aquaculture farms to measure nutrient concentrations within the interstitial water at various depths in the sediment where geoducks had been reared for 5 to 8 y and compared these to nearby reference sites. We also measured the release of these nutrients in the effluent water during commercial geoduck harvest and measured interstitial nutrient concentrations after harvest had occurred.

2. Materials and Methods

2.1. *Site Selection*

We worked at six commercial aquaculture farms within Puget Sound, Washington, USA, selected to be representative of the range of conditions where geoducks are reared (Table 2 and Figure 1). At each farm the area sampled contained geoducks ("Geoduck") of a commercial size that were due to be harvested within our study period in 2008 and 2009; at some sites additional sampling took place after harvest ("Harvest"). At each farm a nearby reference site ("Reference") was selected that was similar in sediment characteristics and topography but was located at a distance (50 to 300 m) and position such that it was not influenced by the cultivated geoducks.

2.2. *Sediment-Water Exchange Rates*

Rates of sediment-water nutrient, oxygen, and N_2-N exchange were measured using sediment incubation techniques (Cornwell and Owens, 2011; Newell et al., 2002) at geoduck and reference locations only at the Thorndyke site. The use of large (19 cm inner diameter) *in situ* chambers to measure fluxes with both sediment and geoducks was attempted but efforts were hindered by high tidal velocities and obvious behavioral differences as the geoducks retracted their siphons with chamber emplacement and operation. In June 2009, we randomly collected 4 sediment cores from within the geoduck bed and 4 additional cores from a reference site located 50 m outside the culture area. Cores did not include geoducks. A 30 cm long transparent acrylic tube (6.35 cm id) was manually pressed into the sediment to a depth of ~ 15 cm and then carefully removed, thereby extracting the sediment core. Each core tube was sealed on the bottom with an o-ring cap, and a rubber stopper was used to cover the top for transport. Within 1 h of collection the stoppers were removed and each tube submerged in an incubation tank (~ 40 L) filled with seawater from the core collection site and regulated at ambient water temperature (16°C). Incubation tanks were placed outdoors in order to maintain sufficient photosynthetically active radiation (PAR) from sunlight to sustain photosynthesis by any benthic microalgae; the flux apparatus attenuates about 20-40% of PAR and the light conditions would best simulate low tide water depths. "Air-lift" aeration pipes (PVC, 2.5 cm id) were inserted into each core tube such that they reached to ~ 3 cm of the sediment surface (Newell et al., 2002). Open tubes were continuously aerated for 2 h before initiation of flux incubations to allow sediments to equilibrate thermally, ensure oxygen saturation, and condition the acrylic so that gas exchange with the plastic was minimal.

Following the 2 h equilibration period an acrylic o-ring lid with a suspended magnetic stir bar was used to seal each core tube. An external magnetic turntable was used to induce rotation of each stir bar in the core tube and thereby maintain well mixed overlying water. Identical tubes without sediment were filled with ambient water and incubated in triplicate to measure water column activity (i.e., blanks). Water samples for initial solute and gas concentrations were collected immediately after sealing the tubes and additional samples were taken at ~ 1 h intervals for 5 time points in the light. When light incubations were completed, the cores lids were removed and each again aerated for 1 hour with the air-lift pipes and an opaque cover to exclude all light was installed. The cores tubes were resealed and an additional 4 time points were sampled under dark conditions. The small volume of water (<30 ml) pumped from the head space at each sampling time was replaced by gravity feeding ambient seawater into the head space such that no air bubbles were introduced.

Water for solute analyses of NH_4^+, NO_x (NO_2^- + NO_3^-), soluble reactive phosphorus (SRP), and dissolved silicate (DSi) was pumped into a 20 ml syringe and immediately filtered (0.45 mm) into plastic vials and stored at -25^0C until analysis. Water samples for gas analysis (O_2, N_2) were collected in glass tubes (7 ml), preserved with 10 ml 50% saturated $HgCl_2$ and held submerged in sub-ambient temperature water until analysis via membrane inlet mass spectrometry (Kana et al., 1994). The head space water volume for each core was calculated from water depth and core area and this was used to convert nutrient concentrations into fluxes:

$$F = \frac{\Delta C}{\Delta t} * \frac{V}{A}$$

Where F is the flux (mmol m^{-2} h^{-1}), $\triangle$C/Dt is the slope of the concentration change in overlying water (mmol L^{-1} h^{-1}), V is the volume of the overlying water (L) and A is the area of the incubated core (m^{-2}). Only those regressions that were significant ($r^2 > \sim 0.8$; $F \leq 0.10$) were used to determine flux rates. Calculations were performed separately for dark and light periods, and positive and negative fluxes were indicative of sediment nutrient release and uptake, respectively. The slopes from the water-only core fluxes were used to adjust the core slopes for water column changes. Fluxes of N_2-N represented the net exchange of N_2-N at the sediment-water interface since the reactions mediating these fluxes (e.g., N_2 fixation, denitrification, anammox) were not parsed out. Samples for chlorophyll a analysis were subcored to a depth of 1 cm with a cut-off and stored at -25 C until analyzed.

2.3. Pore Water Nutrient Collection

We used a combination of three different methods to determine pore water inorganic nutrient concentrations. Each method allowed us to obtain data at different depths in the sediment and integrated over various time scales.

2.3.1 Pore Water Equilibrators

Pore water equilibrators were constructed from a 50 cm long solid PVC block with a series of chambers (~7 mL) bored along its entire length (Hesslein, 1976). Before field deployment, each of these chambers was filled with 0.22 µm filtered ambient seawater and then covered with 0.22 µm pore size filter membrane spread over the front of the entire block. This membrane was tightly clamped in place trapping the water in each chamber. These devices were then fully inserted into the sediment at two locations (three equilibrators in reference areas and three among geoduck culture areas at the Manke farm and three at the unplanted Rogers site). The equilibrators were left in the sediment for 6 d allowing ions to diffuse and equilibrate between the pore water and the chamber. Upon retrieval, the water in each chamber was syringe filtered (0.45 mm) and stored frozen (-25^oC) until analyzed. This approach was restricted in spatial extent and we only used it in initial validation studies to compare with data obtained using the two other techniques for sampling interstitial water at depth over shorter time periods and broader spatial scales.

2.3.2 Interstitial Water Sipper

We constructed a 1.1 m long “sipper” from stainless steel tube (2.4 mm od, 1.8 mm id) with four 0.38 mm holes bored into the lower 5 mm of the shaft (Berg and McGlathery, 2001). This sipper was inserted to known depths (up to 1 m) into the sediment and interstitial water was slowly drawn into a syringe attached by silicon tubing to the steel tube. Water samples (generally 2-5 mL) were immediately syringe filtered (0.45 mm) and stored frozen (-25^oC). These sippers proved to be a relatively easy way to sample interstitial water at discrete depths over very short time scales and along beach transects away from the water.

2.3.3 Piezometers

Standpipe piezometers were used for sampling interstitial water at single discrete depth water and for measuring the position of the water table relative to the sediment surface. We constructed piezometers by cutting standard 1.25 cm id PVC plumbing pipe to length, one end of the pipe was capped, and then six 3 mm holes were drilled within 2 cm of the capped end. Pairs of wells were then inserted to two different depths (~50 to 80 cm depending on site) in the sediment at 4 locations along a transect up the beach at the geoduck aquaculture and reference site. At the aquaculture sites, piezometers were always set in the beach immediately above and below the location where the geoducks were being grown. This was arranged so that we could sample interstitial water before it entered the geoduck area and as it drained down the beach slope from the aquaculture area. Each piezometer was pumped dry and allowed to refill with interstitial water over a period of ~10 min. The water height in each piezometer was then measured using a multimeter to measure changes in resistance between a copper wire placed into the sediment adjacent to the standpipe and another wire that was gradually inserted into the pipe. The point at which a sharp decline in resistance occurred indicated the water level in the pipe and this depth was then measured. Samples of the water were pumped from each pipe, syringe filtered (0.45 mm) into duplicate 7 ml acid washed plastic vials, and stored frozen (-25^{o}C) until analyzed for nutrients. A laser level and measuring tape were used to determine a beach profile of the sediment surface and the level of water in the sediment bed.

2.4. *Harvest Nutrient and Particulate Efflux Rates*

The efflux of dissolved nutrients and suspended particulates during the commercial harvest of geoducks was assessed twice at Thorndyke Bay and once at the Wang-Chelsea site. First, we measured the flow of water from the harvester's water jets by timing the filling of a 20 L carboy. We then collected samples of the water from the adjacent estuary that was used as the source of water being pumped into the sediments (background). During active harvest operations we collected multiple water samples in 20 mL syringes along a transect of surface water (effluent) that was running in rivulets down the beach front back to the estuary. Samples were immediately syringe filtered (0.45 mm) into duplicate 7 ml acid washed plastic vials and stored at -25^{0}C until analysis. For total suspended sediment (TSS) loads in this effluent water, known volumes of water (~ 750 ml) were filtered through 47 mm diameter Whatman GF/F filters which had been heat treated at 450^{0}C and preweighed. Filters were rinsed under vacuum with 2 ml of DI water to remove salts and stored frozen (-25^{0}C). Filters were subsequently dried (24 h at 70^{0}C), weighed, and then heated to 450 ^{0}C to volatilize any organic matter before being reweighed. The Particulate Organic (POM) and Particulate Inorganic Matter (PIM) concentrations were then expressed as mg L^{-1}. Particulates remaining on the filter were extracted with HCL and analyzed for particulate phosphorus (Aspila et al., 1976).

2.5. *Chemical analyses*

A Smartchem 200 discrete analyzer was used for automated inorganic nutrient analysis (NH_4^+, NO_x (= NO_2- + NO_3-), Si, SRP). All methods followed Parsons et al. (1984) and some samples were diluted to allow analysis of all analytes. Dissolved Fe in pore water was analyzed using Ferrozine colorimetry (Gibbs, 1979). Chlorophyll *a* concentrations were measured using an acetone extraction followed by fluorometric analysis (Parsons et al., 1984).

2.6. *Sediment Grain Size*

We collected sediments to a depth of 10 cm from each of the sites to characterize general grain size. Sediment grain size was determined using wet sieving (62 µm) and pipette analysis of the < 62 um fraction, thus determining the proportions of sand, silt and clay (Sweet et al., 1993).

2.7. *Statistical Analysis*

The data from each site was evaluated by one–way ANOVA (SigmaPlot™). Most data was not normally distributed and significance was determined at $P < 0.05$ using either a Kruskal-Wallis one–way ANOVA on ranks or a Mann-Whitney rank sum test. Low numbers of incubation cores limited the ability to discern flux rate differences at the Thorndyke site. For the pore water data, we compared the within site data for each analyte for the Geoduck and Reference environments, and compared those environments with the Harvest data where available.

3. Results

3.1 *Sediment Grain Size*

The intertidal sediments in the various study sites were generally fine-grained sediment (silt + clay), with average percentages at Rogers, Foss-Joemma, Manke and Thorndyke ranging from 0.6-1.9% silt + clay; the Wang-Chelsea site had more fine grained sediment, with silt + clay averaging 14% (Table 2). The low percentages of fine-grained materials indicate relatively large interstitial spaces. In such conditions ion movement is predominantly through direct advective transport processes, rather than diffusive processes (Huettel et al., 1998).

3.2 *Sediment-Water Exchange*

The sediment-water exchange data presented here do not include the direct activities of geoducks since they were not included in the incubations. Differences between geoduck and control locations were not significant for any of the fluxes (Table 3). The net heterotrophic uptake of O_2 in sediment cores was diminished under illumination for both Geoduck and Reference sites. Nitrogen fluxes (NH_4^+, NO_x^- and $N_{2)}$ did not differ significantly between sediments collected from Geoduck and Reference locations when measured under either light or dark conditions (Table 3). In general, ammonium was the largest flux of nitrogen in this system, with higher average effluxes under dark conditions. Average fluxes of NO_x^- were directed into the sediment at both sites and under both light and dark incubations. Average soluble reactive phosphorus flux rates were higher in Geoduck sites during dark incubations than in Reference sites though not significantly. Average rates of DSi release from the sediment to the water column were higher during dark incubations than during light incubations. Sediment chlorophyll *a* concentrations were 78.3±9.6 mg m^{-2} at the Geoduck site and were not significantly different relative to the Reference site (76.4±11.5 mg Chl *a* m^{-2}).

3.3 *Pore Water Chemistry*

The pore water equilibrators provided a fine-scale vertical profile of pore water chemistry relative to the other techniques of pore water collection we used, although the equilibrators were only deployed in sediments in the very lowest level of the intertidal zone. The SRP and NH_4^+ concentrations (Figure 2) indicate very different patterns of N and P enrichment with depth in the three different locations where we deployed them. The Rogers Site did not contain geoducks but showed near-surface enrichment of both SRP and NH_4^+. In contrast the Manke Reference and Geoduck profiles for SRP and NH_4^+ exhibited sub-surface concentration peaks in the top 10-20 cm of the sediment profile. At the Manke geoduck locations, the peak SRP concentrations were generally > 200 mmol L^{-1}, considerably higher than the < 50 mmol L^{-1} SRP concentrations at the Manke Reference site and the < 15 mmol L^{-1} observed at the Rogers Site. While the Rogers and Manke Reference sites had similar NH_4^+concentrations (most samples < 100 mmol L^{-1}), the average Manke Geoduck NH_4^+ concentration was 235 mmol L^{-1}, excluding 3 values > 1,000 mmol L^{-1}. The pore waters were devoid of H_2S, but had concentrations of dissolved Fe that averaged 46±32 mmol L^{-1} in Manke Reference, 34±19mmol L^{-1} in Manke Geoduck, and 6±4 mmol L^{-1} in Rogers sediments using sediment equilibrators (data not shown). The concentrations of DSi in the equilibrators averaged 141, 106 and 375 mmol L^{-1} , respectively, for Rogers, Manke Reference, and Manke Geoduck sites (Figure 2).

A large range of pore water NH_4^+, SRP, NO_x , and DSi concentrations from all sites measured using sippers and well piezometers at Geoduck, Reference, and Harvest sites was observed (Figure 3). . The highest average NH_4^+ concentrations were observed at the Wang-Chelsea site where Geoduck and Harvest sites averaged 485 and 345 mmol L^{-1}, respectively. The next highest average NH_4^+ concentrations were at Thorndyke Geoduck and Cooper Harvest sites (245 and 183 mmol L^{-1} respectively), with the remainder of the data between 1 and 62 mmol L^{-1}. For NH_4^+, the Cooper Harvest data was significantly higher than both the Geoduck and Reference data (Table 4). At Foss-Joemma, the sipper Geoduck and the piezometer Geoduck NH_4^+ data were both significantly higher than found at the Reference site. At Wang-Chelsea, we found significantly lower NH_4^+ concentrations at the Reference site compared with the Geoduck and Harvest pore water concentrations. Using sipper data from all times and all sites, the pore water ammonium concentrations at Geoduck sites were significantly ($P = 0.017$) higher than Reference sites (Figure 4).

The average SRP concentration was 10 mmol L^{-1}, ranging from 2-55 mmol L^{-1}. The average and median Wang-Chelsea Geoduck, Reference and Harvest SRP data were higher than all of the other sites with all but 4 of the sites/treatments having mean concentrations < 10 mmol L^{-1}. The only statistical differences within a site were between Geoduck and Reference sites for both sipper and piezometer data at Foss-Joema (Table 4).

Elevated NO_x concentrations were observed at the Rogers site, the Cooper Reference, and Foss-Joema Reference site (for both sipper and piezometer data). Most concentrations were < 2 mmol L^{-1}, consistent with a reducing environment in which NO_x would likely be denitrified. We observed significant differences between Cooper Reference and Harvest and the Geoduck/Reference pairs at Foss-Joemma (Table 4). Dissolved silicate was highest at the Chelsea-Wang site for Geoduck, Reference, and Harvest, with average concentrations > 450 mmol L^{-1}. The other sites had average DSi concentrations < 138 mmol L^{-1}. The only significant treatment difference was at Cooper, with the Geoduck data significantly lower than the Reference and Harvest data.

3.4 Harvest Nutrient and Particulate Effluxes

The flow rate of water used for a single harvest jet was ~ 2 L s^{-1}. The mass flux of nutrient per harvest jet was determined by multiplying the flow rate by the nutrient concentration. For example, for a concentration of 10 mmol L^{-1} of N or P species, the gross nutrient release would be 72 mmol $h^{-1}hose^{-1}$.

Mean ammonium concentrations (Figure 5) in the rivulets flowing from the active harvest sites were slightly higher than the concentrations observed in the estuarine source water that was pumped from offshore to liquefy the sediments during the geoduck harvest. At Thorndyke, the estuarine source water NH_4^+, SRP, NO_x and Si concentrations were 1.7, 2.3, 0.8, and 77 mmol L^{-1} respectively, while the equivalent data from Wang-Chelsea were 3.3, 1.7, 3.1, and 29.5 mmol L^{-1}. At Cooper, the effluent NH_4^+ concentrations were roughly similar to the pore water concentration, while at Thorndyke and Wang-Chelsea the NH_4^+ concentrations were < 10% of the mean pore water ammonium concentrations. At all sites, the NO_x^- concentrations in the effluent were higher than observed in the porewater. The SRP concentrations in effluent were highest at Cooper, and very low at the other harvest sites. The effluent DSi concentrations were elevated relative to pore water concentrations at Cooper, similar to pore water concentrations at Thorndyke, and much lower than pore water DSi concentrations at Wang-Chelsea.

At Thorndyke andWang-Chelsea during harvest the median and mean (±S.D.) TSS concentrations were 700 and 1104±1053 mg L^{-1} at Thorndyke and 934 and 895±535 mg L^{-1} at Chelsea-Wang (data not shown). The total P concentration on a mass basis averaged 15±4 and 12±3 mmol g^{-1} at Thorndyke and Wang-Chelsea respectively, while the organic fraction of the suspended matter (AFDW) averaged 11±4 and 8±3% for Thorndyke and Wang-Chelsea respectively. On a volumetric basis, the total particulate P concentration in effluent water was variable, with median concentrations of 11 and 10 mmol L^{-1} for Thorndyke and Wang-Chelsea- respectively, concentrations higher than observed for dissolved SRP.

4. Discussion

With declining natural stocks of commercially valuable bivalves worldwide there is increasing recognition for the need for aquaculture to supply a burgeoning demand for seafood (Shumway 2011). While it is clear that there are economic and food security benefits associated with managed cultivation of bivalves there are also ecological consequences associated with increasing the stocking density of bivalves by culturing them in intensive aquaculture farms (Shumway 2011). These consequences can be beneficial if managed correctly or potentially adverse if aquaculture farms are not sited and managed correctly (reviewed by Newell 2004).

In all discussions of bivalve aquaculture it must be remembered, however, that to grow these herbivorous animals from seed to harvest size in open waters results in no additional nutrients being added to the water. The bivalves are feeding on natural phytoplankton that are growing on existing inorganic nutrients sustained by the pool of nutrients in the water column. Aquaculture farms often hold bivalve stocks at higher densities than typical natural populations which can lead to high concentration of fecal material and urine release. This can possibly lead to local nutrient over-enrichment of the surrounding waters, and residual organic matter in the particulate waste can cause adverse effects on the surrounding sediments. In particular, concern has been voiced by citizens of Washington State, USA, about the potential for some of these adverse effects to arise from the cultivation of geoducks in the lower intertidal zone of Puget Sound. The data from this study are used here to provide insight into the processes controlling nutrient balances in geoduck cultivation and to provide a quantitative perspective on nutrient releases relative to nutrient balance of Puget Sound.

Placing the pore water and sediment-water exchange data in a biogeochemical perspective requires consideration of the physical and biological characteristics at each site. These geoduck aquaculture sites are continually submerged for all but a brief time on low water spring tides. Our sampling necessarily occurred during these low tide periods, though our intact core fluxes had overlying water for the incubation. The key finding from the pore water equilibrator data, obtained over a continuous 6-d period with 12 full tidal cycles of immersion and emersion, is that the presence of dissolved iron indicates that the pore waters are devoid of oxygen. The presence of elevated dissolved iron suggests the presence of reducible solid phase Fe-oxides which commonly produce Fe(II) under anaerobic conditions (Murray and Gill, 1978). The absence of fine sediments at all sites except Wang-Chelsea will tend to lead to lower concentrations of metal oxides that are commonly associated with smaller grain size particles, although in other coarse grained marine environments it has been shown that there is active Fe and Mn cycling (Burdige, 1993; Huettel et al., 1998). In no instance in any pore water sample taken from Reference and Geoduck cultivation areas was the distinctive odor of hydrogen sulfide present. The high water table that persisted throughout the low tide period indicates that rates of pore water drainage down the beach slope are slow enough to retain most of the water within the coarse-grained sediments.

Compared to sediments in most estuarine environments, the concentrations of pore water solutes at all sites we surveyed were generally low (Martens et al., 1978). We found mixed evidence for geoduck-related pore water nutrient increases, with the finer-grained Wang-Chelsea site showing higher SRP in all sediments and geoduck enrichment of ammonium. Low to moderate pore water nutrient concentrations lead to low sediment-water exchange rates and lower efflux rates during harvest. Such low pore water nutrient concentrations can result from 1) fast turnover of the pore water by biological or physical processes that are faster than diffusive transport or 2) low rates of organic matter input, either from surficial deposition or infiltration. We believe that the latter explanation is the most plausible given the feeding mode of infaunal geoducks that are only using the sediment as a supportive substrate to provide a refuge from predation. With phytoplankton filtration and ejection of both fecal waste and urine at the sediment-water interface, the feeding mode of geoducks means that there is not a major mechanism whereby particulate or dissolved waste is incorporated directly within the sediments. We frequently observed differing amounts of geoduck fecal strands accumulating in slight depressions on the sediment surface. Once a fecal pellet is deposited to the sediment surface, then the biological processes associated with the abundance of bioturbating infauna, such as various species of amphipods, polychaetes, and the formation of microbial films, all affect rates of incorporation of the fecal pellets into the surficial sediments. Holyoke (2008) reported that biodeposits produced by eastern oysters were rapidly incorporated into fine-grained sediments, such that within 2 d the critical erosional velocity required to resuspend biodeposits placed on ambient sediment had increased to the same level as required to resuspend the ambient sediment particles.

Our estimates of nutrient fluxes from undisturbed sediment cores from Thorndyke Bay are the most realistic way to assess rates of nutrient exchange between interstitial pore water and the nutrient pool in the water column, though direct nutrient inputs from geoduck urine are not included. The sediment NH_4^+ fluxes in this environment were > 20 times lower than found in other clam aquaculture environments (Nizzoli et al. 2007). We would expect a much higher NH_4^+ efflux if geoducks were included.

Assuming a CO_2 efflux to O_2 uptake ratio of 1, the dark efflux of SRP in geoduck environments is similar that expected for algal decomposition (Nixon 1981). Much lower SRP yields were observed for control sites suggesting the retention of remineralized P onto particles. At Thorndyke, the fine-grained particles released by the harvest jetting of the sediment averaged 15±4 umol g^{-1} total P or about 0.05% P. A possible source for this sedimentary phosphorus is iron oxide-bound inorganic P attached to particles filtered from suspension by geoducks and then voided in their biodeposits. These particles can then become incorporated in the coarse sediments by normal bioturbation processes, and in deeper sediments, where oxygen is depleted, iron reduction could result in enhanced SRP release.

For all fluxes, high core heterogeneity is reflective of the spatial variability in the amount of fecal material deposited to and ultimately incorporated into the sediments. As in other shallow water coastal environments, the actively growing benthic microalgae attenuated the fluxes of dissolved inorganic nutrients (Newell et al. 2002, Risgaard-Petersen, 2003; McGlathery et al., 2007; Nizzoli et al. 2007). Our results add to the growing body of evidence that nutrient cycling in shallow water environments cannot be fully understood without considering nutrient uptake by the benthic microalgal community.

With the exception of DSi concentrations in harvest nutrient effluent, the nutrient concentrations in effluent water returning to the adjacent estuarine waters were generally low to moderate. This is not surprising because pore water nutrient concentrations were not especially high. The Cooper site had the highest pore water nutrient concentrations of NH_4^+, with median values > 200 mmol L^{-1} in geoduck intact and harvested sites; the NH_4^+ in the efflux was < 10% of the pore water concentration. The SRP concentrations in effluxes were quite low, even at the Wang-Chelsea site where pore water SRP fluxes were highest. Similarly, effluent DSi concentrations were generally somewhat smaller than pore water concentrations.

In general, the release of pore water nutrients in the harvest effluent was low. In the case of NH_4^+, NO_x^-, and DSi, this suggests that water from the jet, while efficient at liquefying the sediment for geoduck removal, does not mix very completely with the bulk pore water. Low SRP yield during harvest may result from rapid oxidation of Fe(II) in pore water as aerobic water is introduced to the sediment, resulting in a co-precipitation of inorganic P with Fe(III) oxyhydroxides within the sediment. Thus large scale sediment disruption results in precipitation of SRP, despite relatively high pore water SRP concentrations and undisturbed effluxes. Direct comparisons to other processes that disrupt sediments are difficult, though observations of nutrient releases during dredging activities suggest that efficient releases of pore water and adsorbed NH_4^+ can occur (Cornwell and Owens 2011).

Any elevated sediment-water exchange of SRP from geoduck aquaculture beds will lead to minimal increases in the production of algae in Puget Sound because there is already an abundant amount of P imported in the saline water entering from the continental shelf. Instead, as in other estuarine systems, nitrogen is the main nutrient limiting algal production in Puget Sound (Bernhard and Peele 1997). Although nitrogen releases are more likely to have an impact on algal production, such releases need to be considered in a whole ecosystem perspective (i.e. Newell et al., 2004; 2005). The N filtered by geoducks is derived from within the estuary and any releases of N directly by geoducks or in adjacent sediment via microbial processes is "recycled" N. In order for geoducks to enhance algal growth, the efficiency of the aquaculture release of NH_4^+ or NO_x^- must be increased beyond that expected from other fates of algal-derived N, perhaps through diminishment of denitrification.

Although the N cycling processes such as nutrient remineralization, denitrification, and N burial have not been comprehensively examined in lower Puget Sound, recent work has identified the major sources of terrestrial N inputs. For lower Puget Sound, Mohamedi et al. (2011) estimated riverine input of 2720 kg N d^{-1} and a waste water treatment plant input of 2950 kg N d^{-1} (Table 5). They also estimated per capita septic N inputs of 7.3 g $person^{-1}$ day based on 261 L d^{-1} of waste water, effluent concentrations of 2.2 mmol L^{-1} and a 10% denitrification loss for homes within 150 m of the tidal water. Our estimate of total man-hours harvesting geoducks in the Puget Sound are ~7810 h y^{-1} or 21.4 h d^{-1}, equivalent to ~ 28 g N d^{-1} for the entire system. This is ~ 0.001% of the daily annual load from streams or wastewater plants. These comparisons are not meant to suggest that terrestrial inputs and harvest effluent inputs are directly comparable, but rather to scale the size of effluent recycling inputs to some N input estimates. Also, the timing of harvesting is tied into market demand and tidal level, so nutrient inputs may be proportionately higher for short periods of time.

In summary, we found that the cultivation of geoducks at even high densities of ~15 m^{-2} leads to generally low to moderate levels of accumulation of inorganic N, P, and Si in the pore waters of the sediment. Our findings of low amounts of inorganic nutrients release into adjacent water suggest that the harvest process has a negligible impact. Thus the concern that geoduck harvest may release such large amounts of DIN and DIP to stimulate an algal bloom is not supported. Overall, the magnitude of nutrients released by current levels of geoduck aquaculture is an inconsequential fraction of the Puget Sound nutrient balance.

Acknowledgments

This was funded by a grant (project R/GD-1b) from the Washington Sea Grant Program, University of Washington, pursuant to the geoduck aquaculture research account created under Washington state law (RCW 28B.20.476). The views expressed herein are those of the author(s) and do not necessarily reflect the views of the funders. We are especially grateful to Drs. P Sean McDonald, Glenn VanBlaricom, Joth Davis, and George Waldbusser for technical and logistical assistance and constructive discussions.

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Tables and Figures

Table 1. Geoduck culture area and harvest parameters. These data are adapted from Anchor QEA 2011, with an alternate estimate of geoduck harvest acreage based on density and individual geoduck numbers. Information on output per harvester, average geoduck weight and area of geoducks per unit area provided by Paul Taylor, personal communication.

Parameter	Value	Note
Production of farmed geoduck	637,302 kg y^{-1} live weight	Anchor Qea (2011)
Individual clam harvest	936,675 individuals	Based on 1.5 lb per clam
Total acreage – all ages	144 ha	Based on 7 year grow out
Yearly harvest area	21 ha 5 ha	Anchor QEA (2011) Based on 18.8 geoducks m^{-2}
Output per harvester	81.6 kg h^{-1}	Concentrated on low tide events
Total harvester hours	7810 h	Calculated here
Average harvest time per day	21.4 h	Calculated here

Table 2. Locations, times and activities for Geoduck nutrient work. The average and standard deviation of the fine grain size data (silt + clay) for each site is indicated, parentheses indicate number of grain size analyses.

Name	Latitude and Longitude	Sample Period	Pore Water Technique	Harvest Efflux	% Fine Grained
Rogers	N 47°14.94 W 122°49.70	July/Aug 2008	Equilibrators, Sippers, Wells		1.9±1.0 (3)
Foss-Joemma (“FJ”)	N 47°13.70 W 122°49.21	July/Aug 2008	Sippers, Wells		0.6±0.8 (13)
		Oct 2008	Sippers		
Manke	N 47°12.12 W 122°50.50	July/Aug 2008	Equilibrators, Sippers, Wells		1.2±0.5 (15)
		November 2009	Sippers		
Cooper Point (“Cooper”)	N 47°08.33 W 122°56.01	Aug 2008	Sippers	1 time	No data
Thorndyke Bay (“Thorndyke”)	N 48°48.61 W 122°44.18	June 2009	Sippers	2 times	1.2±0.3 (7)
Wang-Chelsea (“WC”)	N 47°07.73 W 122°57.60	June 2009	Sippers	1 time	14.0±3.7 (16)

Table 3. Fluxes (mean ± S.D.; µmol m^{-2} h^{-1}) of gases (oxygen, di-nitrogen) and inorganic nutrients (ammonium, nitrate plus nitrite, soluble reactive phosphorus, dissolved silicate) measured in incubations of sediment collected from 4 sites at the Reference location (no Geoducks) and 4 sites within a Geoduck bed at Thorndyke. Positive values indicate a flux out of the sediment to the overlying water; negative values indicate flux into the sediment.

Flux (µmol m^{-2} h^{-1})	**Sediment Collection Site**			
	Light Incubation		*Dark Incubation*	
	Geoduck Bed	Reference	Geoduck Bed	Reference
Oxygen	-426.1 ± 524.9 (4)	36.8 ± 521.4 (4)	-1271.4 ± 453.9 (4)	-706.2 ± 366.7 (4)
N_2-N	-36.3 ± 49.2 (2)	3.7 ± 75.1 (4)	32.2 ± 6.8 (3)	38.0 ± 29.4 (3)
NH_4^+	42.6 ± 45.0 (4)	-20.9 ± 3.5 (2)	112.4 ± 174.3 (2)	53.4 ± 77.5 (3)
$NO_2^- + NO_3^-$	-12.3 ± 3.8 (4)	-10.2 ± 1.1 (4)	-13.1 ± 5.2 (4)	-7.6 ± 4.3 (3)
SRP	8.4 ± 4.4 (3)	5.9 ± 7.6 (3)	82.0 ± 65.9 (3)	16.2 ± 69.3 (3)
Silica	255.8 ± 229.2 (3)	12.1 ± 104.2 (4)	429.4 ± 353.2 (3)	51.7 ± 157.5 (3)

Table 4. ANOVA results for Geoduck, Control and Harvest Pore water chemistry. Only results that are significant are included ($P < 0.05$). Three types are data are included, Reference (R), Geoduck (D) and Harvest (H) and the direction of the differences in means are indicated. Harvest data is only available for Cooper and WC. Total numbers of samples are in the Figure 3 legend.

Site	**Sample Type**	**NH_4^+**	**SRP**	**NO_x^-**	**DSi**
Manke	Sipper	-	-	-	-
Manke	Well	-	-	-	-
Cooper	Sipper	H>R, H>G	H>R, H>G	R>G	R>G, H>G
FJ	Sipper	G>R	-	R>G	-
FJ	Well	G>R	-	R>G	-
WC	Sipper	G>R, H>R	-	-	-
Thorndyke	Sipper	-	-	-	-

Table 5. Basis for N input/output estimates.

Input/Output	**Data Type**	**Value**	**Units**	**Source**
Septic	Per CapitaWater N Inputs	0.0073	kg person^{-1} d^{-1}	Mohamedali et al. (2011)
Wastewater	Lower Puget Sound	2950	kg d^{-1}	Mohamedali et al. (2011)
Riverine	Lower Puget Sound	2720	kg d^{-1}	Mohamedali et al. (2011)
Geoduck Harvest	Harvest Water Flux	7200	L h^{-1} hose^{-1}	This study
	Harvest Water N	0.182	mg N L^{-1}	This study
	Harvest N Flux	1.31	g N h^{-1} hose^{-1}	This study
	Harvest Time – Puget Sound	21.4	h d^{-1}	Table 1
	Harvest N Flux	0.028	kg N d^{-1} system^{-1}	This study
Proportions	Harvest/Wastewater	0.0009	%	This study
	Harvest/River Input	0.0010	%	This study

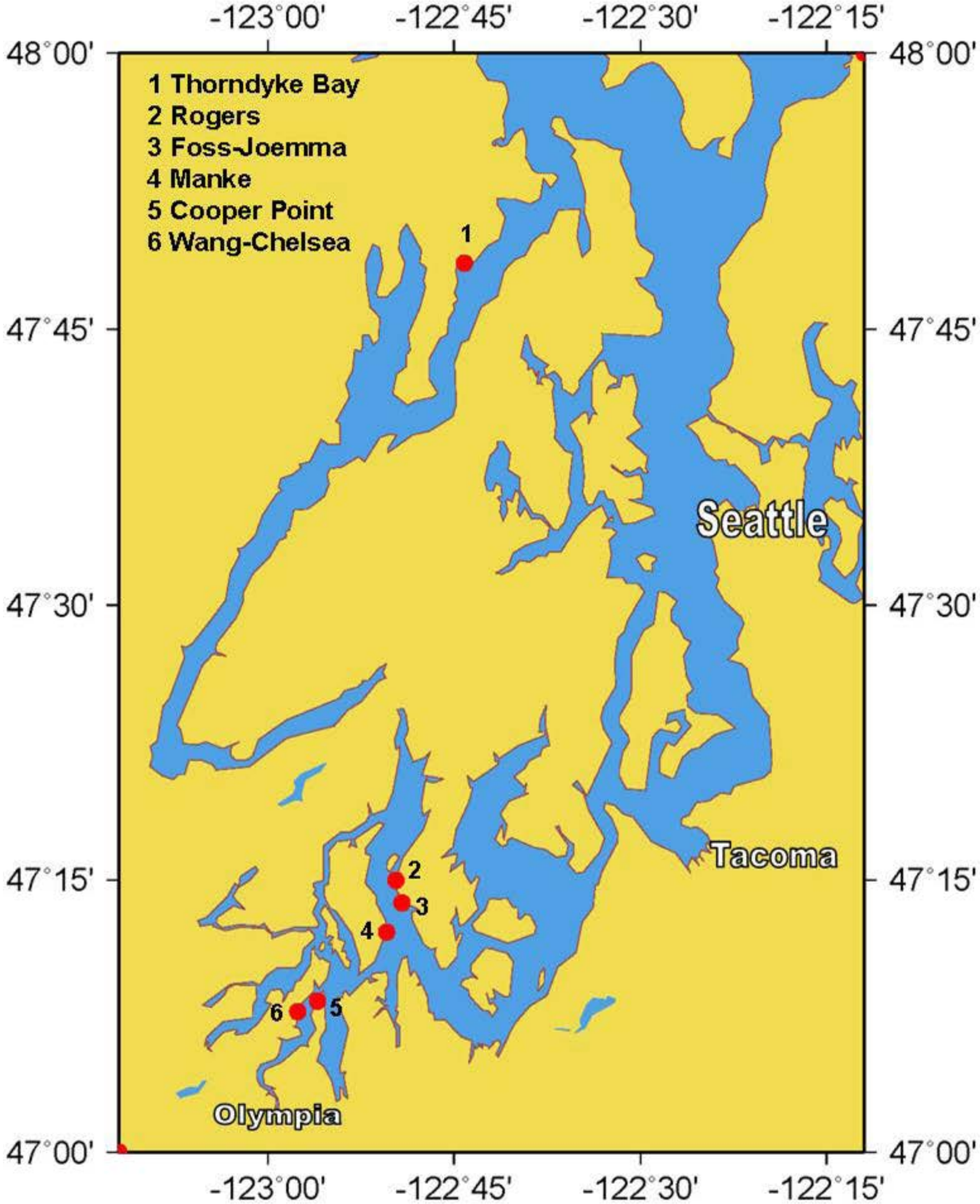


Figure 1. Location of sampling sites in Puget Sound.

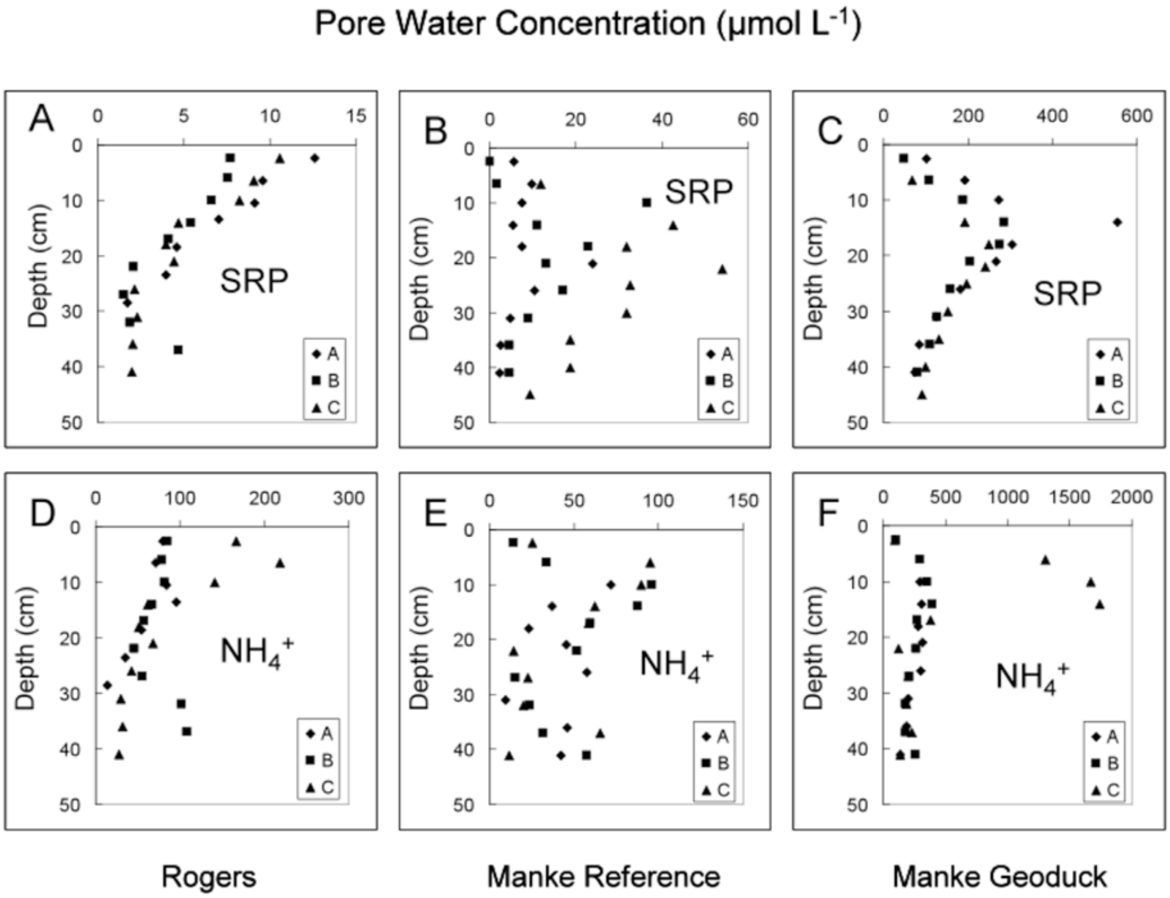
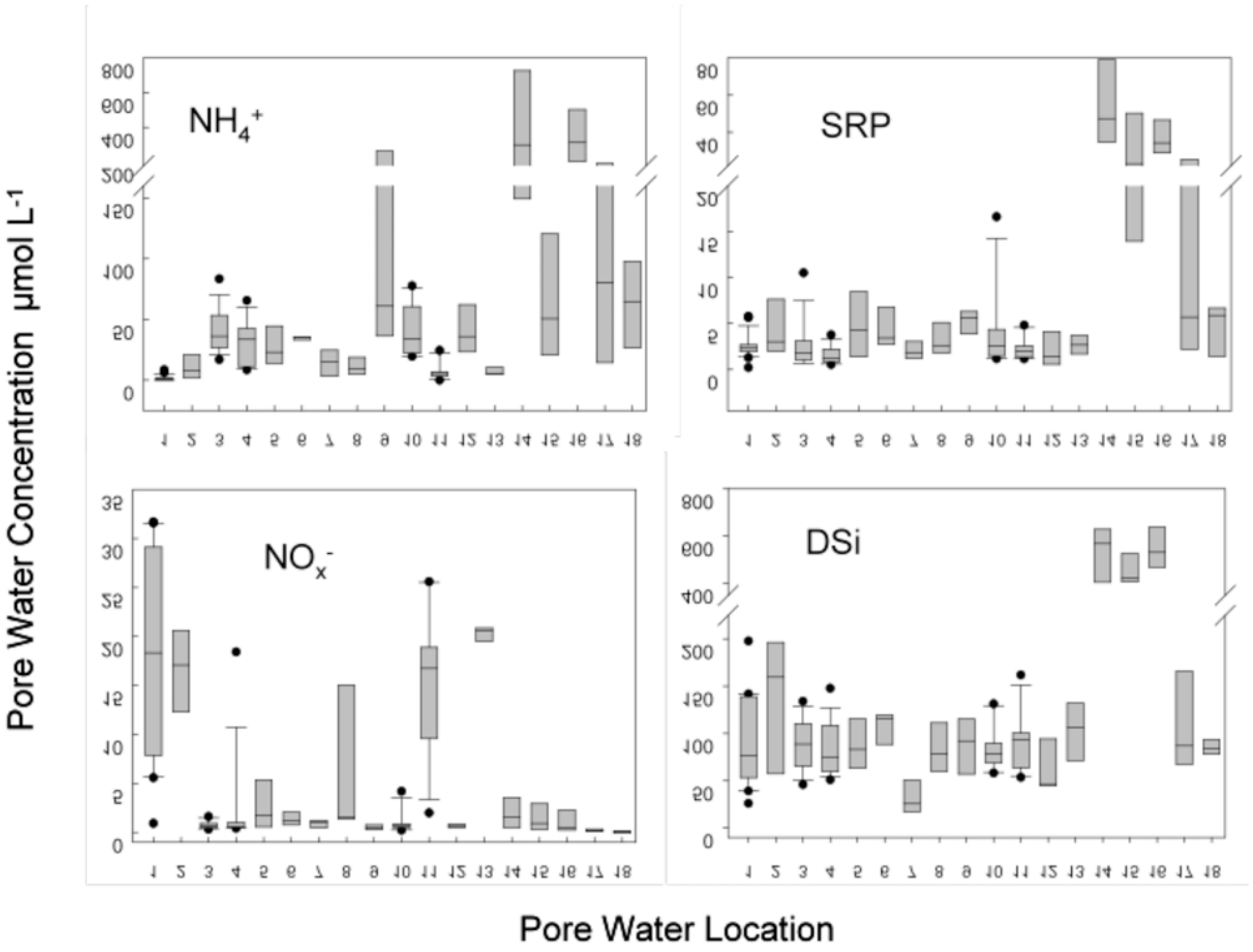


Figure 2. Pore water SRP and ammonium profiles at Rogers, the control site at Manke and the Manke site with geoducks present. The data for 3 co-located pore water equilibrators (A-C) are shown in each panel. Note the scale differences for nutrient concentrations.



1	Rogers Sipper (23)
2	Rogers Well (4)
3	Manke Sipper Geoduck (14)
4	Manke Sipper Reference (15)
5	Manke Well Geoduck (8)
6	Manke Well Reference (6)
7	Cooper Sipper Geoduck (5)
8	Cooper Sipper Reference (6)
9	Cooper Sipper Harvest (6)
10	FJ Sipper Geoduck (11)
11	FJ Sipper Reference (11)
12	FJ Well Geoduck (7)
13	FJ Well Reference (8)
14	WC Geoduck (6)
15	WC Reference (7)
16	WC Harvest (5)
17	Thorndyke Geoduck (7)
18	Thorndyke Reference (7)

Figure 3. Nutrient concentration box plots for 18 different locations within 6 intertidal sites used in this study. The cross bar within the shaded box is the median, the shaded area represents the 25-50 percentile distribution of the data. The data for each single bar in the box plot is taken from all data collected at that site, with a predominance of sample depths at 45 cm. The total number of analyses represented in each bar is shown in parentheses.

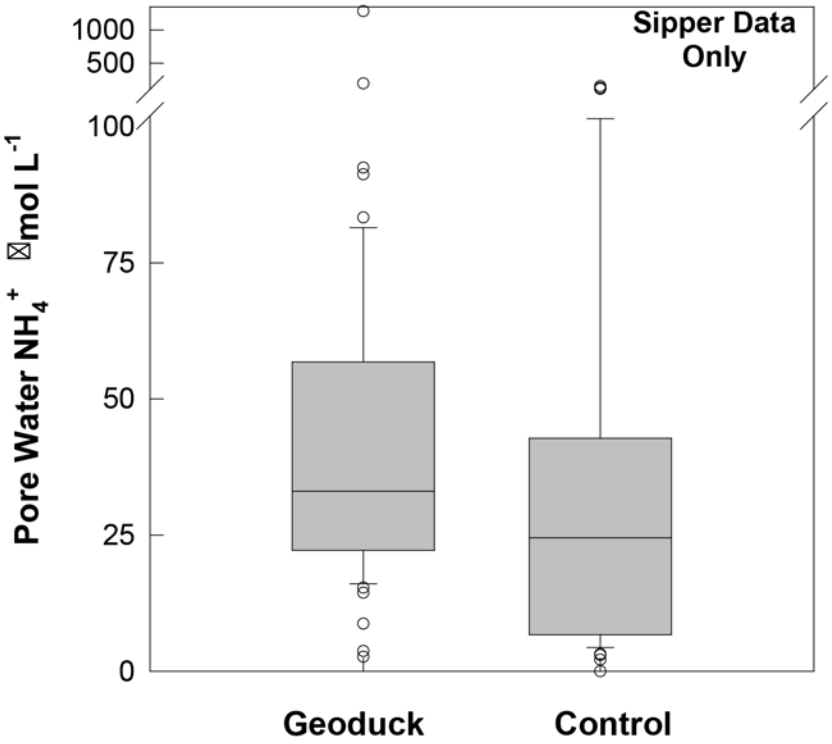


Figure 4. Box plot of all pore water ammonium data for Reference (N = 47) and Geoduck (N = 56) locations. The Geoduck NH_4^+ concentrations were significantly greater than Reference locations (P = 0.013, Kruskal-Wallis one way analysis on ranks).

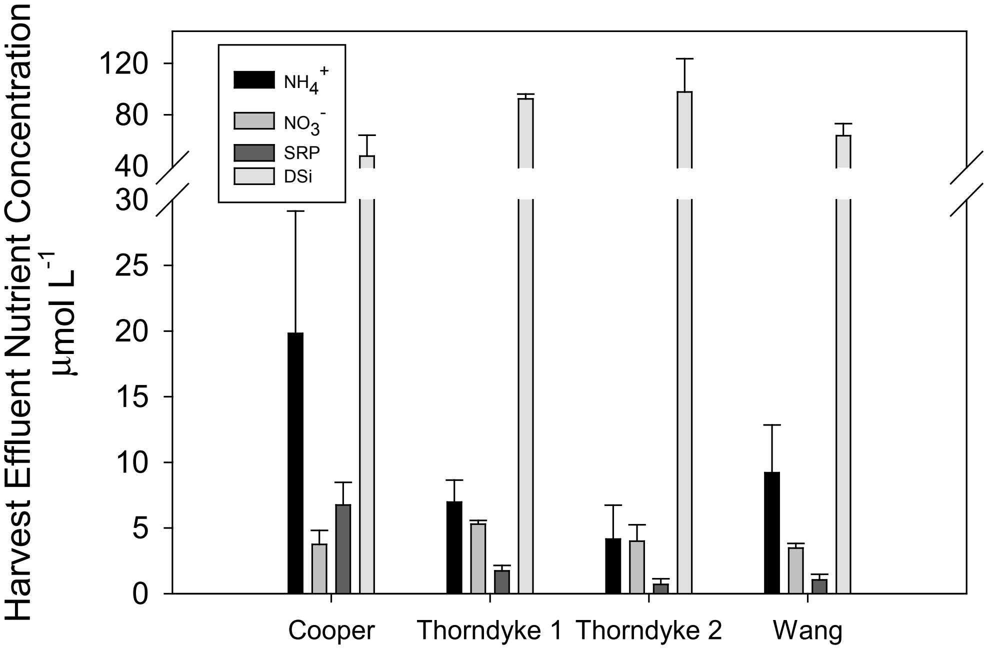


Figure 5. Nutrient concentrations in surface water after geoduck harvest. At Cooper Point, residual water in small harvest-created depressions was sampled, while at the other sites and times, samples were collected along the length of the rivulets as water from the jets used for harvest flowed downslope to open water.

Appendix IV

Temporal and spatial variability of native geoduck (*Panopea generosa*) endosymbionts in the Pacific Northwest

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Abstract

Lucrative commercial cultivation of Pacific geoduck (*Panopea generosa*) has developed in the United States within the last 20 years making it one of the most economically important commercial shellfish species harvested for export. Aquaculture of the species exists in close proximity to native populations, but very little is known about the health of native populations. Baseline information on endosymbiont identification, prevalence, intensity and geographic distribution are necessary to facilitate management and/or mitigation of potential disease interactions between cultured and natural shellfish stocks. A survey of Pacific geoduck (*Panopea generosa*) parasites from three natural populations in Washington state, USA (Totten Inlet, Thorndyke Bay, Freshwater Bay) was conducted in 2008 – 2010. Histopathology of 634 animals was used to explore trends of parasite presence and identify potential environmental factors (site distribution, collection depth, and season) that influence parasite assemblages. Endosymbionts observed upon histological examination included: *Rickettsia*-like organisms (RLO) in the ctenidia (*n* = 246), an unidentified metazoan parasite in the siphon epithelium (*n* = 220), microsporidia-like species in the intestine (*n* = 103), siphon muscle (*n* = 28) and ova (a *Steinhausia*-like parasite; *n* = 99). This study reveals the presence of three microsporidia-like organisms (including *Steinhausia*-like parasites), not previously described in geoduck clams. Assemblages of most parasites showed strong seasonal variations and site-specific distributions throughout the year. RLO presence may be driven by seasonal elevated temperatures and was extremely common at Freshwater Bay. Metazoans and microsporidia were common in South Puget Sound and exhibited high infection intensity year-round. Spawning season drove *Steinhausia*-like parasite presence with no spatial driver. Baseline information on natural parasite levels, distribution, and infection loads complements ongoing monitoring of natural geoduck population dynamics and provides crucial information to evaluate future disease events should they occur.

Key words: *geoduck, disease, parasite, shellfish, Washington state, USA*

Introduction

Baseline information on the health status and prevalence of parasites and diseases in wild populations is necessary to understand potential interactions between wild and farmed shellfish, such as spill-over (e.g. farmed to wild) and spill-back effects (e.g. wild to farmed) (Daszak et al. 2000). Parasites and diseases present at low densities in wild populations may elevate to epidemic status due to the increases in population density or shifts in environmental conditions within culture settings (May et al. 1981). Shellfish transport has been long thought to potentially spread disease within wild and cultured populations. Strict shellfish transportation regulations exist as important management tools to help control disease interactions and prevent further transmission. Movements of shellfish stock or seed, may pose a significant threat to native populations, especially if animals are not properly monitored for disease or parasite presence. Unmonitored stock transport by growers or scientists and ballast discharge are suspected modes of transmission for some of the major shellfish diseases including bonamiasis of the Asian oyster (*Crassostrea ariakensis*) (Carnegie et al. 2008), Denman Island disease of the European oyster (*Ostrea edulis*) (Gagné 2009) and two diseases, *Haplosproidium nelsoni* (or multinucleated sphere unknown (MSX)) and *Perkinsus marinus*, in the eastern oyster (*Crassostrea virginica*) (Burreson et al. 2000; Burreson & Ford 2004; Ford & Smolowitz 2007).

The Pacific geoduck (*Panopea generosa* Gould, 1850) is a large, burrowing hiatellid clam found in low intertidal and subtidal sediments throughout the Northeast Pacific coast including the USA (Alaska, Washington state, California), Canada (British Columbia), and Mexico (North Baja Pacific Coast). Geoduck clams are one of the most economically important commercial shellfish species harvested for export (Hofmann et al. 2000; Bower & Blackbourn 2003). A commercial Washington state geoduck fishery initiated in 1970 became highly lucrative in the 1990s through live exports to Asia; subsequent commercial cultivation of the species was developed in response to additional market demands. Washington state is at the forefront of geoduck aquaculture, which currently occurs in close proximity to wild geoduck aggregations targeted in the commercial fishery.

Few studies have been conducted regarding parasite load, natural distribution patterns, and epizootics specific to geoducks. However, this clam is known to experience several morphological abnormalities including warts, pustules, discoloration of the periostracum and infectious agents such as protozoas and *Rickettsia*-like prokaryotes (Kent et al. 1987; Bower & Blackbourn 2003). The ongoing evolution of the geoduck aquaculture industry presents a unique opportunity to evaluate and potentially mitigate negative effects of cultured-wild interactions in geoduck clams. To enhance our understanding of disease ecology within native geoduck populations, a comprehensive histopathological survey of three sites in Washington state was initiated in southern Puget Sound, Hood Canal and the Strait of Juan de Fuca. These areas represent locations of natural geoduck aggregations where native populations reside within close proximity to cultured geoduck stocks. The goal of this study was to (1) explore trends of parasite presence within wild geoduck populations, and (2) identify geographic patterns (site and collection depth) and seasonal trends in the diversity of parasite assemblages. Information on parasite distribution (spatial and temporal) and abundance, coupled with the host response to infection, will provide needed baseline data for future species management and assist in future research regarding the impact of these diseases on Northwest populations of Pacific geoducks.

Methods

Sample Collection and Histology

A target of 60 Pacific geoducks that ranged in size from 80 - 225 mm (mean = 141 ± 31.13 mm, mean ± SD) were randomly collected by Washington Department of Fish and Wildlife divers at two depth strata from three natural populations in Washington state, USA over multiple seasons during a two year period. Sites included Totten Inlet (Latitude: 47.1697 Longitude: -122.9617) (*n* = 224), Thorndyke Bay (Latitude: 47.8042 Longitude: -122.7344) (*n* = 173), and Freshwater Bay (Latitude: 48.1439 Longitude: -123.5848) (*n* = 237) (Fig. 1). To capture the presence of parasites more prevalent in warmer or colder seasons, animals were collected during the following months: October 2007 and July 2008 to represent warmer periods and May 2007, February 2009, and April 2009 to represent cooler periods. Water depth was determined using mean lower low water (MLLW), or the average value of lower low water height each tidal day observed over the National Tidal Datum Epoch by the National Oceanic and Atmospheric Administration (NOAA). Collection depths were either shallow (10 – 30 ft MLLW) or deep (30 – 70 ft MLLW). Freshwater Bay geoducks were only aggregated in shallow depths at time of sampling and therefore were not collected in deep water.

Animals were dissected within 24 hours of harvesting. Length, width and depth of shells were taken. Three 2-3 mm cross-sections were excised from each animal to obtain tissues from the following organs: siphon, ctenidia, labial palps, mantle, heart, digestive organs, and gonad. Any gross lesions were recorded and sections were removed for histological processing and future molecular characterization. All tissue samples were preserved in Davidson's solution for 24 hours and stored in 70% ethanol until processed for routine paraffin histology (Shaw & Battle 1957; Luna 1968). Deparaffinized tissue sections were stained with hematoxylin and eosin and examined for parasite presence by light microscopy. If warranted, specific stains for bacteria or fungi detection such as Gram stain or Periodic Acid Schiff stain (PAS) were prepared (Luna 1968).

Observed pathogens were grouped into broad taxonomic categories: *Rickettsia*-like organisms (RLO), microsporidia-like organisms (MLO), and metazoan parasites. For each category, tissue sections were assigned a semi-quantitative score of 0 – 4 per field of view (0 = no parasites, 1= few parasites (< 10), 2 = small numbers of parasites (11 – 20), 3 = moderate numbers of parasites (21 – 30), 4 = large numbers of parasites (> 30)). The parasite data set consisted of 634 geoducks and 5 tissue sections (ctenidia, siphon muscle, siphon surface epithelium, intestine, and ova) containing five parasite categories: [1] RLO (ctenidia), [2] metazoa (siphon external epithelium), and MLO in the [3] siphon muscle, [4] intestine, and [5] ova. A parasite abundance matrix was organized into unique animal identification numbers described by parasite taxa and environmental variables: harvest depth (shallow, deep), season collected (Winter = December – February; Spring = March – May; Summer = June – August; Fall = September - November), and site (Thorndyke Bay, Totten Inlet, Freshwater Bay).

Statistical Analysis

Generalized linear models (GLM) were created with the binomial family distribution and the logit link function and employed to test significance of terms (site, collection depth, season) associated with geoduck parasite presence or absence. Residual scaled deviance values were used to measure goodness of fit of the final GLM models. Tukey's Honest Significant Difference tests were employed for pairwise comparisons of parasite frequency according to the model of best fit. Kruskal-Wallis one-way analysis of variance tests (ANOVA) were used to compare ranked parasite intensities among sites and seasons. The Chi square test was used to test for differences in parasite prevalence between depth strata. Post hoc pairwise comparisons of Kruskal-Wallis ANOVAs were performed using Dunn's method. GLMs, ANOVAs, Chi square, and Tukey's Honest Significant Difference tests were performed using R software 2.11.1 (R Development Core Team 2012). Post hoc analyses were performed with SigmaPlot software version 11.0 (Systat Software, Inc.).

Results

Parasite morphology and characterization

The most common geoduck parasites observed upon histological examination included: a RLO in the ctenidia (Fig. 2a) (39%), an unidentified metazoan in siphon external epithelium (Fig. 2b) (35%), a Steinhausia-like parasite (SLO) in the ovum (Fig. 2c) (16%), and MLOs in the intestine (Fig. 2d) (16%) and siphon muscle (Fig. 2e, f) (4%) (Table 1). RLOs were characterized by the presence of basophilic inclusions that stained violet with hematoxylin and eosin within the ctenidia epithelium (Fig. 2a) and were Gram negative. Inclusions were spherical and measured 13.22 + 0.85 µm (mean + s.d.) in maximum dimension (n = 5); individual RLOs were too small to measure. No host response was observed in association with RLO infections. Metazoa within the siphon epithelium were characterized as multicellular organisms surrounded by an eosinophilic keratin-like cuticle, some of which contained ova, and measured 128.81 + 49.48 µm in length and 74.04 + 36.57 µm in width (n = 15; Fig. 2b). Steinhausia-like microsporidians were observed within oocytes and were characterized by the presence of spherical eosinophilic inclusion bodies and sporocysts that contained numerous 1-2 µm basophilic spores (Fig. 2c). No host response was observed in association with the Steinhausia-like infections. Two spherical stages of MLOs were observed in inflammatory lesions within the intestinal submucosa. The larger merogonic stage measured 4.89 + 1.16 µm (n = 15) and the smaller spore-like stages measured 0.85 + 0.28 µm (n = 15) and were found in intracytoplasmic sporocysts of hemocytes (Fig. 2d). Multifocal inflammatory lesions that contained several sporocysts of a MLO were observed in the siphon musculature of some geoduck. Sporocysts measured a mean of 13.43 + 3.5 µm (n = 20) and contained 4-15 spores (mean = 6.8 + 2.8 spores per sporocyst; n = 20), which measured a mean of 2.91 + 0.47 µm (n = 15; Fig. 2e). The spores stained PAS positive and were not acid-fast.

Overall parasite prevalence and intensity

Parasite intensity was measured using a semi-quantitative score of 1 – 4 (see above) (Fig. 3). Parasite prevalence varied among seasons for all parasites except for the SLO ($X^2 = 0.44$, df = 1, $p > 0.05$). RLO prevalences were higher in geoduck collected in the shallow depths ($X^2 = 4.8$, df = 1, $p < 0.05$). Siphon MLOs were only observed in shallow collection depths. Both the intestinal MLO and metazoan parasites were more prevalent at the deeper collection depths ($X^2 = 26.99$, df = 1, $p < 0.001$; $X^2 = 58.28$, df = 1, $p < 0.001$, respectively). Overall infection intensities differed by season (Kruskal-Wallis H statistic = 60.385, df = 3, $p < 0.001$).

Rickettsia-like Organism

The most commonly encountered parasite was a RLO within ctenidial epithelia, which was observed in 39% of the sampled geoducks (Fig. 2a; Table 1). RLO prevalence was highest in Freshwater Bay (62%) relative to both Thorndyke Bay (35%) and Totten Inlet (19%) (Fig. 4d; Table 2). Although overall seasonal trends in RLO prevalence were not determined due to significant interactions between season and site (Table 1), seasonal trends in RLO infection intensity varied within Freshwater and Thorndyke Bays (Freshwater Bay: $H = 41.23$, df = 2, $p < 0.001$; Thorndyke Bay: $H = 15.08$, df= 2, $p < 0.001$; Totten Inlet: $H = 2.70$, df = 2, $p > 0.05$; Fig. 3d; Table 2). Over all sites, RLO intensities varied among seasons with the highest intensities observed in summer (2.13 ± 0.14 parasite intensity score) and winter (1.75 ± 0.75) (Table 1). No significant difference in RLO infection intensity was detected among sites ($H = 3.09$, df = 2, $p > 0.05$; Fig. 3d; Table 2).

Metazoan parasites

Metazoan parasites were observed in the siphon epithelium of 35% of the geoducks sampled in this study (Fig. 2b; Table 1). Overall seasonal trends in metazoan prevalence were not determined due to significant interactions between season and site (Table 1). Prevalence of siphon metazoa varied among sites with the highest levels observed in geoducks from Totten Inlet (57%) and Thorndyke Bay (46%) relative to only 9% of Freshwater Bay (overall: $H = 53.65$, df = 2, $p = < 0.001$; Fig. 4). Similar seasonal trends in metazoan prevalence were observed in geoducks from Freshwater and Thorndyke Bays where summer prevalence exceeded those of all other seasons (Table 2). Animals from both sites exhibited similar prevalence patterns of metazoan parasites; no seasonal trend was observed in Totten Inlet animals (Fig. 4a; Table 2). Across all sites, metazoan infection intensity was significantly lower in the spring compared to winter and summer seasons (winter: Dunn's Multiple Comparison Q statistic = 2.83, $p < 0.05$; summer: $Q = 2.72$, $p < 0.05$; Fig. 3a; Table 1). Totten Inlet geoducks had higher intensity metazoan infections (3.26 ± 0.11) relative to those in animals from both Freshwater (1.60 ± 0.26) and Thorndyke Bays (2.03 ± 0.14; $p < 0.05$), which were similar to one another ($Q = 1.16$, $p > 0.05$).

Steinhausia-like Organism

SLO parasites were observed in oocytes of 16% of total geoducks sampled in this study (Fig. 2c; Table 1). Mean prevalence (28 - 33%) and intensity (1.08 ± 0.06 – 1.26 ± 0.08) of SLO infection were similar among sites (intensity: $H = 2.12$, df = 2, $p > 0.05$; Table 2). Site was not a significant term in the final GLM for SLO presence ($F = 1.12$, df = 2, $p > 0.05$). Across all sites, SLO prevalence was highest in the winter (70.7%) and spring (58.0%) relative to summer (14.3%) and fall (1.9%) ($p < 0.05$; Fig. 4e; Table 1). Differences in SLO parasite infection intensity by season were not detected ($H = 2.06$, df = 2, $p > 0.05$; Fig. 3e).

Intestinal Microsporidia-like Organism

Intestinal MLOs were observed in 16% of all geoducks sampled in this study (Fig. 2d; Table 1); no overall seasonal trends in prevalence were observed ($F = 0.94$, df = 3, $p > 0.05$; Fig. 4b; Table 1). Prevalence varied among locale with the most infections observed in Totten Inlet animals (34%) ($p < 0.05$) relative to those from Thorndyke Bay (17%) and Freshwater Bay (4%; Fig. 4b), which were similar to one another ($p = 0.16$; Fig. 4b; Table 2). Mean infection intensity was similar among sites ($H = 4.94$, df = 2, $p > 0.05$; Fig. 3b; Table 2). Infection intensities varied with season across all sites ($H = 14.34$, df = 2, $p < 0.05$; Fig. 3b; Table 1): Fall intensity (2.46 ± 0.20) was higher than spring (1.75 ± 0.16) and summer (1.73 ± 0.15), but significantly exceeded that observed in winter when the lowest mean infection intensity (1.47 ± 0.19) was observed ($Q = 3.33$, $p < 0.05$).

Siphon Microsporidia-like Organism

Siphon MLOs were observed the least frequently (4%) of all characterized parasites encountered in geoducks sampled in this study (Fig. 2e, f; Table 1); no overall seasonal trends in prevalence or intensity were observed ($p > 0.05$; Fig. 3c, 4c; Table 1). Overall prevalence was similar among seasons and ranged from 0% in winter to 9.9% in summer (Table 1). Prevalence of the siphonal MLOs varied among sites: 9% of Totten Inlet animals and 6% of those from Thorndyke Bay were infected, while no MLOs were observed in the siphon of Freshwater Bay geoduck (Fig. 4c; Table 2). Mean overall infection intensity was high (2.79 ± 0.19) and was similar among seasons ($H = 4.7$, df = 2, $p > 0.05$; Fig. 3c; Table 1). Siphon muscle MLOs were observed in the highest infection intensities at Totten Inlet (2.67 ± 0.26) and Thorndyke Bay (3.00 ± 0.30) and intensity differences were nonsignificant between the two sites (Mann-Whitney U Statistic = 75, $p > 0.05$; Table 2).

Discussion

This study revealed five morphologically distinct endosymbionts of natural Pacific geoduck populations in the Pacific Northwest: a RLO in the ctenidia, an unidentified metazoan in the siphon epithelium, *Steinhausia*-like sp. in oocytes, and two other microsporidia-like organisms within siphon muscle and intestinal submucosa. This is the first report of microsporidia-like parasites, including *Steinhausia*-like parasites, in geoduck clams. This study provides an initial characterization of endoparasites in wild Puget Sound geoduck populations and suggests that seasonal and geographic differences in distribution and infection intensity should be taken into account when moving animals among locales.

Putative identification and seasonal distribution of geoduck parasites

Intracytoplasmic rickettsia-like colonies (inclusion bodies) are commonly observed in a variety of molluscan species worldwide, such as oysters, abalone, and clams including the geoduck clam (Elston 1986; Fries & Grant 1991; Friedman et al. 2000; Bower & Blackbourn 2003). RLOs were the most common geoduck parasite (39%) observed in this study. Microscopic examination revealed that RLO prevalence peaked in warmer months (fall sampling) with the highest infection intensity observed during summer months. This finding suggests that elevated temperature may be an important driver of RLO presence in geoduck clams and complements experimental trials of other *Rickettsia* investigations in invertebrate species (e.g. Moore et al. 2000; Friedman et al. 2002; Braid et al. 2005; Vilchis et al. 2005). Transmission experiments of one *Rickettsia*-like organism, "*Candidatus* Xenohaliotis californiensis", in abalone (*Haliotis spp.*) indicate that elevated seawater temperature significantly enhanced parasite transmission and accelerated progression of the disease (Moore et al. 2000; Friedman et al. 2002; Braid et al. 2005; Vilchis et al. 2005). In geoduck populations, RLO reproduction may also increase with elevated temperature and lead to the trends observed.

In the present study, metazoan infections in geoduck clams were present year-round in high intensity at all sites and seasons other than those from Freshwater Bay, where both prevalence and intensity were low. The relatively high occurrence and elevated infection intensities observed may be the result of an accumulation of these parasites over time (Rohde 1984); age data from future studies are necessary to confirm this prediction. Geoducks are known to be one of the longest living bivalve molluscs, and in fact, Bureau et al. (2002) used growth rings, verified as annual by the bomb radiocarbon signal (Vadopalas et al. 2011), to estimate the age of one geoduck clam at 168 years. Animals collected in this study were recruits and assumed to be collected at random with respect to age. Although shell length was collected for all specimens, shell length is poorly correlated with age after asymptotic length is attained at age 5 - 15 years (Goodwin & Pease 1991; Hagen & Jaenicke 1997; Hoffmann et al. 2000; Campbell et al. 2004).

Microsporidian infections have not been previously identified in geoduck clams. Presently, microsporidia have only been reported in oysters, mussels, and cockles from Europe, Australia, California and the eastern United States (Figueras et al. 1991; Comtet et al. 2003; Graczyk et al. 2006). Of the three MLOs observed in geoduck clams in our study, only those observed within oocytes (SLO) were morphologically consistent with a known microsporidian genus previously observed in oocytes of some bivalve species. This parasite was morphologically similar to members of the genus *Steinhausia*, such as *S. mytiloyum* that parasitizes oocytes of mussels (*Mytilus galloprovincialis*) (Figueras et al. 1991; Graczyk et al. 2006).

The other microsporidia-like parasites identified in geoduck intestine and siphon muscle do not possess all of the classic characteristics of microsporidia (Garcia 2002). Microsporidia are obligate intracellular protists that form spores (Garcia 2002). Like several other taxa, the life cycle of microsporidia includes an asexual reproduction (merogony) and sexual reproduction via the production of spores, the infectious stage responsible for host-to-host transmission (Garcia 2002). Both of these stages were observed in geoduck. However, the two life stages were not always observed within the same individual. Of all geoduck examined with either intestinal or siphon muscle MLO parasites, nine were observed with both MLO life stages (7%). The intestinal MLO parasites in geoduck had a plasmodium-like morphology, which may represent meronts, while the siphon muscle MLO contained spore-like stages. Although the spores stained PAS positive, typical of microsporidia, they were not acid-fast, one of the characteristics of the microsporidia taxon (Garcia 2002), suggesting that these parasites may belong to another taxon or are distantly related to known microsporidia. Both MLO parasites elicited a host inflammatory response in infected tissues; the potential of these parasites to influence host health in not known.

Seasonal fluctuations have been long known to influence endoparasite presence in marine hosts (Noble 1957; Rohde 1984; Couch 1985). Relatively high intensity microsporidian infections were observed in geoduck siphons and intestinal epithelia year-round; no clear temporal or spatial environmental driver was detected. The highest prevalence of SLO infections was observed in geoduck during colder months (February through May), while SLO parasites in warmer months were rarely observed. This observation is consistent with the annual oocyte maturation cycle in geoduck clams (Goodwin et al. 1979). Gametogenesis begins in spring months and peaks in June and July (Goodwin 1976; Sloan & Robinson 1984; Campbell & Ming 2003). The female spawning season is reported to be shorter compared to males, occurring August through October (Goodwin 1976); however, recent observations suggest that reproduction starts in late winter with evidence of spawning in March followed by simultaneous spawning of both male and female geoduck in Puget Sound in June and July (Friedman & Vadopalas, unpubl. data). Of geoduck cases with SLO parasites, infection intensity was generally low, possibly due to elimination by the host when oocytes are released during spawning. Vertical transmission of *Steinhausia* is suspected to occur in *M. galloprovincialis*, which may explain the perpetuation of infection within the geoduck population year after year (Bower et al. 1994).

Spatial distribution of geoduck parasites

The Puget Sound is a series of interconnected, fjord-type channels connected to the Northeast Pacific Ocean by the Strait of Juan de Fuca. This large estuarine environment has a massive land-water interface with fluctuations in freshwater, organic matter, nutrients, and sediments from land and urbanized areas (Emmet et al. 2000). The sites selected for this study represent geoduck populations from two of the five major basins of the Sound - Thorndyke Bay (Hood Canal) and Totten Inlet (South Sound) - and one site from the Strait de Juan de Fuca, Freshwater Bay. Seawater conditions vary among these sites (Herlinveaux & Tully 1961; Thompson 1994; Newton et al. 2002; Moore et al. 2008).

Spatial differences in parasite communities were evident, especially between Freshwater Bay and Totten Inlet. Freshwater Bay and Totten Inlet exhibited the greatest differences in parasite abundance and infection intensity of the parasite taxa described in this study while, generally, Thorndyke Bay exhibited intermediate parasite abundance and infection intensity. Intestinal MLO and metazoan parasites were observed in highest prevalence at Totten Inlet (mean 63%) and showed the lowest abundance at Freshwater Bay (mean 9%). In contrast, trends in RLO prevalence were the inverse of those observed for metaozoan and intestinal microsporidia: Totten Inlet exhibited the lowest RLO prevalence (mean 19%), while RLOs were commonly observed in Freshwater Bay (mean 62%). Sample site did not influence presence of the SLO, which was limited to reproductively active female geoduck regardless of site. Similarly, siphon muscle microsporidian parasites were generally of low prevalence or absent at all sites. Drivers of the distinct spatial patterns observed among the locations sampled in this study are unclear but may be linked to environmental and hydrographic conditions unique to these locales.

In addition to physiological tolerances of these parasites to environmental variation, host density and spatial population aggregation can influence parasite dispersal in marine species (Blower & Roughgarden 1989). Geoducks are commonly found in discontinuous aggregate populations that vary in population density (Goodwin & Pease 1991), which could affect parasite ranges and distribution within Puget Sound. Further, host factors, such as feeding rate and diet, may also contribute to the variation in parasite distribution and accumulation in filter-feeding bivalves (Ford & Tripp 1996; Ford et al. 1999).

Conclusions

We revealed the presence of several previously unreported parasites in Puget Sound geoduck clams. Parasite presence in geoduck populations was significantly influenced by spatiotemporal differences in Puget Sound. Reasons for the differences in parasite assemblages may be attributed to host physiology and density, seasonality of infective stages of parasites, temperature shifts, or localized environmental factors (e.g., currents, freshwater input, mixing, nutrient availability) at each sampling location.

Parasite presence is ultimately dependent on both the environment of the host and the microenvironment of the parasite. Management of future disease outbreaks in geoducks will benefit from the baseline knowledge gathered in this study. To fully assess the potential risks of geoduck diseases continued exploration of individual parasite distributions, virulence and physiological tolerances is needed. Gathering further information about geoduck endosymbiont life cycles and host-parasite interactions can assist in future fishery management decisions regarding geoduck aquaculture and stock movement.

Acknowledgments

Special thanks to Bob Sizemore and the Washington Department of Fish and Wildlife, Celia Barton and divers of the Washington state Department of Natural Resources (WDNR), Kelly Toy and the Jamestown S'Klallam tribe, Doug Morrill and the Lower Elwha Klallam tribe, Joth Davis (Baywater, Inc.), Seattle Shellfish, and Taylor Shellfish Farms, Inc. We thank Drs. Ralph Elston (AquaTechnics, Inc.) and Frank Morado (NOAA)for histology assistance, Cyrus Ma, Nate Wight, and Adrianna Santa-Cruz for sampling assistance, Robyn Strenge for data organization, Julian Olden for statistical assistance, and Colleen Burge and Lisa Crosson for editorial comments. This research was funded by a grant (project R/GD-2) from the Washington Sea Grant Program, University of Washington, pursuant to the geoduck aquaculture research account created under Washington state law (RCW 28B.20.476) and, in part, by the Washington Sea Grant Program, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce (USDC), grant number NA04OAR4170032 to Dr. Carolyn S. Friedman and by the National Marine Aquaculture Initiative, NOAA, USDC, grant number NA07OAR4170450 to Dr. Carolyn S. Friedman. Additional funding provided by WDNR and the University of Washington's School of Aquatic and Fishery Sciences. The views expressed herein are those of the authors and do not necessarily reflect the views of the funders, NOAA, or any of its subagencies. The U.S. government is authorized to reproduce and distribute for governmental purposes.

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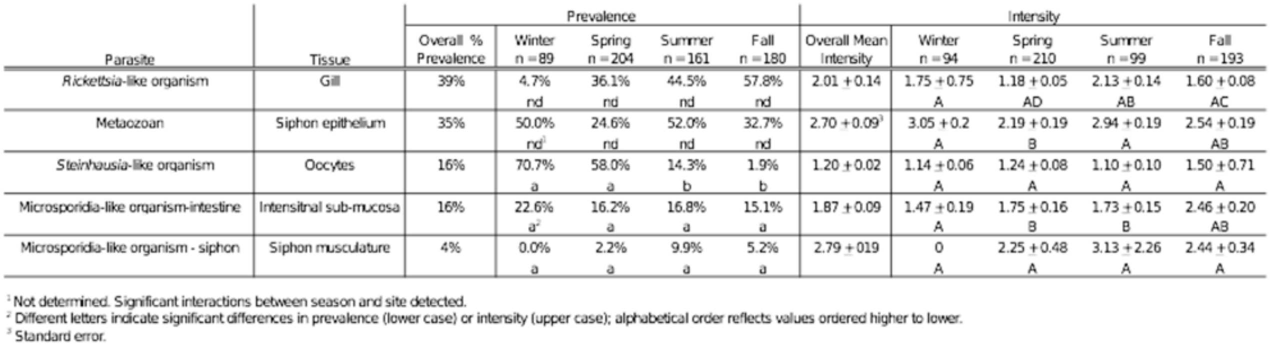
Tables and Figures

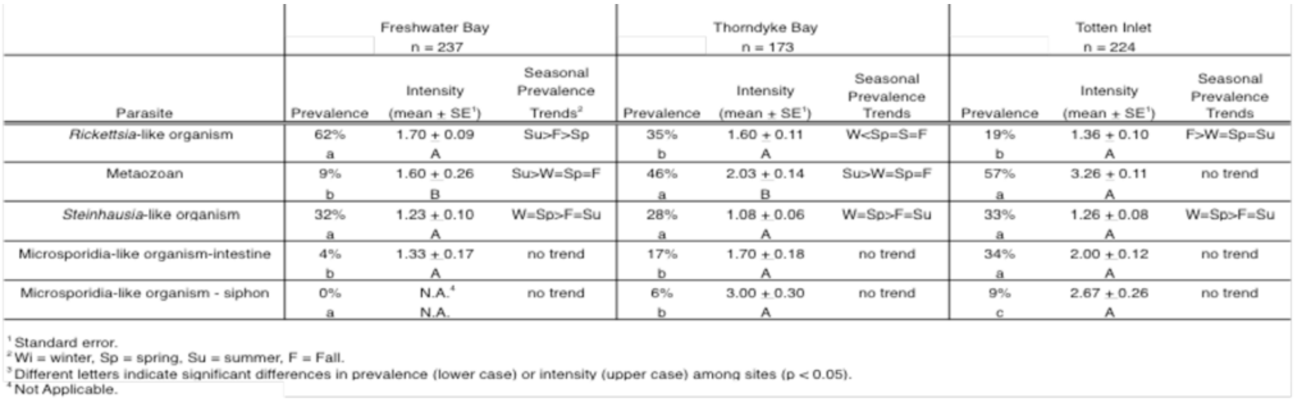
Table 1. Overall mean parasite prevalence and intensity in natural populations of Washington state P. generosa. Parasite intensity is based on a semi-quantitative score of 0 - 4 parasite intensity: 0 = no parasites, 1= few parasites (< 10), 2 = small numbers of parasites (11 – 20), 3 = moderate numbers of parasites (21 – 30), 4 = large numbers of parasites (> 30). 1Not determined. Significant interactions between season and site detected. 2Different letters indicate significant differences in prevalence (lower case) or intensity (upper case); alphabetical order reflects values ordered higher to lower. 3Standard error.



		Prevalence					Intensity				
Parasite	Tissue	Overall % Prevalence	Winter n = 89	Spring n = 204	Summer n = 161	Fall n = 180	Overall Mean Intensity	Winter n = 94	Spring n = 210	Summer n = 99	Fall n = 193
Rickettsia-like organism	Gill	39%	4.7% nd	36.1% nd	44.5% nd	57.8% nd	2.01 ± 0.14	1.75 ± 0.75 A	1.18 ± 0.05 AD	2.13 ± 0.14 AB	1.60 ± 0.08 AC
Metaozoan	Siphon epithelium	35%	50.0% nd[1]	24.6% nd	52.0% nd	32.7% nd	2.70 ± 0.09[3]	3.05 ± 0.2 A	2.19 ± 0.19 B	2.94 ± 0.19 A	2.54 ± 0.19 AB
Steinhausia-like organism	Oocytes	16%	70.7% a	58.0% a	14.3% b	1.9% b	1.20 ± 0.02	1.14 ± 0.06 A	1.24 ± 0.08 A	1.10 ± 0.10 A	1.50 ± 0.71 A
Microsporidia-like organism-intestine	Intensitnal sub-mucosa	16%	22.6% a[2]	16.2% a	16.8% a	15.1% a	1.87 ± 0.09	1.47 ± 0.19 A	1.75 ± 0.16 B	1.73 ± 0.15 B	2.46 ± 0.20 AB
Microsporidia-like organism - siphon	Siphon musculature	4%	0.0% a	2.2% a	9.9% a	5.2% a	2.79 ± 019	0 A	2.25 ± 0.48 A	3.13 ± 2.26 A	2.44 ± 0.34 A

[1] Not determined. Significant interactions between season and site detected.
[2] Different letters indicate significant differences in prevalence (lower case) or intensity (upper case); alphabetical order reflects values ordered higher to lower.
[3] Standard error.

Table 2. Parasite prevelance and intensity among sites and seasons. 1Standard error. 2Wi = winter, Sp = spring, Su = summer, F = Fall. 3Statistical difference among sites ($p < 0.05$). 4Not Applicable.



	Freshwater Bay n = 237			Thorndyke Bay n = 173			Totten Inlet n = 224		
Parasite	Prevalence	Intensity (mean ± SE[1])	Seasonal Prevalence Trends[2]	Prevalence	Intensity (mean ± SE[1])	Seasonal Prevalence Trends	Prevalence	Intensity (mean ± SE[1])	Seasonal Prevalence Trends
Rickettsia-like organism	62% a	1.70 ± 0.09 A	Su>F>Sp	35% b	1.60 ± 0.11 A	W<Sp=S=F	19% b	1.36 ± 0.10 A	F>W=Sp=Su
Metaozoan	9% b	1.60 ± 0.26 B	Su>W=Sp=F	46% a	2.03 ± 0.14 B	Su>W=Sp=F	57% a	3.26 ± 0.11 A	no trend
Steinhausia-like organism	32% a	1.23 ± 0.10 A	W=Sp>F=Su	28% a	1.08 ± 0.06 A	W=Sp>F=Su	33% a	1.26 ± 0.08 A	W=Sp>F=Su
Microsporidia-like organism-intestine	4% b	1.33 ± 0.17 A	no trend	17% b	1.70 ± 0.18 A	no trend	34% a	2.00 ± 0.12 A	no trend
Microsporidia-like organism - siphon	0% a	N.A.[4] N.A.	no trend	6% b	3.00 ± 0.30 A	no trend	9% c	2.67 ± 0.26 A	no trend

[1] Standard error.
[2] Wi = winter, Sp = spring, Su = summer, F = Fall.
[3] Different letters indicate significant differences in prevalence (lower case) or intensity (upper case) among sites ($p < 0.05$).
[4] Not Applicable.

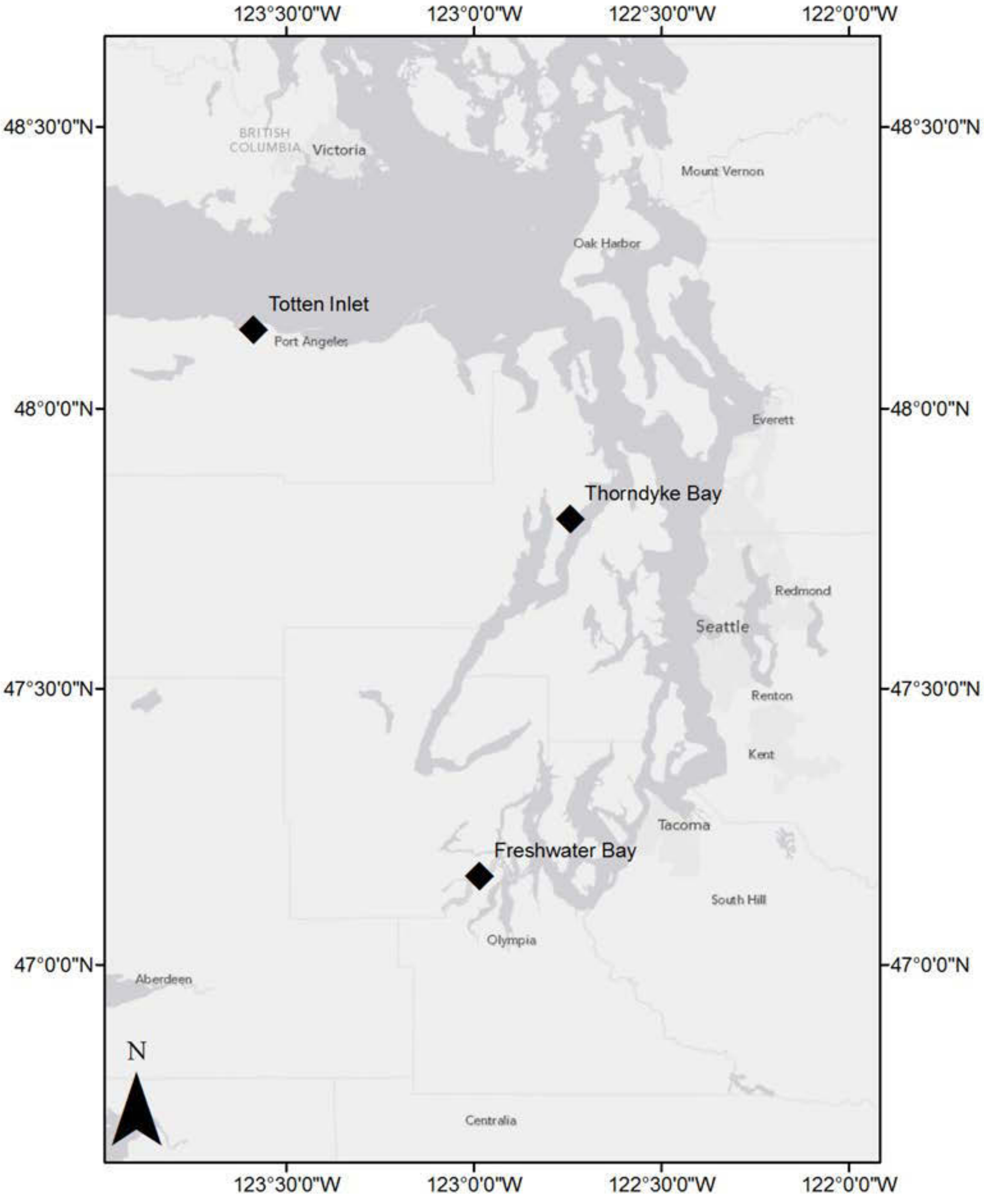


Figure 1. Geoduck sampling sites in Washington state.

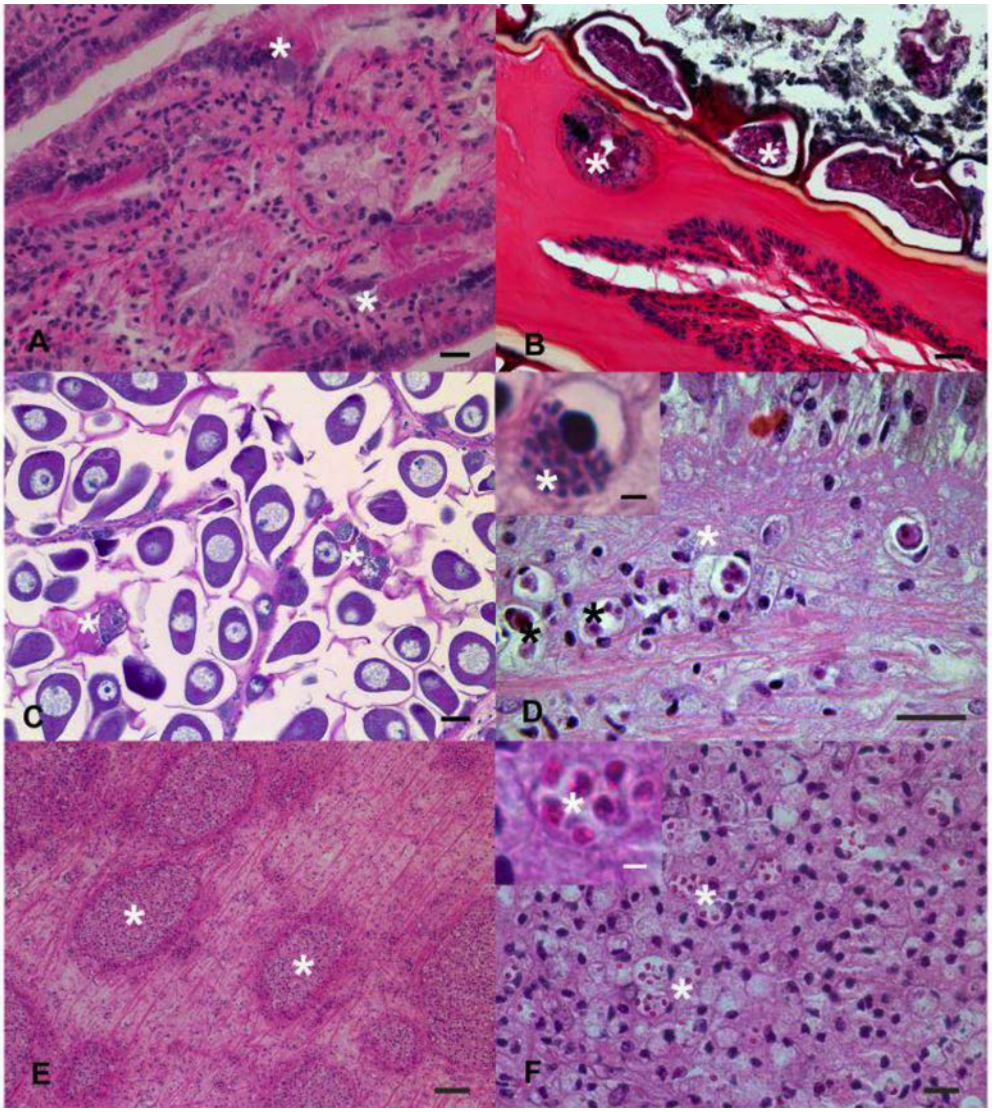


Figure 2. Commonly observed parasites in wild geoduck clams in Washington state. An asterisk denotes parasite presence in each photo. A. Rickettsia-like inclusion bodies in geoduck ctenidia tissue; bar = 13 µm. B. Metazoan parasites; bar = 25 µm. C. Steinhausia-like microsporidian with oocytes ; bar = 25 µm. D. MLO parasites within intestinal submucosa illustrating meronts (black asterisk) and spores (white asterisk and inset image); bar = 20 µm and inset bar = 2 µm. E. Low magnification illustrating the multifocal nature of the MLO within siphon musculature; bar = 50 µm. F. High magnification of siphonal MLOs; bar = 8 µm; inset bar = 2 µm . Stained with H&E.

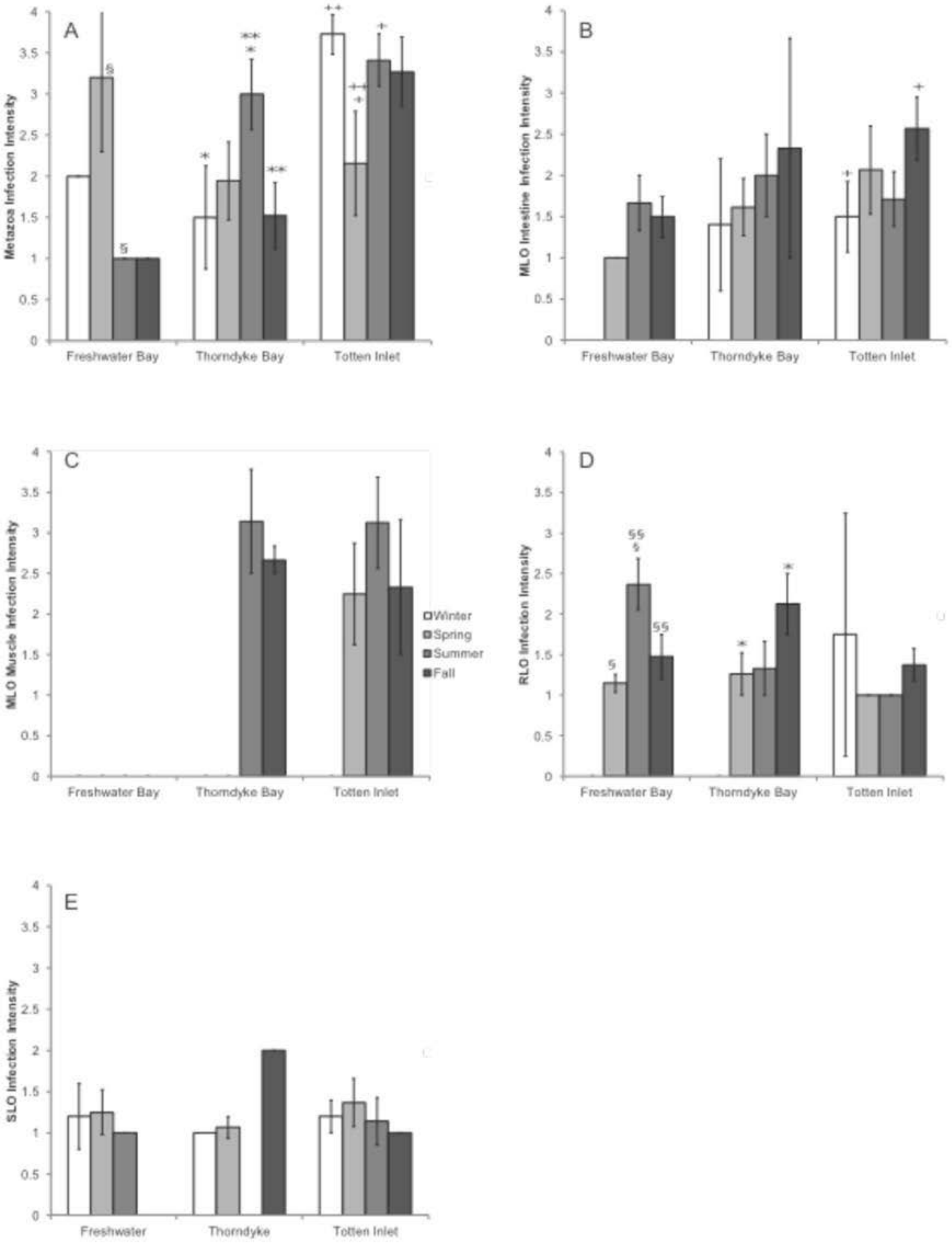


Figure 3. Infection intensity in P. generosa by site and season. Parasite groups: metazoa (A), intestinal microsporidia (MLO intestine; B), siphon muscle microsporidia (MLO muscle; C), Rickettsia-like organism (RLO; D), and Steinhausia-like organism (SLO; E) observed from histology in geoduck clams collected from Freshwater Bay, Thorndyke Bay, and Totten Inlet. Error bars represent 95% CI. § = Freshwater Bay pairwise comparisons indicating significant difference between seasons; * = Thorndyke Bay pairwise comparisons indicating significant difference between seasons; + = Totten Inlet pairwise comparisons indicating significant difference between seasons.

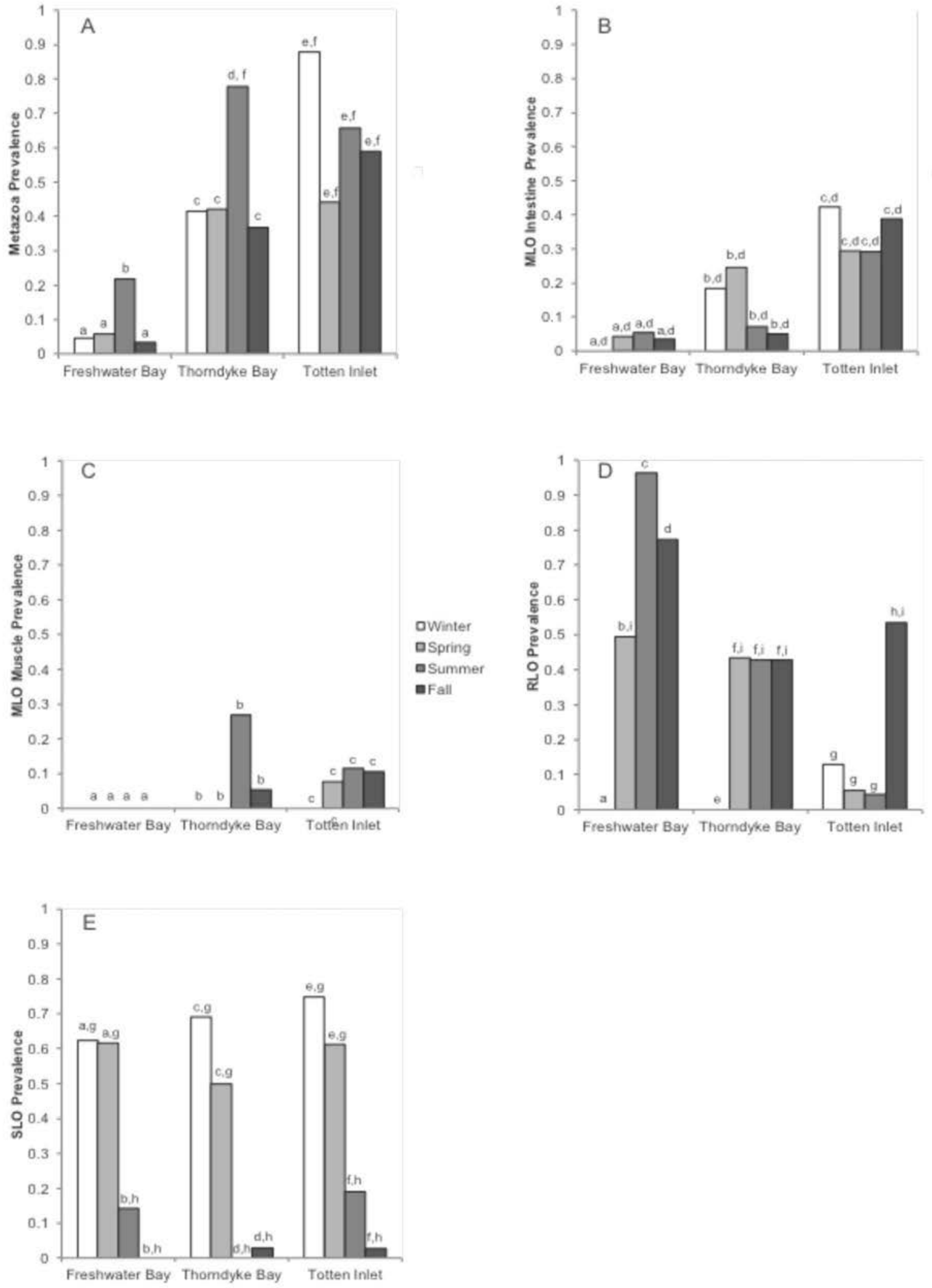


Figure 4. Proportions of parasite groups: metazoa (A), intestinal microsporidia (MLO intestine; B), siphon muscle microsporidia (MLO muscle; C), Rickettsia-like organism (RLO; D), and Steinhausia-like organism (SLO; E) observed from histology in geoduck clams collected from Freshwater Bay, Thorndyke Bay, and Totten Inlet. Error bars represent 95% CI.

Appendix V

Changes in seagrass (*Zostera marina*) and infauna through a five-year crop cycle of geoduck clams (*Panopea generosa*) in Samish Bay, WA

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Abstract

A 0.5 ha farm of geoduck clams (*Panopea generosa*) in Samish Bay, WA, was surveyed 13 times from April 2008 to April 2013 for traits of eelgrass (*Zostera marina*), sediment organic content, and infauna. Simultaneously, samples were collected in a stratified random design in an eelgrass meadow adjacent to the farm. The sampling period spanned the harvest of adult clams (July 2008), the installation (July 2008) and removal (July 2010) of nets and tubes for predator protection, and additional growout. At the beginning of the study (coinciding with the end of one crop cycle), the presence of cultured adult geoducks had little effect on eelgrass density or biomass. Harvest slightly reduced these traits, but the main difference between the farmed and unfarmed areas arose a year later, when *Z. marina* disappeared from the farm following the biofouling of overlying nets. One year after the nets were removed, *Z. marina* seedlings recolonized the farm. In the adjacent meadow, eelgrass near the farm differed from eelgrass far from the farm primarily in summer, when shoots closer to the farm were more dense. Infaunal diversity and abundance, measured in spring only, were lower in the farm than in the unfarmed area following harvest, even when differences in *Z. marina* were accounted for. This single-site case study may inform the consideration of interaction between food production and rooted aquatic vegetation, as well as scientifically based buffer zones.

Key words: *Bivalve aquaculture, artificial structure, disturbance–recovery, habitat complexity, spillover effects*

Introduction

As the shellfish aquaculture sector continues to develop, it is crucial to understand how this industry interacts with the systems that support it. Many studies have documented the effects of bivalve aquaculture on cultivated grounds and adjacent habitats (Forrest and Creese 2006, Munroe and McKinley 2007, Whitley and Bendell-Young 2007, Bouchet and Sauriau 2008), and these effects can include altered habitat structure, sediment character, and infaunal assemblages. The effects of bivalve aquaculture are of particular interest when aquaculture sites occur near habitats subject to protection and conservation, such as seagrass meadows.

Interaction between shellfish aquaculture and seagrasses yields a range of effects that depend upon culture practices and environmental context. In eastern Tasmania, Crawford et al. (2003) found no obvious effects of subtidal longline oyster (*Crassostrea gigas*) aquaculture on a mixed meadow of *Heterozostera tasmanica* and *Halophila australis*. In northern New Zealand, Bulmer et al. (2012) found reduced *Zostera muelleri* density directly underneath hanging baskets of *C. gigas*, but no large-scale effects of aquaculture on the seagrass. Experimental work in southwestern Washington State, USA, determined that on-bottom culture of *C. gigas* can have negative effects on eelgrass (*Zostera marina*) through space competition, and that the intensity of these effects depends upon oyster density (Wagner et al. 2012).

Aside from the initial impact of bivalve aquaculture on seagrasses, recovery time also depends upon culture practices, environmental factors, and the traits of the affected seagrass. Neckles et al. (2005) found variable rates of recovery in *Z. marina* after commercial harvest of *Mytilus edulis* by dragging, but sites that suffered more intense dragging activity had not fully recovered 7 years after harvest. Other species have demonstrated the ability to recover more rapidly: Park et al. (2011) found that the harvest of Manila clams removed or buried all *Zostera japonica* shoots within the farmed area, but noted recovery of *Z. japonica* density and biomass 3 months after harvest. The wide range of initial impacts and recovery times following interaction between bivalve aquaculture and seagrasses suggests that further research is necessary to evaluate emerging practices in shellfish aquaculture.

Commercial geoduck clam (*Panopea generosa*) aquaculture is a growing industry on the west coasts of the United States and Canada. In 2010 and 2011, geoduck (from wild harvest and aquaculture) represented 2.9 to 3.1% of United States clam landings by weight, but 31 to 37% of the total value of clam landings (Lowther 2011). The lucrative nature of this fishery has inspired interest in expanding the use of tidelands for geoduck aquaculture. The commercial geoduck aquaculture cycle comprises distinct periods of activity, including harvest, reseeding, the installation of predator protection structures, the subsequent removal of those structures, and several years of additional grow-out before the clams reach marketable size. To date, the separate and cumulative effects of these activities on different components of intertidal systems are largely unknown (Straus et al. 2007).

In this single-site case study, I examined the ecological consequences of the *P. generosa* aquaculture cycle at one commercial geoduck farm in northern Washington State, USA. Although clams are not typically planted into *Z. marina* in Washington State, the response of eelgrass to geoduck aquaculture is relevant because this protected seagrass can recruit into cultivated beds, and because cultivation could influence nearby eelgrass. I compared eelgrass traits, sediment organic content, and infaunal abundance and diversity in and outside of a geoduck farm. Sampling in the unfarmed area followed a design that enabled the evaluation of any changes related to distance from the farm. Although this investigation encompassed one site only, these data may allow management to better understand the effects of geoduck aquaculture activity on intertidal systems within and nearby geoduck farms, improving the decision-making process in the leasing of public tidelands.

Materials and Methods

Fisk Bar is an intertidal site in Samish Bay, WA (48°36'N, 122°26'W), hosting a geoduck farm approximately 140m by 36m and extensive surrounding eelgrass meadows (Fig. 1). A crop of geoducks was planted on Fisk Bar in the summer of 2002, with an intended harvest date of summer 2008. Although the farm was not a *Z. marina* meadow when geoducks were planted in 2002, eelgrass colonized the farmed area between 2002 and 2008. This circumstance afforded a unique opportunity to explore the effects of commercial geoduck aquaculture on an important type of intertidal habitat. I conducted an initial survey on 04/08/08, prior to harvest, and an additional 12 surveys over the following 5 years as the farm entered the next crop cycle.

Aquaculture activity

In May and June of 2008, geoducks were harvested from the Fisk Bar farm via high-volume seawater "stingers" used to liquefy sediment and remove geoducks at low tide. By early July, the farm was reseeded with juvenile geoducks and predator protection structures. These structures included PVC pipe tubes 10cm in diameter, installed at a density of approximately 10 per square meter. Three juvenile geoducks were planted into each tube. The tubes were then covered with anchored nets spanning the entire farm ('blanket' nets). In July of 2009, the nets were replaced due to heavy biofouling. In July of 2010, all nets and tubes were removed from the Fisk Bar farm.

Field surveys

For each survey, I sampled from 25 quadrats within the farm and 25 quadrats within the unfarmed area. I positioned quadrats (0.5m x 0.5m) with coordinates assigned in advance: within the farm, quadrat placement was wholly random, whereas within the unfarmed area, quadrat placement followed a stratified random design, in order to sample more heavily towards the farm boundary (5 quadrats each within 0 to 3m, 3 to 9m, 9 to 21m, 21 to 45m, and 45 to 93m of the farm; Fig. 1). Within each quadrat, I counted the number of *Z. marina* vegetative shoots. I then collected sediment, infauna, and *Z. marina* samples for laboratory analysis. Between April of 2008 and April of 2013, I conducted 13 surveys (on 04/08/08, 07/29/08, 11/12/08, 04/26/09, 07/18/09, 11/04/09, 04/30/10, 08/09/10, 11/05/10, 04/20/11, 07/28/11, 04/09/12, and 04/28/13).

Zostera marina samples

For quadrats with *Zostera marina* present, I collected all *Z. marina* from one quadrant of the quadrat, rinsing samples in the field. In the laboratory, I counted the number of vegetative shoots collected in each sample. I then haphazardly selected 20 vegetative shoots, and measured sheath length for each (when less than 20 vegetative shoots were sampled, I measured sheath length for all collected shoots). I washed all collected shoots to remove epiphytes, and then divided each shoot into above- and below-ground components. I dried the divided shoots at 60°C for 48 hours, and weighed them to determine above- and below-ground biomass per area. For each survey, I measured between 288 and 701 shoots, for a total of 6,010 analyzed shoots across 12 surveys.

Sediment samples

For all quadrats, I used a trowel to sample from the top 2cm of sediment in a second quadrant of the quadrat. In the laboratory, I dried sediment samples at 60°C for at least 48 hours. For each sample, I then combusted a 30g subsample in a muffle combustion furnace, collecting pre- and post-combustion weights in order to calculate sediment organic content. For each survey, I collected between 45 and 50 sediment samples, for a total of 716 analyzed sediment samples across 12 surveys.

Infauna samples

For all quadrats, I collected a ~1,000cm^3 core of sediment from a third quadrant of the quadrat. In the field or the laboratory, I wet-sieved each core over a 500µm mesh, and transferred the remaining material into 10% buffered formalin solution in order to fix specimens. After 24 hours, I rinsed each sample with ethanol over a 500µm mesh, followed by transfer to a 70% ethanol solution for long-term storage. I stained each sample with Rose Bengal, waited at least 24 hours, and commenced debris sorting under a dissection microscope, isolating preserved organisms from detritus. Finally, I examined sorted specimens under a dissection microscope at high power, and identified each organism to the lowest possible taxonomic level. I summarized infaunal invertebrate data using univariate metrics of total abundance, taxa richness, and Shannon-Weiner diversity (H') for each core. I performed these analyses on 10 cores from the farmed area and 10 from the unfarmed area for each spring survey (04/08/08, 04/26/09, 04/30/10, and 04/20/11) for a total of 80 analyzed infauna samples.

Statistical analysis

Because of seasonal and crop-cycle variation, I analyzed data from each survey separately, to compare eelgrass traits, sediment organic content, and infauna abundance and diversity between the unfarmed and farmed areas at each date. Because eelgrass was initially patchy within the farm, only those quadrats having eelgrass were included in the analysis of eelgrass density and above- and below-ground biomass. Each unfarmed–farmed comparison was tested by t-test for the following response variables: eelgrass density, above-ground biomass per area, below-ground biomass per area, sediment organic content, and infaunal abundance, taxa richness, and H'.

Using data from the unfarmed area, I further analyzed two eelgrass traits — density and above-ground biomass — by comparing the most distant sampling zone (within 45 to 93m of the farm) pairwise to each zone closer to the farm. These eelgrass traits were selected as most relevant to discussions of buffer zones, given implications of shoot density and biomass for habitat complexity and primary production.

I used a linear mixed-effects model to evaluate infaunal responses to changes in eelgrass, as opposed to other aspects of farming. I included quadrat-specific eelgrass biomass (the sum of above- and below-ground biomass) and farmed/unfarmed origin as fixed effects in this analysis, as well as their interaction. I included survey date as a random effect.

For all comparisons, alpha-levels for significance were set at 0.05, which is conservative for multiple comparisons associated with zone-by-zone contrasts.

Results

Zostera marina

Prior to harvest (04/08/08), *Z. marina* was patchily distributed within the farm (being present in 52% of quadrats). Where eelgrass was present, *Z. marina* was not distinguishable between the farmed and unfarmed areas in density (Fig. 2) or above-ground biomass (Fig. 3). However, *Z. marina* in the unfarmed area had 49% lower below-ground biomass than eelgrass in the farm (Fig. 3).

Immediately following harvest (07/29/08), eelgrass remained patchily distributed within the farm (being present in 64% of quadrats), but where it was present, *Z. marina* was now 78% more dense in the unfarmed area than in the farm (Fig. 2). Above- and below-ground biomass comparisons similarly showed higher values in the unfarmed area than in the farm (Fig. 3). Eelgrass was no longer present on the farm 1 year after harvest (07/18/09; Fig. 2), following a period of heavy biofouling on the blanket nets.

Between April and July of 2011, the Fisk Bar farm was recolonized by *Z. marina*. Although eelgrass density was very low in the farm (07/28/11; Fig. 2), I found small numbers of shoots throughout. The recolonizing plants persisted through the winter, as *Z. marina* adult shoots were present in the farm on 04/09/12. 2012 and 2013 each saw a small year-over-year increase in shoot density within the farm, although the proportion of occupied quadrats did not show the same trend.

On a zone-by-zone basis within the unfarmed area, eelgrass in the zone closest the farm was sometimes but not always different from eelgrass in the zone furthest from the farm ($45 < x < 93$m). Particularly in summer, *Z. marina* reached higher densities closer to the farm (Fig. 4). Across all but one survey date, eelgrass above-ground biomass was similar throughout the unfarmed area (Fig. 5).

Sediment

Prior to harvest, there was no difference in sediment organic content between the farmed and unfarmed areas (Fig. 6). Immediately following harvest, the unfarmed area had 13% higher sediment organic content than the farm (Fig. 6). This pattern persisted until 04/09/12, when sediment organic content was higher in the farm than the unfarmed area (Fig. 6). Sediment organic content remained higher in the farm than the unfarmed area on 04/28/13 (Fig. 6).

Interestingly, linear regressions show that sediment organic content in the unfarmed area was significantly higher (at $\alpha = 0.05$) near the farm on two summer surveys (07/18/09 and 08/09/10; $R^2 = 0.32$ and 0.23, respectively). Springtime sediment organic content showed a significant response to plot (unfarmed/farmed), but no response to quadrat-specific eelgrass biomass or to the interaction of these factors (Table 1).

Infauna

Before harvest, the unfarmed and farmed areas showed no difference in infaunal abundance or taxa richness, but the unfarmed area did exhibit higher H' (Fig. 7). In the years following harvest, the unfarmed area showed higher infaunal abundance and taxa richness than the farm, and maintained higher H' (except in 2009; Fig. 7).

Infaunal abundance and taxa richness showed a significant response to plot (unfarmed/farmed), but no response to quadrat-specific eelgrass biomass or to the interaction of these factors (Table 1). Infaunal H' showed no response to plot, quadrat-specific eelgrass biomass, or their interaction (Table 1).

Discussion

Based on the pre-harvest survey (04/08/08) in which *P. generosa* were present throughout the farm, adult geoducks at aquaculture densities appeared to have little influence on traits of *Z. marina* on Fisk Bar. These results are consistent with findings from South Puget Sound, where eelgrass density was 30% lower in summer when geoducks were added, but was not consistently different (Ruesink and Rowell 2012). Following harvest, *Z. marina* density was 44% lower in the farm than in the unfarmed area (Fig. 2), a magnitude of disturbance less than the 75% density reduction following harvest in South Puget Sound (Ruesink and Rowell 2012).

The most profound consequences of the crop cycle on Fisk Bar were associated with biofouling of the blanket nets used to protect geoducks from predators. A thick mat of ulvoid algae recruited to the nets in the winter and early spring of 2009, and almost certainly reduced light availability for plants below. Prior to the loss of *Z. marina* in the farm, I witnessed significant declines in shoot size. In retrospect, these changes may have indicated stress by light limitation. A similar pattern emerged when Hauxwell et al. (2001) subjected *Z. marina* to experimental shading under a macroalgal canopy, and noted reduced shoot size and density prior to eelgrass loss. Seagrasses generally are sensitive to shading, whether from phytoplankton, macrophytes, or artificial structures (Duarte 2002).

The first signs of recovery for eelgrass began 1 year after the removal of tubes and nets, and continued evidence for recovery appeared in the following year. *Z. marina* was lost from the farm between 04/26/09 and 07/18/09, but a small number of new shoots appeared within the farm between 04/20/11 and 07/28/11. *Z. marina* remained within the farm, at low densities, in 2012 and 2013. Thus, current geoduck aquaculture practices do not appear to have made this site unsuitable for later recolonization by eelgrass. The recruitment of new plants in the farm was likely through seeds and seedlings, as new shoots were often too far from the unfarmed area to be the product of vegetative propagation. Hauxwell et al. (2001) similarly noted the recovery of eelgrass from seed following the removal of shading macroalgae.

The temporal pattern of differences in infaunal assemblages mirrored the pattern of differences in eelgrass traits: infaunal abundance, richness, and diversity were lower in the farm across the post-harvest surveys (Fig. 7). Structured habitats on estuarine tideflats typically have higher abundance and diversity of benthic fauna (Ferraro and Cole 2011), and seagrasses in particular are known to enhance infaunal abundance and diversity (Lee et al. 2001), so one might expect that any differences in infaunal assemblages between the unfarmed area and the farm would arise from differences in eelgrass. On Fisk Bar, however, infaunal abundance, richness, and diversity were poorly predicted by quadrat-specific *Z. marina* biomass (Table 1), suggesting that the effects of geoduck aquaculture on infauna were not mediated solely through eelgrass.

It is possible that geoducks themselves affect neighboring infauna; Ruesink and Rowell (2012) found that the presence of geoducks led to increased porewater ammonium, and experimental enrichment of porewater ammonium has been shown to reduce recruitment in some infaunal species (Engstrom and Marinelli 2005). It is also possible that installed nets and tubes affect the recruitment or post-recruitment survival of infaunal species; Danovaro et al. (2002) found that artificial reefs in the Mediterranean had negative effects on local infaunal abundance. Although this study cannot pinpoint the mechanism(s) behind the differences in infaunal assemblages on Fisk Bar, it can offer site-scale information regarding the effects of geoduck aquaculture on infauna, whose responses can be idiosyncratic. To provide but one example of the capricious nature of infaunal response, aquaculture of a single bivalve species (*Mytilus edulis*) has been found to have a negative effect (Chamberlain et al. 2001), no effect (Danovaro et al. 2004), or a positive effect (Callier et al. 2008) on infaunal diversity.

In other studies of aquaculture harvest, changes in sediment characteristics are generally shorter-lived and of lesser magnitude than changes in biota (Kaiser et al. 1998, Cesar and Frid 2009). On Fisk Bar, sediment organic content was lower inside the farm across all but one of the post-harvest surveys, until this pattern was reversed on 04/09/12 (Fig. 6). Sediment organic content and seagrasses commonly exhibit a positive relationship (de Boer 2007), so one might expect that any differences in sediment organic content between the farmed and unfarmed areas would arise from differences in eelgrass. However, on Fisk Bar, sediment organic content was poorly predicted by quadrat-specific *Z. marina* biomass (Table 1). Furthermore, sediment organic content on 04/09/12 and 04/28/13 was higher in the farmed area, despite very low *Z. marina* density in the farm at that date (Figs. 2 and 6). Together, these results suggest that the effects of geoduck aquaculture on sediment organic content were not solely mediated through eelgrass.

The difference in sediment organic content between the farmed and unfarmed areas on 07/29/08 (Fig. 6) suggests that harvest reduced sediment organic content on Fisk Bar, perhaps through the movement of sediment by seawater stingers. This difference in sediment organic content generally increased in magnitude until 08/09/10, when nets and tubes were removed, and decreased thereafter (Fig. 6). This pattern could indicate that nets and tubes reduced sediment organic content within the farm. Bottom-seated cylinders can cause sediment scour due to interaction of the wave boundary layer with the cylinder (Sumer et al. 2001), and this dynamic could have been at work around each of the thousands of tubes installed in a geoduck farm. All evidence for such scour had disappeared by 04/09/12, less than 2 years after the removal of nets and tubes, when sediment organic content was actually higher inside the farm (Fig. 6).

Natural resource management often considers buffer zones for human activities that could have 'spillover' effects on aquatic habitats (Washington Administrative Code 173-26-221). The stratified random sampling of the unfarmed area in this study allowed the evaluation of the magnitude and duration of spillover effects from the geoduck farm. The results show that eelgrass traits differed with distance from the farm at particular times, both before and after harvest. Prior to harvest, *Z. marina* density did not vary as a function of distance to the farm, but eelgrass near the farm had lower above-ground biomass (Figs. 4 and 5). During each summer following harvest, *Z. marina* showed higher shoot densities nearer the farm (Fig. 4). Eelgrass bordering the farm also had lower above-ground biomass in summer, though this trend was not consistently significant (Fig. 5).

Because geoduck aquaculture on Fisk Bar effectively formed a distinct meadow edge where none had existed before, one might expect that any observed differences with distance from the farm would reflect patterns often witnessed from the edge to the center of a meadow. However, Bowden et al. (2001) found lower *Z. marina* density near the meadow edge (see Bologna and Heck 2002 for similar results in *Thalassia testudinum*), while Olesen and Sand-Jensen (1994) and Peterson et al. (2004) found no effect of distance-to-edge on shoot density or size. Since denser shoots near the Fisk Bar farm do not reflect previous findings on typical 'edge effects', these spatial patterns could be a product of interaction with the geoduck farm. Alternatively, or concomitantly, these spatial patterns could result from environmental variation: shoots in the farthest zone were slightly deeper than shoots near the farm, and *Z. marina* size and density can change with tidal elevation (Keller and Harris 1966, Ruesink et al. 2012).

Just as eelgrass differed with distance-to-farm predominantly in summer, sediment organic content was higher near the farm on two summer surveys (see Results), even as the farm itself had lower sediment organic content. This pattern could reflect increased particle capture and/or increased production by *Z. marina* near the farm.

I approached the interaction of geoduck aquaculture and its intertidal environment through a longitudinal study of multiple response variables in and outside of a single farm over one crop cycle. The colonization of the Fisk Bar farm by *Z. marina* during the previous crop cycle afforded a unique opportunity to examine the effects of geoduck aquaculture on eelgrass at realistic scales. The most dramatic effect was the loss of eelgrass within the farm (Fig. 2), likely due to shading by blanket nets. *Z. marina* recolonized the farm 1 year after the removal of nets and tubes. In keeping with previous work on aquaculture disturbance (Kaiser et al. 2006), it appears that *Z. marina* may take several more years to recover its pre-harvest density in the farm. Seedling germination was essential to recovery, given the size of the blanket nets. *Z. marina* shoots near the farm were more dense in summer (Fig. 5), which could indicate spillover effects. Farming practices reduced infaunal abundance and diversity on Fisk Bar, and temporarily reduced sediment organic content. Differences in eelgrass could not account for these effects (Table 1).

This case-study was limited to a single site, and the patterns of change witnessed across the geoduck crop cycle on Fisk Bar may not be generalizable to other contexts. With this limitation in mind, these data may prove useful for management decisions regarding the siting of geoduck farms relative to eelgrass meadows, and for bounding expectations regarding the duration and intensity of geoduck aquaculture effects on eelgrass, sediment, and the infaunal community.

Acknowledgements

I thank Dr. Jennifer Ruesink for her advice, support, and patience, from the initial design of this study to the composition of this manuscript. As field and laboratory technicians, Isaac Horwith, Katrina Van Raay, and Johannah Verhulst were integral to this work. Taylor Shellfish provided logistical support and access to their tidelands: I extend special gratitude to Danny Lomsdalen and Bernardo Ibarra Solis. The surveys of Fisk Bar depended upon help from more than two dozen colleagues and friends, and I am grateful to each one. Funding for this study was provided by Washington Sea Grant, the Washington State Department of Natural Resources, and the University of Washington Department of Biology.

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Table 1. Mixed-effects linear models on springtime sediment organic content and univariate metrics of infauna in the farmed and unfarmed areas.

Response	Fixed effects	F statistic	P value
Sediment organic content	Quadrat-specific *Z. marina* biomass	0.47	0.64
	Plot (Unfarmed/farmed)	4.89	<0.01*
	Z. marina biomass x Plot	0.74	0.46
Infaunal abundance	Quadrat-specific *Z. marina* biomass	0.50	0.62
	Plot (Unfarmed/farmed)	1.43	0.16
	Z. marina biomass x Plot	0.67	0.51
Infaunal taxa richness	Quadrat-specific *Z. marina* biomass	0.63	0.53
	Plot (Unfarmed/farmed)	3.99	<0.01*
	Z. marina biomass x Plot	0.21	0.84
Infaunal H'	Quadrat-specific *Z. marina* biomass	0.36	0.72
	Plot (Unfarmed/farmed)	4.18	<0.01*
	Z. marina biomass x Plot	1.01	0.32

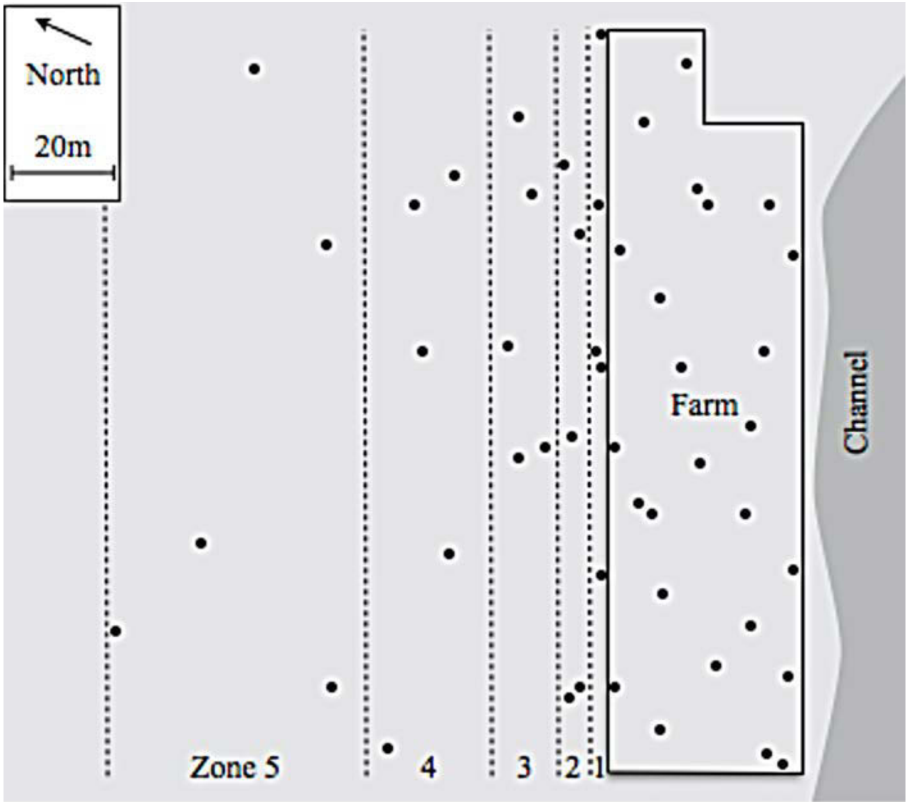

Figure 1: Schematic of Fisk Bar site, showing the farm, surrounding unfarmed areas, and the adjacent channel used for access. Light gray areas are above -2 MLLW; dark gray areas are below -2 MLLW. Dots indicate the placement of quadrats for a hypothetical survey.

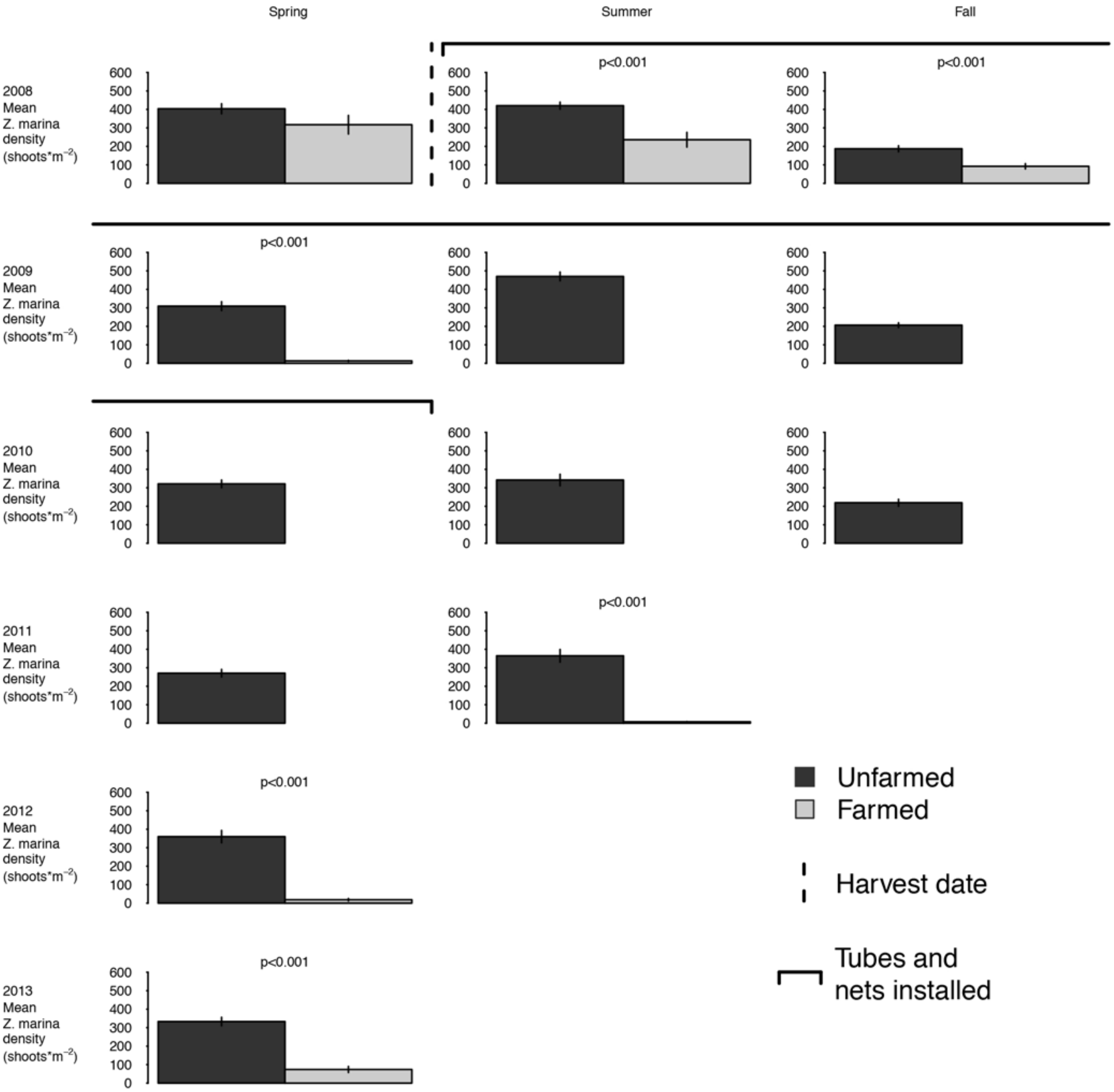

Figure 2: Within-patch eelgrass density over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 9 to 25). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at α= 0.05) in within-patch eelgrass density.

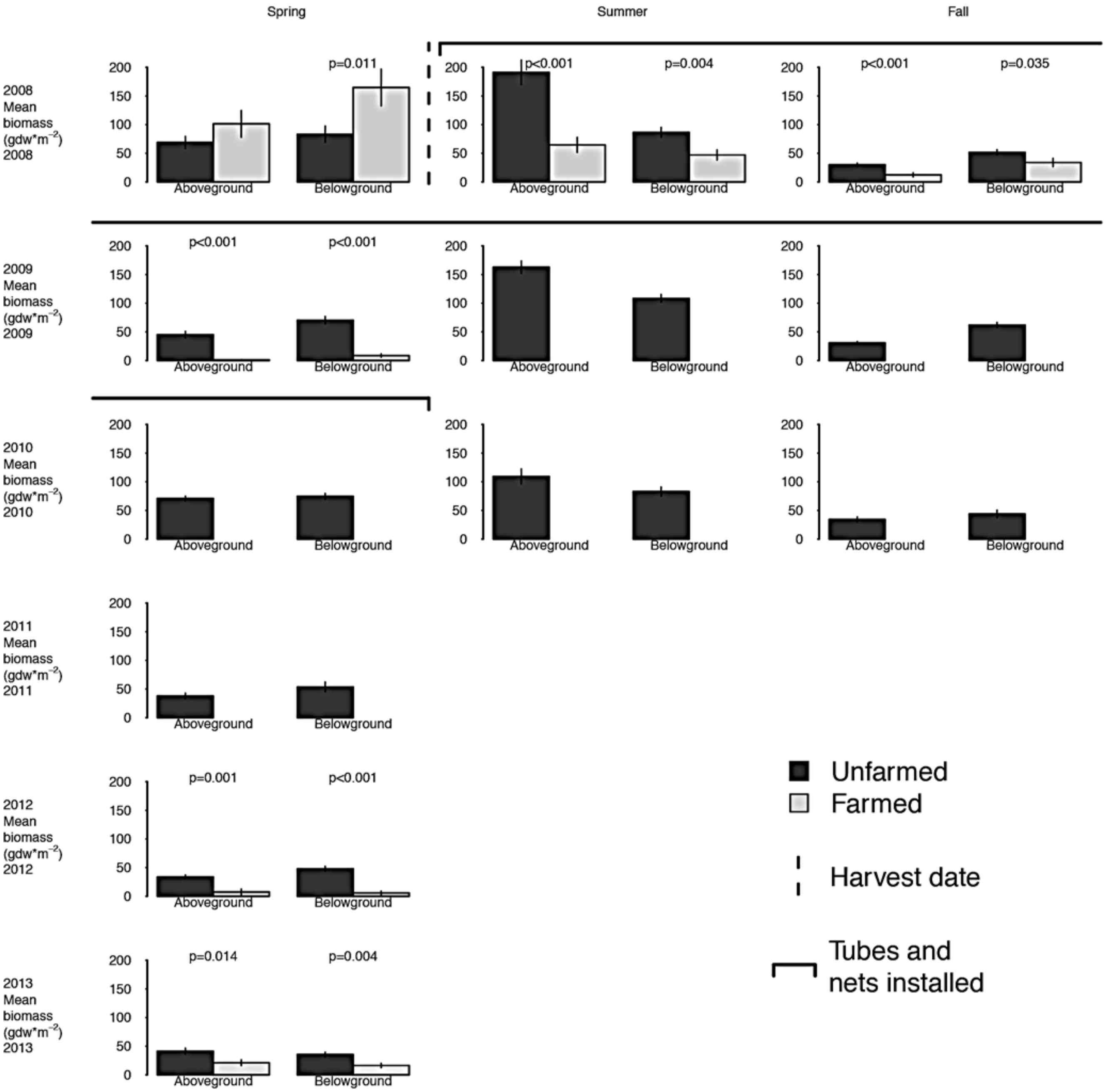

Figure 3: Eelgrass above- and below-ground biomass over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 9 to 25). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at $\alpha = 0.05$) in eelgrass above- or below-ground biomass.

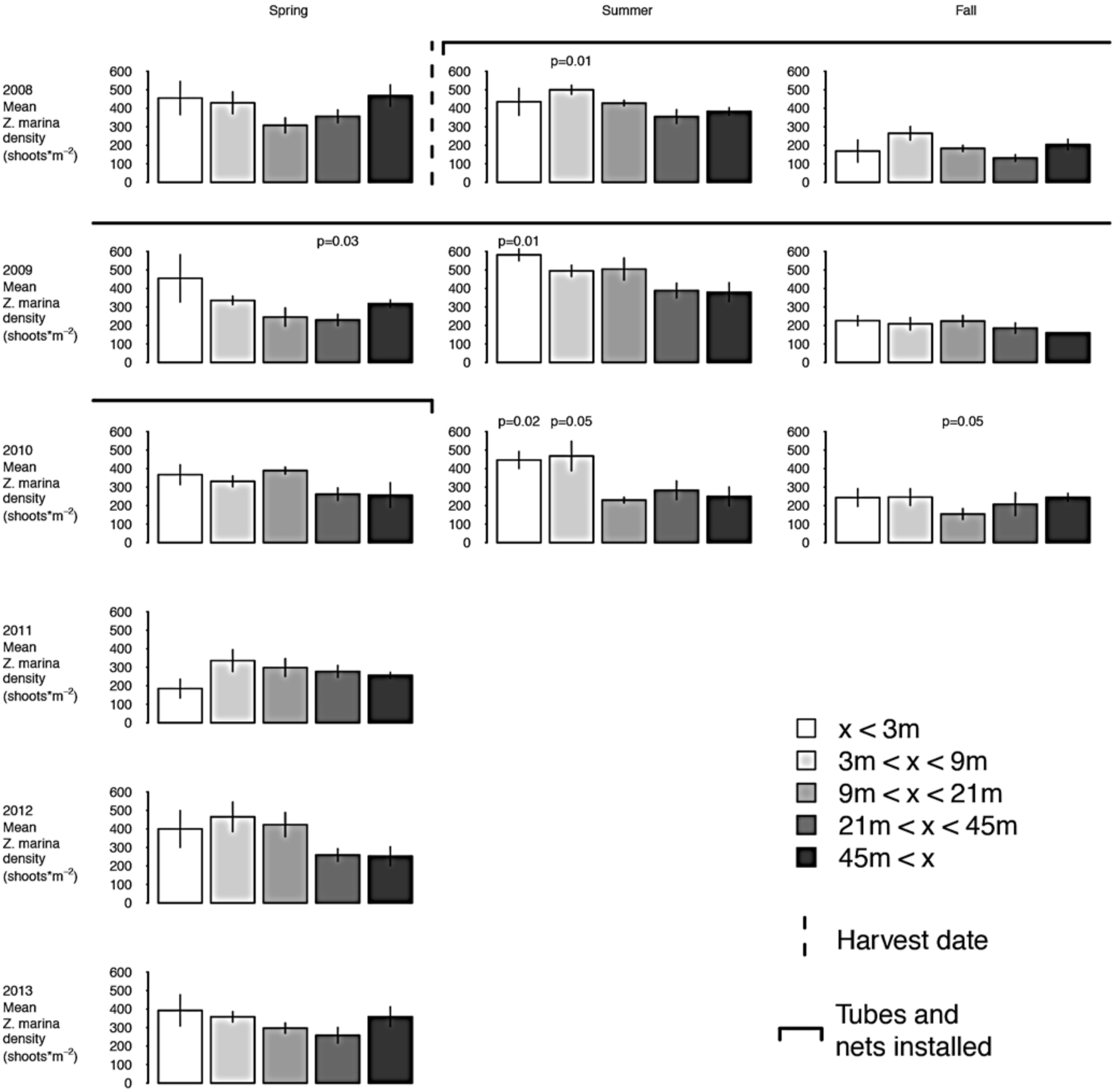

Figure 4: Eelgrass density over time across the five zones of the unfarmed area of Fisk Bar. Bars indicate standard error (n = 3 to 5). Within each survey date, p-values are given for each zone showing a significant difference (at $\alpha = 0.05$) in eelgrass density from the zone furthest from the farm (45m < x).

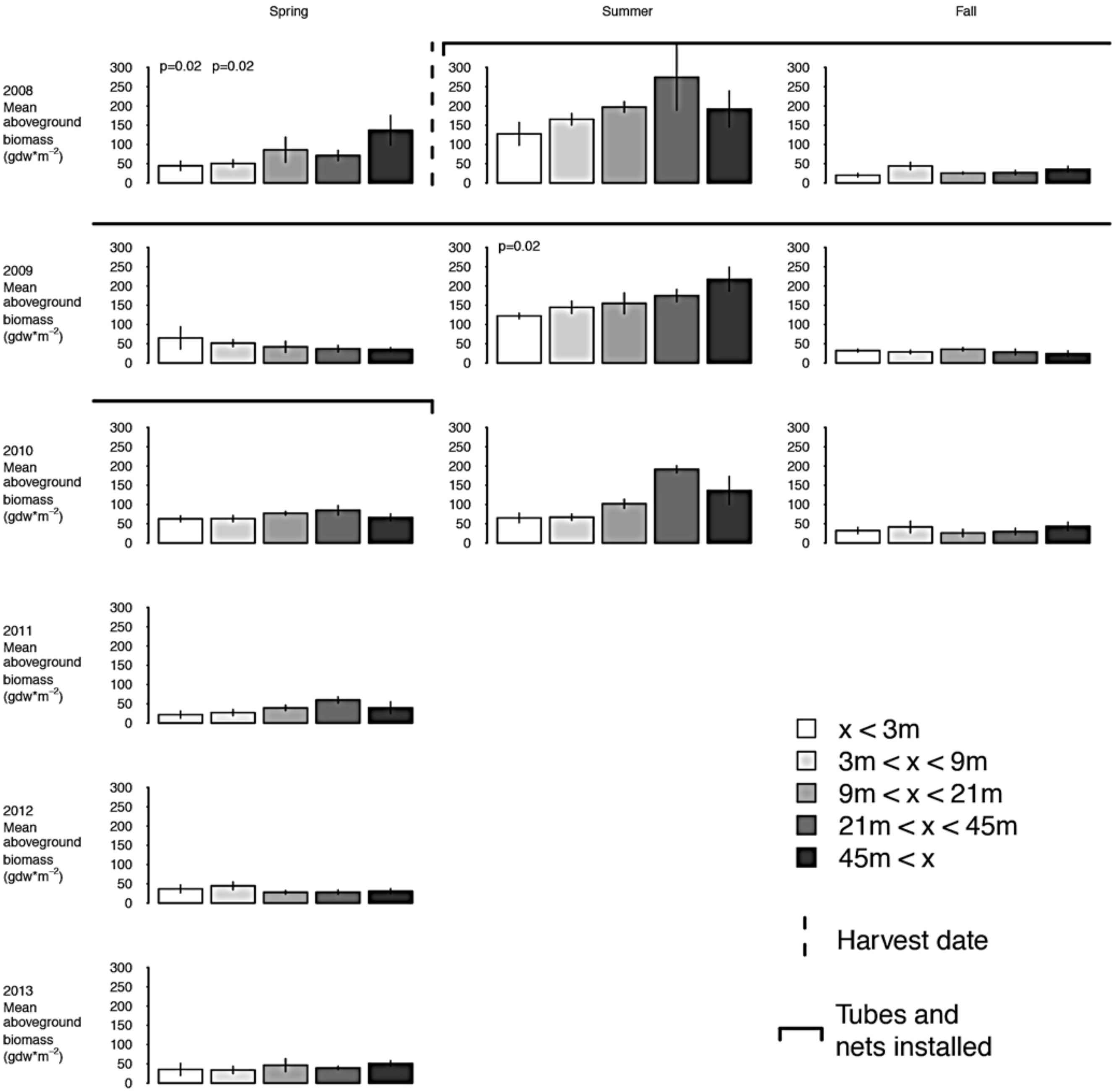

Figure 5: Eelgrass above-ground biomass over time across the five zones of the unfarmed area of Fisk Bar. Bars indicate standard error (n = 3 to 5). Within each survey date, p-values are given for each zone showing a significant difference (at $\alpha = 0.05$) in eelgrass above-ground biomass from the zone furthest from the farm (45m < x).

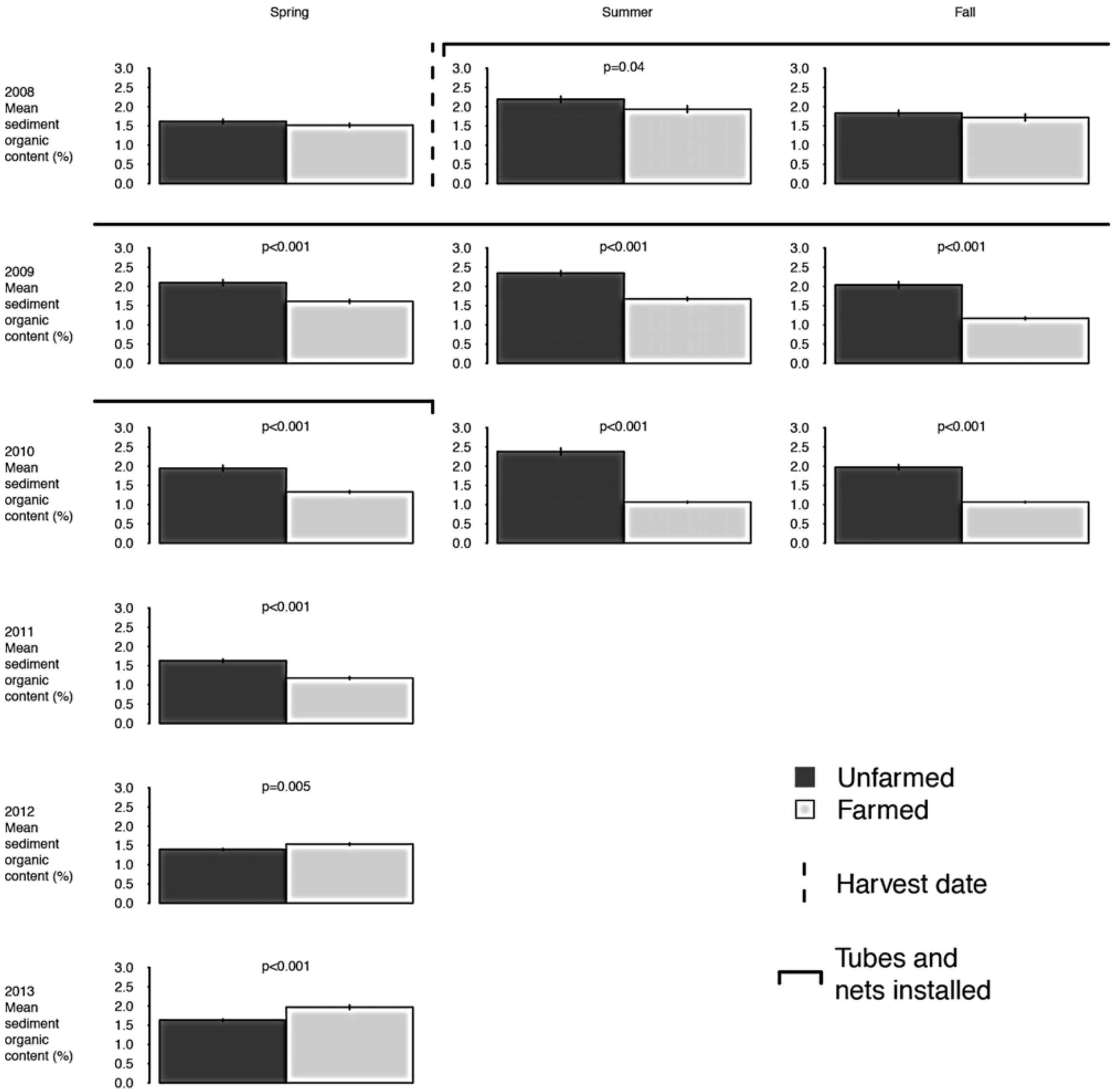

Figure 6: Sediment organic content over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 24 to 25). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at $\alpha = 0.05$) in sediment organic content.

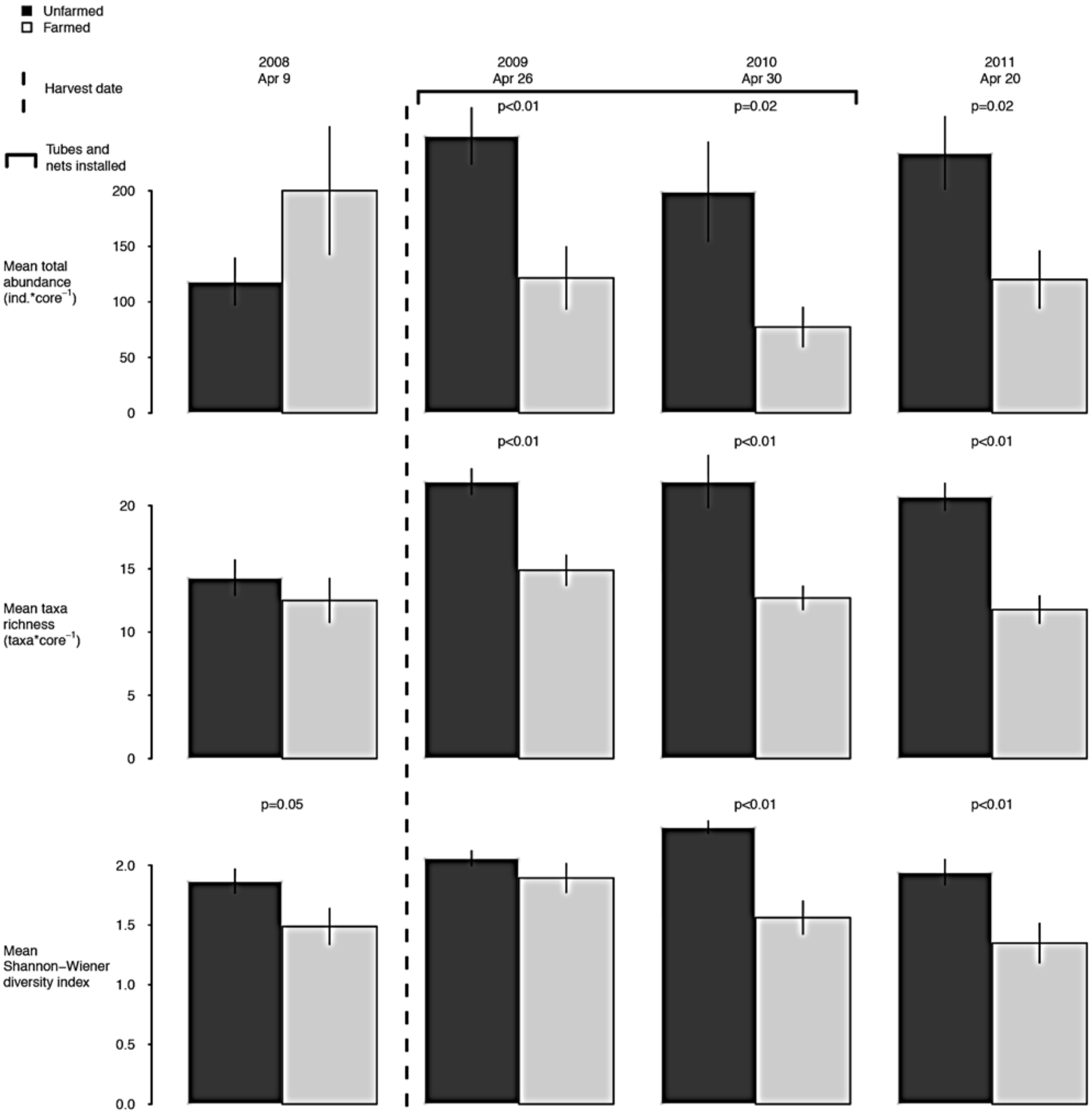

Figure 7: Infauna abundance, richness, and diversity over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 10). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at $\alpha = 0.05$) in the given infaunal character.