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).



FINAL REPORT

Publication and Contact Information

This report is available on the Washington Sea Grant website at wsg.washington.edu/geoduck

For more information contact:



Washington Sea Grant

University of Washington 3716 Brooklyn Ave. N.E. Box 355060 Seattle, WA 98105-6716

206.543.6600

wsg.washington.edu seagrant@uw.edu

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Primary	Investigators/
Report A	Authors

Jeffrey C. Cornwell Carolyn S. Friedman P. Sean McDonald Jennifer Ruesink Brent Vadopalas Glenn R. VanBlaricom

Contributing Scientists

David Armstrong

Lisa M. Crosson
Jonathan Davis
Elene M. Dorfmeier
Tim Essington
Paul Frelier
Aaron W. E. Galloway
Micah J. Horwith
Perry Lund
Kate McPeek
Roger I. E. Newell
Julian D. Olden
Michael S. Owens
Jennifer L. Price

Kristina M. Straus

Washington Sea Grant Staff

Penelope Dalton Marcus Duke David G. Gordon Teri King Meg Matthews Robyn Ricks Eric Scigliano Raechel Waters Dan Williams

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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.

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;
- 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;
- 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;
- 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;
- genetic interactions between cultured and wild geoducks, including measurement of differences between cultured and wild geoducks in term of genetics and reproductive status; and
- 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

SHB 2220 required a review of all available scientific Presearch 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 20131 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.

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.

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.

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

Background

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

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

report.

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 Van-Blaricom, 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-controlimpact" (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.

Geoduck Aquaculture Research Program

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

bservations 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

esident and transient macrofauna communities respond Addifferently 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 porewater 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 porewater 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 = noparasites, 1 = few parasites (<10), 2 = small numbers of parasites (11 - 20), 3 = moderate numbers of parasites <math>(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 Lthe 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

he investigators revealed the presence of several previ-Lously 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. Aboveand 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.

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

ivalves in culture may alter nutrient cycling and affect Becological 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 sub-

tidal 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.

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)

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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.