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January 6, 2017

Via Email: pamela.sanguinetti@usace.army.mil
U.S. Army Corps of Engineers
Seattle District
Regulatory Branch
ATTN: Pamela Sanguinetti
Post Office Box 3755
Seattle, WA 98124-6904

RE: Nationwide Permit 48 Commercial Shellfish Aquaculture Activities
Pacific Coast Shellfish Growers Association Comments

Dear Ms. Sanguinetti:

Thank you for providing an opportunity to comment on the U.S. Army Corps of Engineers ("Corps") Seattle District's proposed regional conditions ("Regional Conditions") for Nationwide Permit 48 ("NWP 48") as identified in the November 30, 2016 Special Public Notice.[1] We are submitting these comments on behalf of the Pacific Coast Shellfish Growers Association ("PCSGA"). PCSGA, founded in 1930, represents over 100 shellfish growers in Alaska, Washington, Oregon, California and Hawaii. PCSGA is headquartered in Olympia, Washington, and it works on behalf of its members on a broad spectrum of issues, including environmental protection, shellfish safety, regulations, technology, and marketing. Members of PCSGA grow a wide variety of healthful, sustainable shellfish including oysters, clams, mussels, and geoduck.

A. Regional Condition 1 Should Be Revised to Eliminate Unnecessary Paperwork

Regional Condition 1 states that a project's pre-construction notification ("PCN") must include a complete description of a proposal and that if an operator intends to undertake unanticipated changes that require Corps authorization, the operator must contact the Corps to request and receive written approval.

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[1] The November 30, 2016 Special Public Notice clarifies an earlier notice dated November 23, 2016 and states, among other things, that proposed regional general conditions 10 and 14 will not be applicable to NWP 48.

PCSGA has no objection to the general requirements that shellfish farmers submit descriptions of their projects and update these descriptions if a farmer wants to undertake unanticipated changes. However, for NWP 48 to operate as general permits are intended—to allow activities with minimal adverse effects to proceed with little or no delay—Regional Condition 1 should be revised to eliminate any need for farmers to submit, and for the Corps to review, unnecessary or duplicative paperwork. Many shellfish farmers have submitted previous paperwork to obtain coverage for their projects under the 2007 and/or 2012 version of NWP 48. In addition, many farmers have or will submit additional paperwork to the Corps to obtain coverage under the recent programmatic Endangered Species Act ("ESA") and Magnusson-Stevens Fishery Conservation and Management Act ("MSA") consultation completed for shellfish activities in Washington State inland marine waters. Accordingly, by the time the 2017 version of NWP 48 is effective, the Corps will already possess complete descriptions for the vast majority of commercial shellfish farms. Given the volume of projects that will seek authorization under the 2017 version of NWP 48, it would be an enormous waste of time and resources—for both shellfish farmers and Corps personnel—to require shellfish farmers to repackage and resubmit information that is already in the Corps' possession. Therefore, Regional Condition 1 should allow shellfish farmers to satisfy the PCN requirement by submitting a simple letter requesting authorization under NWP 48 in those cases where the Corps already possesses a complete description of the project.

Regional Condition 1 should also be clarified to explicitly address and acknowledge the need for growers to make changes to their farms throughout the course of the authorization. Shellfish farming is a dynamic business. Environmental, market, and technological conditions are continually changing, and growers need the ability to quickly adapt—whether that means changing the species being cultivated, modifying culture techniques, or implementing new technology. While shellfish farmers should provide the Corps with sufficient information for the Corps to understand what is proposed and to confirm that the activities fall within the scope of NWP 48, growers should not be required to request and obtain permission for making changes to their farms when such changes fall within activities authorized by NWP 48. For example, since NWP 48 covers oyster, clam, mussel, and geoduck aquaculture, including on- and off-bottom culture activities, a farmer should be able to obtain coverage for all of these species and culture techniques and make changes at any time throughout the life of the authorization without requesting permission for each change. It appears Regional Condition 1 implicitly allows for this needed flexibility by stating "The PCN must include a complete description of the NWP 48 activities anticipated to occur *over the duration of the permit authorization* within the applicant's project area." (Emphasis added). However, providing additional, explicit direction on this point will help ensure that both shellfish farmers and Corps personnel have a clear and consistent understanding with respect to what growers may include in their authorization requests and when additional authorization is needed.

Considering the foregoing, we respectfully request that Regional Condition 1 be revised as follows (additions are in underline):

> 1. The PCN must include a complete description of the NWP 48 activities anticipated to occur over the duration of the permit authorization within the applicant's project area. If a complete description of the proposal has previously

> been provided, the operator may satisfy the PCN requirement by referencing such information and requesting permit authorization. An operator may indicate in its PCN that multiple species of shellfish and multiple culture techniques may be utilized and changed throughout the effective period of this NWP. If an operator intends to undertake unanticipated changes to the commercial shellfish aquaculture operation during the effective period of this NWP beyond those indicated in the PCN, and those changes require Department of the Army authorization, the operator must contact the district engineer to request a modification of the NWP verification and receive written approval prior to commencing work. If the Corps does not deny such a modification request within 14 days, it shall be deemed approved.

Adopting these revisions will address the concerns raised above and also help advance numerous national and state laws and policies that recognize the importance of protecting and increasing domestic aquaculture production, including the following: the National Aquaculture Act of 1980 (passed in response to findings that the nation has potential for significant aquaculture growth, but that this growth is inhibited by many scientific, economic, legal, and production factors);[2] the National Oceanic and Atmospheric Agency ("NOAA") Marine Aquaculture Strategic Plan FY 2016-2020 (noting the nation currently faces a rising seafood trade deficit of more than $14 billion, despite possessing the world's largest Exclusive Economic Zone, posing a risk to our food security);[3] NOAA's Marine Aquaculture Policy (reaffirming that aquaculture is important to maintain healthy and productive ecosystems, restore endangered species, restore and conserve habitat, create employment and business opportunities, and enable the production of safe and sustainable seafood);[4] NOAA's National Shellfish Initiative (designed to increase populations of bivalve shellfish in recognition of the broad suite of economic, social, and environmental benefits they provide);[5] the National Ocean Council's National Ocean Policy Implementation Plan (emphasizing aquaculture production will benefit from streamlined federal permitting);[6] Washington State Bush and Callow Acts (legislation passed in early statehood to encourage the development and expansion of shellfish farming within the state);[7] Washington State Shoreline Management Act (identifying aquaculture as a preferred, water-dependent use that can have long-term benefits and protect the resources and ecology of the shoreline);[8] Washington State Aquaculture Marketing Act (encouraging the development and expansion of aquaculture in the State in recognition of the important economic benefits this use provides);[9]

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[2] 16 U.S.C. § 2801(a). The National Aquaculture Act of 1980 is codified at Title 16, Chapter 48 of the United States Code.

[3] NOAA Fisheries Marine Aquaculture Strategic Plan FY 2016-2020, p. 3 (http://www.nmfs.noaa.gov/aquaculture/docs/aquaculture_docs/noaa_fisheries_marine_aquaculture_strategic_plan_fy_2016-2020.pdf).

[4] NOAA Marine Aquaculture Policy, p. 1 (http://www.nmfs.noaa.gov/aquaculture/docs/policy/noaa_aquaculture_policy_2011.pdf).

[5] National Shellfish Initiative Fact Sheet, p. 1. (http://www.nmfs.noaa.gov/aquaculture/docs/policy/natl_shellfish_init_factsheet_summer_2013.pdf).

[6] National Ocean Policy Implementation Plan, p. 3 (https://www.whitehouse.gov/sites/default/files/national_ocean_policy_implementation_plan.pdf).

[7] Wash. ESHB 2819 (2002 c 123 § 1).

[8] RCW 90.58.020; WAC 173-26-241(3)(b).

[9] RCW 15.85.010.

Washington Shellfish Initiative (encouraging shellfish farming in Washington State in recognition of its critical importance to the state, and with a key goal of improving permitting processes to maintain and increase sustainable aquaculture).[10]

It bears emphasizing that while protecting and increasing aquaculture production is in the national interest, this is particularly important in Washington State, which currently leads the country in the production of farmed clams, oysters, and mussels. Revising Regional Condition 1 as shown above will ease administrative burdens for all parties involved and help growers implement needed changes to their farms in a timely manner without sacrificing any environmental protections.

B. Regional Condition 2 Should Be Clarified

PCSGA understands that Regional Condition 2 is intended to only apply to the harvest of clams using hydraulic escalator harvester equipment—very specific equipment that uses water jets, a digging scoop, and a conveyor belt to harvest clams from the substrate—and not to other equipment for clam harvest. PCSGA has no objection to this exclusion and notes that hydraulic escalator harvester equipment for clams is not commonly used in Washington State. However, to ensure Regional Condition 2 is consistently interpreted, we request that the condition be clarified as follows:

> 2. The harvest of clams by means of hydraulic escalator harvester equipment is not authorized by this NWP. This exclusion does not apply to the harvest of clams using any other equipment, including mechanical harvest equipment that does not use water jets and a conveyor in the harvest process.

C. NWP 48 Should Be Reissued without Additional Regional Conditions

No additional Regional Conditions, including but not limited to General Regional Conditions 10 and 14, should be imposed beyond those currently proposed. There is extensive scientific support for the finding that commercial shellfish aquaculture operations have minimal individual and cumulative adverse environmental impacts, much of which is identified in the Federal Register notice and the draft Decision Document for NWP 48.[11] Additional scientific information demonstrating shellfish aquaculture has minimal adverse to beneficial impacts is presented in PCSGA's comment letter on the draft 2012 version of NWP 48.[12] As discussed in more detail in that letter, shellfish help clean water by filtering suspended materials, and shellfish and aquaculture gear provide beneficial, structured habitat.[13] Shellfish beds can help provide

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[10] Washington Shellfish Initiative, p. 1 (http://pcsga.org/wprs/wp-content/uploads/2013/04/Washington-Shellfish-Initiative.pdf). *See also* Washington Shellfish Initiative – Phase II Work Plan pp. 10-11 (http://www.governor.wa.gov/sites/default/files/ShellfishWorkPlan.pdf).

[11] Proposal to Reissue and Modify Nationwide Permits, 81 Fed. Reg. 35186 (June 1, 2016); Draft Decision Document, Nationwide Permit 48 (https://www.regulations.gov/document?D=COE-2015-0017-0049).

[12] Appendix A (attachments not included).

[13] *Id.*, pp. 2-4.

mitigation and are increasingly being utilized in environmental restoration projects across the nation.[14]

Since PCSGA submitted its prior comment letter on the 2012 version of NWP 48, additional scientific information has been released confirming shellfish aquaculture has minimal adverse to beneficial environmental impacts. Washington Sea Grant concluded its multi-year study regarding the environmental impacts associated with geoduck aquaculture, and it issued a final report to the state Legislature in November 2013.[15] That report concludes that the limited disruptions associated with geoduck aquaculture are within the range of natural variation experienced by benthic communities in Puget Sound. Highlights from the final report include:

- Geoduck harvest practices have minimal impacts on benthic communities of infaunal invertebrates, with no observed "spillover effect" in habitats adjacent to cultured plots, suggesting that disturbance is within the range of natural variation experienced by benthic communities in Puget Sound.

- Differences in the structure of mobile macrofauna communities between planted areas with nets and tubes and nearby reference beaches do not persist once nets and tubes are removed during the grow-out culture phase.

- Nutrients released from a typical commercial geoduck operation are low and localized effects are likely to be negligible.

- Geoduck aquaculture practices do not make culture sites unsuitable for later colonization by eelgrass.

Many of the findings reached by the Washington Sea Grant geoduck research program have been published in peer-reviewed journals, including the following articles: Glenn R. VanBlaricom et al., *Ecological effects of the harvest phase of geoduck (Panopea generosa Gould, 1850) aquaculture on infaunal communities in southern Puget Sound*, Washington, Journal of Shellfish Research Vol. 34, No. 1, pp. 171-87 (2015); P. Sean McDonald et. al, *Effects of geoduck (Panopea generosa Gould, 1850) aquaculture gear on resident and transient macrofauna communities of Puget Sound*, Washington, Journal of Shellfish Research Vol. 34, No. 1, pp. 189-202 (2015); McPeek et. al, *Aquaculture Disturbance Impacts the Diet but not Ecological Linkages of a Iniquitous Predatory Fish*, Estuaries and Coasts (Nov. 8, 2014). In fact, in April, 2015 a special issue of the Journal of Shellfish Research was published for the express purpose of disseminating the most current information on geoduck aquaculture, which has experienced a dramatic increase in scientific study in recent years. These studies demonstrate that, similar to other forms of shellfish aquaculture, properly managed geoduck farms do not have significant environmental impacts.

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[14] *Id.*, pp. 6-7.

[15] Appendix B. Washington Sea Grant submitted an additional report to the Washington State Legislature in December 2015. This report provides results from a research program that the legislature directed to promote and manage shellfish aquaculture in Washington State. Results from this research program include, among other things, a finding that aquaculture can mitigate eutrophication in western South Puget Sound. See Appendix C.

Additional studies have also been performed on the interaction of shellfish farming and eelgrass. This information, along with a review of prior studies on the relationship between shellfish farms and eelgrass and the ecosystem services offered by shellfish beds, is presented in the attached technical report from Confluence Environmental Company.[16] Highlights from that report include the following findings:

- The most comprehensive analysis of factors that drive landscape-scale changes to eelgrass indicated that aquaculture operations have minor impacts to eelgrass cover (less than 1.5% of the total predicted eelgrass density in any year). This level of reduction is within the range of existing natural disturbances to the system (e.g., winter storms) to which eelgrass is inherently adapted. When the functional value of eelgrass and shellfish are combined, and the landscape matrix of habitats is considered, it is possible that a broader ecosystem perspective would find benefits from the presence of aquaculture.[17]

- Native eelgrass exhibits a stable and possibly increasing trend in distribution and abundance in areas like Willapa and Humboldt bays where oysters have been actively farmed for over 100 years and are currently used by commercial growers. This indicates that eelgrass is resilient to the level of shellfish aquaculture activities in these estuaries.[18]

- Many effects of shellfish aquaculture can be considered a positive interaction with eelgrass and the surrounding habitat. Shellfish can help protect shorelines from erosion by stabilizing sediments and dampening waves. This may benefit eelgrass, as eelgrass has been known to expand into areas after sediments are stabilized.[19]

- Shellfish aquaculture plots may recruit eelgrass through three main mechanisms: (1) recruitment of floating seeds as they travel singly or within detached reproductive shoots; (2) providing superior conditions for seed germination; and (3) increasing the survival of seedlings by increasing light levels, nutrients, and protecting against erosion.[20]

- Shellfish aquaculture beds can also reduce desiccation of eelgrass beds by expelling water when the tide recedes and creating micro-habitats.[21]

- Commercial geoduck aquaculture has been shown to facilitate the establishment of eelgrass beds in areas previously devoid of eelgrass, and eelgrass recovered and continued to thrive after disturbances from farm activities occurred.[22]

- Commercial shellfish beds provide broader ecosystem services, including helping to control nutrient loading to water bodies by filtering and removing nutrients from the

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[16] Appendix D (Eelgrass and Shellfish Aquaculture – Review of Relevant Scientific Literature on Ecological Functions and Interactions, Confluence Environmental Company, July 29, 2016).

[17] *Id.*, p. 5.

[18] *Id.*, p. 6.

[19] *Id.*, p. 6.

[20] *Id.*, pp. 6-11.

[21] *Id.*, pp. 14-15.

[22] *Id.*, pp. 7-8.

> water column, enriching sediment; supporting the food web; and providing structured habitat, thereby increasing species abundance and diversity.[23]

Finally, as discussed previously, the Corps, National Marine Fisheries Service ("NMFS"), and U.S. Fish and Wildlife Service ("USFWS") have recently completed a programmatic ESA and MSA consultation for shellfish activities in Washington State inland marine waters ("Programmatic Consultation"). Dozens of conditions or limitations are identified in the Programmatic Consultation documents, and the vast majority of commercial shellfish farms authorized in Washington State under NWP 48 will likely obtain ESA and MSA coverage under the Programmatic Consultation.[24] Projects that obtain individual ESA and MSA coverage will likely be required to comply with similar conditions and, at the very least, must address the underlying substantive concerns that support the conditions. These conditions, in addition to the conditions and limits in NWP 48 and the numerous other regulations and programs with which shellfish farmers must comply, are more than adequate to ensure that the individual and cumulative effects associated with activities authorized under NWP 48 in Washington State remain no more than minimal.[25]

For these reasons, PCSGA respectfully requests the Corps to reissue NWP 48 in Washington State without any additional Regional Conditions beyond those already identified by the Seattle District, and that Regional Conditions 1 and 2 be revised as discussed above.

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PCSGA thanks you for your consideration of these comments. Please do not hesitate to contact us at (206) 588-4188 if you have any questions.

Sincerely,

Samuel W. Plauché
Jesse G. DeNike

JGD:cml
Attachments
cc: Margaret P. Barrette, PCSGA Executive Director

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[23] *Id.*, pp. 10-18.

[24] The Programmatic Consultation documents include an October 2015 Programmatic Biological Assessment (http://www.nws.usace.army.mil/Portals/27/docs/regulatory/160907/Shellfish%20PBA_%20Oct30_2015_final.pdf), a Biological Opinion prepared by USFWS (http://www.nws.usace.army.mil/Portals/27/docs/regulatory/160907/USFWS_Final%20BiOp_AQ%2020160826.pdf), a Biological Opinion prepared by NMFS (http://www.nws.usace.army.mil/Portals/27/docs/regulatory/160907/NMFS_2016_09-02_WA%20Shellfish%20Aquaculture_WCR-2014-1502.pdf), and a revised Incidental Take Statement and Biological Opinion Errata by NMFS.

[25] As an illustration of these extensive regulations and programs, see Appendix E (Existing Permitting Processes and Supplemental Narrative).

Appendix A

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Amanda M. Stock

April 18, 2011

Via Email and Federal eRulemaking Portal
Mr. Dave Casey
Mr. David Olson
U.S. Army Corps of Engineers
ATTN: CECW-CO-R
441 G Street, NW.
Washington, DC 20312-1000

RE: Pacific Coast Shellfish Growers Association Comments on Proposal to Reissue and Modify Nationwide Permit 48

Dear Messrs. Casey and Olson:

On behalf of the Pacific Coast Shellfish Growers Association ("PCSGA"), thank you for providing an opportunity to comment on the U.S. Army Corps of Engineers' ("Corps") proposal to reissue and modify Nationwide Permit 48 ("NWP 48"). PCSGA, founded in 1930, represents over 100 shellfish growers in Alaska, Washington, Oregon, California and Hawaii. Members of PCSGA grow a wide variety of healthful, sustainable shellfish including oysters, clams, mussels, scallops and geoduck. As a representative of both private and tribal shellfishing interests, PCSGA submits the following comments on the Corps' proposal.

Shellfish Science Background

Every five years, the Corps utilizes collected data and institutional knowledge to reevaluate the potential impacts of activities authorized by NWPs. The scientific studies and reports that have been generated since the Corps' original issuance of NWP 48 support expanded coverage under NWP 48. Those studies and reports confirm that the environmental effects of shellfish farming are not only minimal, but in many cases beneficial. Indeed, the ecosystem services that are provided by shellfish farming, including water filtration, refuge, benthic-pelagic coupling and erosion reduction, are generally recognized as improving water quality and wildlife habitat, making shellfish beds a component of numerous watershed restoration efforts across the United States. The establishment of new and expanded commercial shellfish beds should be facilitated under NWP 48.

Provision of Fish and Wildlife Habitat

Shellfish and shellfish aquaculture gear provide three-dimensional habitat that is used by numerous species for refuge, foraging and predator avoidance. Benthic invertebrate and fish species richness and abundance is often found to be higher on cultured shellfish beds than that found on unstructured habitats lacking cultured shellfish stocks. (Dumbauld et al. 2000; Peterson et al. 2003; Dealteris et al. 2004; Pinnex et al. 2005; Powers et al. 2007; Tallman and Forester 2007; Grabowski and Peterson 2007). The overall ecological result is greatly enhanced biodiversity in shellfish habitat compared to surrounding areas of the seabed. (Atlantic States Marine Fisheries Commission ("ASMFC") 2007). Shellfish also provide structured habitat for attachment by macroalgae, mussels, and barnacles, which in turn provide protection and/or food for crab, outmigrating juvenile salmon, and various species of amphipods. Hence, oysters and other reef-forming shellfish can be considered "bioengineers" because they create habitat that allows many additional species to thrive. (Jones et al. 1994, 1997; ASMFC 2007).

Species abundance, biomass and diversity are all increased in shellfish habitat compared to open mud or eelgrass-dominated habitat. As part of a programmatic effort to estimate estuarine habitat values, Ferraro and Cole (2001) conducted estuary-wide studies in Washington's Willapa Bay and Oregon's Tillamook Bay, in 1996 and 1998. Their research determined benthic macrofauna-habitat relationships for eight intertidal habitats in Pacific Northwest estuaries, including native eelgrass, Japanese eelgrass, Atlantic cordgrass, bottom culture Pacific oyster, mud, sand, and unharvested subtidal habitat. On average, bottom-cultured oysters provided the highest value habitat for the greatest abundance of species.

Studies conducted in Pt. Judith Pond, a tidal estuary in Southern Rhode Island, found that shellfish aquaculture gear used for the grow-out phase of the American oyster has substantially greater habitat value than a shallow non-vegetated seabed, and has habitat value at least equal to and possibly superior to submerged aquatic vegetation. (Dealteris et al. 2004). Similarly, aquaculture gear used to cultivate geoduck, including PVC tubes and either individual net caps or area-wide predator nets, is heavily utilized for settlement by barnacles, hydrozoans, Manila clams, cockles, benthic copepods, redrock crabs, kelp crabs and macroalgae. (Davis Report 2011, attached). Research indicates a greater presence of juvenile fish and mobile invertebrates in areas covered by area-wide anti-predator nets. (Powers et al. 2007). A number of studies show that infauna and smaller epibenthic invertebrates that comprise the prey of small flatfish and larger invertebrates generally are similarly available or even more abundant in sediment surrounding the tubes compared to such biota in unfarmed reference areas. (Fleece et al. 2004; Pearce 2007).

Studies have shown that shellfish may also increase recruitment of floating eelgrass seeds, either as they travel as single seed, or within detached reproductive shoots. Entrapment can be facilitated by the structure provided by shellfish beds, and since eelgrass seed is a common food item for crustaceans, shellfish can provide refuge for seeds, providing for higher survival. (Wigand and Churchill 1988). By filtering seawater and increasing sediment organic content, shellfish provide optimum conditions for seed germination. Shellfish may also increase the survival of seedlings, which have very high mortality rates, by increasing light levels, nutrients, and protecting against erosion and herbivory. (Orth et al. 1994; Ruckelshaus 1996).

Habitat provided by the presence of oyster aquaculture has been shown to provide some of the ecosystem services provided by native seagrass as it relates to the provision of structured habitat in west coast estuaries. (Dumbauld et al. 2009). Indeed, given the variety of species and complex interactions associated with the three-dimensional structure formed by shellfish beds, they can rightfully be considered "essential fish habitat" as defined in the Magnuson Stevens Conservation and Management Act. (Coen et al. 1999).

For many of the reasons mentioned above, shellfish beds are identified as "priority habitat" in Washington State, which is a habitat type with unique or significant value to one or more species.[1] Shellfish beds, both commercial and recreational, also are expressly defined as critical saltwater habitats.[2] Critical saltwater habitats "require a higher level of protection due to the important ecological functions they provide."[3] Because of the important habitat that shellfish beds provide, local jurisdictions in Washington State are required to include policies and regulations in their shoreline management programs "to protect critical saltwater habitats and should implement planning policies and programs to restore such habitats."[4]

Improved Water Quality and Nutrient Cycling

Bivalves concentrate suspended materials contained in the water column, ingesting a portion of the material as nutrition, and helping to transfer the load of suspended materials from the water column to the benthos. (Davis Report 2011, attached). Unlike other forms of aquaculture, shellfish aquaculture does not require the addition of feed or other organic materials to the water column. Bivalves therefore have the potential to greatly improve the quality of the overlying seawater by reducing the organic load associated with upland development and by grazing down plankton that have grown in excess due to high nutrient loading associated with urban runoff, developed shorelines, inadequately treated sewage and pet waste. The presence of shellfish aquaculture in the Puget Sound serves as a primary deterrent to further degraded water quality due to large assemblages of bivalves serving as a functional filtration system for some components associated with nitrification of urbanized shorelines by human development. (Davis Report 2011, attached).

Bivalves perform a critical environmental service through their ability to cycle nutrients in a phenomenon called "benthic-pelagic coupling." As bivalves consume phytoplankton in the water column, including nitrogen, phosphorous and carbon, these and other nutrients are conveyed to the benthos. This filter-feeding function of cultured bivalves also improves water quality, which in turn causes a reduction in turbidity of the water column, allowing more light penetration and higher rates of photosynthesis for macroalgae and seagrasses. This has a positive impact on submerged aquatic vegetation. (Meyer and Townsend 2000). In addition, the

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[1] Washington Administrative Code ("WAC") 173-26-020(24).

[2] WAC 173-26-221(2)(c)(iii)(A).

[3] WAC 173-26-221(2)(c)(iii)(A).

[4] WAC 173-26-221(2)(c)(iii)(B).

benthic biodeposits of nitrogen, phosphorous and carbon from bivalves in essence fertilizes eelgrass and other sea grasses, facilitating their increased growth. (Peterson and Heck 1999, 2001).

The Biological Opinion prepared by the National Marine Fisheries Service ("NMFS") for NWP 48 activities in Washington State likewise concluded that shellfish contribute to improved water quality and conditions favorable to submerged aquatic vegetation:

> Shellfish aquaculture activities are also reported, however, to contribute to water clarity via the filter feeding of cultured mollusks, removing phytoplankton from the water column. Such improved water quality can also contribute to improving habitat for the establishment of [submerged aquatic vegetation] (e.g. eelgrass). The presence of oyster shell habitat can also provide better habitat for the establishment of eelgrass than soft, bioturbated substrates (Dumbauld and Wyllie-Echeverria 2003).

NMFS Bi-Op at 39 (2009). *See also id.* at 52 ("Eelgrass growth is likely accelerated in areas where the plants are co-mingled with bottom-growing shellfish.").

Geoduck Cultivation

Since NWP 48 was issued, a significant amount of research has been undertaken with regard to the potential environmental effects of geoduck cultivation. Although much of that research is still underway, preliminary data suggest that geoduck aquaculture has only a minor and temporary impact on the surrounding environment. Legislation passed by the Washington State Legislature in 2007 required the University of Washington Sea Grant Program to commission a series of scientific research studies to examine the possible effects of aquaculture techniques and practices in and around the Puget Sound. The Sea Grant research currently is focused on determining: 1) Geochemical and ecological consequences of disturbance associated with geoduck aquaculture operations in Washington; 2) baseline information on pathogens to improve understanding of geoduck health and management of wild and cultured stocks; and 3) the effect of geoduck aquaculture on soft-sediment tideflats and eelgrass meadow habitats.

While the research on these issues is still proceeding, Sea Grant issued a report on April 12, 2011, summarizing its progress. The report documents the following tentative observations:

- The total abundance and diversity of infauna and epifauna indicate no significant effect of geoduck harvest.
- Preliminary analyses of shore survey data in areas near geoduck aquaculture have not indicated differences in use of habitats by juvenile salmonids.
- Nutrients (nitrogen and phosphorus) released from a typical commercial geoduck operation into Puget Sound are so low, that even in a small, poorly-flushed embayment, the level of input is unlikely to result in any local change in water quality.
- The effects of harvest on sediment elevation are temporary.

- Harvesting activities produce effects where eelgrass was recruited to the area after geoducks were planted; however, it is possible that the farmed area presents a suitable habitat to eelgrass once geoduck aquaculture gear has been removed.

Additional research on the effects of geoduck harvesting was presented recently at the National Shellfisheries Association's 103rd Annual Meeting in Baltimore on March 27-31, 2011. Jennifer Price and colleagues from the University of Washington presented their current findings on the effects of *intertidal* geoduck harvest events. Their presentation abstract concludes:

> As of yet we have seen little evidence to indicate that activities associated with geoduck aquaculture cause significant long term damage or disruption to benthic ecosystems on the intertidal sand flats of southern Puget Sound.

Similarly, Wenshan Liu and Chris Pearce of Fisheries and Oceans Canada assessed the potential benthic impacts of *subtidal* geoduck clam harvesting. They examined various sediment qualities, infaunal diversity and numbers, and suspended sediment levels. They also examined potential effects on a nearby eelgrass bed. Their presentation abstract concludes:

> Sample processing and data analysis are currently underway, but preliminary evidence suggests that impacts appear to be relatively limited in time and space.

Further, the Washington Department of Ecology recently promulgated rules that regulate the manner in which geoduck aquaculture may be conducted. Under these rules, local governments are encouraged to require geoduck farmers to obtain a land use permit before commencing farm activities.[5] As part of the land use permit process, local jurisdictions are required to analyze each proposed geoduck farm under the Washington State Environmental Policy Act ("SEPA"). One such review recently was conducted in Pierce County, Washington. The review spanned the course of several years and multiple reports were generated evaluating the potential environmental impacts of a proposed geoduck farm. Pierce County ultimately issued a determination that the proposed geoduck farm was not likely to result in any probable significant adverse environmental impacts. Several of the scientific reports submitted to Pierce County are attached to this comment letter.[6] These reports address issues relating to the potential of geoduck aquaculture gear and harvesting techniques to impact local geomorphology, the potential of geoduck aquaculture practices to result in biological impacts, genetic issues concerning cultivated geoduck, and the potential toxicological effects of geoduck aquaculture gear on the environment. All reports unequivocally concluded that the proposed geoduck farm would not result in any significant adverse environmental impacts.

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[5] These regulations are located at section 173-26-241 of the Washington Administrative Code.

[6] The eight attached reports were prepared by Jeffrey Fisher, Ph.D.; Jonathan P. Houghton, Ph.D.; Rosalind Schoof, Ph.D.; Rita Schenck, Ph.D.; Jonathan Davis, Ph.D.; and Phil Osborne, Ph.D.

Shellfish as Mitigation for Human Activities

As previously described, shellfish aquaculture has the potential to greatly influence the quality of the overlying seawater by reducing the organic load associated with human activities. For example, cultured geoducks in Puget Sound remove phytoplankton and other organic material and sequester nitrogen as protein in their tissues, and upon harvest, the nitrogen is removed from the water. This is an important ecosystem service provided by bivalve culture and helps to counteract increasing human inputs of total nitrogen into Puget Sound. (Davis Report 2011, attached). Similarly, in a recent study funded by the Environmental Protection Agency and administered by the National Fish and Wildlife Foundation, researchers estimated that the harvest of 1 million eastern oysters results in the removal of 132 kg of total nitrogen. The study states that in terms of nutrients removed per unit area, "oyster harvest is an effective means of nutrient removal compared with other nonpoint source reduction strategies." (Higgens et al. 2011).

Shellfish habitat can also offset the effects of erosion resulting from increased population growth and associated residential, recreational and industrial development. Due to their three-dimensional structure, shellfish beds can serve as a natural breakwater, stabilizing shorelines and reducing the amount of suspended sediment in the adjacent waters. This can result in improved water clarity and protection for seagrasses and other species. (Myer et al. 1997). Further, intertidal oyster reefs can form a protective breakwater for fringing marshes, retarding shoreline erosion. (Coen and Fischer 2002; Coen and Bolton-Warberg 2005).

Beyond shoreline stabilization, restored oyster reefs may provide additional ecosystem services, including seston filtration, benthic-pelagic coupling, refuge from predation, creation of feeding habitat, and nesting habitat. (Coen et al. at 2007). Restored oyster reefs have been demonstrated to function at an equivalent or greater level than natural reefs with decapods, crustaceans, and fish quickly colonizing the available substrate. (Meyer and Townsend 2000). Indeed, recent research finds that sustainable oyster aquaculture may reintroduce some of the ecosystem services that were lost with the decline of wild oyster populations. (Higgins et al. 2011). Some researchers suggest that because oyster grow-out cages provide good-quality habitat for fishes typically associated with hard-bottom habitats, habitat restoration programs for these fishes should consider using grow-out cages alongside other types of artificial reefs. (Tallman and Forrester 2007).

For these reasons, shellfish are increasingly being utilized in environmental restoration projects across the United States. Ecosystem modeling and mesocosm studies have indicated that restoring shellfish populations to even a modest fraction of their historic abundance could improve water quality and aid in the recovery of seagrasses. (Newell and Koch 2004; Ulanowicz and Tuttle 1992; Peterson and Heck 1999). The Corps itself recognizes the value of shellfish as mitigation by permitting shellfish seeding activities for environmental restoration efforts under NWP 27. The Corps also is involved in its own shellfish restoration projects, such as its hugely successful oyster reef project in the Great Wicomico River as part of the overall Chesapeake Bay Native Oyster Restoration Program.

Other restoration gains involving shellfish include increasing bivalve numbers in the Great South Bay of Long Island, New York. During the 1970s, hard clams filtered 40 percent of the entire volume of Great South Bay each day. (ASMFC 2007). Due to declines in shellfish populations, that percentage dwindled to 1 percent. As of this year, the Bay has been stocked with over 3.5 million adult clams. Along with eight government agencies, scientific experts, and community interests, The Nature Conservancy is developing a long-term plan to restore a robust self-sustaining hard clam population to Great South Bay by 2020.

In Maryland, oyster reef restoration is being coupled with submerged underwater vegetation restoration in Harness Creek, South River. Existing oyster reefs will be augmented with additional oyster spat to increase the filtering capacity of the reefs. Once the enlarged oyster reefs provide increases in water quality, bay grass restoration plantings will take place inshore of the reefs.

And in a cutting-edge experiment in Quartermaster Harbor off Vashon Island, Washington, an area plagued by low levels of dissolved oxygen and fish die-offs, the Puget Sound Restoration Fund recently installed a mussel raft for the purpose of measuring the amounts of nutrients mussels remove from the water column.

Scientific studies also demonstrate that shellfish cultivation can be significantly increased for both restorative and commercial purposes without raising concerns of exceeding carrying capacity. In a recent study of the Narragansett Bay in Rhode Island, where the number of shellfish farms doubled within six years, researchers concluded that cultured oyster biomass could be increased 625 times without exceeding the ecological carrying capacity. (Byron et al. 2011). At the ecosystem level in places like the Chesapeake Bay, even a dramatic increase of oyster aquaculture in the Bay would still only be a fraction of harvest levels just prior to the widespread impacts of overharvesting and disease. (Higgins et al. 2011). Thus, carrying capacity has been shown not to be a limiting factor in expanding existing or establishing new shellfish farms.

Proposed NWP 48 Reissuance

As stated in its notice of Proposal to Reissue and Modify Nationwide Permits, the NWP program allows the Corps to authorize activities with minimal adverse environmental impacts in a timely manner and protect the aquatic environment. This in turn allows the Corps to focus its limited resources on more extensive evaluation of projects that have the potential to cause environmentally damaging adverse effects. As the brief summary of shellfish science above demonstrates, commercial shellfish farms, both individually and cumulatively, cause only minimal adverse environmental effects. Biological Opinions prepared for the Corps by the NMFS and the U.S. Fish and Wildlife Service for NWP 48 activities in Washington State are consistent with that conclusion. Moreover, any minimal adverse environmental effect caused by commercial shellfish aquaculture activities is more than offset by the beneficial ecosystem services such activities provide.

PCSGA supports the Corps' continued coverage of existing commercial shellfish aquaculture activities under NWP 48. PCSGA also supports the additional coverage under NWP 48 for expanded project areas with pre-construction notification ("PCN"). Many shellfish farmers have struggled with the submission of new permit applications for relatively small expansions of their existing operations. This additional coverage for such expansions is consistent with the purpose of the NWP program and will greatly lessen the regulatory burden on small businesses. Finally, PCSGA supports the Corps' proposed removal of reporting requirements for those activities that do not require PCN. The removal of this requirement will allow shellfish growers to spend more time on their farms rather than in their offices preparing unnecessary paperwork.

New Farm Coverage

The Corps' notice states that it is seeking comments on whether to reissue NWP 48 with modifications to authorize new commercial shellfish aquaculture activities or to issue a separate NWP to authorize only new commercial shellfish aquaculture activities. PCSGA supports the establishment of new farms under NWP 48 with PCN. Providing coverage under NWP 48 for new commercial shellfish activities is consistent with national policy. The National Aquaculture Act of 1980 declares that aquaculture development is in the national interest, and that Federal agencies must address barriers to aquaculture development. Coverage under a NWP to establish new shellfish farms is consistent with that mandate. Additionally, both the National Oceanic and Atmospheric Administration ("NOAA") and the U.S. Department of Commerce recently issued draft aquaculture policies. The purpose of both NOAA's and the Department of Commerce's policies, in part, is to enable or support "the development of sustainable marine aquaculture." This support includes eliminating unnecessary paperwork and expenses related to new shellfish aquaculture activities that have minimal adverse environmental impacts.

As previously described, commercial shellfish aquaculture is likely to have a net beneficial impact on the environment. Although commercial shellfish aquaculture may result in some temporary disruption to the environment, such impacts are minor and/or temporary and are offset by the ecosystem services introduced by shellfish farms and provided over the course of the entire culture cycle. Perhaps for this reason bivalve aquaculture is considered one of the most ecologically sustainable types of aquaculture. (Shumway et al. 2003). Indeed, the Monterey Bay Aquarium's Seafood Watch places farmed clams, oysters, scallops and mussels in the "Best Choices" category. Further, the Food and Water Watch Smart Seafood Guide recommends U.S. farmed oysters, mussels and clams to conscientious consumers.

PCSGA firmly believes that all new shellfish aquaculture activities should qualify for coverage under the NWP program. Nevertheless, if the Corp determines that such coverage should be limited in this NWP cycle, PCSGA would suggest the following as the maximum limitations that should be placed on new farm coverage under NWP 48:

- Cover all farms that are 10 acres or less in size. Subsequent expansion of these new farms should be authorized under NWP 48 with PCN.

- New farms covered under NWP 48 should not utilize floating aquaculture. While floating aquaculture provides significant environmental benefits, we also recognize that floating systems provide unique issues, such as potential navigational impacts, that may at times need to be reviewed individually.

PCSGA recommends that these limitations on coverage of new shellfish aquaculture under NWP 48 be applied only in this NWP cycle, and reconsidered with the reissuance of NWP 48 in five years. PCSGA is confident that an evaluation of new farm coverage under NWP 48 in the next NWP cycle will justify further expansion of coverage.

PCN for Change in Culture Methods

PCSGA supports the Corps' proposal to remove the PCN requirement for a change in species being cultivated. The transfer and import of species is an issue that is closely monitored by state regulatory authorities to ensure the health of local populations. The species being cultivated should not be of importance to the Corps, except to the extent cultivation practices may change from species to species.

With regard to the Corps' PCN requirement related to changes in culture methods, most changes are environmentally insignificant and notice to the Corps is not warranted. A primary example of this is the change from bottom culture of oysters to off-bottom culture of oysters. Off-bottom culture of oysters does not implicate the same navigational related concerns as floating aquaculture because off-bottom culture takes place in intertidal areas, above the extreme low tide line. Indeed, oyster growers need the flexibility to convert from bottom culture to off-bottom culture to better respond to disasters like the *Deepwater Horizon* oil spill. For PCSGA peers in the Gulf of Mexico, the removal of as many regulatory burdens as possible is essential to facilitating much-needed recovery of the industry. PCSGA recommends the Corps remove the PCN requirement for changes in culture methods, with the exception of conversion to floating aquaculture, defined as culture that is located on a floating structure anchored below the extreme low tide line.

Clarification Regarding PCN

PCSGA recommends the reissued NWP 48 clarify that the filing of a new PCN is not required for any activities that were covered by a PCN submitted under the 2007 version of NWP 48. Submission of PCN documentation is costly and time-consuming. This is particularly true for small shellfish farming operations with limited resources; most farming operations fall under this category. Submission of this redundant documentation is also a burden on the Corps; the Corps has taken so much time to review the thousands of PCNs that were issued under the 2007 Nationwide Permit that as of today, over four years after issuance of the permit, the Corps has yet to actually complete the implementation process for that permit on the West Coast. PCSGA fully supports the comments submitted to the Corps on this issue by the U.S. Small Business Administration, Office of Advocacy on April 7, 2011.

For these reasons, PCSGA requests that PCN submittal under the reissued NWP 48 not be required for any activity described in a PCN previously submitted to the Corps.

* * * * * * * * * *

PCSGA thanks you for your consideration of these comments. Please do not hesitate to contact us at (206) 488-5188 if you have any questions.

Sincerely,

Samuel W. Plauché
Laura C. Kisielius

LCK:tat
Attachments
cc: Margaret P. Barrette, PCSGA Executive Director

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Appendix B

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).

Sea Grant
Washington

University of Washington,
Seattle, Washington

FINAL REPORT

Publication and Contact Information

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

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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)

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Ruesink and Horwith

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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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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 semi-diurnal 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.

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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.

*•••: $p<0.001$; ••: $0.001\leq p<0.01$; •: $0.01\leq p<0.05$; NS: $p\geq 0.05$.

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

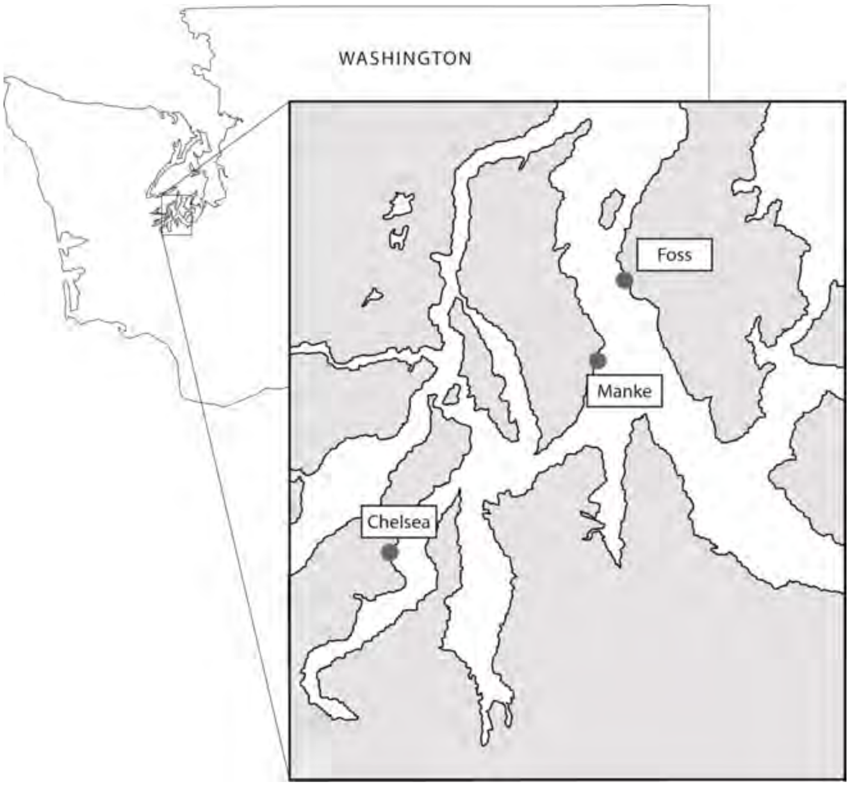

Figure 1. Locations of study sites in southern Puget Sound, Washington USA. Coordinates (latitude and longitude) for each site are provided in text. Shaded areas are land, white areas are water.

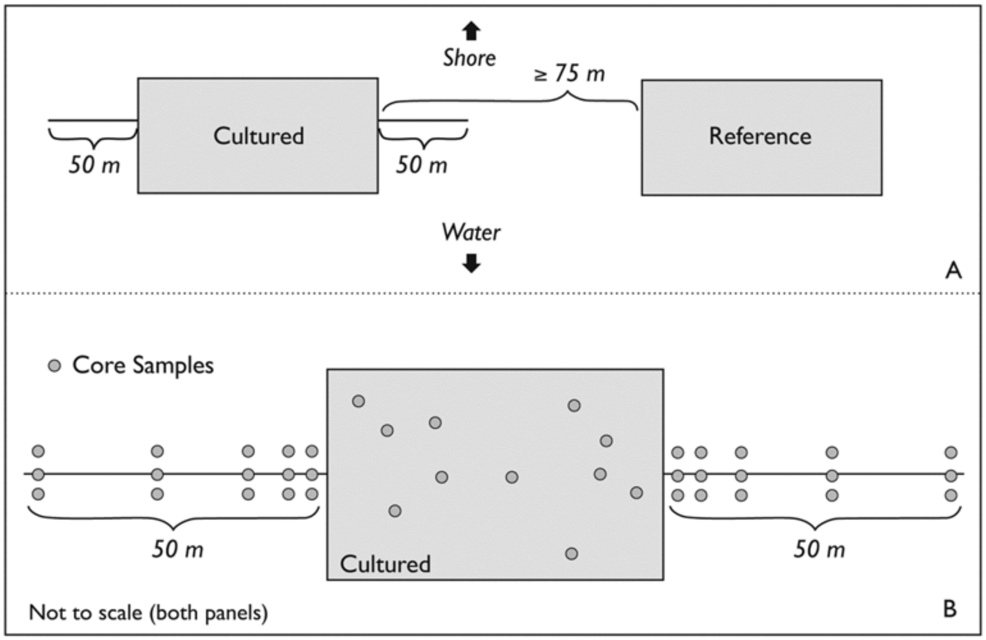

Figure 2. Diagram of physical layout (plan view) used for each of the three study areas. A: Relative positions of cultured and reference plots at each site, and placement of extralimital transects at Foss (only one transect was established at Manke and Chelsea, respectively). B: Example random placement 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.

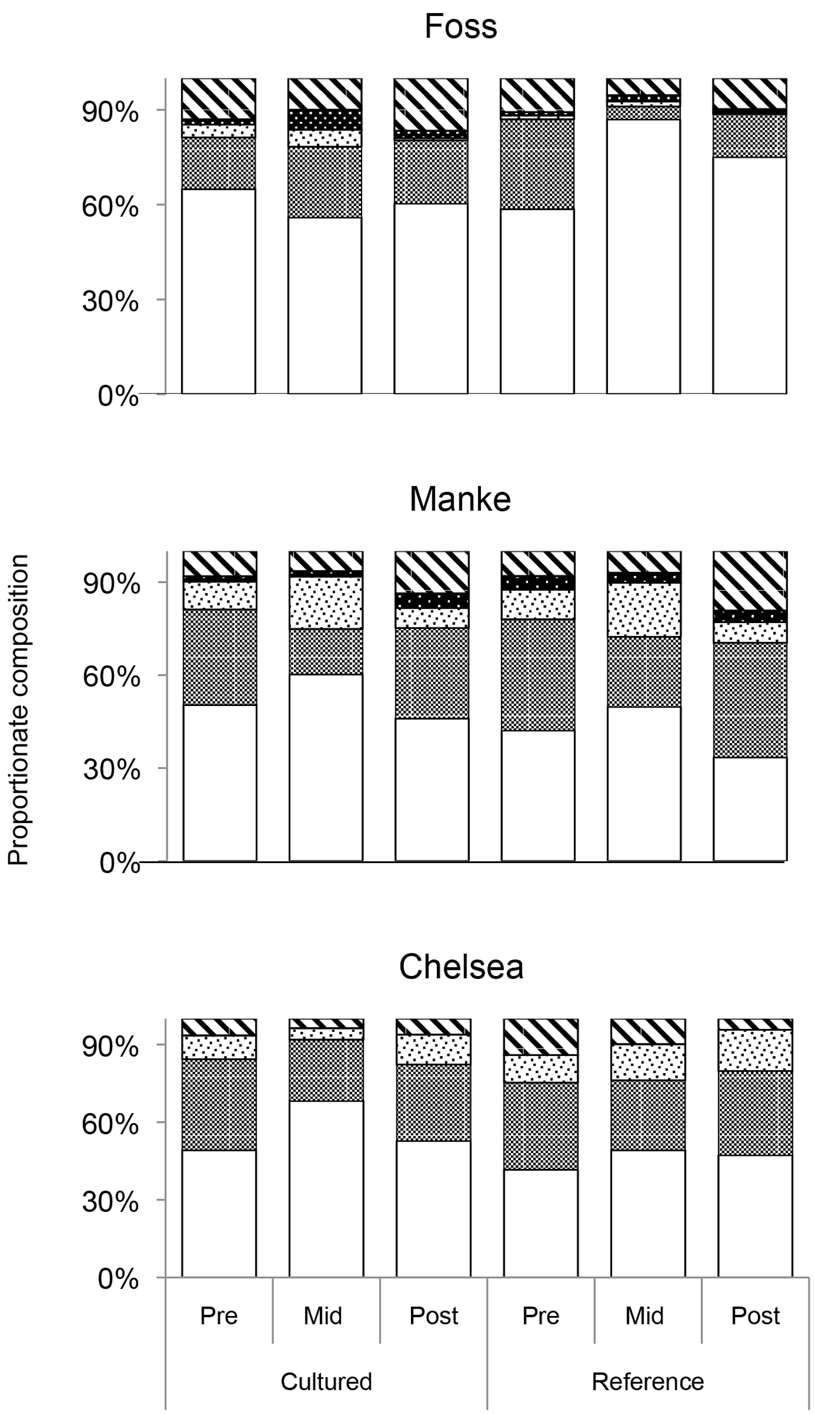

Figure 3. Taxonomic composition of all infauna summed, as proportions of numbers of individuals in samples, in cultured and reference plots during pre-harvest, mid-harvest, and post-harvest states at each study site. In each plot taxonomic categories are, from bottom to top, polychaetes, crustaceans, 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.

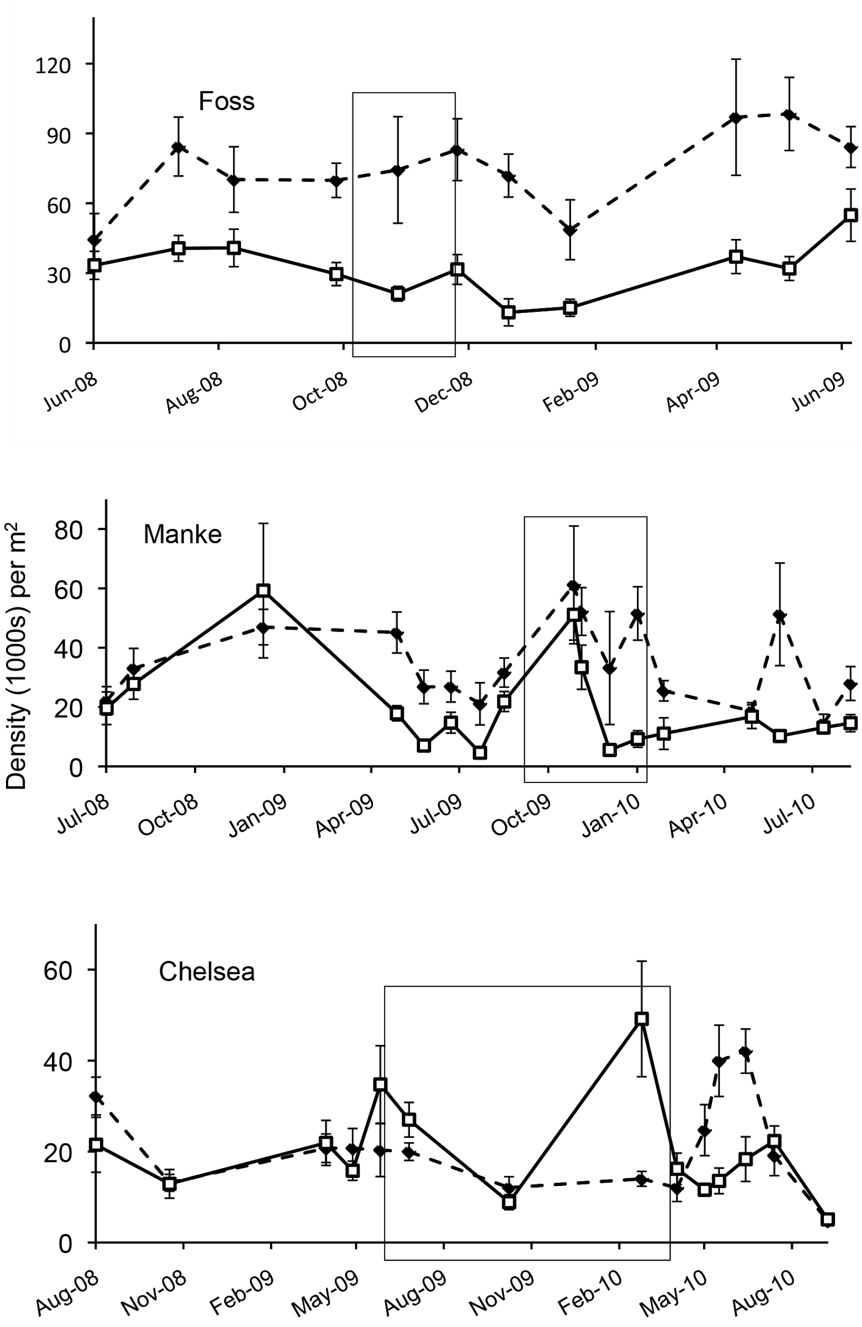

Figure 4. Mean densities of all infauna summed, as thousands of individuals per m^2 (± one standard error) from samples on each plot for each sampling date at each study site. Data from cultured plots are shown with white boxes and solid lines, and from reference plots with black diamonds and dashed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

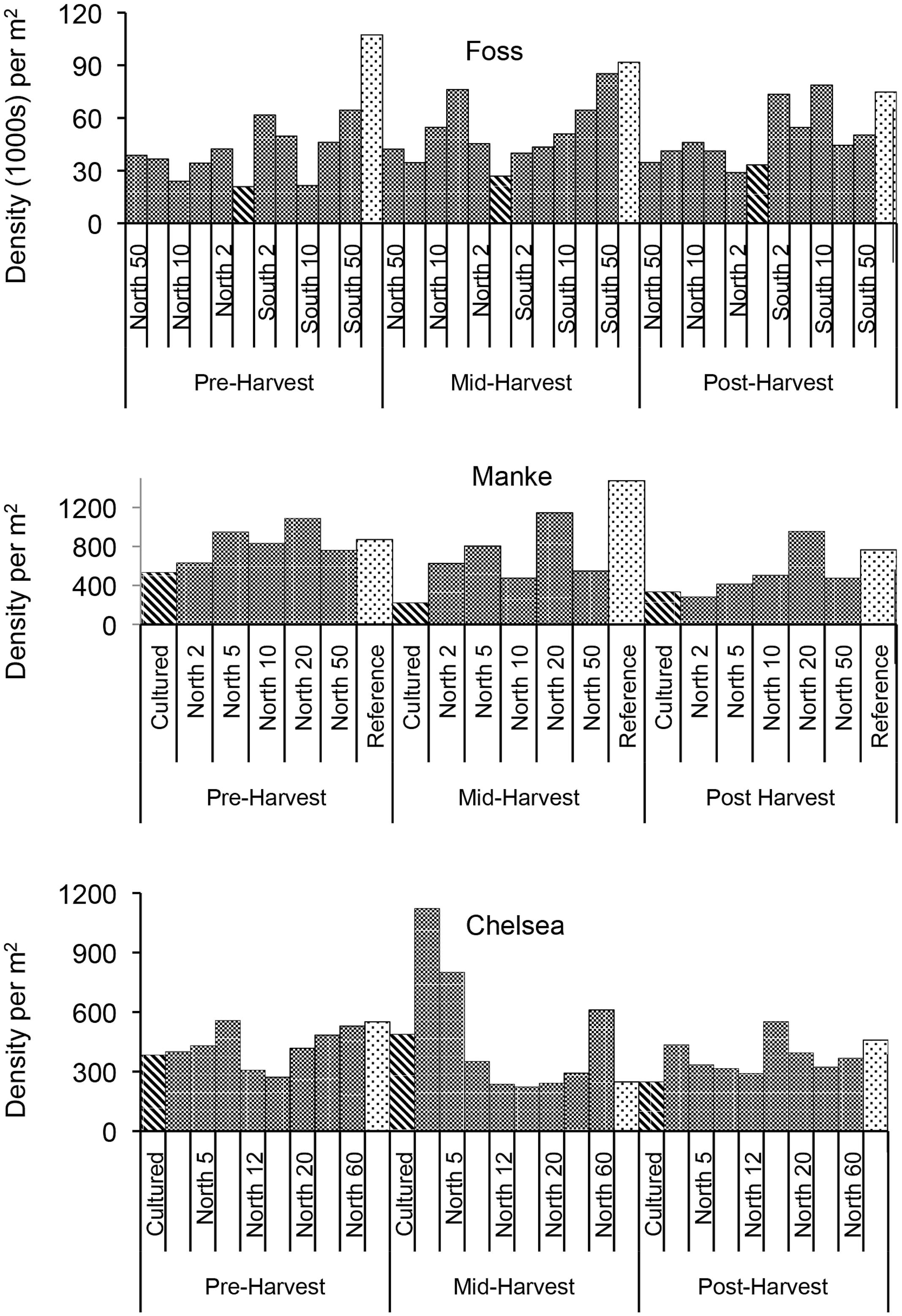

Figure 5. Mean densities of all infaunal organisms summed, as individuals per m², from samples in cultured and reference plots and on extra-limital transects at each distance, within harvest states. Diagonally-hatched bars represent densities within cultured plots, coarsely-stippled white bars reference 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.

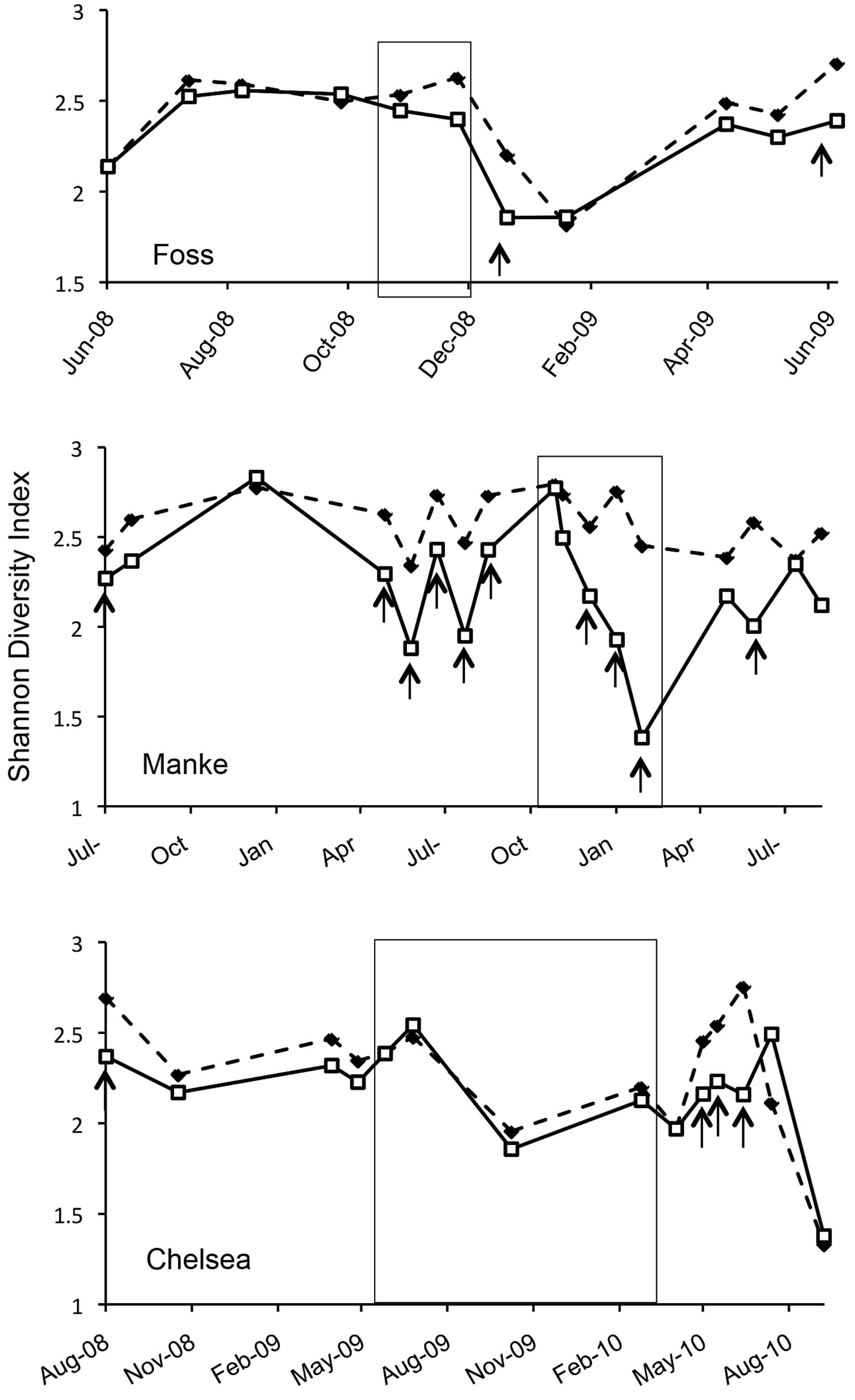

Figure 6. Shannon diversity index values from samples on each plot for each sampling date at each study site. Data from cultured plots are shown with white boxes and solid lines, and from reference plots with black diamonds and dashed lines. Arrows indicate sample dates with significant differences between 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.

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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

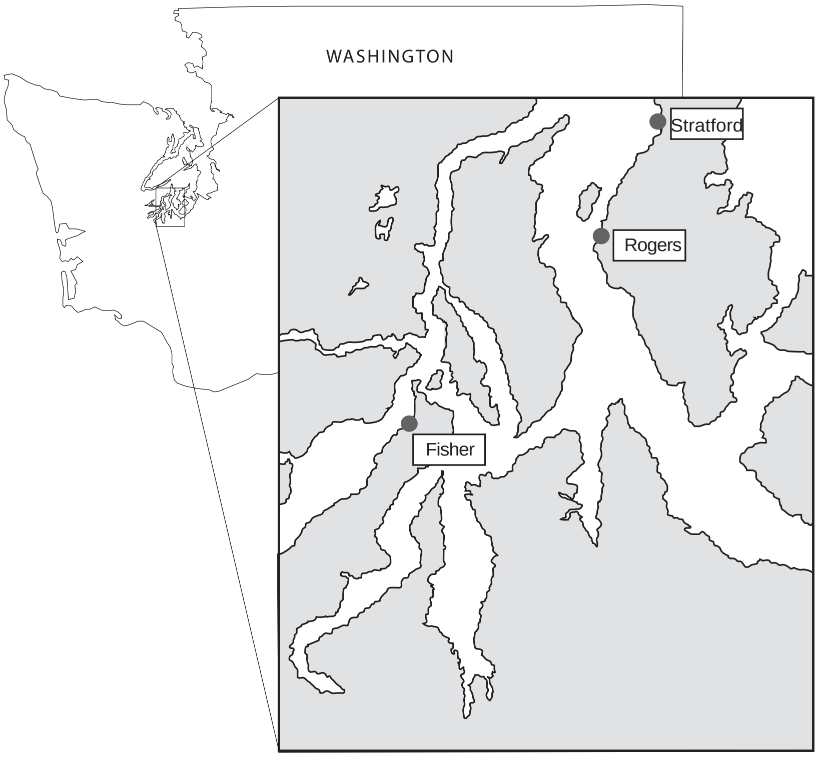

Figure 1. Locations of study sites in south Puget Sound, Washington, USA. Inset map shows the region of interest; most geoduck aquaculture in Washington State occurs within the area demarcated by the box.

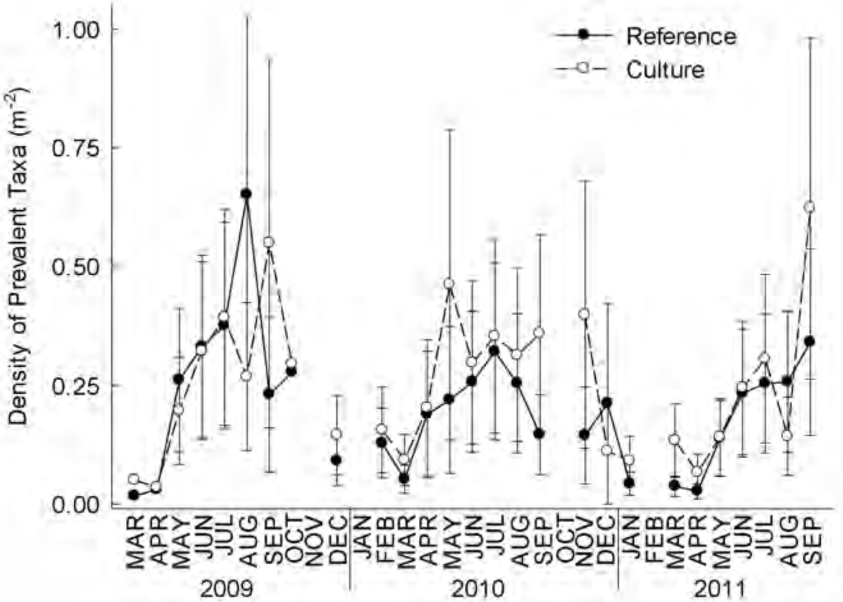

Figure 2. Density of prevalent taxa in SCUBA surveys of transient macrofauna (fish and invertebrates) defined as species present in at least 10% of surveys. Data were collected on culture plots (culture) and adjacent reference areas (reference) at three sites in southern Puget Sound during SCUBA surveys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

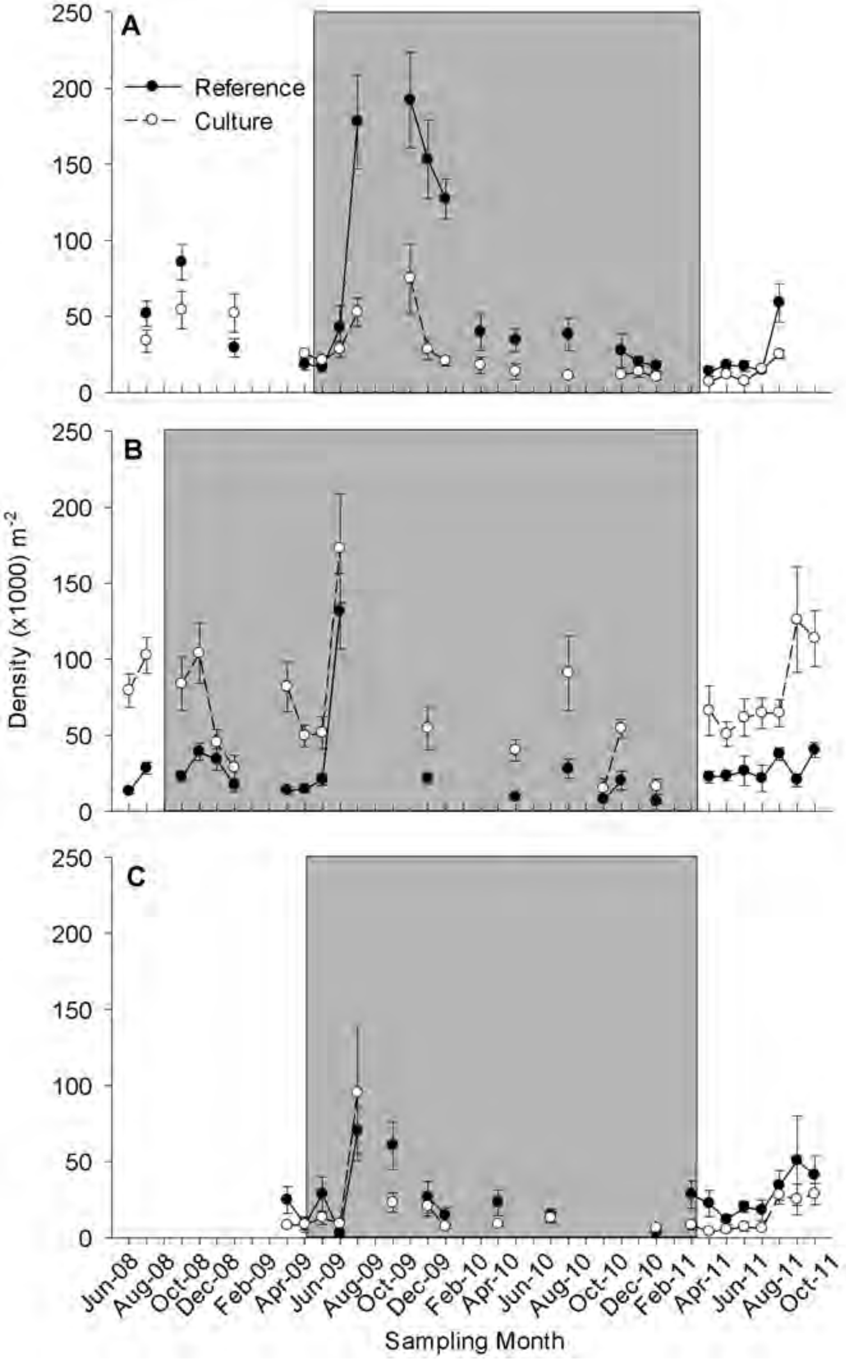

Figure 3. Density (in 1000s per m^2) of total taxa in surveys of resident macrofauna (infauna and epifauna). Data were collected on culture plots (culture) and adjacent reference areas (reference) at three sites in southern Puget Sound: Fisher (A), Rogers (B), and Stratford (C). Shaded areas illustrate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

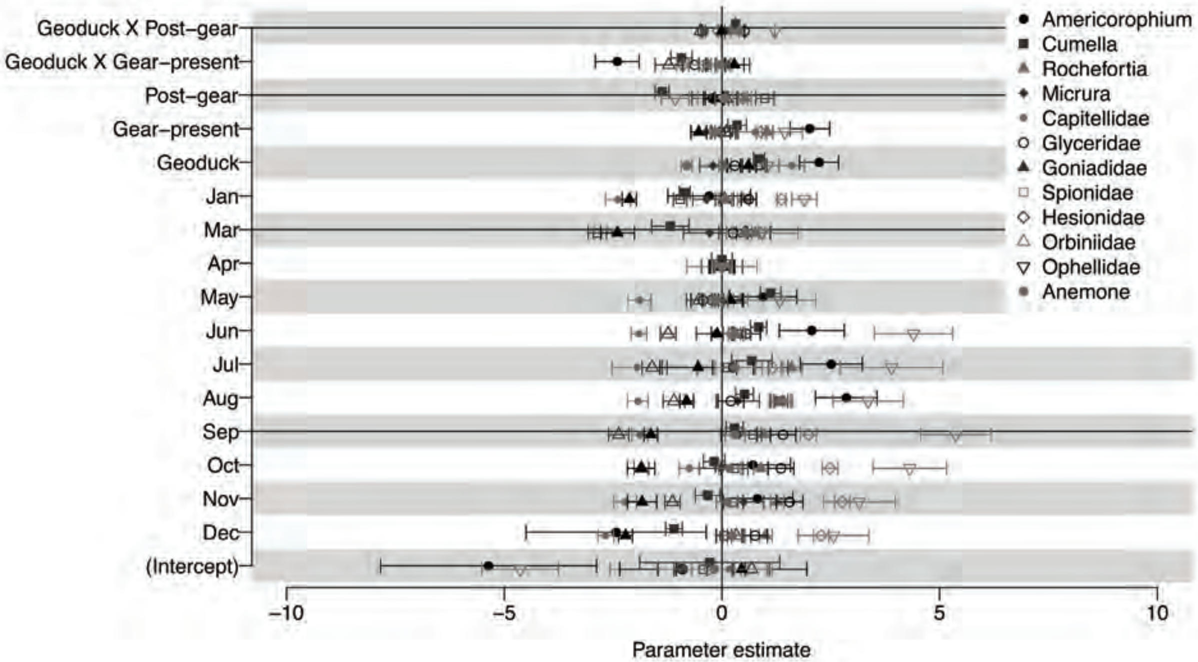

Figure 4. Parameter estimates and 95% confidence intervals for generalized linear mixed models of selected macroinfauna. The models included main effects of month of sampling, plot type (geoduck culture or reference area), phase (pre-gear, gear-present, post-gear), and their interaction, as well as random 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.

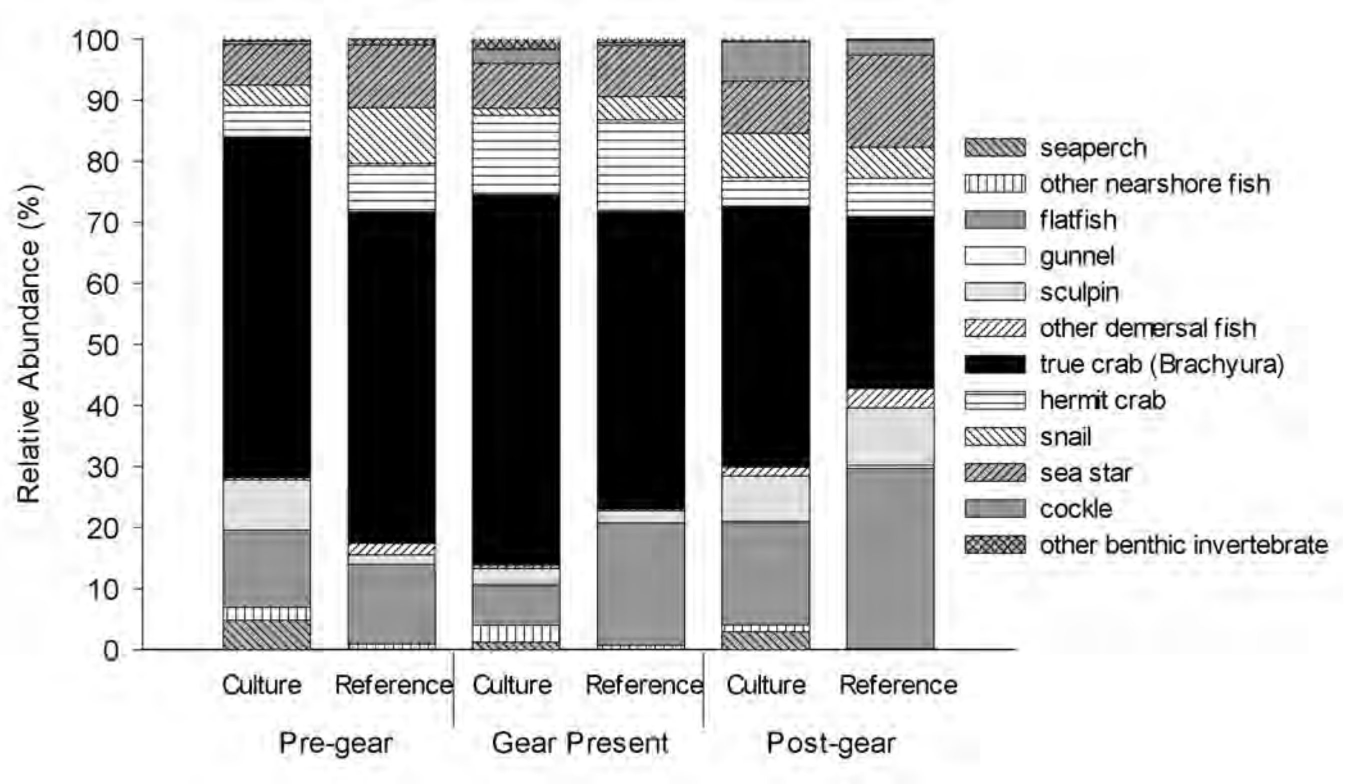

Figure 5. Relative abundance of 10 functional groups of transient fish and macroinvertebrates on geoduck culture plots (culture) and adjacent reference beaches (reference) during SCUBA surveys at three sites in southern Puget Sound (2009-2011). Data are presented in three April-October periods comprising 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.

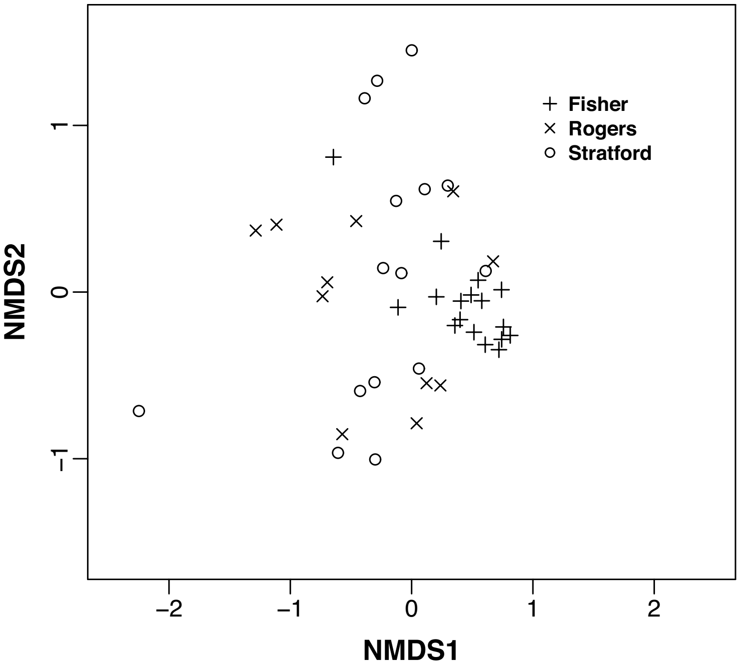

Figure 6. Two-dimensional NMDS ordination of SCUBA surveys at reference areas during 2010, which corresponds to when aquaculture gear was in place (gear-present) on the culture sites. Stress=17.24. Stress tested statistically significant under the Monte Carlo randomization approach ($p<0.01$). A goodness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

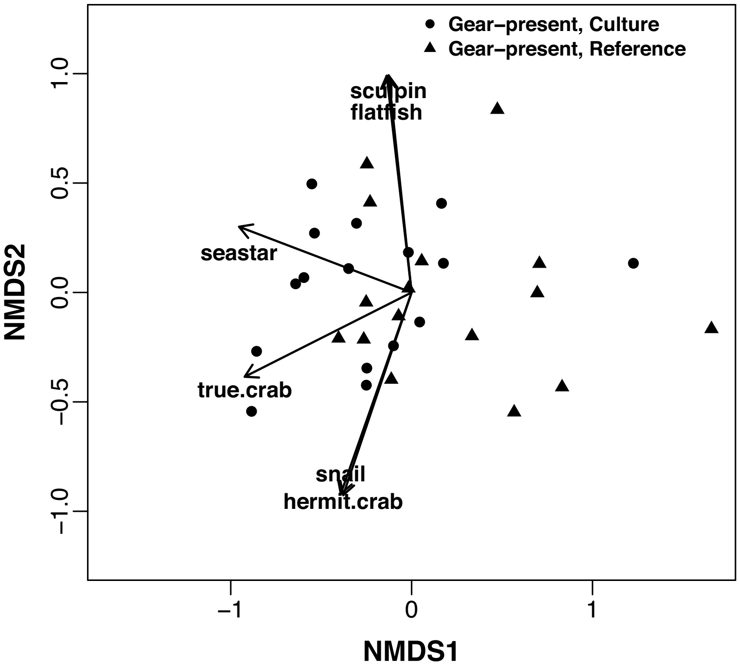

Figure 7. Two-dimensional NMDS plot of SCUBA surveys at culture plots (green circles) and reference areas (orange triangles) when aquaculture gear was in place (gear-present). Functional group vectors shown are those with $p<0.05$. Stress=13.87. Stress value tested statistically significant under the 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 ($p<0.05$).

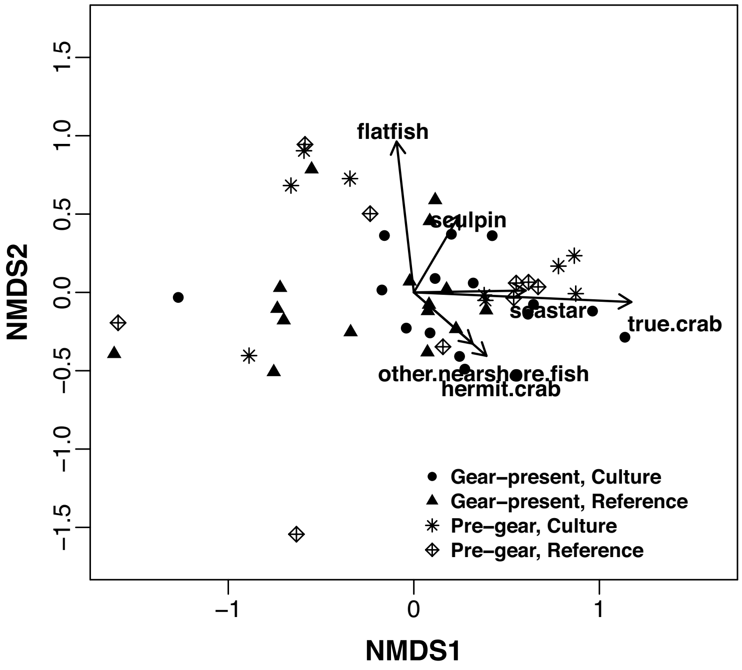

Figure 8. Two-dimensional NMDS plot of SCUBA surveys at culture plots and reference areas prior to deployment of aquaculture gear (pre-gear) and when aquaculture gear was in place (gear-present). Functional group vectors shown are those with $p<0.05$. Stress=14.498. Stress value tested statistically 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 ($p<0.05$).

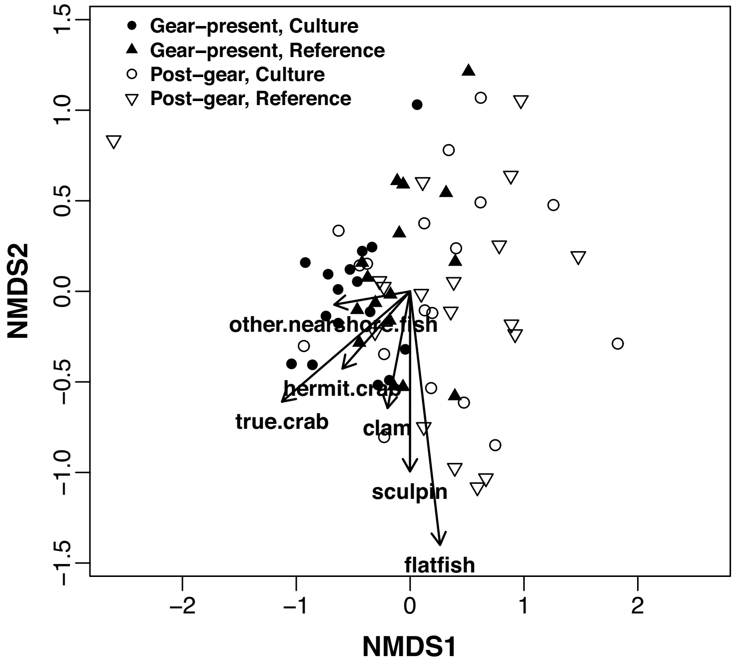

Figure 9. Two-dimensional NMDS plot of SCUBA surveys at culture plots and reference areas when aquaculture gear was in place (gear-present) and after gear was removed (post gear). Functional group vectors shown are those with $p<0.05$. Stress=18.08. Stress value tested statistically significant under 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^{o}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}), $\Delta C/Dt$ 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°C) 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°C). 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

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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

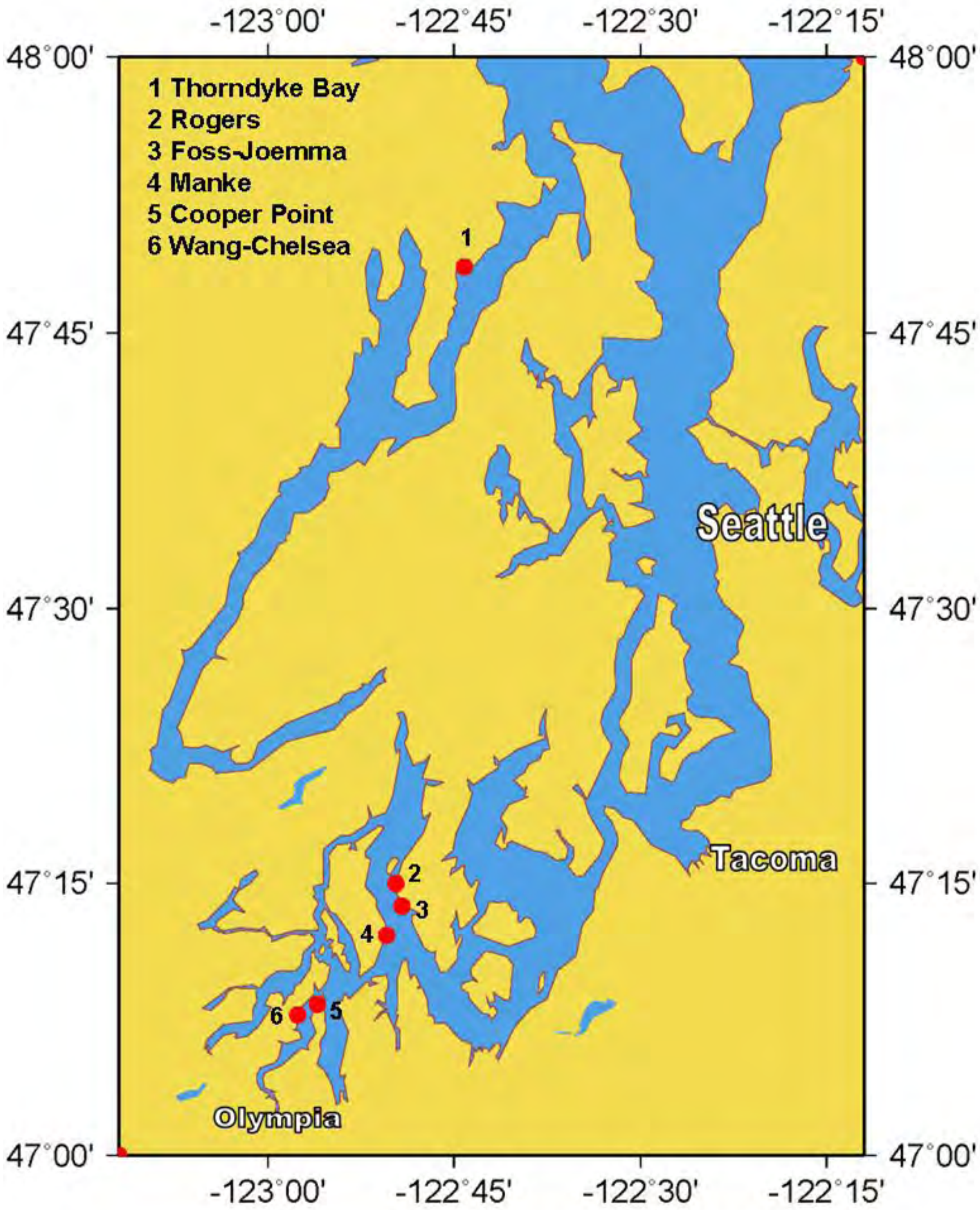


Figure 1. Location of sampling sites in Puget Sound.

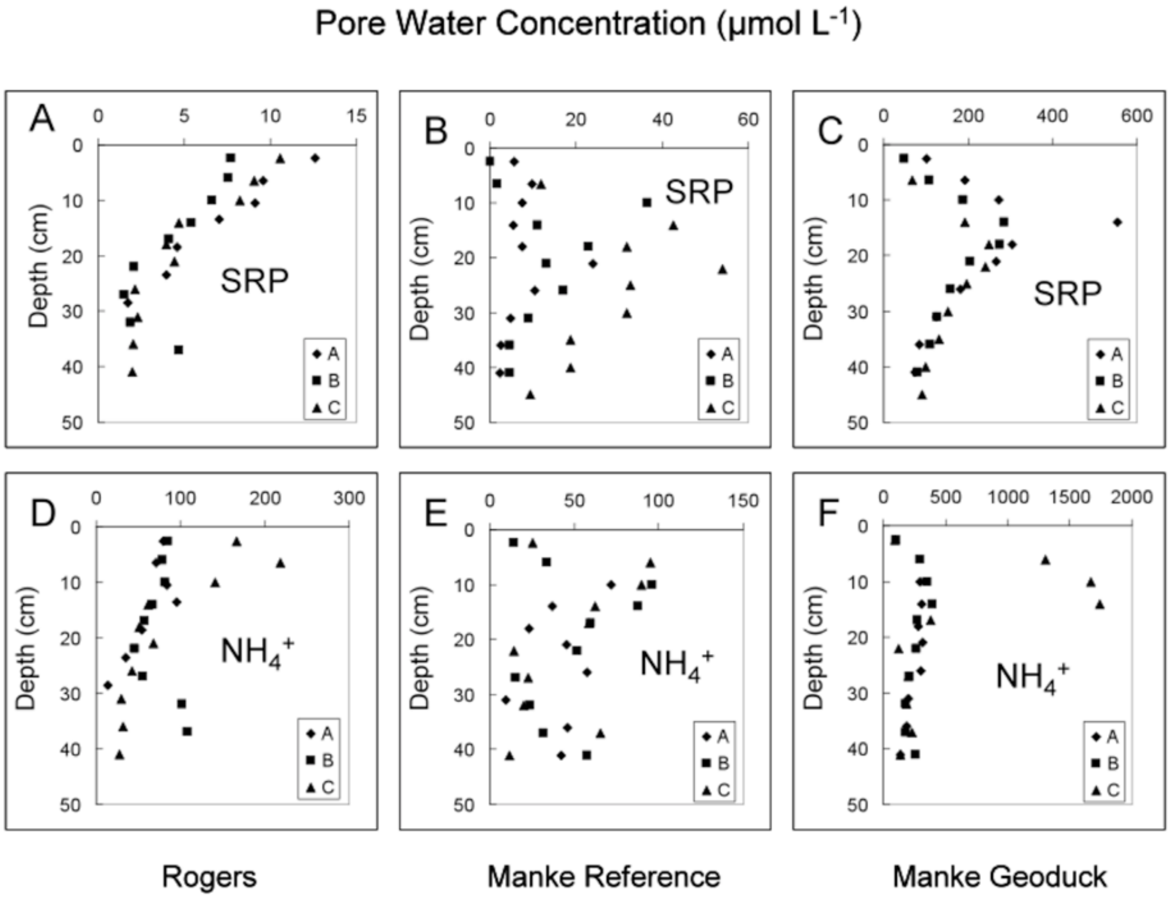
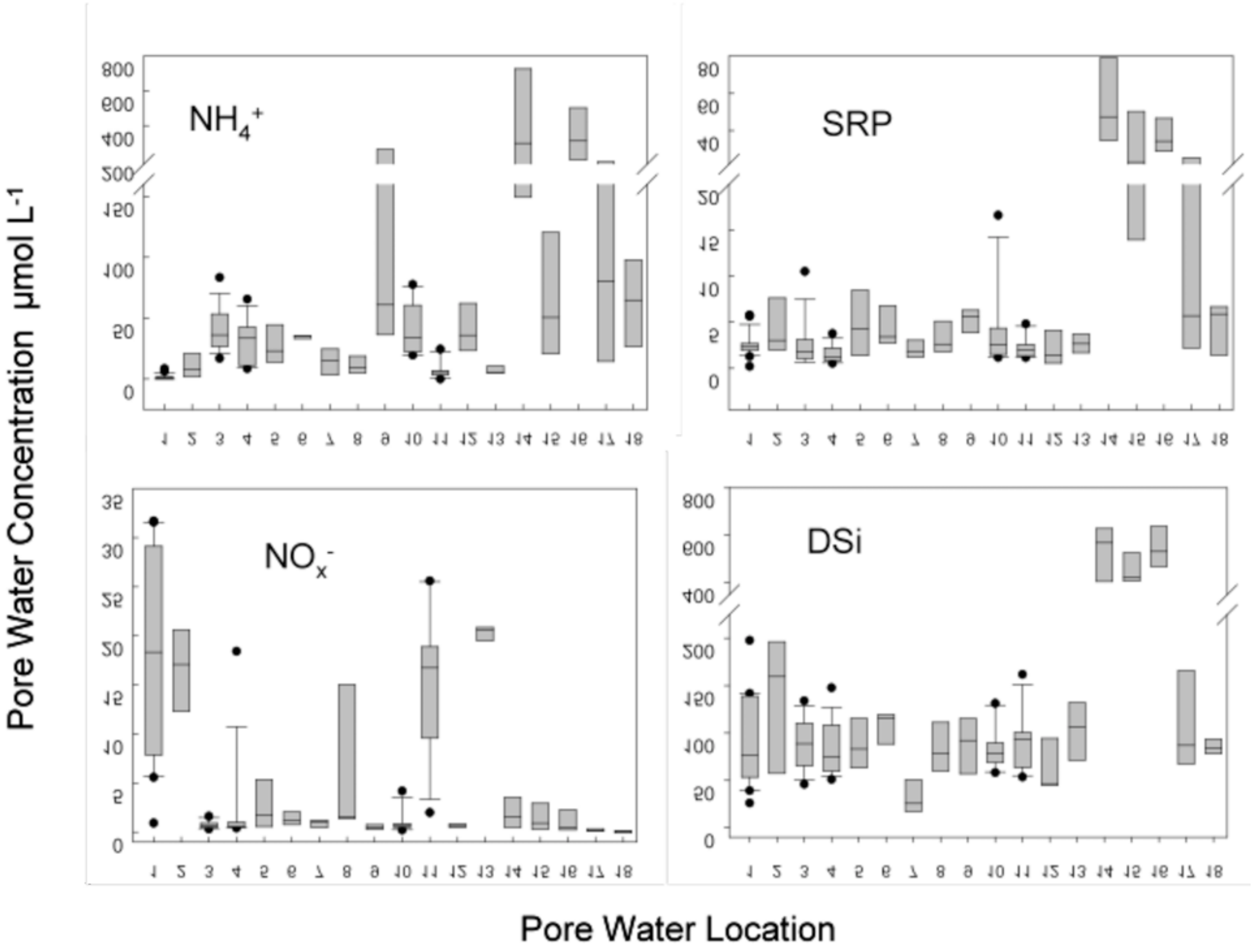


Figure 2. Pore water SRP and ammonium profiles at Rogers, the control site at Manke and the Manke site with geoducks present. The data for 3 co-located pore water equilibrators (A-C) are shown in each panel. Note the scale differences for nutrient concentrations.



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.

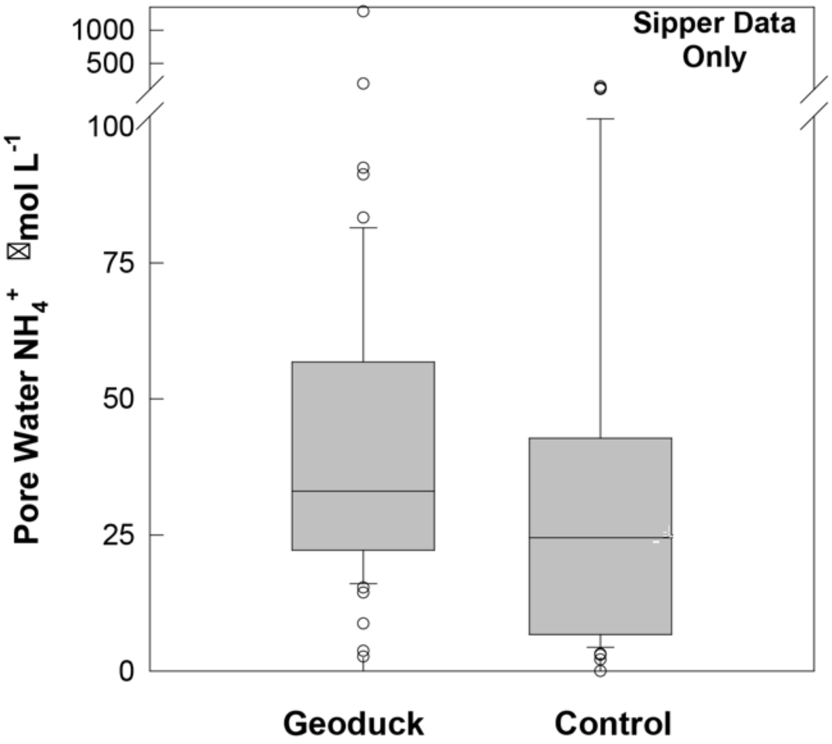


Figure 4. Box plot of all pore water ammonium data for Reference (N = 47) and Geoduck (N = 56) locations. The Geoduck NH_4^+ concentrations were significantly greater than Reference locations (P = 0.013, Kruskal-Wallis one way analysis on ranks).

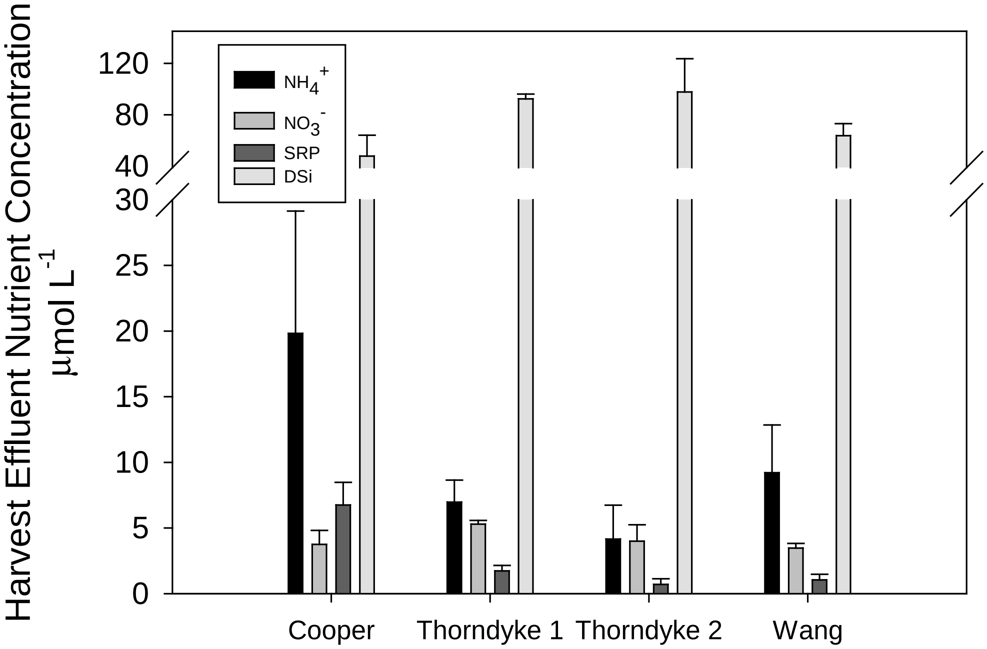


Figure 5. Nutrient concentrations in surface water after geoduck harvest. At Cooper Point, residual water in small harvest-created depressions was sampled, while at the other sites and times, samples were collected along the length of the rivulets as water from the jets used for harvest flowed downslope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

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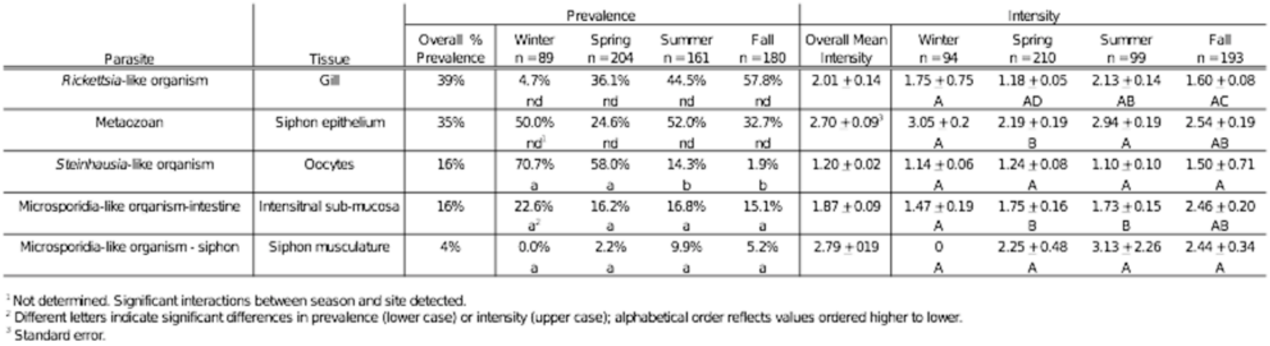
Tables and Figures

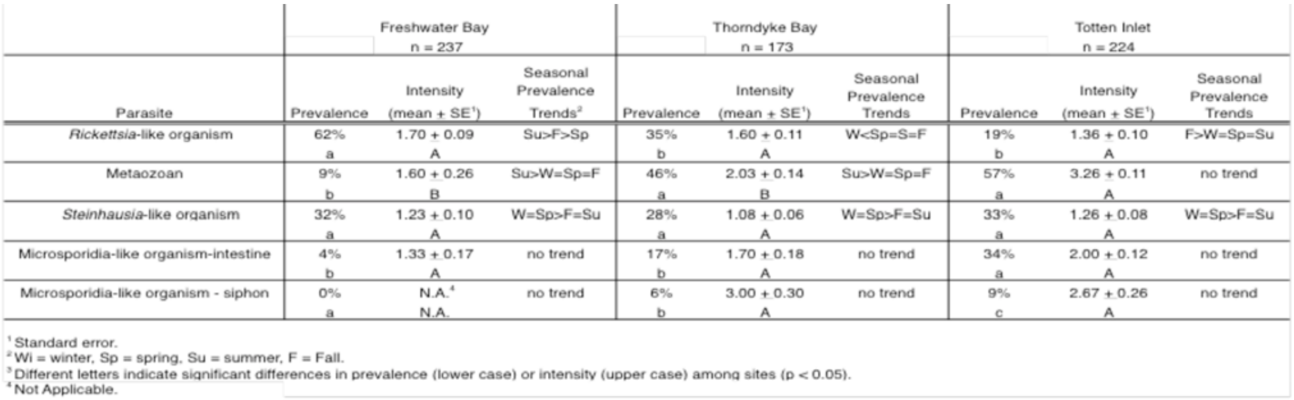
Table 1. Overall mean parasite prevalence and intensity in natural populations of Washington state P. generosa. Parasite intensity is based on a semi-quantitative score of 0 - 4 parasite intensity: 0 = no parasites, 1= few parasites (< 10), 2 = small numbers of parasites (11 – 20), 3 = moderate numbers 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.

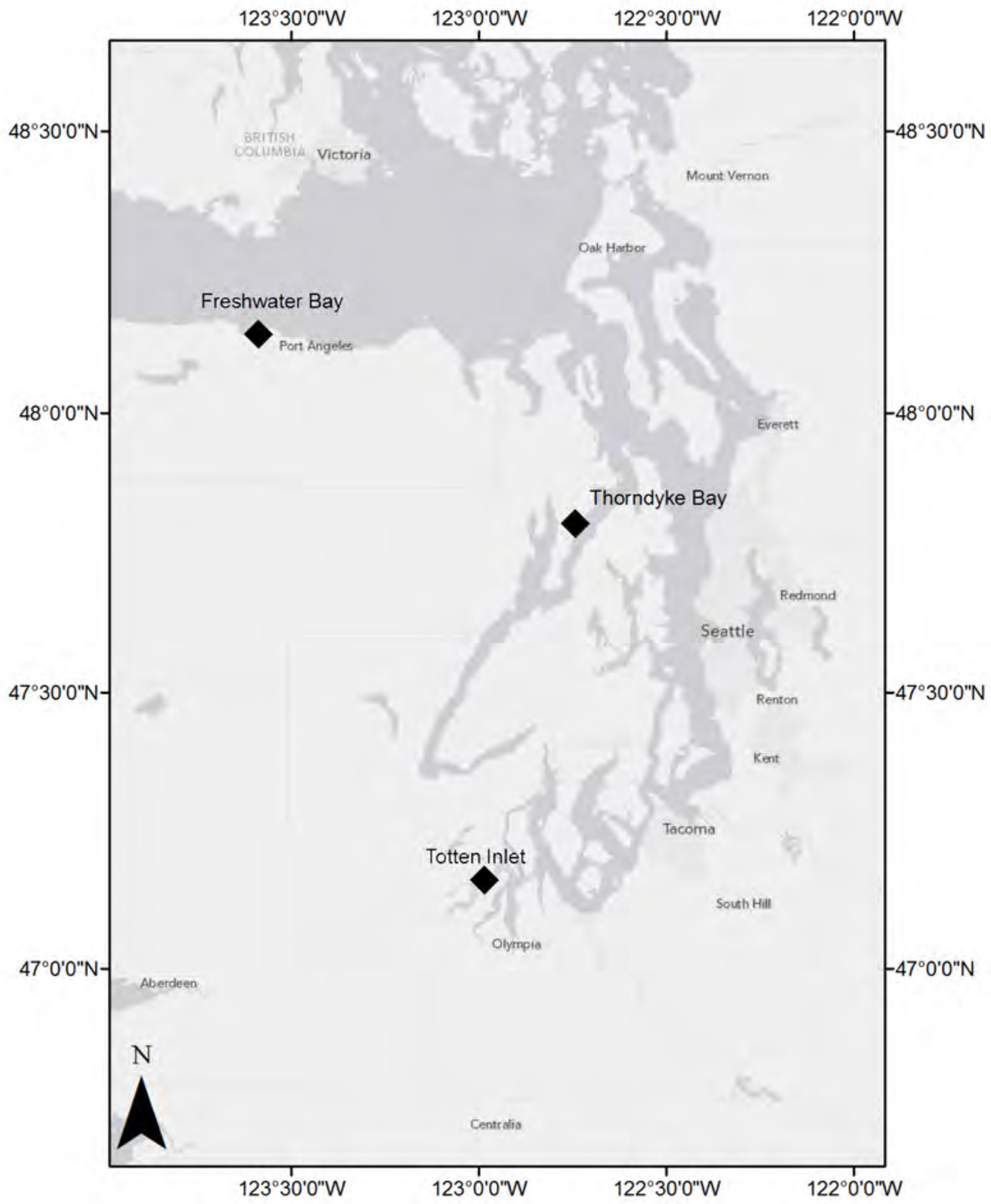


Figure 1. Geoduck sampling sites in Washington state.

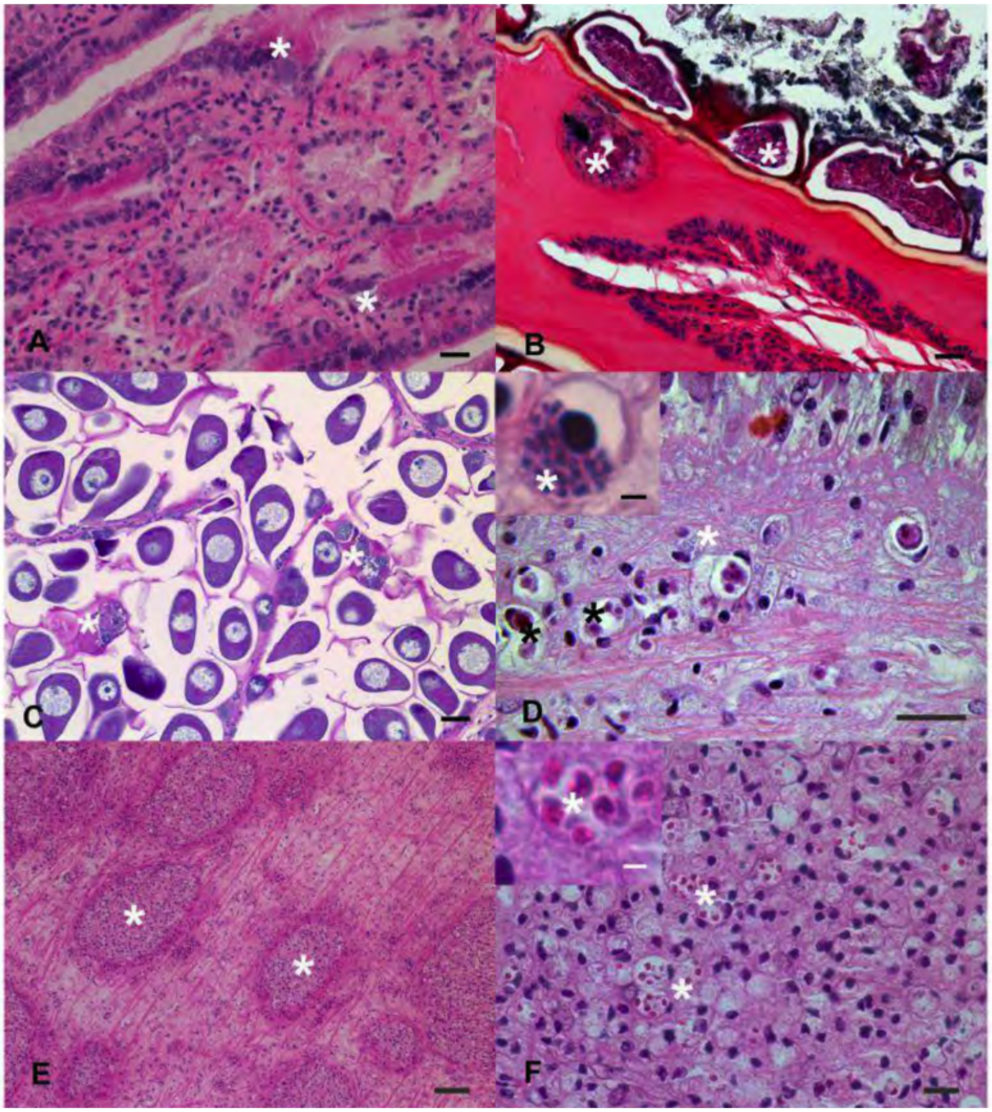


Figure 2. Commonly observed parasites in wild geoduck clams in Washington state. An asterisk denotes parasite presence in each photo. A. Rickettsia-like inclusion bodies in geoduck ctenidia tissue; bar = 13 µm. B. Metazoan parasites; bar = 25 µm. C. Steinhausia-like microsporidian with oocytes ; bar = 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.

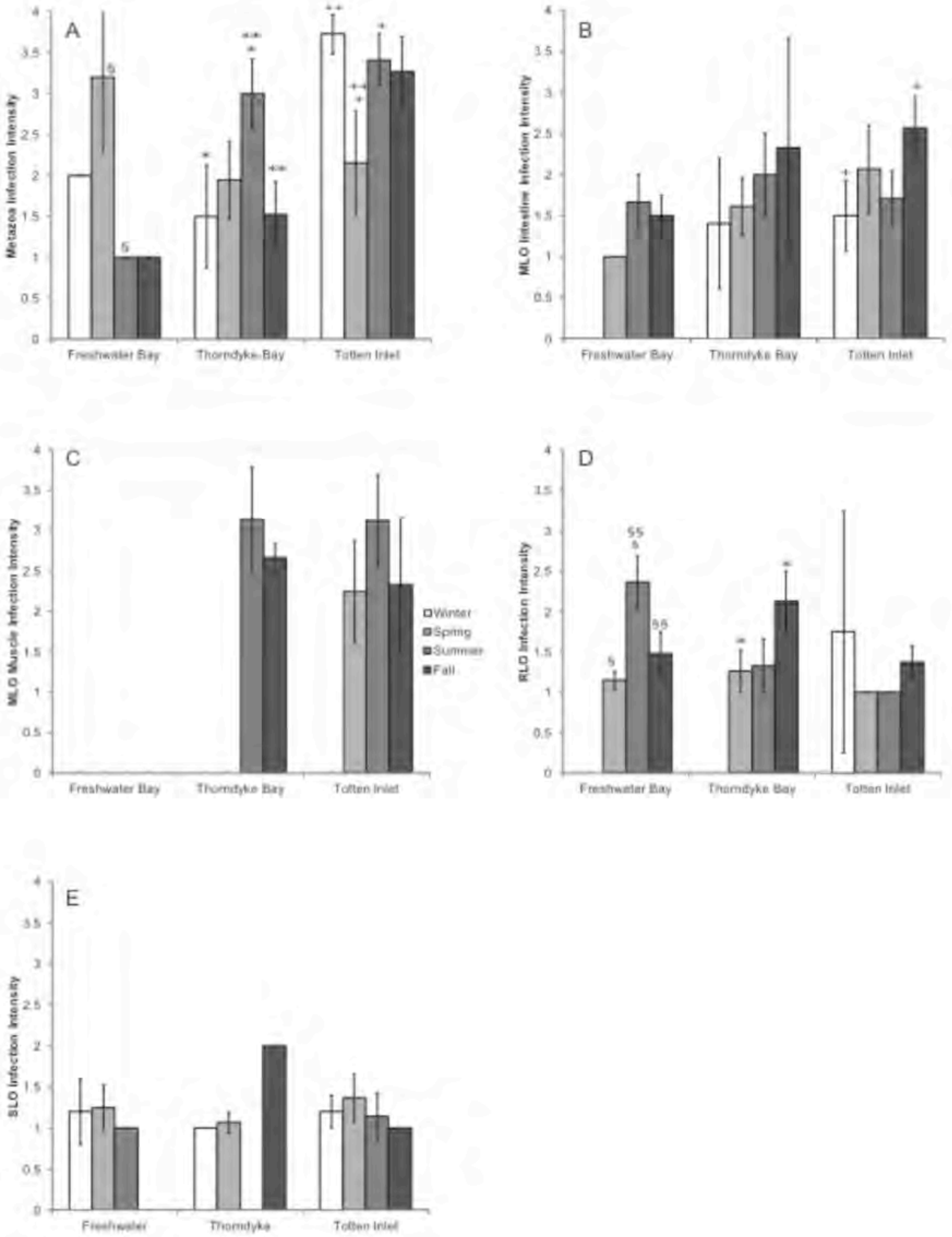


Figure 3. Infection intensity in P. generosa by site and season. Parasite groups: metazoa (A), intestinal microsporidia (MLO intestine; B), siphon muscle microsporidia (MLO muscle; C), Rickettsia-like organism (RLO; D), and Steinhausia-like organism (SLO; E) observed from histology in geoduck clams collected 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.

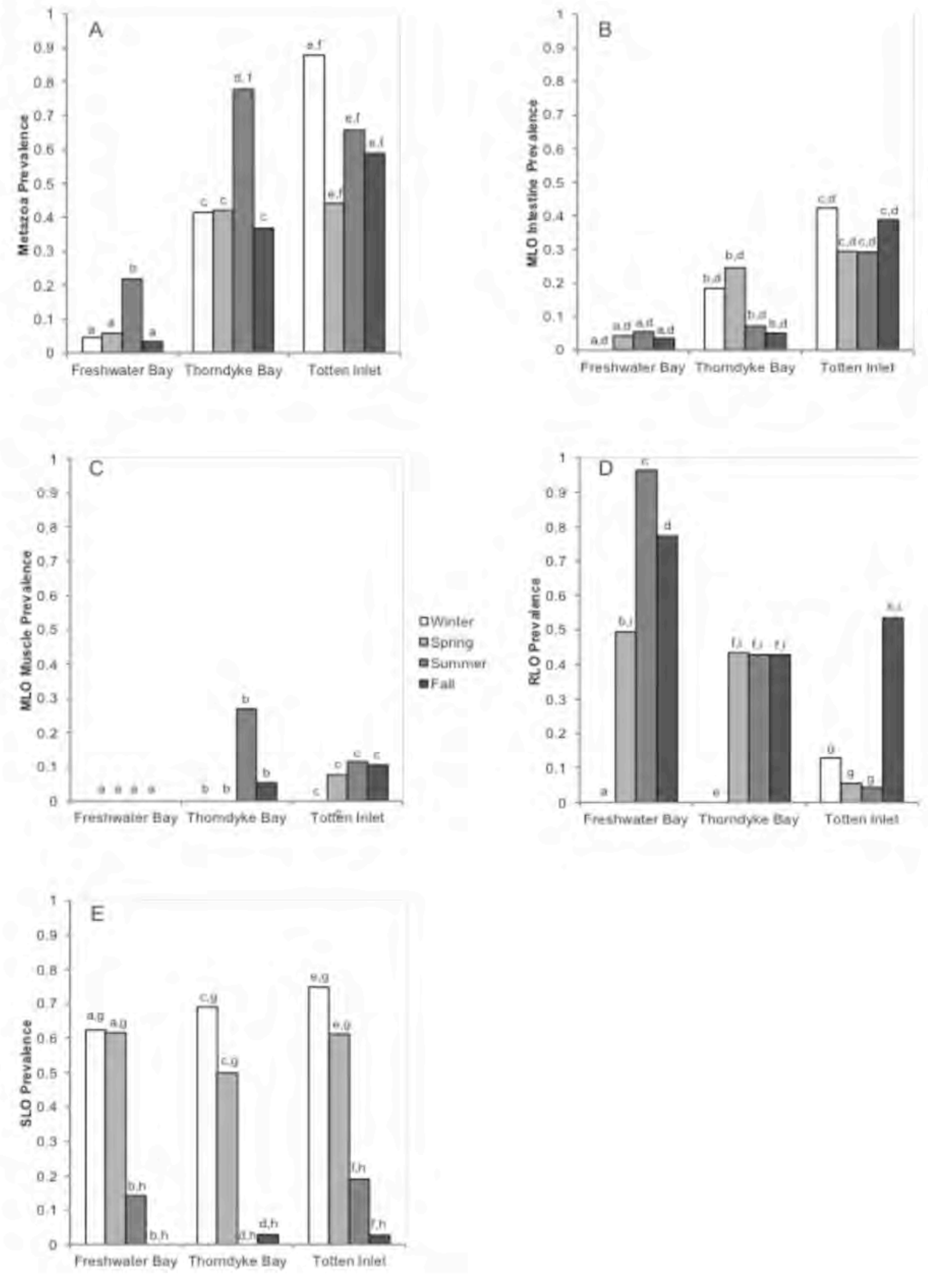


Figure 4. Proportions of parasite groups: metazoa (A), intestinal microsporidia (MLO intestine; B), siphon muscle microsporidia (MLO muscle; C), Rickettsia-like organism (RLO; D), and Steinhausia-like organism (SLO; E) observed from histology in geoduck clams collected from Freshwater Bay, Thorndyke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

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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

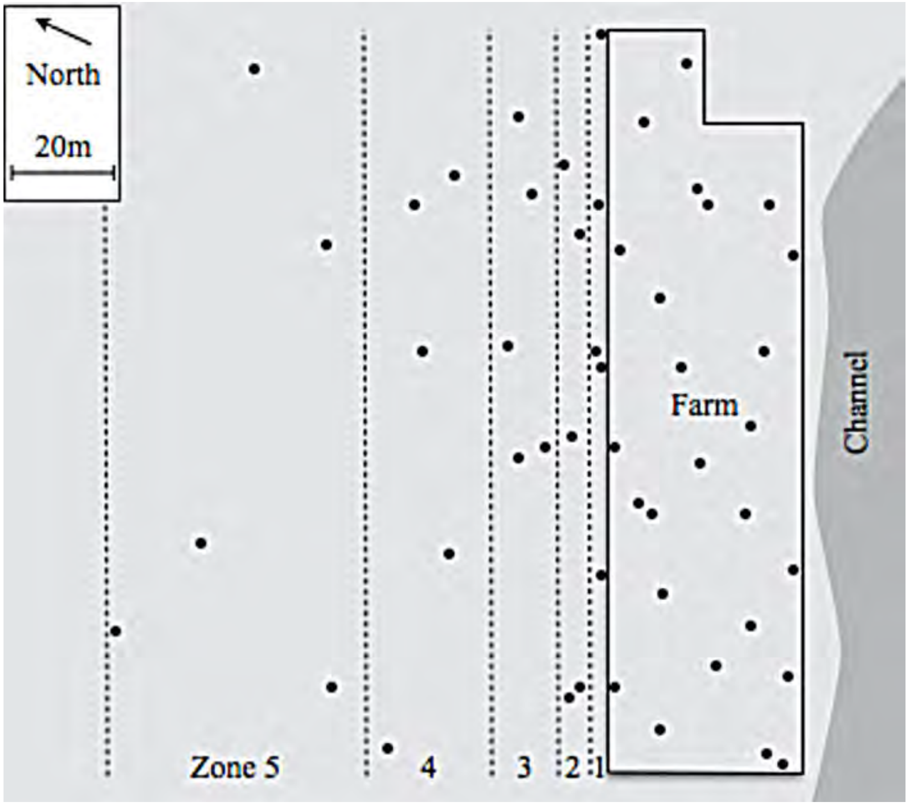

Figure 1: Schematic of Fisk Bar site, showing the farm, surrounding unfarmed areas, and the adjacent channel used for access. Light gray areas are above -2 MLLW; dark gray areas are below -2 MLLW. Dots indicate the placement of quadrats for a hypothetical survey.

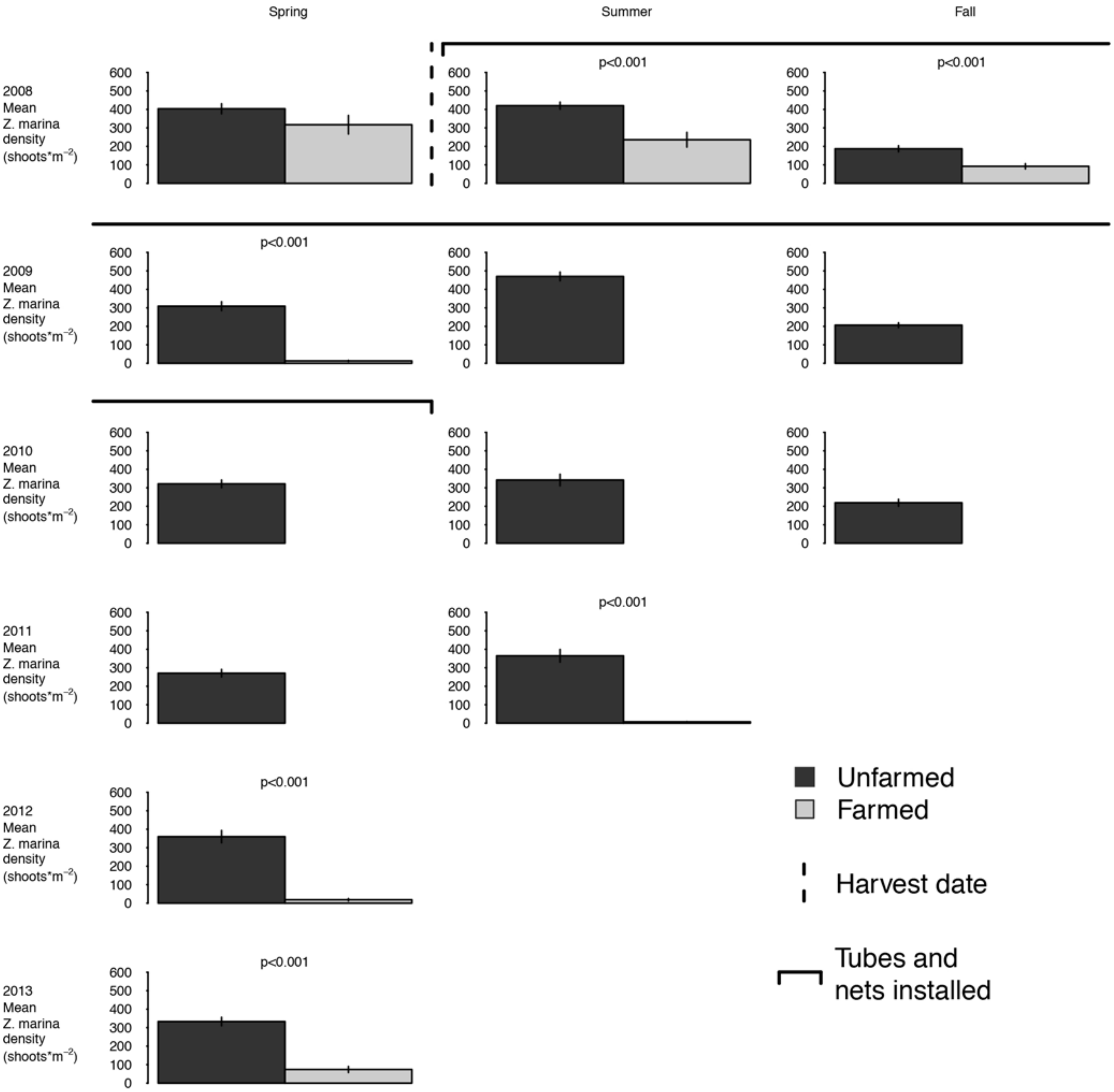

Figure 2: Within-patch eelgrass density over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 9 to 25). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at α= 0.05) in within-patch eelgrass density.

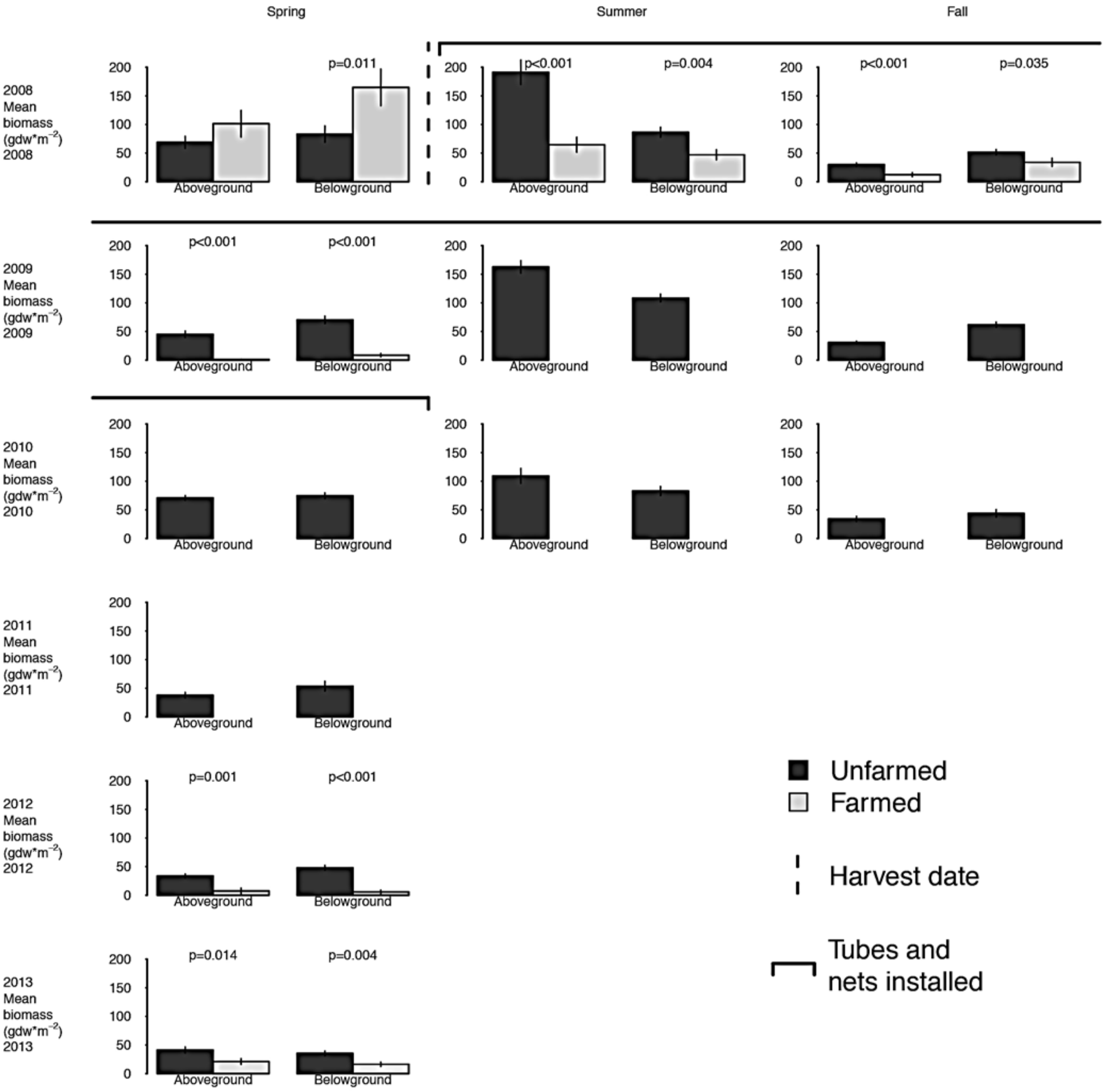

Figure 3: Eelgrass above- and below-ground biomass over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 9 to 25). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at $\alpha = 0.05$) in eelgrass above- or below-ground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

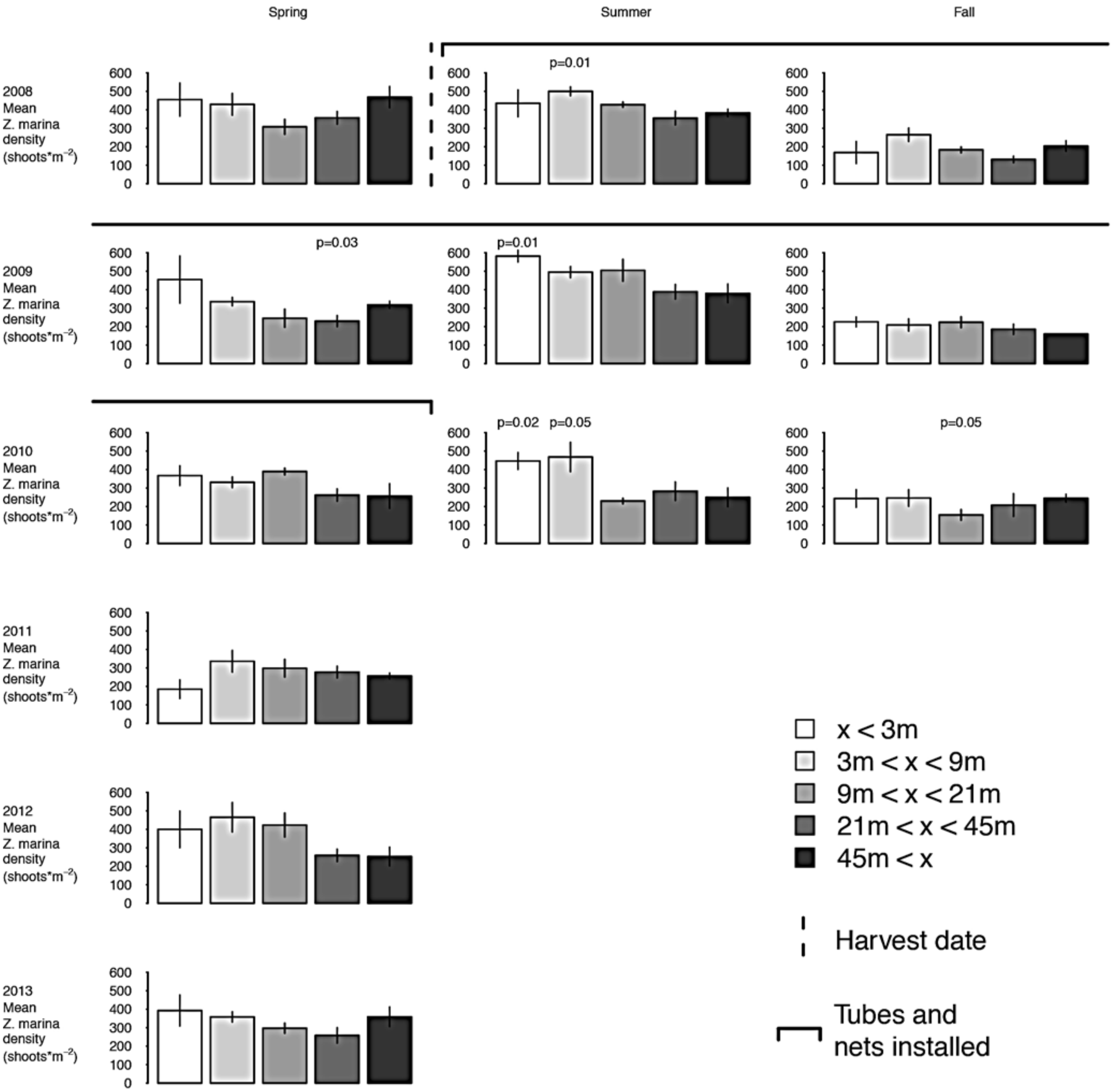

Figure 4: Eelgrass density over time across the five zones of the unfarmed area of Fisk Bar. Bars indicate standard error (n = 3 to 5). Within each survey date, p-values are given for each zone showing a significant difference (at $\alpha = 0.05$) in eelgrass density from the zone furthest from the farm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

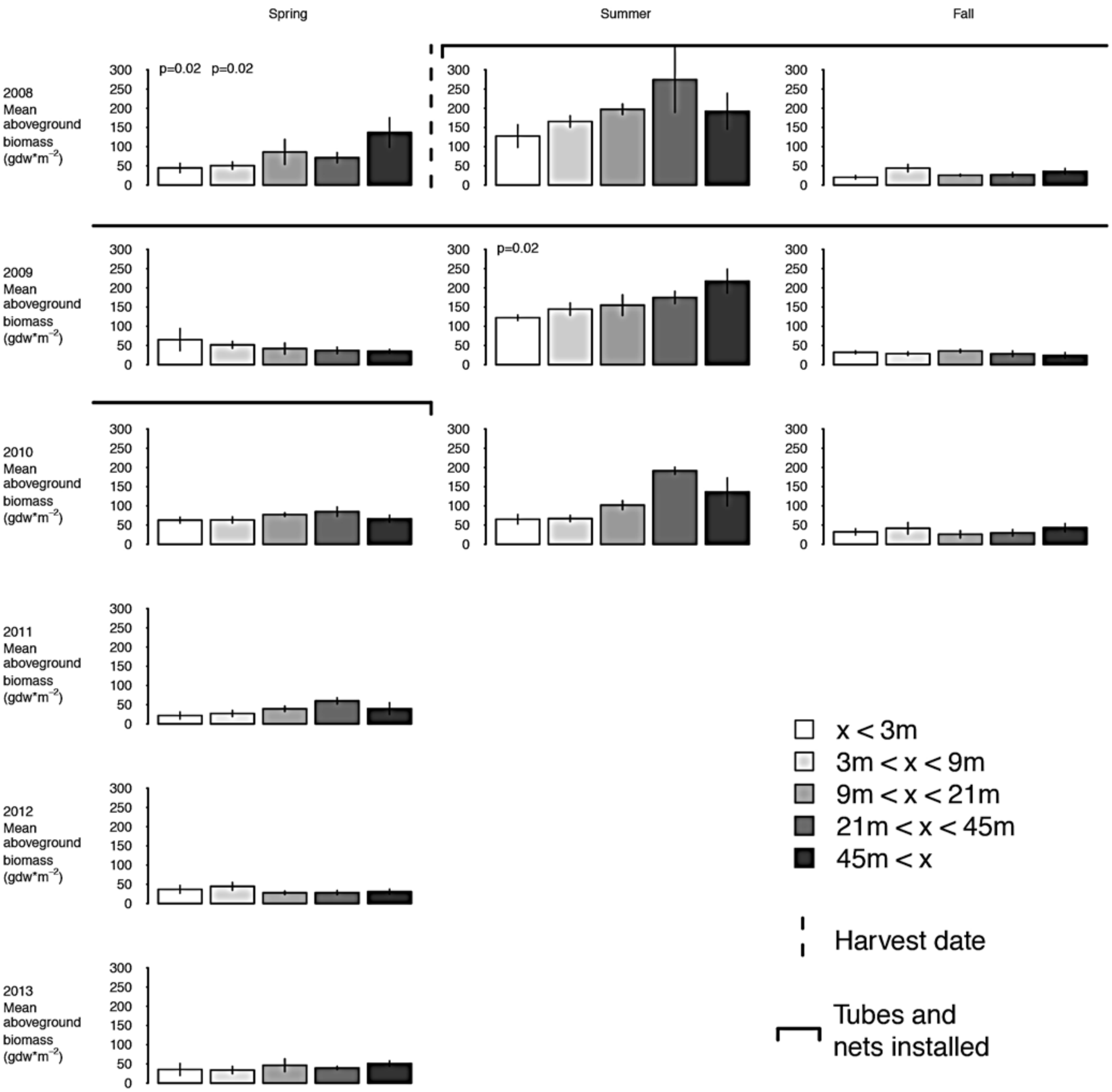

Figure 5: Eelgrass above-ground biomass over time across the five zones of the unfarmed area of Fisk Bar. Bars indicate standard error (n = 3 to 5). Within each survey date, p-values are given for each zone showing a significant difference (at $\alpha = 0.05$) in eelgrass above-ground biomass from the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

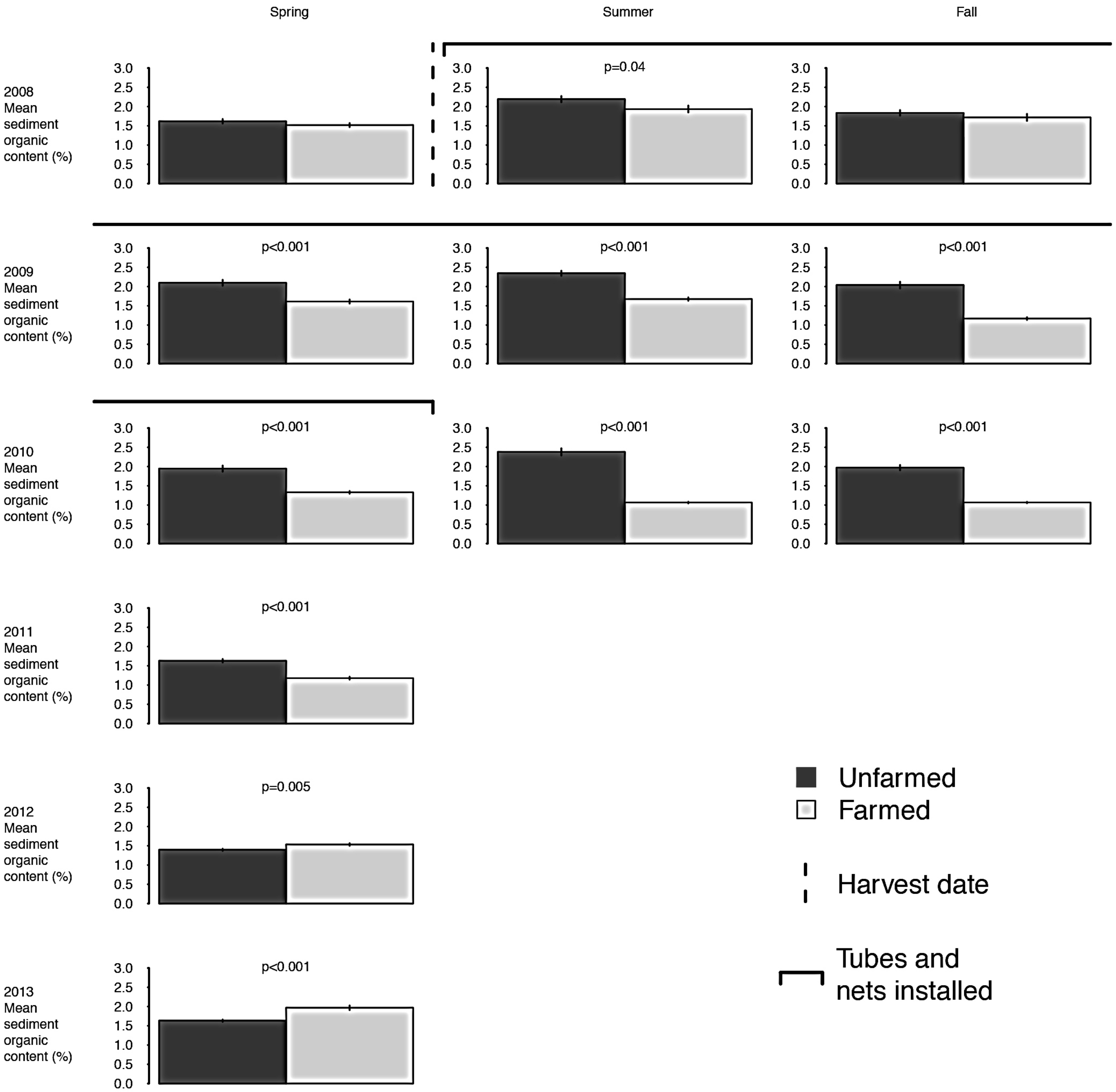

Figure 6: Sediment organic content over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 24 to 25). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at $\alpha = 0.05$) in sediment organic content.

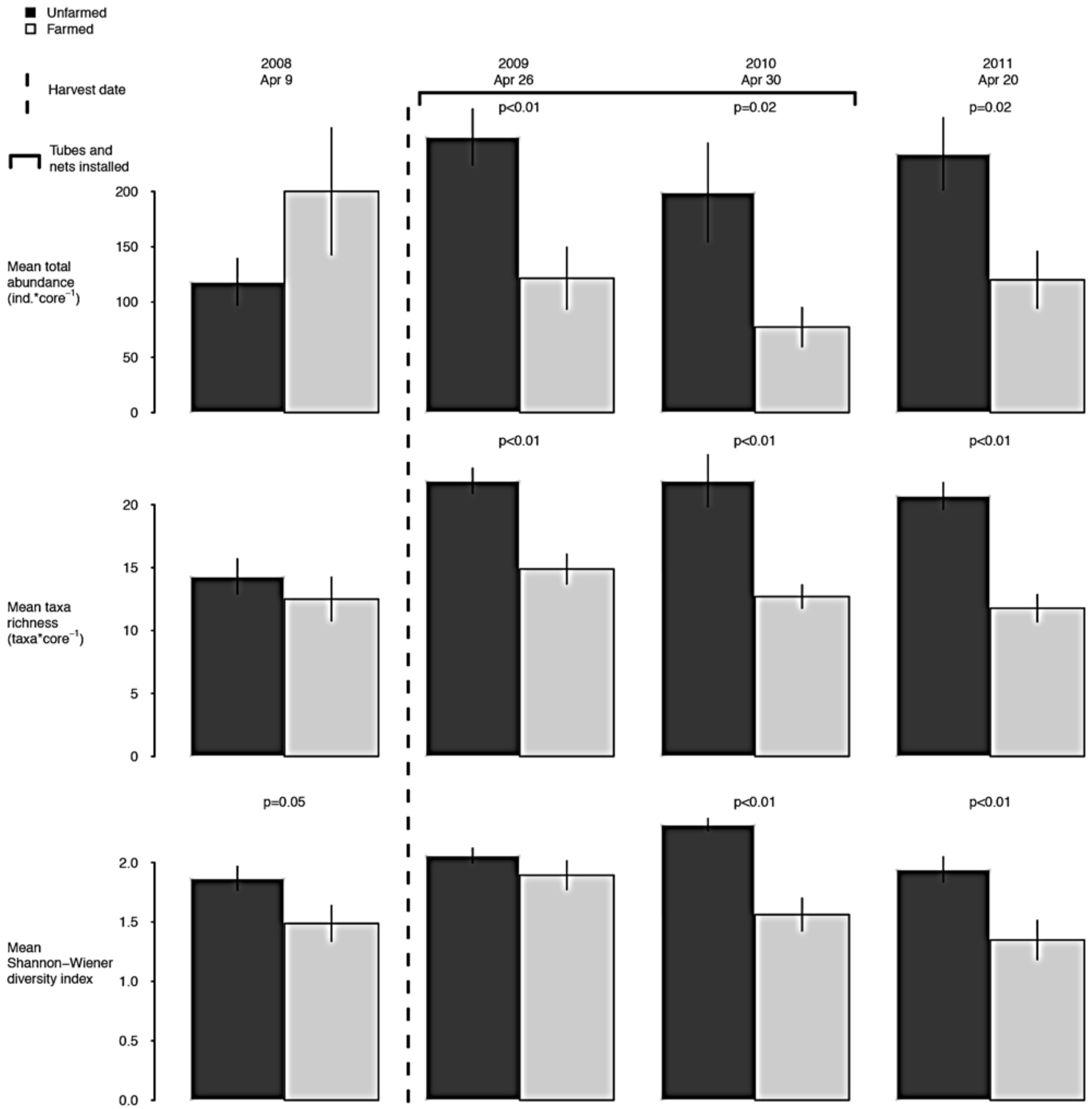

Figure 7: Infauna abundance, richness, and diversity over time in the unfarmed and farmed areas of Fisk Bar. Bars indicate standard error (n = 10). P-values are given for each date in which the unfarmed and farmed areas showed a significant difference (at $\alpha = 0.05$) in the given infaunal character.

Appendix C

Shellfish Aquaculture in Washington State

Final Report to the Washington State Legislature

December 2015

Washington Sea Grant

Shellfish Aquaculture in Washington State

Final Report to the Washington State Legislature • December 2015

This report provides the final results of Washington Sea Grant studies conducted from July 1, 2013 to November 30, 2015 on the effects of evolving shellfish aquaculture techniques and practices on Washington's marine ecosystems and economy. Funding provided by a proviso in Section 606(1) of the adopted 2013 – 2015 State Operating Budget.

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Contents

Overview

Shellfish aquaculture is both culturally significant and economically important to Washington communities, and in many locations interest exists in expanding production. To promote and manage shellfish aquaculture in a sustainable manner, it is essential to understand the potential ecological and economic effects, both positive and negative, of evolving aquaculture practices. At the direction of the Washington State legislature in 2013, Washington Sea Grant initiated a research program to assess and develop tools and resources that could help growers, managers, and other coastal residents address a range of issues. The research program included an economic analysis, three pilot modeling studies, and an overview of spatial data approaches:

1. An economic trend analysis of Washington shellfish production and value (p. 1) details the economic contribution of shellfish aquaculture to different coastal areas in Washington and to the state. The analysis underscores the contribution of aquaculture to generating revenue in the state economy. Results from this work should help guide future development of economic studies and social science on the state aquaculture industry.
2. An ecosystem model of Central Puget Sound (p. 15) was developed to explore the potential influence of aquaculture on the environment and, alternatively, how environmental changes affect aquaculture. In this region, sufficient data are available to build a quantitative ecosystem model, which can be used to explore different management scenarios. For example, a finding from the model that aquaculture gear had stronger ecosystem impacts than the farmed geoduck themselves points to development of innovative gear and new culture techniques as a promising approach for minimizing impacts.
3. Relying on limited data, qualitative food web models of South Puget Sound and Willapa Bay (p. 35) can be used to identify whether shellfish populations or other food web members are likely to increase or decrease given a particular management or environmental scenario. The models for Willapa Bay, for instance, indicate that ocean acidification could potentially result in fewer Manila clams but more eelgrasses and phytoplankton. The models were relatively simple to build and can be easily refined using alternative scenarios.
4. An oceanographic study advances development of a high-resolution circulation model for South Puget Sound (p. 59). In a preliminary analysis, the model suggests that aquaculture may have the capacity to control phytoplankton concentrations in localized areas. The results strongly encourage further investigation of both the possible downstream effects on other consumers of phytoplankton and a possible role for aquaculture in mitigating eutrophication (which can be associated with water quality issues) in western South Puget Sound. The model also has a wide range of other potential applications and could be an important first step towards better prediction of seawater oxygen and acidity levels in South Puget Sound.
5. A framework and data assessment for spatial decision support in aquaculture (p. 71) can further the development of tools to support decisions such as where to site shellfish farms. A decision support study outlines the framework and includes an assessment of publicly available spatial data in Washington State that will likely be relevant. It provides a starting point for growers, managers, and researchers interested in developing spatial tools to weigh the potential ecological, social, and economic tradeoffs involved in farm placement.

SHELLFISH AND THE WASHINGTON ENVIRONMENT

Commercial shellfish cultivation has taken place in Washington waters since the mid-1800s and has evolved in terms of the species farmed, methods used, product markets, and acreage under cultivation. Today Washington State is the nation's leading producer of farmed clams, oysters, and mussels. The 2011 Washington Shellfish Initiative estimated that state shellfish growers directly and indirectly employ more than 3,200 people and provide an estimated total economic contribution of $270 million. Production includes hatcheries, nurseries, farms, and processing, distributing, wholesale, and retail operations. In addition to their commercial importance, shellfish are central to tribal cultures and economies and contribute to recreational opportunities and tourism.

Shellfish are an important component of marine ecosystems, and environmental changes and stressors can affect shellfish aquaculture production. For example, the Washington coast is especially vulnerable to ocean acidification (OA), a change in ocean chemistry that interferes with shell development in some marine organisms and which may potentially affect both cultured species and marine food web dynamics. Harmful algal blooms and aquatic invasive species also continue to pose serious threats to shellfish resources and seafood product safety. Meanwhile, climate change has introduced additional variability in environmental parameters like water temperature, contributing to and interacting with other changes.

Shifts in Washington's coastal environment have been coupled with growing human populations that affect coastal water quality and put additional pressure on regional shellfish resources. Approximately 65 percent of state residents live in coastal counties, and the Puget Sound region alone is expected to grow almost 35 percent, to five million people, by 2040. The complex challenges facing shellfish managers and growers have spurred interest in more comprehensive, ecosystem-based research that integrates environmental, social, economic, and institutional information.

2013-2015 SHELLFISH AQUACULTURE RESEARCH PROGRAM

Housed in the UW College of the Environment, Washington Sea Grant is a federal–university partnership that conducts research, education, and outreach to address Washington's coastal and marine issues and needs. In 2013, the Washington State Legislature directed Washington Sea Grant to conduct a two-year scientific research program specifically addressing state concerns related to shellfish aquaculture. The legislative language specified that funding be used to:

> … commission scientific research studies that examine possible negative and positive effects, including the cumulative effects and the economic contribution, of evolving shellfish aquaculture techniques and practices on Washington's economy and marine ecosystems. The research conducted for the studies is not intended to be a basis for an increase in the number of shellfish harvesting permits available and should be coordinated with any research efforts related to ocean acidification.

As a first step, Washington Sea Grant convened a series of scoping sessions with researchers and faculty from the University of Washington, Washington Ocean Acidification Center, and National Oceanic and Atmospheric Administration (NOAA). Based on the recommendations of session participants, a research team was assembled to develop a scope of work for the program. Although shellfish are cultured throughout Washington State marine waters, a decision was made to build on completed and ongoing research studies, focusing on program components that complemented those studies and had potential to leverage one another. This approach maximized use of existing scientific data and the geographic overlap among research program components. The resulting scope of work focused on three shellfish growing areas: Willapa Bay, Central Puget Sound, and South Puget Sound.

In March 2014, scientists with expertise in ecosystem function and ecology were asked to provide external peer reviews of the proposed research scope of work. The document was then revised in response to their comments and suggestions. The final scope included four research components in which a variety of modeling tools and approaches were used to study potential interactions between aquaculture and the environment: Puget Sound ecosystem and circulation models, qualitative food web analyses, and a synthesis of data relevant to aquaculture siting. A fifth component examined regional trends in the economic contribution of shellfish aquaculture and provides a foundation for future economic analyses.

Work on all program components commenced in May 2014. In August 2014, the research team held a workshop at The Evergreen State College with participants representing tribes, environmental groups, county planners, state and federal agencies, scientists, shellfish growers, and legislative staff. The workshop provided a forum for the team to present the goals of the research and initial work products, and for participants to provide feedback that informed the development of models and scenarios. In June 2015, chapter manuscripts were distributed to subject experts for external review, and revised and finalized by November 2015.

Consistent with the direction from the legislature, the research team's products and results are not intended to provide a basis for either increasing the availability of shellfish harvesting permits or restricting the extent or intensity of shellfish aquaculture in Washington waters. Several program components involved development of modeling tools and required the team to make a variety of assumptions about ecosystem properties. Considering those assumptions when examining model results, the team focused on evaluating general patterns and relative changes rather than precise numerical outputs. However, the models should prove useful for (1) identifying ecosystem species and attributes that may be sensitive to aquaculture practices; (2) evaluating how marine systems, including aquaculture, respond to environmental change; and (3) informing monitoring and research priorities. Products and results should lead to new insights into the ecosystem services provided by and the carrying capacity of shellfish aquaculture in Washington state.

PRODUCTS AND RESULTS

Patterns in the Economic Contribution of Shellfish Aquaculture

Kevin Decker

Understanding the economic contribution of shellfish aquaculture at the regional level is important for industry and policy decisions. In this analysis, cultured shellfish production and value were examined over time to assess economic trends based on an evaluation of seven geographical areas: South Puget Sound, Central Puget Sound, North Puget Sound, Hood Canal, Strait of Juan de Fuca, Grays Harbor and, Willapa Bay.

Because of differences in the species cultivated and in market price among species, the proportional contribution of weight versus value among areas can vary greatly. Overall, the analysis indicated that Pacific County is more dependent on shellfish aquaculture than any other county in the state. An analysis of revenue, expenses, profits, and state leases indicates an average of more than one dollar in profit for each pound of shellfish produced and $510 in annualized profit for each acre under production. The analysis highlights important differences in the economic contribution of shellfish aquaculture in the seven regions examined, but further work is needed, particularly with regard to consistent and accurate reporting of production and the value of the ecosystem services provided by shellfish in Washington State.

Evaluating Trophic and Non-Trophic Effects of Shellfish Aquaculture in the Central Puget Sound Food Web

Bridget Ferriss, Jonathan Reum, P Sean McDonald, Dara Farrell, Chris Harvey

Models of interactions between aquaculture and the environment are important for evaluating potential impacts of either environmental change or different management scenarios on cultivated species and the larger ecological community. If sufficient information is available, quantitative food web models like Ecopath with Ecosim (EwE) can be used. The models represent the main predator–prey relationships in a food web, but can be modified to include other types of relationships as well. For instance, farmed shellfish beds may have artificial structures that can increase or decrease densities of some species.

A recently developed EwE model of Central Puget Sound was updated to include commercial geoduck farms, and relationships representing the effect of geoduck anti-predator structures on several species were incorporated based on inferences from prior studies. The model suggests that, at a basin scale, the food web can support a substantial increase in geoduck aquaculture over current production levels, with only minor changes in the biomass of individual species. Nearly all the observed changes were due to the effects of predator exclusion devices as opposed to the effects of geoduck grazing on phytoplankton or acting as prey to other species. Within the model framework, increased geoduck culture resulted in higher biomass densities of surfperches, nearshore demersal fishes, and small crabs, and lower densities of seabirds, flatfishes, and certain invertebrates (e.g., predatory gastropods and small crustaceans). Such modeling exercises can help identify species that may be particularly sensitive to aquaculture expansion and warrant additional research and monitoring.

Qualitative Network Models in Support of Ecosystem Approaches to Aquaculture Production: Potential Applications to Management and Climate Change

Jonathan Reum, Bridget Ferriss, P Sean McDonald, Dara Farrell, Chris Harvey

Ecosystem-based approaches to managing aquaculture require understanding the potential ecological outcomes associated with expanding or changing aquaculture practices, and qualitative models can play an important role in this capacity. Qualitative models require basic information for forecasting abundance changes. When formally analyzed, the potential qualitative response of the entire community to an increase or decrease in one or more species can be predicted. Like quantitative food web models, qualitative models can help screen management actions for potentially unexpected outcomes or identify tradeoffs in species responses. And qualitative models have much lower data requirements compared with quantitative models.

Qualitative models were developed for South Puget Sound and Willapa Bay that describe relationships between the major cultivated species and the ecological community. For South Puget Sound, the analysis highlighted potential tradeoffs between species based on different management scenarios and actions. For example, under some scenarios, increased cultivation of one shellfish species may indirectly reduce abundance of another. For Willapa Bay, the potential effects of OA were examined. Several species responded consistently, both negatively (e.g., Manila clam) and positively (e.g., phytoplankton and eelgrasses), across a range of scenarios corresponding with different potential direct impacts of OA. Qualitative models can help identify species that strongly influence the response of the community as whole, highlight areas for future research, and summarize and integrate diverse information sources. With little additional effort, qualitative models could be developed for other areas of the state and tailored to address a wide range of questions.

An Oceanographic Circulation Model for South Puget Sound

Neil Banas, Wei Cheng

Shellfish production is dependent on phytoplankton supply, which in turn is strongly influenced by water circulation patterns. In addition, a host of other processes that affect shellfish production, including pollutant dispersal and the supply of wild larvae, depend principally on water circulation patterns. To help address these and other issues, researchers developed a new, high-resolution (200 meters) circulation model for South Puget Sound. The model was used to examine patterns for water exchange and residence-time.

In general, the surface waters in each of the major inlets in South Puget Sound disperse throughout the basin in only a few days, mainly toward the deep central channels and Main Basin. A map depicting the time required for cultured shellfish to reduce the standing stock of phytoplankton by 50%, given their inlet-scale densities, was estimated and compared with the map of water residence time. Preliminary results suggest that aquaculture may control phytoplankton concentrations in Henderson, Eld, Totten, Hammersley, and Case inlets, and Oakland Bay. This strongly encourages further investigation of both the possible downstream effects on other consumers of phytoplankton and a possible role for aquaculture in mitigating eutrophication (associated with water quality issues) in western South Puget Sound.

Geographic Information System Approaches and Spatial Datasets Relevant to Shellfish Aquaculture Siting in Washington State

Dara Farrell, Jonathan Reum, Bridget Ferriss, P Sean McDonald, Dara Farrell, Chris Harvey

Shellfish aquaculture is often just one of several competing uses for the coastal environment, and spatial analyses can help growers and managers identify tradeoffs between potential production at a given site and other economic, social, or ecological considerations. To assess and facilitate application of spatial approaches, investigators reviewed a framework to develop a farm siting geographic information system (GIS) decision support tool. The framework draws upon the most current peer-reviewed literature on GIS applications to shellfish farm siting. In addition, publicly available spatial datasets were identified for Washington State that may be relevant to future analyses. The datasets vary in terms of quality and spatial coverage and resolution, and are grouped under the following five themes: current aquaculture, physical, production, ecological and social. Datasets that are unavailable but that could prove useful for future spatial analyses were also noted. For instance, spatial data on areas that are currently and actively cultivated are unavailable; data on phytoplankton standing stocks and productivity are also largely absent. The framework and inventory of key datasets provide a starting point for developing a focused spatial research program and should be valuable to researchers, managers, and growers alike.

Patterns in the Economic Contribution of Shellfish Aquaculture

Kevin Decker, Washington Sea Grant

SUMMARY

Shellfish have been cultivated in Washington State for more than 160 years. While shellfish aquaculture production around the state has evolved and output increased, analyses of its economic contribution to the state have been sparse. Production output and pricing through 2013 was used to conduct a longitudinal analysis to assess the economic contribution of shellfish aquaculture to Washington State at a regional and state level. The analysis specifically focuses on seven regions: South Puget Sound, Central Puget Sound, North Puget Sound, Hood Canal, Strait of Juan de Fuca, Grays Harbor, and Willapa Bay (Figure 1). It revealed trends that are relevant for industry and policy analysis and provided additional metrics to highlight differences at the regional and county levels. For example, Pacific County's economy is more dependent on shellfish aquaculture than any other county in the state. Pricing for Pacific oyster, Manila clam, and mussels has historically been relatively stable, but geoduck prices have been much more volatile. Owing to differences in market price at the species level, there can be big differences between the proportional contributions of pounds versus value for a region. An analysis of average revenue, expenses, and profits reveals an average of $1.08 in profit for each pound of shellfish produced and $510 in profit for each acre under production (annualized). Revenue to the state from leasing tidelands for shellfish aquaculture varies from year to year based on a percentage of production, and it reached almost $1 million in 2013. Data on the value of ecosystem services provided by aquaculture continues to be limited, and additional research is needed to ensure this value is considered in the larger analysis of economic value to Washington State.

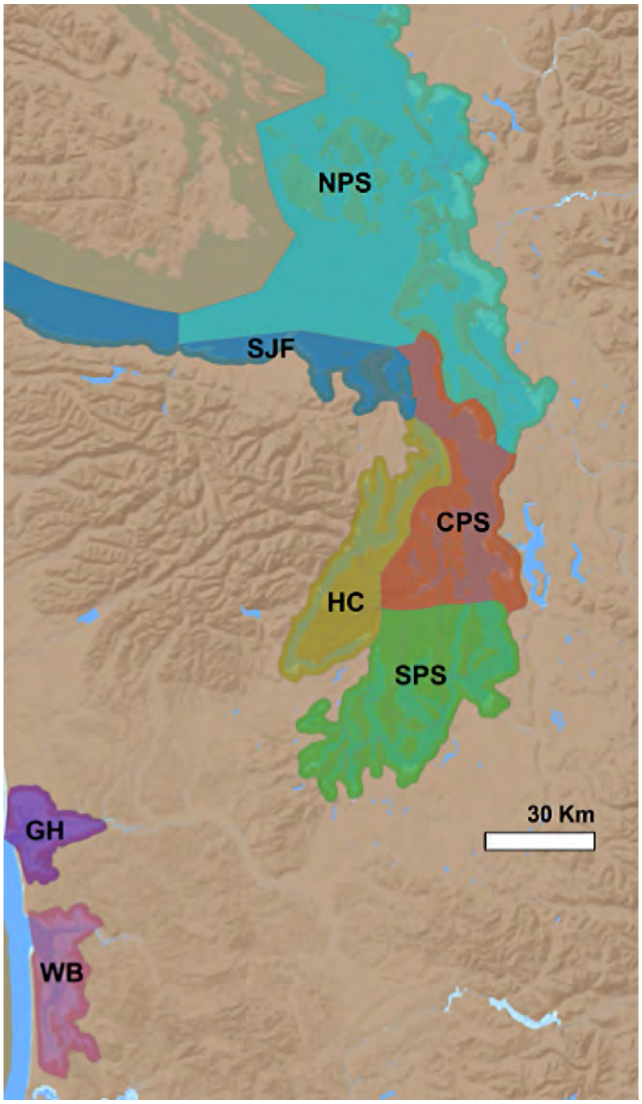

Figure 1. Map of shellfish aquaculture production regions. Delineations were based on Washington Department of Fish & Wildlife (WDFW) aquaculture area codes used for fisheries management. Production was divided into seven regions: (1) South Puget Sound, (2) Central Puget Sound, (3) North Puget Sound, (4) Hood Canal, (5) the Strait of Juan de Fuca, (6) Willapa Bay, and (7) Grays Harbor.

INTRODUCTION

The farming of oysters, clams, mussels, and geoduck in the cold, nutrient-rich, clean waters of the Pacific Northwest is a long-standing tradition and an important cultural and economic part of rural coastal communities. Shellfish farming has evolved over time, relying more and more on hatchery technology to produce the seed needed for cultivation. A handful of large-scale hatcheries produce seed shipped to numerous nurseries prior to outplanting. The nurseries allow vulnerable seed a chance to grow larger, giving it a better chance of survival after final planting. Shellfish growers also have adapted their practices to address a number of environmental challenges including ocean acidification, harmful algal blooms, water pollution, and nearshore habitat alterations.

Consumer preferences and markets have shifted as well, allowing shellfish producers to innovate with new species and new techniques for production. Key cultured species include the Pacific oyster (*Crassostrea gigas*), Kumamoto oyster (*Crassostrea sikamea*), Eastern oyster (*Crassostrea virginica*), Olympia oyster (*Ostrea lurida*), Manila clam (Venerupis philippinarum), geoduck (*Panopea generosa*), and mussels (*Mytilus trossulus* and *M. galloprovincialis*). Pacific oysters, once grown for mostly the shucked meat market, now are being tumbled and flipped to form deep-cupped oysters for the half shell market. The product mimics the deep cup of the highly prized Kumamoto oyster, whose seed availability is limited. Some harvested species such as littleneck (*Leukoma staminea*), eastern softshell clam (*Mya arenaria*), and horse clams (*Tresus nuttallii* and *T. capax)* are from wild stocks whose juveniles have settled on farmed beaches and are harvested alongside cultured product. Not all tidelands are suitable for cultivating all species, so shellfish growers optimize their production farming tidelands with compatible species.

To meet the growing demand for seafood, Washington shellfish products are sold throughout the United States and exported worldwide with primary markets in Canada and Hong Kong. Currently, Washington is the leading U.S. producer of farmed bivalves with recent annual sales of nearly $150 million. Virginia is second with annual sales of $41 million, followed by Connecticut with harvests valued at $28 million (USDA 2014).

Including indirect output from industries that support aquaculture and induced output resulting from money spent in the community by aquaculture employees and supporting industries, a Northern Economics (2013) report estimated that shellfish aquaculture contributed $184 million to Washington's economy in 2010. The report also estimated the total number of jobs from shellfish aquaculture at around 1,900 and the number of indirect and induced jobs at 810. A higher number of 3,200 direct and indirect jobs was reported by the Washington Shellfish Initiative (2011). In 2010, direct aquaculture industry wages of $37 million and an additional $40 million in indirect and induced wages were paid for a Washington State total of $77 million (Northern Economics 2013).

AQUACULTURE PRODUCTION AND VALUE

To achieve a more comprehensive understanding of the economic contribution of the shellfish aquaculture industry to Washington State, it is important to understand regional differences and how the industry has changed over time. This analysis addressed these topics by evaluating regional differences in production and value using the most recent data as well as trends in historical data. It looked at production at the state level and for each of seven regions defined for this analysis (Figure 1): South Puget Sound, Central Puget Sound, North Puget Sound, Hood Canal, Strait of Juan de Fuca, Grays Harbor, and Willapa Bay. Regional delineations were based on existing aquaculture codes used by the Washington Department of Fish and Wildlife (WDFW) for fishery management. Quantity and value of shellfish aquaculture over time were examined to identify trends and a similar analysis was completed for value. The latter identified the most valuable species statewide and by region as well as changes in species value over time.

One important consideration in the analysis was the use of WDFW aquaculture production data. WFDW issues aquatic farm permits (WAC 220-69-243) and requires growers to submit accurate records showing the quantity of products sold and to provide that information quarterly. However, WDFW does not verify the production numbers submitted and there is little incentive for growers to provide accurate information to the agency. For these reasons, industry and WDFW generally consider production numbers submitted to WDFW to be underreported (B Kauffman, WDFW, personal communication). Despite this shortcoming, the WDFW data are the most comprehensive and accurate available for analysis and the only data available that have been gathered consistently over time to allow for a longitudinal analysis. All tables and figures for state and regional production and value were created from WDFW production data.

Washington State

Historical trends: Figure 2 summarizes 28 years of shellfish production data for Washington State. From 1986 until 1998, total shellfish aquaculture production stayed relatively stable, between 11.7 and 15.1 million pounds. Between 1998 and 2005, total production increased considerably, reaching a peak in 2005 at 24.9 million pounds. After 2005, production leveled off again, decreasing to a low in 2011 of 22.5 million pounds. Pacific oyster, Manila clam, and mussels have continued to be the three primary staples of shellfish aquaculture production, with the Pacific oyster maintaining the highest production by species in Washington State. Manila clam production was almost equal to Pacific oyster production in 2012, but a drop in Manila clam production in 2013 increased the gap slightly.

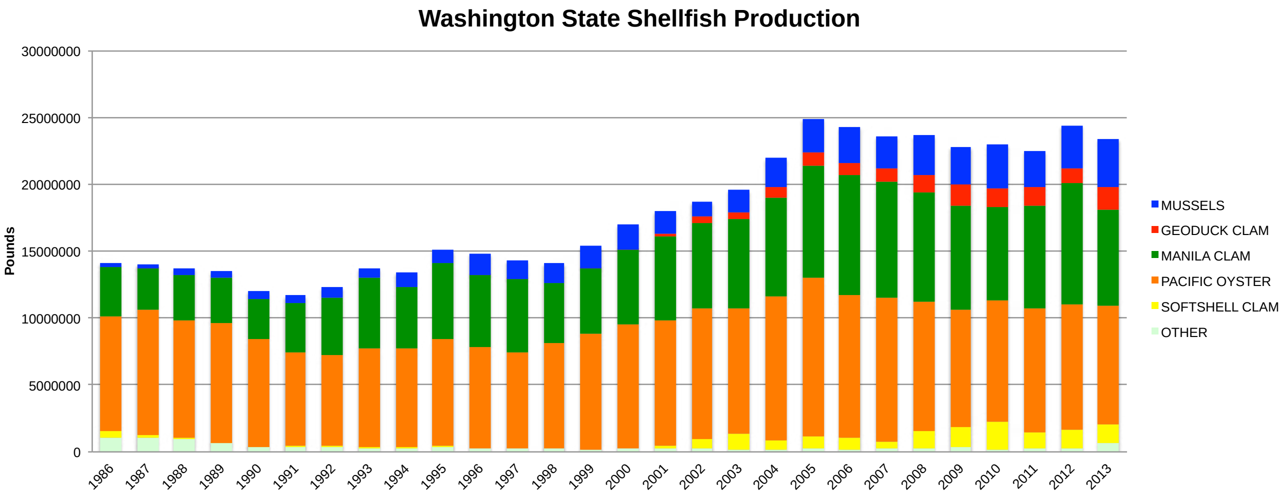

Figure 2. Washington shellfish aquaculture production by species, 1986–2013.

Current production and value: Table 1 and Figure 3 provide more detailed information on species production and value for 2013 and show that Pacific oyster accounted for 38% of total production and total value. With the expansion of the Pacific oyster market from primarily shucked meats to shucked meats and half shell, an increase in total value is expected as consumer interest in flipped and tumbled Pacific oyster takes hold. Manila clam culture accounted for 31% of production and 19% of the value. Geoduck production was previously only a very small part of total production, but it has increased substantially and now accounts for 7% of the total pounds produced and 27% of the total value for the state. In 2013, WDFW estimates for total output from shellfish aquaculture were 23.4 million pounds and $91.9 million in value.

State and regional summary: Table 2 provides summaries of production and value for 2013, indicating that South Puget Sound is the top producing region with 37% of total production and almost 58% of total value. Willapa Bay is second with 25% of production and almost 17% of the value. Species importance varies between Puget Sound and the Pacific coast, with the Sound primarily producing Manila clam and coast mainly producing Pacific oyster.

Table 1. Weight and value of Washington shellfish aquaculture production by species, 2013 (percentages are rounded to the nearest whole number for all tables).

	Weight		Value	
Species	**Pounds**	**Percentage**	**Dollars**	**Percentage**
Mussels	3,655,551	16	7,940,408	9
Geoduck clam	1,613,114	7	24,482,209	27
Manila clam	7,259,401	31	17,451,985	19
Pacific oyster	8,793,138	38	34,853,940	38
Softshell clam	1,419,509	6	454,198	<1
Other	664,905	3	6,738,647	7
Total	23,405,618	100	91,921,390	100

Table 2. Regional summary of 2013 Washington aquaculture production and value.

	Production		Value	
Region	**Pounds**	**Percentage**	**Dollars**	**Percentage**
South Puget Sound	8,664,322	37	53,230,541	58
Central Puget Sound	5,253	<1	19,411	<1
North Puget Sound	3,926,994	17	7,311,343	8
Hood Canal	3,490,795	15	11,566,475	13
Strait of Juan de Fuca	155,467	<1	455,587	<1
Willapa Bay	5,948,216	25	15,567,786	17
Grays Harbor	1,209,895	5	3,956,918	4
Total	23,400,942	100	92,108,061	100

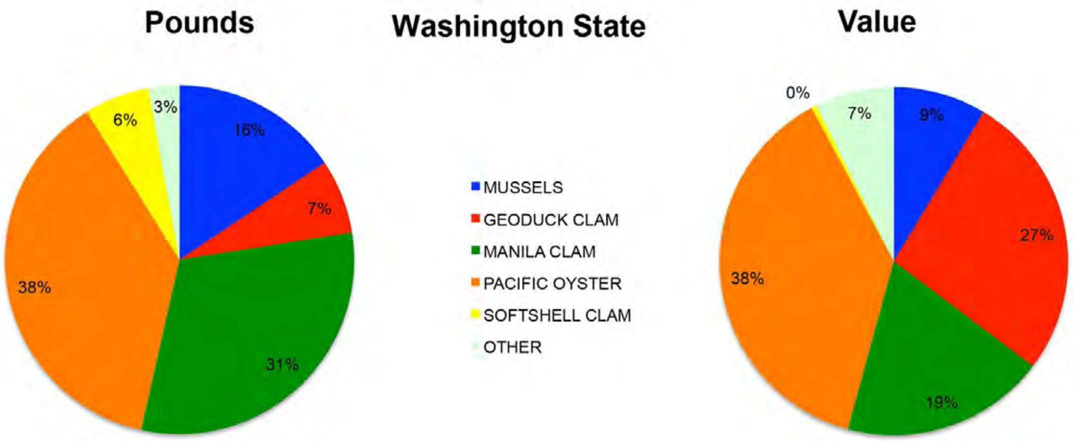

Figure 3. Percentages for 2013 harvest weight and value of Washington shellfish species.

South Puget Sound

Historical trends: As indicated in Figure 4, long-term data for South Puget Sound show the Manila clam has been the primary cultured species in terms of landings but production has decreased since peak reported landings in 2006. Until recently, Pacific oyster was the second most produced species but was surpassed by geoduck in 2010 and mussels in 2011. Geoduck production began to increase in 2000 and has maintained a mostly upward trajectory. Two native species, Olympia oyster and littleneck clam, continue to be a small part of overall landings.

Current production and value: More detailed 2013 information provided in Table 3 and Figure 5 shows that South Puget Sound has low levels of production for butter clam (*Saxidomus gigantea*), cockle (*Clinocardium nuttallii*), European flat oyster (*Ostrea edulis*), Kumamoto oyster, Eastern oyster, horse clams, littleneck clam, and Olympia oyster. Together, these species made up less than one percent of the total landings in terms of weight and value in 2013. While geoduck clams accounted for only 18% of pounds produced, they contributed 44% of the regional value. In addition to cultured product, there was a substantial wild harvest of geoduck clams in South Puget Sound. According to WDFW catch records, in 2013 the wild geoduck harvest totaled 479,739 pounds, valued at $3.6 million. Wild harvest from all Washington Department of Natural Resources (WDNR) geoduck tracts are strictly managed and a tract is left fallow for many years during natural tract recovery. Manila clam had the highest production by weight (42%) of production, but only accounted for 16% of total value.

Table 3. Weight and value of South Puget Sound production by species, 2013.

	Weight		Value	
Species	**Pounds**	**Percentage**	**Dollars**	**Percentage**
Mussels	1,767,688	20	4,615,502	9
Eastern oyster	140,628	2	1,953,601	4
Geoduck clam	1,573,169	18	23,648,591	44
Kumamoto oyster	118,826	1	2,901,719	5
Manila clam	3,654,315	42	8,546,063	16
Pacific oyster	1,342,967	15	11,472,384	22
Other	66,729	<1	92,678	<1
Total	8,664,322	100	53,230,541	100

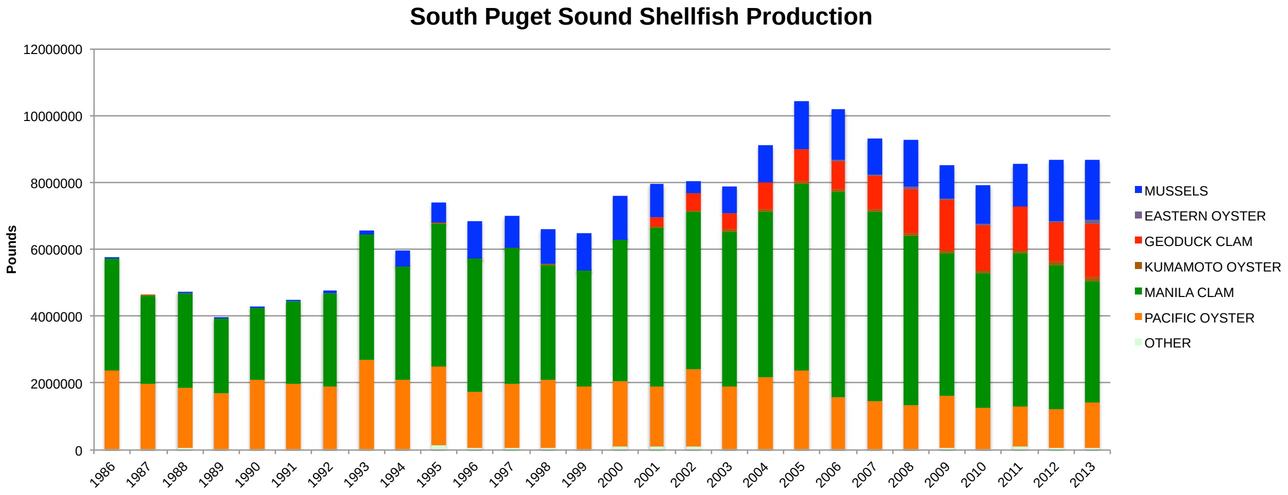

Figure 4. South Puget Sound shellfish aquaculture production by species, 1986–2013.

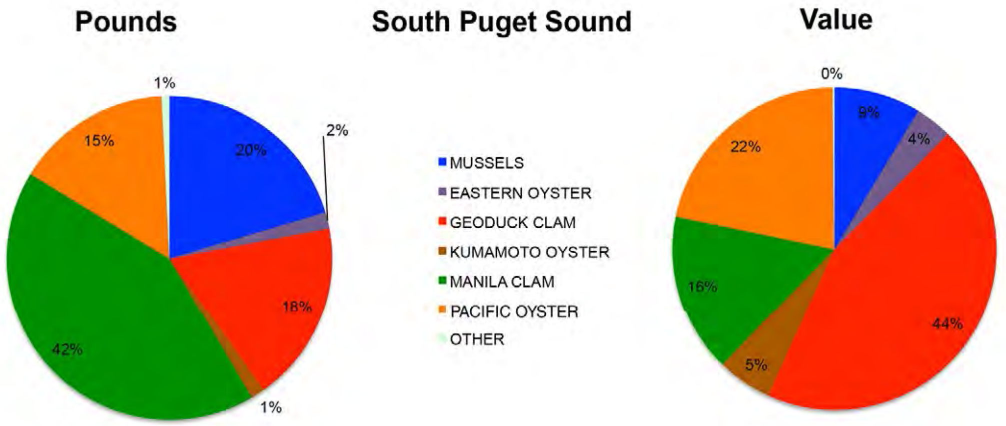

Figure 5. Percentages for 2013 harvest weight and value of South Puget Sound shellfish species.

Central Puget Sound

Historical trends: As shown in Figure 6, Central Puget Sound produced primarily Pacific oyster between 1986 and 1995. Production reached a peak of just over 560 thousand pounds in 1989 and dropped precipitously in 1995. Since 1995, only very small amounts of shellfish aquaculture have been attributed to Central Puget Sound as that region is defined for this analysis.

Current production and value: As shown in Table 4 and Figure 7, Central Puget Sound is currently producing just two species: Manila clam, which accounts for 87% of production and 64% of value; and Pacific oyster, which accounts for the remaining 13% of production and 36% of value. The 2013 production of all species in the region accounted for less than one percent of state production and value.

Table 4. Weight and value of Central Puget Sound production by species, 2013.

	Weight		Value	
Species	**Pounds**	**Percentage**	**Dollars**	**Percentage**
Manila clam	4,570	87	12,339	64
Pacific oyster	683	13	7,072	36
Total	5,253	100	19,411	100

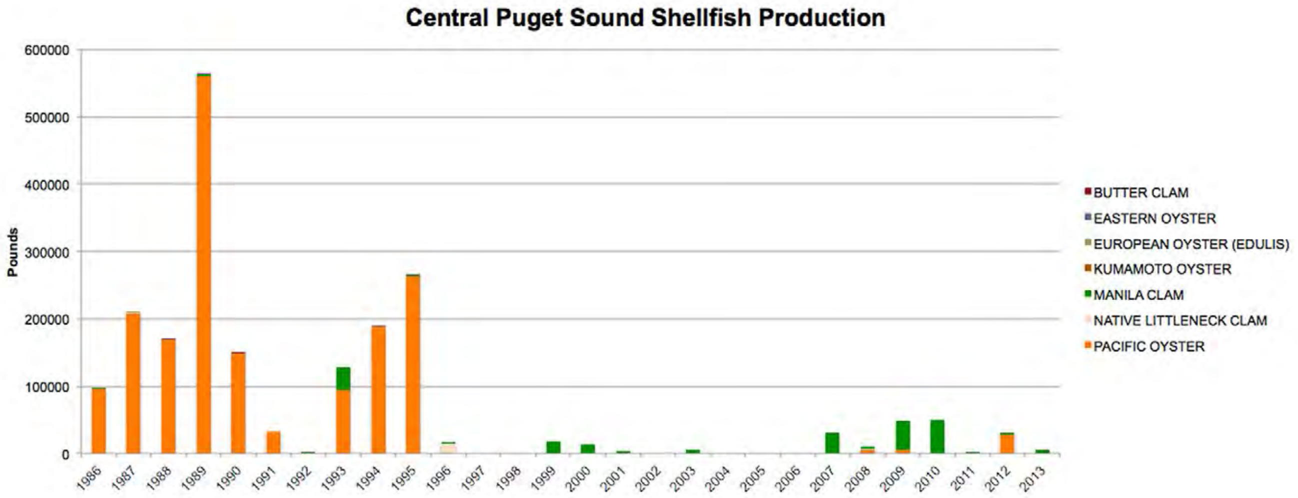

Figure 6. Central Puget Sound shellfish aquaculture production by species, 1986–2013.

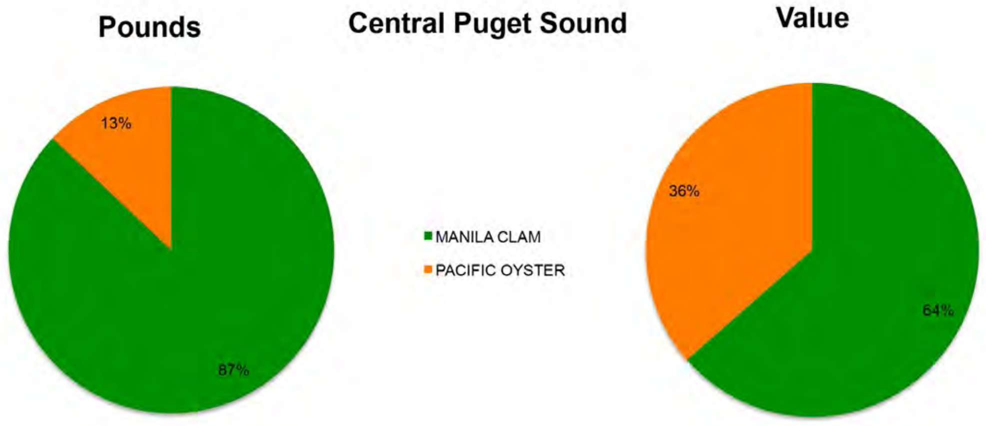

Figure 7. Percentages for 2013 harvest weight and value of Central Puget Sound shellfish species.

North Puget Sound

Historical trends: As Figure 8 summarizes, North Puget Sound primarily lands mussels and softshell and Manila clams. The region saw consistent growth in cultured mussels from 1998 until 2010, followed by a dramatic decrease in 2011. Dropping from 1.8 million pounds in 2010 to 447 thousand pounds in 2011, mussels rebounded back to 1.47 million pounds by 2013. Softshell clams peaked in 2010, with production of 2.1 million pounds, dropped to 920 thousand by 2012, and bounced back slightly to 1.4 million pounds by 2013.

Current production and value: As Table 5 and Figure 9 show, in 2013 mussels topped North Puget Sound production in terms of weight and value. Softshell clams contributed 36% of the harvest but only 6% of the value, while Pacific oyster culture accounted for only 6% of production but 23% of the value.

Table 5. Weight and value of North Puget Sound production by species, 2013.

	Weight		Value	
Species	**Pounds**	**Percentage**	**Dollars**	**Percentage**
Mussel	1,473,464	38	2,497,982	34
Eastern oyster	56,356	1	413,231	6
Manila clam	751,062	19	2,063,479	28
Pacific oyster	226,404	6	1,688,582	23
Softshell clam	1,419,304	36	454,146	6
Other	404	<1	193,920	3
Total	3,926,994	100	7,311,343	100

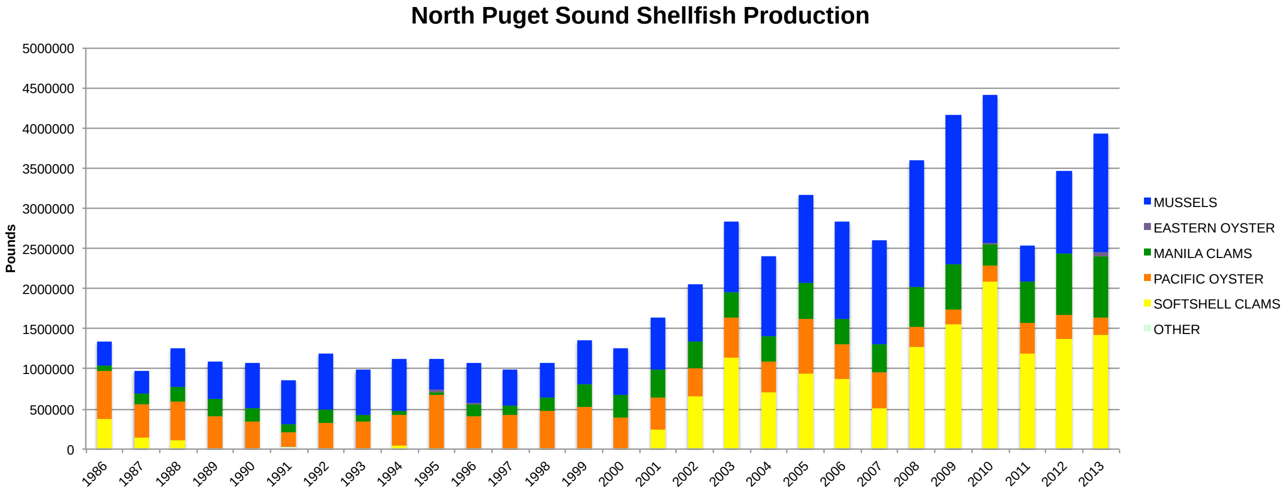

Figure 8. North Puget Sound shellfish aquaculture production by species, 1986–2013.

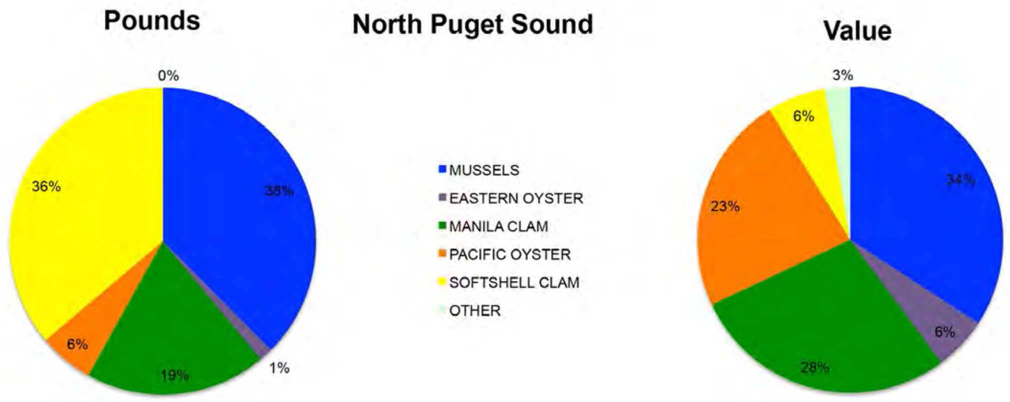

Figure 9. Percentages for 2013 harvest weight and value of North Puget Sound shellfish species.

Hood Canal

Historical trends: As indicated by Figure 10, Manila clam and Pacific oyster have been the staple species of shellfish aquaculture in Hood Canal since 1989. In 2010, a mussel culture operation expanded into the region and mussels have continued to be important for the region since then. Native littleneck clams were harvested in large numbers between 1986 and 1988, at which point production dropped from 389 thousand pounds to just under 4 thousand pounds in 1989. Manila clams have seen the steadiest growth in production over time, experiencing 41% growth during the last 10 years. In 2013, Pacific oyster production increased significantly from 711 thousand pounds to 1.3 million pounds.

Current production and value: Because of the growth in Pacific oyster production, the species accounted for 39% of total 2013 production and 46% of the year's value as shown in Table 6 and Figure 11. The Manila clam continued to be the primary species by weight, accounting for 47% of regional production and 40% of the value. Mussels accounted for 12% of production and 7% of value. While geoduck contributed less than one percent of production, its high prices accounted for 6% of the total value.

Table 6. Weight and value of Hood Canal production by species, 2013.

	Weight		Value	
Species	**Pounds**	**Percentage**	**Dollars**	**Percentage**
Mussels	414,000	12	826,027	7
Geoduck clam	29,212	<1	639,698	6
Manila clam	1,657,173	47	4,674,670	40
Pacific oyster	1,344,865	39	5,311,618	46
Other	45,545	1	114,461	<1
Total	3,490,795	100	11,566,475	100

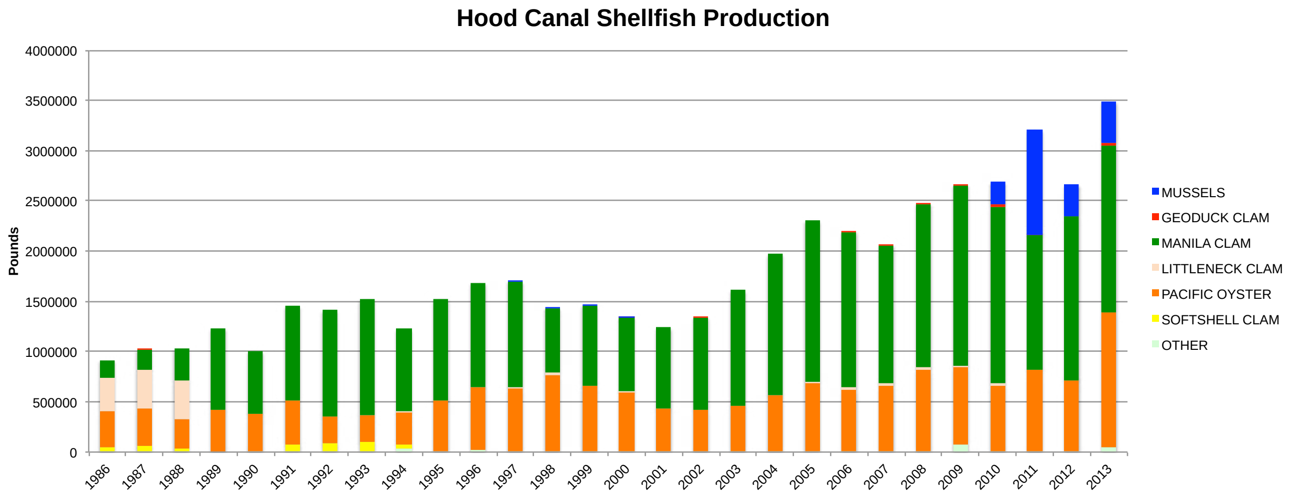

Figure 10. Hood Canal shellfish aquaculture production by species, 1986–2013.

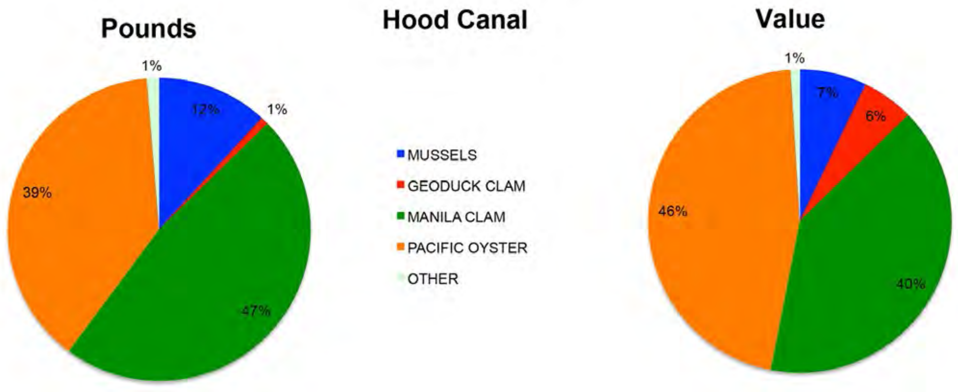

Figure 11. Percentages for 2013 harvest weight and value of Hood Canal shellfish species.

Strait of Juan de Fuca

Historical trends: As indicated in Figure 12, the Strait of Juan de Fuca saw a substantial decrease in production between 1986 and 2013, from more than 743 thousand pounds down to 155 thousand pounds. In 1986, the native littleneck clam was the primary species harvested, but it became less of the proportional mix over time. Butter clam, geoduck, Manila clam, and Pacific oyster have also been important species for the region. Geoduck production first started in the region in 2006 and has continued at a low level since then, with no production in 2008

Current production and value: The more detailed 2013 information provided for the Strait in Table 7 and Figure 13 confirms a total harvest of 155 thousand pounds. While geoduck accounted for less than 7% of total production, it accounted half the value for the region. Because WDFW provided no value for the region's geoduck production, value was extracted based on the average price per pound for Hood Canal during this same period. Butter clams accounted for 21% of harvest but less than one percent of the value for the region.

Table 7. Weight and value of Strait of Juan de Fuca production by species, 2013.

	Weight		Value	
Species	**Pounds**	**Percentage**	**Dollars**	**Percentage**
Butter clam	32,791	21	9,542	2
Geoduck clam	10,329	7	226,205	50
Manila clam	54,163	35	96,435	21
Littleneck clam	20,737	13	21,438	5
Pacific oyster	31,610	20	99,836	22
Other	5,837	4	2,128	<1
Total	155,467	100	455,587	100

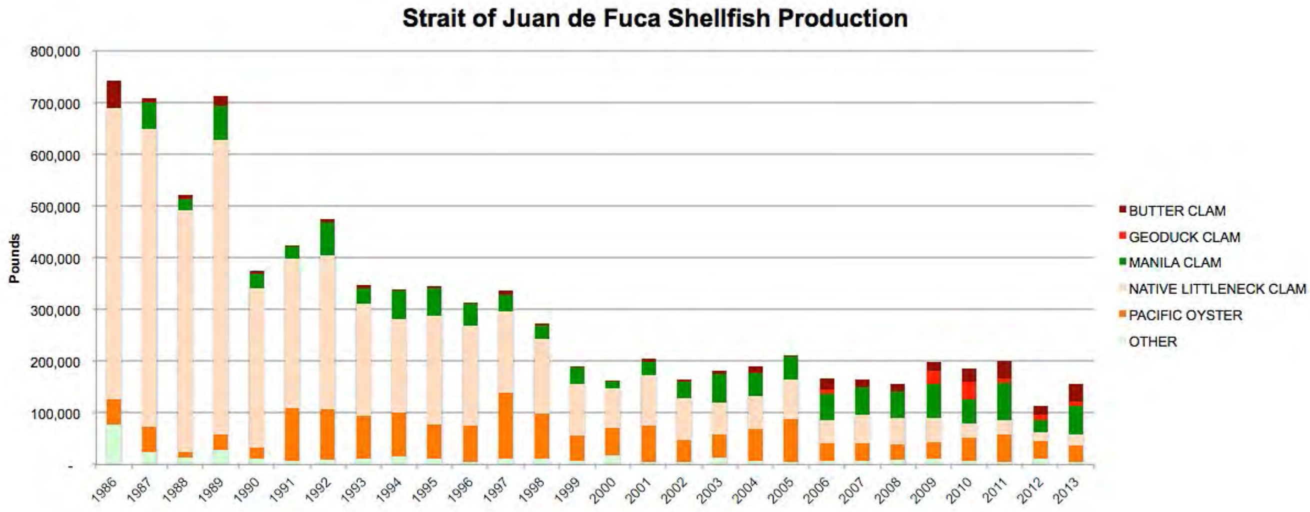

Figure 12. Strait of Juan de Fuca shellfish aquaculture production by species, 1986–2013.

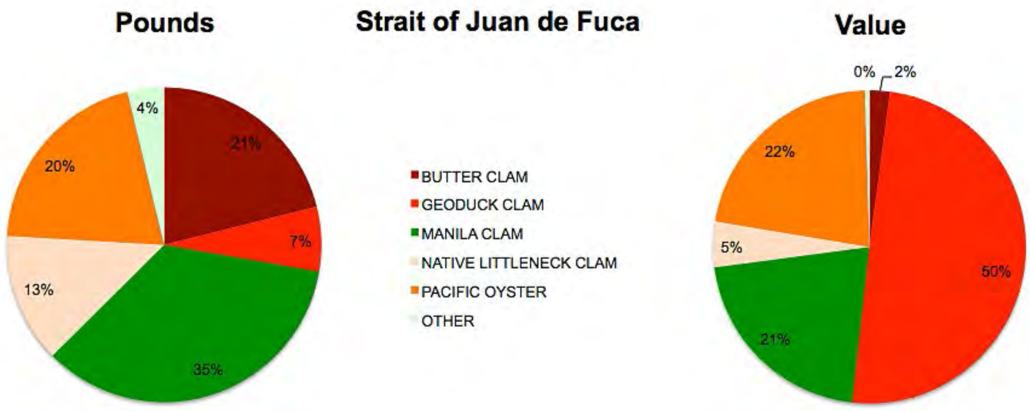

Figure 13. Percentages for 2013 harvest weight and value of Strait of Juan de Fuca shellfish species.

Willapa Bay

Historical trends: Figure 14 shows Willapa Bay production for two primary species, Manila clam and Pacific oyster. Manila clam production appears to have leveled off between 1.1 and 1.2 million pounds. Pacific oyster production has been a little more variable: it peaked in 2007 with 7 million pounds produced and $15.8 million in value. Since 2008, production has gone up and down in alternating years.

Current production and value: As Table 8 and Figure 15 demonstrate, in 2013 Willapa Bay production was dominated by Pacific oyster and Manila clam, accounting for 78% and 19% of total production, respectively. Mussels and Kumamoto oysters were negligible contributors to overall production, but the Eastern oyster accounted for almost 3% of production and 8% of the value.

Table 8. Weight and value of Willapa Bay production by species, 2013.

	Weight		Value	
Species	**Pounds**	**Percentage**	**Dollars**	**Percentage**
Eastern oyster	177,451	3	1,229,106	8
Manila clam	1,135,168	19	2,051,032	13
Pacific oyster	4,635,525	78	12,286,434	79
Other	72	0	1,211	<1
Total	5,948,216	100	15,567,786	100

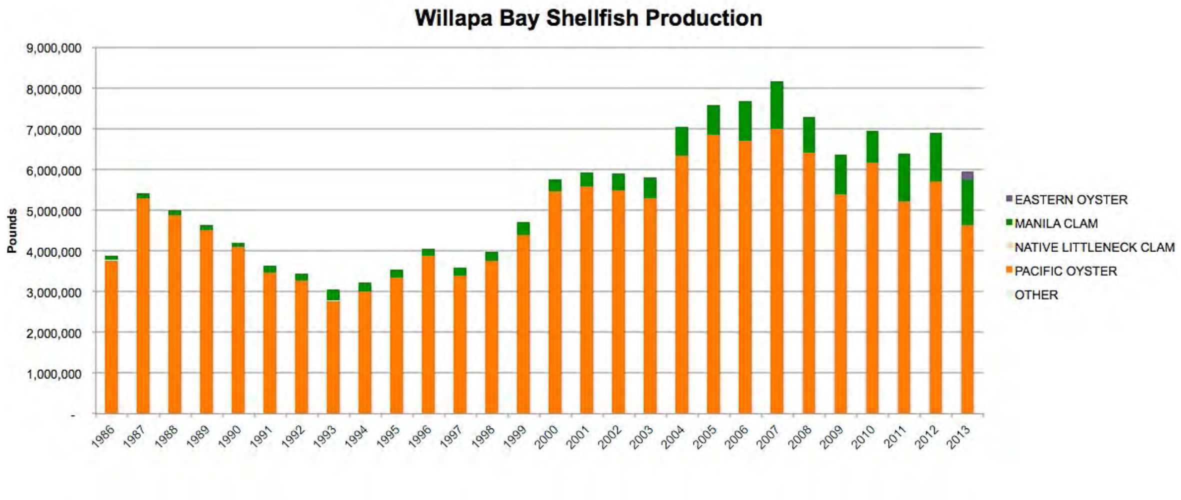

Figure 14. Willapa Bay shellfish aquaculture production by species, 1986–2013.

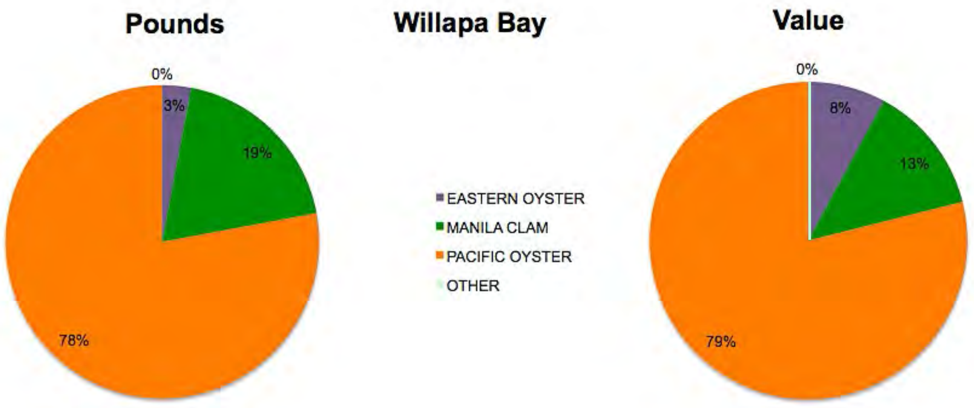

Figure 15. Percentages for 2013 weight and value of Willapa Bay shellfish species.

Grays Harbor

Historical trends: As shown in Figure 16, Grays Harbor production relied almost exclusively on the Pacific oyster, with small amounts of cockles harvested between 1994 and 1997 and Manila clam cultivated in 2004, 2012, and 2013. In 2011, Pacific oyster harvests increased to an all-time high of 1.6 million pounds with a value of $5.3 million.

Current production and value: Total Grays Harbor production in 2013 was 1.2 million pounds valued at $3.9 million. Manila clam contributed less than one percent by weight and value to total production (Table 9).

Table 9. Weight and value of Grays Harbor production by species, 2013.

	Weight		Value	
Species	Pounds	Percentage	Dollars	Percentage
Manila clam	2,950	<1	7,965	<1
Pacific oyster	1,206,945	>99	3,948,953	>99
Total	1,209,895	100	3,956,918	100

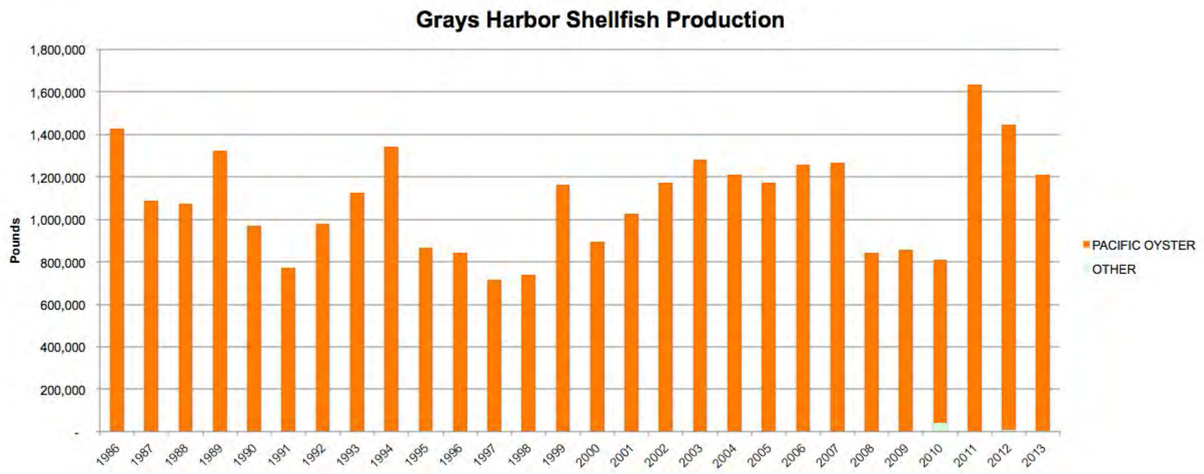

Figure 16. Grays Harbor shellfish aquaculture production by species, 1986–2013.

OTHER ECONOMIC CONSIDERATIONS

Contribution to Regional Economies

Shellfish aquaculture is important, particularly in rural counties, because it helps to diversify local economies and provides jobs. Estimates of economic contribution reflect the proportional contribution of shellfish aquaculture relative to the whole economy at county or state levels. While this value demonstrates the importance of an industry, it also highlights potential economic risk if the industry reduces business activities, relocates, or closes down.

The contribution of shellfish aquaculture to a county's economy was calculated using the 2010 gross domestic product (National Ocean Economics Program 2015) and county-level aquaculture output from the Northern Economics (2013) report. Combining information from the two data sources facilitated determination of the proportional contribution of shellfish aquaculture to each county. Results indicated that Pacific County was the most aquaculture-dependent county in the state with almost 20% of its economy relying on aquaculture. By contrast, the contribution in other counties and for Washington as a whole was less than five percent. While this value seems quite small, it reflects the complexity and size of the overall economy upon which counties and the state rely.

Price Stability

Evaluating the price for shellfish aquaculture species over time helps to assess its stability or volatility. All prices were derived from WDFW production and value numbers and converted to 2013 dollars. Prices for the primary species produced in Washington are provided in Figures 17, 18, and 19. Figure 17 shows that mussels and Pacific oyster have had relatively stable prices. Although Eastern and Kumamoto oyster prices have been more volatile, they provided a higher price per pound. Prices for Kumamoto oysters have been particularly variable, experiencing jumps in 2006 and 2008, then dropping in 2010 to stabilize around $23 per pound. In 2013, Eastern oysters experienced a slight drop in price.

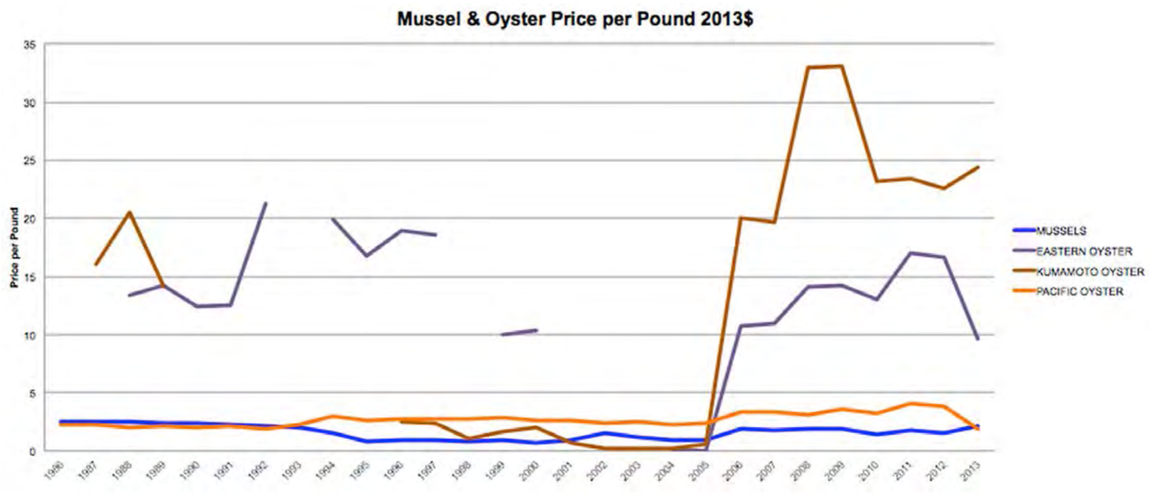

Figure 17. Average price in 2013 dollars for mussels and oysters in Washington, 1986–2013.

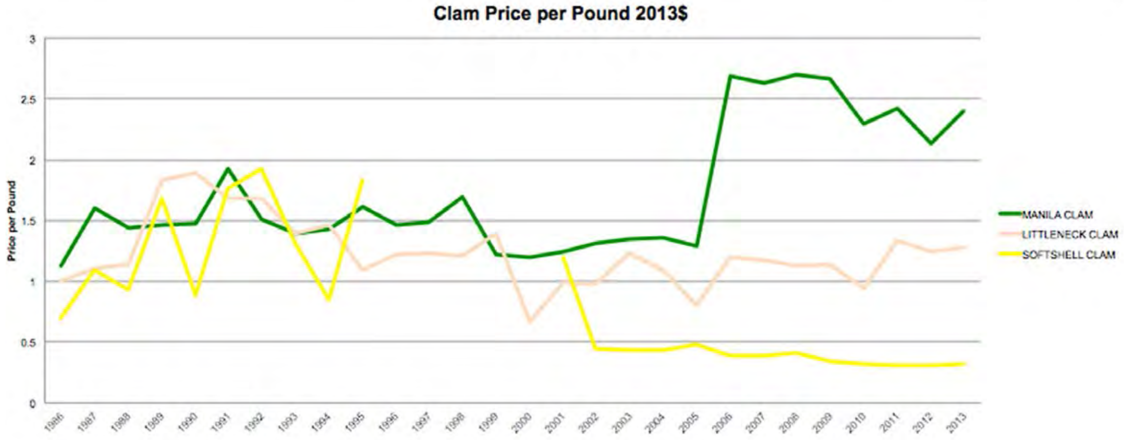

Figure 18. Average price in 2013 dollars for clams in Washington, 1986–2013.

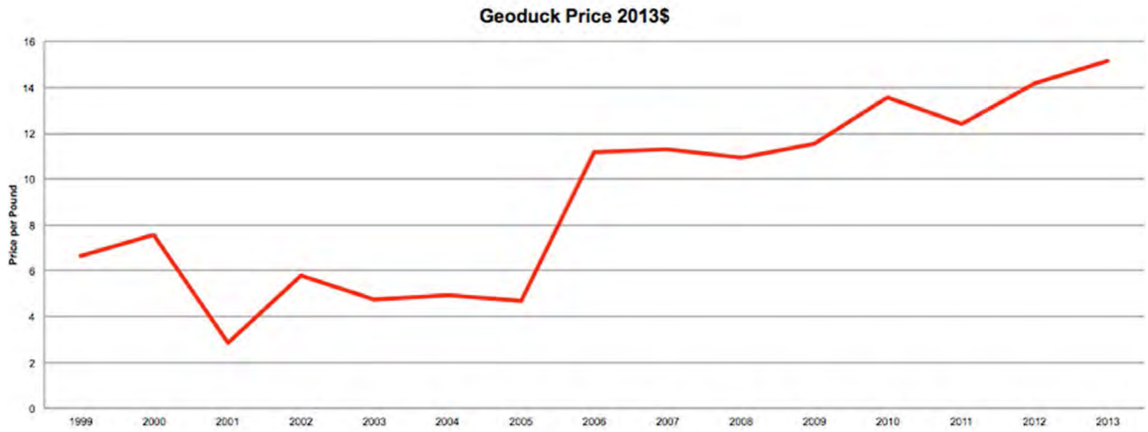

Figure 19. Average price in 2013 dollars for geoduck in Washington, 1999–2013.

Manila, littleneck, and softshell clams had similar pricing through the 1990s but began to diverge in the early 2000s (Figure 18). Now, there are relatively large price differences between them, with Manila providing the highest price, followed by littleneck and softshell. The price of littleneck clams has been relatively stable over time. The Manila clam has experienced a doubling in price — $1.29 in 2005 to $2.69 in 2006 — and has maintained prices around that level ever since. Softshell clam experienced high volatility and intermittent pricing until 2002, where prices stabilized between $0.31 and $0.48 (Figure 18) and a robust commercial fishery took hold. Geoduck has a significantly higher price than any of the other species being produced in large quantities, and pricing for geoduck appears to be continuing its upward trajectory (Figure 19).

Price stability for each species was evaluated by calculating the coefficient of variation (CV) of price, a unit-less measure that facilitates direct comparison between species over time (Table 10). A higher CV indicates a higher level of volatility in the price. Because the CV is unit-less, a doubling of the value indicates that the price is twice as volatile. The price difference was calculated by subtracting the average price during 2004–2008 from the average price during 2009–2013 using 2013 real prices. These two recent 5-year averages were used to provide price smoothing and to demonstrate the long-term pricing trend.

Overall, pricing volatility decreased between the two time periods, indicating that prices have been stabilizing. Kumamoto oysters had the highest price volatility between 2004 and 2008, and cockles between 2009 and 2013.

Industry Contribution

On average, the shellfish aquaculture industry generates $4.75 in revenue, $3.67 in expenses, and $1.08 in profit for every pound of shellfish produced (Table 11). Comparatively, for every acre under production, there is $5,497 in revenue, $4,987 in expenses, and $510 in profit (Table 11). The values are statewide averages, aggregated owing to data limitations. They may vary widely for an individual firm based on the species produced, production method, location, firm size, and level of vertical integration. These factors could substantially affect the individual firm's revenue, profit, and expenses, and wide variation in these values would be expected.

Tideland Lease Revenue

The WDNR leases state tidelands for the production of shellfish aquaculture. Lease amounts are based on a percentage of production and fluctuate from year to year. Lease revenue data include subtidal and intertidal leases but do not include physical structures — such as docks, moorings, or piers — or WDFW leases. Lease revenue information provided in Table 12 was obtained from WDNR's lease management system, NaturE. Pacific and Grays Harbor counties have 1,622 acres under lease that produce an average rental fee of $93 per acre. The Puget Sound region has fewer acres under agreement, but generates more lease revenue: The average in Puget Sound is about $1,900 per acre, twenty times more than Pacific and Grays Harbor counties. An estimated 2,288 acres are in production in Grays Harbor County and 17,288 acres in Pacific County, for a total 19,576 acres (Northern Economics 2013). This indicates that approximately 8% of farmed acreage in Pacific and Grays Harbor counties is leased from the state, and the remaining acreage is privately owned or leased. Puget Sound has an estimated 10,085 acres under production, 436 of which are leased from the state. This translates to about 4% of the tidelands in Puget Sound being leased from WDNR for shellfish aquaculture. The two coastal counties also have more total agreements and more acres covered in each agreement. In Washington State, an estimated 7% of shellfish production takes place on tidelands leased from WDNR.

Table 13 provides five years of data on state revenue generated by tideland leases for shellfish aquaculture. In 2010, WDNR received more than $1 million in lease revenue, but the amount dropped the subsequent year to less than half that sum. By 2014, revenue regained a level that was nearly equivalent to revenue generated in 2010.

Table 10. Average price per pound for each species and coefficient of variation (CV) for 2004–2008 and 2009–2013, and average price per pound difference between the two time periods. All prices in dollars. Created from WDFW production data.

Species	2004–2008 Average Price	2004–2008 CV	2009–2013 Average Price	2009–2013 CV	Average Price Difference
Mussels	1.50	0.36	1.75	0.17	0.25
Butter clam	0.60	0.23	0.52	0.53	-0.08
Cockle clam	0.25	0.03	0.59	0.59	0.34
Eastern oyster	7.18	0.93	14.12	0.21	6.94
Geoduck clam	8.60	0.40	13.37	0.11	4.76
Kumamoto oyster	14.71	0.96	25.35	0.17	10.64
Manila clam	2.13	0.35	2.38	0.08	0.25
Littleneck clam	1.08	0.15	1.19	0.13	0.11
Olympia oyster	70.15	0.53	131.64	0.25	61.49
Pacific oyster	2.89	0.19	3.33	0.26	0.43
Softshell clam	0.42	0.09	0.32	0.04	-0.10
Total	2.68	0.28	3.25	0.10	0.57

Table 11. Revenue, expenses, and profit for the shellfish aquaculture industry based on per pound and per acre units. Created from Northern Economics (2013).

	Dollars per Pound	Dollars per Acre
Revenue	4.75	5,497
Expenses	3.67	4,987
Profit/Income	1.08	510
Total	24.4 million pounds	18,450 farmed acres

Table 12. Statewide, Pacific and Grays Harbor counties, and Puget Sound aquaculture lease information. Source: Washington Department of Natural Resources (WDNR).

	Pacific and Grays Harbor Counties	Puget Sound	Statewide
WDNR Lease revenue	$150,781	$828,511	$979,292
Acres under lease	1622	436	2,058
Total leases	70	51	121
Average acres/lease	23	9	17
Revenue/acre	$93	$1,900	$476

Table 13. Statewide tidelands lease revenue, 2010–2014.

Year	Statewide WDNR tideland lease revenue for shellfish aquaculture
2014	$979,292
2013	$644,870
2012	$645,147
2011	$505,334
2010	$1,023,567

Ecosystem Services

Ecosystem services are the benefits provided to people from nature (Millennium Ecosystem Assessment 2005). Owing to the difficulty in valuing ecosystem services, many of them are frequently not considered when assessing economic value or contribution, and this may result in underestimation of the overall importance of these services. Shellfish, for example, play a key role in coastal ecosystems, contributing multiple services and providing value beyond their market price. While it is important for Washington State to recognize the economic value provided by ecosystem services from shellfish, limited work has been done and more is needed.

In general, ecosystem services can be separated into the four broad categories of provisioning, regulating, habitat or supporting, and cultural (Millenium Ecosystem Assessment 2005). Key ecosystem services that may be provided by shellfish include the following (adapted from Brumbaugh and Toropova 2008):

- Provisioning — subsistence and commercial fisheries, aquaculture, fertilizer and building materials, and jewelry and other decoration
- Regulating — protection of coastlines from storm surges and waves, water quality maintenance, reduction of shoreline erosion, and stabilization of submerged land by trapping sediments
- Habitat or supporting — nursery habitats and cycling of nutrients
- Cultural — tourism and recreation, and as a symbol of coastal heritage

Provisioning services are ecosystem services that describe the material or energy outputs from ecosystems. For shellfish, food and habitat provisioning are among the most widely cited services (Soto et al. 2008) and the economic value of food in particular is relatively easy to measure. However, remaining categories of ecosystem services are much more difficult to measure and remain largely unquantified for shellfish in Washington State.

Regulating services are those that act as regulators of other variables or processes in the ecosystem. For instance, as filter feeders, bivalves remove particulates including phytoplankton from the water column, which can help combat symptoms of eutrophication that primarily result from excessive nitrogen loading in coastal waters. In Oakland Bay, nitrogen removal through shellfish harvest amounted to 11.7 metric tons per year, or 0.87% of the total nitrogen loading from all sources (Steinberg and Hampden 2009). In Puget Sound, nitrogen removal by bivalves was 62 metric tons per year, or 0.04% of the total nitrogen load (Steinberg and Hampden 2009). Estimated value of the benefits to water quality from nitrogen removal ranged from $25,300 to $815,400 (2007 dollars) in Oakland Bay (Burke 2009) based on a replacement cost methodology. Similar methods could be used to extrapolate the economic value of nitrogen removal across the state. Changes to the costs of existing technology or the development of new technology could dramatically change the value of shellfish for nitrogen removal.

Shellfish aquaculture can also provide structured habitat, which can benefit species of commercial or conservation value. A single square meter of oyster reef may provide as much as 50 square meters of surface area, which provides attachment points and shelter for various plants and animals (Bahr and Lanier 1981). Oyster reefs attract a variety of species, resulting in complex interactions; these reefs are considered essential fish habitat (Coen et al. 1999).

Cultural ecosystem services are nonmaterial benefits people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences, as well as the identity and sense of place of an area provided by the ecosystem. Cultural ecosystem services from shellfish in Washington State can be seen in South Bend, which is known as the "Oyster Capital of the World," and Oysterville, which was named because of the rich oyster beds of Willapa Bay. These cities are symbolic of the region's heritage. Cultural ecosystem services are difficult to characterize and especially difficult to measure with an economic value (Chan et al. 2012, Donatuto and Poe 2015). This difficulty often results in their omission from decision making. Regardless of the framework used, it is important to include cultural services when assessing the economic contribution of shellfish to Washington State.

CONCLUSION

Washington State continues to be a national leader in shellfish aquaculture, and the industry continues to grow and innovate. The shellfish aquaculture industry is an important element of the overall Washington State economy. The industry provides needed revenues and jobs to the coastal economies of which it is a part; for example, it contributes as much as 20% of the total economy for Pacific County. Each region has a unique mix of species and the industry contributes varying levels of economic value to each region. Based on the geographic delineations used in this report, South Puget Sound generates more production and value than any other region: 37% of total harvest weight and almost 58% of the industry's value. Manila clam, Pacific oyster, and mussels continue to be important species for the entire Puget Sound, but geoduck is becoming more important to the region. Willapa Bay relies primarily on the production of Pacific oyster and Manila clam, and Grays Harbor relies almost exclusively on Pacific oyster. The Puget Sound region, particularly South Puget Sound, is increasing its reliance on geoduck, which introduces additional risk and price volatility to those growers in the region. Grays Harbor and Willapa Bay's reliance on Pacific oyster and Manila clam provides a level of stability to these regions, since the pricing for these species tends to be much less volatile.

Shellfish aquaculture is a profitable industry and can provide economic opportunities for those seeking entry. While future expenses are difficult to predict, pricing seems relatively consistent, adding some stability to the revenue side of the industry. Washington benefits from the taxes on revenue and jobs generated by the industry and also from revenue paid directly to the state to lease tidelands for production. Puget Sound generates substantially more lease revenue than other regions — more than five times the revenue of Grays Harbor and Willapa Bay combined. Since revenues are based on a percentage of production, a growing shellfish aquaculture industry means growing lease revenues for the state. In addition to the more explicit monetary contributions from the industry, ecosystem services should be considered in any analysis that seeks to evaluate the economic contribution of shellfish aquaculture.

In order to accurately assess the economic contribution of shellfish aquaculture to Washington State, an ongoing, accurate, and consistent data-gathering process is needed. Future research should address data limitations, the primary barrier to comprehensive and accurate representation of the industry. Additionally, production of shellfish does not exist in isolation. There are additional economic benefits from shellfish aquaculture created from secondary products and services such as shucking and packing houses, transport, manufacturing of prepared oyster products, and retail sales (Northern Economics 2013). These benefits are not captured in this report, and additional research into their economic contribution to Washington State would provide a more comprehensive picture of the contribution of the overall industry than that provided by only production. There is also a substantial knowledge gap in assessing the economic value of the ecosystem services provided by shellfish aquaculture. It is important to understand if these services differ from the services provided by natural stock and restored sites, as well as the effects of these services on value. Future research should focus on identifying and quantifying the value of ecosystem services and assessing changes.

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Evaluating Trophic and Non-Trophic Effects of Shellfish Aquaculture in the Central Puget Sound Food Web

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ABSTRACT

Expansion of the shellfish aquaculture industry may affect the structure and dynamics of coastal estuarine food webs. To better understand potential food web tradeoffs, trophic and non-trophic interactions (e.g., habitat facilitation, predator refuge) were incorporated into a food web model of Central Puget Sound to predict the potential effects of an increase in geoduck (*Panopea generosa*) aquaculture. At a basin scale, the food web can support at least a 120% increase in geoduck aquaculture over current production levels (based on landings of 10,546 kilograms in 2012), with only minor changes in individual species' biomass or metrics of ecosystem resilience. The non-trophic effects of increased geoduck aquaculture, related to the influence of anti-predator structure, had a stronger influence on the food web than the trophic role of cultured geoducks as filter feeders and prey to other species. Increased geoduck culture caused substantial increases in biomass densities of surf perches, nearshore demersal fishes, and small crabs, and decreases in seabirds, flatfishes, and certain invertebrates (e.g., predatory gastropods and small crustaceans). This study identifies species that should be a priority for additional empirical research and monitoring related to bivalve aquaculture interactions, including demersal fishes, small crustaceans, and seabirds. It also provides insights into the benefits and challenges of incorporating habitat-related data into a food web model. Understanding these relationships can inform management decisions by clarifying tradeoffs in ecosystem functions and services in Puget Sound, and can facilitate estimation of direct and cumulative effects of bivalve aquaculture at a food web scale.

INTRODUCTION

Bivalve aquaculture is a rapidly growing, global industry that occurs primarily in coastal waters and depends upon functioning, productive ecosystems. Interactions between cultured bivalves and the environment can vary with species, growout method, harvest and maintenance disturbance regimes, and development scale (Dumbauld et al. 2009, Simenstad and Fresh 1995). In regions with high bivalve densities and water retention times, bivalves may locally deplete phytoplankton (Asmus and Asmus 1991, Banas et al. 2007), potentially reducing symptoms of eutrophication (Zhou et al. 2006). However, bivalve aquaculture may also alter the composition of benthic communities (Cheney et al. 2012, Dubois et al. 2007, Dumbauld et al. 2009, Simenstad and Fresh 1995) and influence the abundance and distribution of higher trophic level animals such as seabirds (Connolly and Colwell 2005, Faulkner 2013, Zydelis et al. 2009). Understanding these potential interactions is important to sustainably manage industry expansion and is critical for supporting ecosystem-based management approaches to aquaculture development (Cranford et al. 2012, NRC 2010).

Food web models, such as Ecopath with Ecosim (EwE; Christensen and Walters 2004, Polovina 1984), are useful tools for addressing resource management issues in an ecosystem context. To date, applications of EwE to bivalve aquaculture have been restricted to modeling the trophic relationships of bivalves as filter feeders and prey to other species (Byron et al. 2011a, Jiang and Gibbs 2005, Leloup et al. 2008). However, bivalve aquaculture may also have important non-trophic effects. Changes in pelagic–benthic coupling, competition for space, prey concentration, predator refuge, and altered habitat structure (either biogenic structure or gear structure) may change the behavior of species and influence interspecific interactions (see review by Dumbauld et al. 2009; NRC 2010). The potential non-trophic effects of aquaculture are widely documented but are often difficult to incorporate into traditional food web models.

Mediation functions are a tool within Ecosim that simulate the influence of a third (mediating) variable on predator–prey interactions, following Wootton's (1994) definition of an interaction modification. Mediation functions can be used to describe non-trophic interactions between species or between species and habitats within a food web modeling framework (Ainsworth et al. 2008, Espinosa-Romero et al. 2011, Ma et al. 2010, Plummer et al. 2013). For example, mediation functions can be applied to systems in which shellfish farms modify the vulnerability of prey to predators through facilitation (e.g., concentrating prey, thereby increasing predation) or protection (e.g., refuge that decreases predation). The mediation effect is the enhancement or dampening caused by the shellfish farm on predator–prey interactions (Christensen et al. 2000). Widespread use of mediation functions is limited by the dearth of knowledge of their functional shape and the strength of the mediating relationships (Harvey 2014), and they typically require regionally specific, empirical data to paramaterize. McDonald et al.'s (2015) study on the interaction of geoduck aquaculture and the surrounding community provides the data needed to overcome these limitations.

Presently, geoduck (*Panopea generosa*) is the most valuable shellfish cultivated in intertidal Washington State. Recent reported landings have approached 589,670 kilograms with an estimated value of $18,500,000 (2010 aquaculture landings estimates, Washington Department of Fish & Wildlife [WDFW]). As suspension feeders, geoducks have a direct trophic effect on phytoplankton, but non-trophic effects resulting from the cultivation process may also influence community members (McDonald et al. 2012, McDonald et al. 2015, Price et al. 2012). Geoduck aquaculture production occurs on a five- to seven-year cycle. In the early phase of the cycle, a common practice is to protect newly outplanted juvenile geoduck (i.e., seed) from predators by placing them inside vertically oriented sections of polyvinyl chloride (PVC) tube (10-15 centimeters diameter) inserted into the tideflat; the tubes are then covered with netting to eliminate predator access (McDonald et al. 2015). Initial stocking density is typically 20-30 clams per square meter (VanBlaricom et al. 2015). These anti-predator structures are removed after approximately two years, once the clams have reached a size and depth that minimize most predation (McDonald et al. 2015). Market-sized geoducks are eventually harvested individually by hand in the sixth or seventh year in a process of liquefaction whereby a harvester uses a hose to inject large volumes of low-pressure water into sediments around the clam to loosen and extract it (VanBlaricom et al. 2015). In Puget Sound, Washington, McDonald et al. (2015) and VanBlaricom et al. (2015) showed that anti-predator structure and disturbance resulting from harvest of cultured geoducks, respectively, can suppress some benthic species while promoting others; thus, culture practices likely have important mediation effects. Empirical data from such studies can help evaluate the effects of geoduck aquaculture expansion on the food web and assess the relative importance of trophic versus non-trophic interactions on the community in a single modeling framework.

This study revised and expanded a previously published EwE model of the Central Puget Sound (Harvey et al. 2012a) to help evaluate the potential ecological effects of geoduck aquaculture expansion. Central Puget Sound is the largest of four subbasins that compose Puget Sound, a major fjordal system located in Washington State (Figure 1). Currently, Central Puget Sound supports significantly less geoduck harvest relative to other major shellfish-producing regions in Washington State, but the potential to develop geoduck culture further exists. In this study, investigators examined the potential effects of geoduck aquaculture on the Central Puget Sound ecosystem. Specific goals were to (1) explore the potential influence of trophic and non-trophic interactions on biomass predictions in a food web model and (2) identify potential community and ecosystem responses to increased geoduck farming. First an existing, dynamic, mass-balanced food web model of Central Puget Sound was modified to include cultured shellfish functional groups and mediation functions were added that captured the non-trophic effects of geoduck culture on the surrounding food web. Subsequently, the potential trophic and non-trophic effects of expanded geoduck aquaculture on community structure were calculated under varying scenarios of expansion.

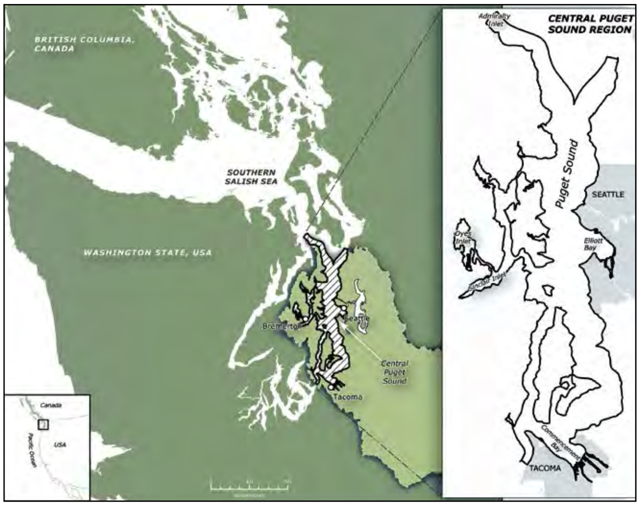

Figure 1. Map of Central Puget Sound, the spatial domain for the Ecopath with Ecosim (EwE) model, and the rest of the southern Salish Sea, as well as catchment areas (lightly shaded) that feed directly into Central Puget Sound. Inset shows Puget Sound in more detail (Harvey et al. 2012a).

MATERIALS AND METHODS

Model Development

A recently parameterized EwE model of Central Puget Sound (Harvey et al. 2012a) was modified to incorporate ecological relationships between geoduck aquaculture and the larger food web. The Central Puget Sound model domain drains a total area of 35,500 km^2, encompassing all marine habitat between the Tacoma Narrows (47.2681°N, 122.5506°W) in the south to Whidbey Island (47.9013°N, -122.3778°W) in the north (Figure 1). Central Puget Sound includes intertidal habitats dominated by sand, gravel, and occasional eelgrass or algal habitats and mud-bottomed subtidal habitats that exceed depths of 250 m in some areas (Figure 1). In addition, the region includes large bays and numerous pocket estuaries, and it receives freshwater inputs from moderately sized rivers (Cedar, White, and Green rivers).

As a general overview, investigators first revised the EwE model to include additional taxonomic detail regarding nearshore biota relevant to intertidal bivalve aquaculture. Next, they incorporated mediation functions into the model that corresponded to the non-trophic effects of geoduck culture on other species. The functions were directly informed by field experiments and observations (McDonald et al. 2015) and corresponded to mediation effects that reduced the vulnerability of certain species to predation (i.e., predator refuge) or increased the search rate of predators (i.e., habitat exclusion). Last, investigators ran scenarios in Ecosim simulating increased geoduck aquaculture.

The Ecopath model (Christensen and Pauly 1992, Polovina 1984) balances biomass gains and losses for each functional group using the following expression:

$$B_i \cdot \left(\frac{P}{B}\right)_i \cdot EE_i = BA_i + Y_i + \sum_{j=1}^{n} B_j \cdot \left(\frac{Q}{B}\right)_j \cdot DC_{ij} \qquad \text{Equation (1)}$$

where the biomass (B), production to biomass ratio (P/B) and ecotrophic efficiency (EE) (the fraction of production used in the system) of prey group *i* are balanced with the biomass accumulation (*BA*) and mortalities due to fisheries (*Y*) of prey group (*i*), and predation by all groups *j*. Predation mortality is calculated using the biomass of all predator groups *j*, the consumption to biomass ratio (*Q/B*) of all predator groups, and the fraction of group *i* in the diet of each group *j* (*DC*). Ecopath uses matrix inversion to calculate one parameter (often *B* or *EE*) for each group based on inputs of the other parameters such as diet, production, consumption, and mortality rates.

Ecosim adds a temporal dynamic to the food web model, allowing biomass of functional groups to change based on trophic dynamics, harvest, other mortality, immigration, and emigration. A set of differential equations are solved in Ecosim based on the following form:

$$\frac{dB_i}{dt} = g_i \sum_j Q_{ji} - \sum_j Q_{ij} + I_i - (M_i + F_i + e_i)B_i \qquad \text{Equation (2)}$$

where $dB_i \cdot dt^{-1}$ represents the growth rate of group *i*. Biomass increases with net growth efficiency (g_i), total consumption of group *i* (Q_{ji}), and immigration (I_i). Biomass decreases with predation mortality (Q_{ij}) by all predators on group *i*, non-predation mortality (M_i), fishing mortality (F_i), and emigration (e_i).

The Central Puget Sound model was revised to include additional detail in nearshore functional groups and cultured geoduck groups (Table 1 and Appendix tables 1–3, p. 27). Specifically, migratory shorebirds (e.g., dunlins, great blue herons), small brachyuran crabs, and red rock crab were added. Also, the existing infaunal bivalve group was divided into two groups: large- and small-bodied bivalves. Large-bodied bivalves consisted principally of species of interest to recreational and commercial harvesters (e.g., butter clam, horse clam, heart cockle). Small-bodied bivalves included those not targeted by commercial or recreational harvest (e.g., purple *Transennella* and amethyst gem clam).

Cultured geoducks were added as a multistanza group to separate the stages in which anti-predator structure is present (years 1 to 2), anti-predator structure is absent (years 3 to 5), and harvest occurs (years 6 to 7). The Central Puget Sound standing stock biomass was calculated based on the 2012 aquaculture landings estimate of 10,546 kilograms (WDFW) and an average geoduck weight of 0.7 kilograms at harvest. Estimated natural mortality rate is 50% from outplanting to harvest, with half the mortality occurring in the first 2 years (B Phipps, Taylor Shellfish, J Gibbons, Seattle Shellfish, personal communication). The von Bertalanffy growth equation was used to calculate individual growth (maximum length = 158 millimeters, length at maturity = 75 millimeters, k = 0.19; Bradbury and Tagart 2000, Calderon-Aguilera et al. 2010), and logistic growth was used to estimate number of geoducks over time. von Bertalanffy growth was used to keep consistent with the Ecopath biomass calculations for multi-stanza groups. Density (metric tons per square kilometer (t km^{-2})) was determined by dividing these biomass estimates by the product of total area in Central Puget Sound (757.08 square kilometers (km^2); Harvey 2012a) and proportion of that area in the 0- to 10-meter depth range (0.14 km^2; Harvey 2012a). The resulting densities are 5.3 t km^{-2} (year 1-2), 9.7 t km^{-2} (year 3-5), and 5.03 t km^{-2} (year 6-7). The density would be largely underestimated in planted areas and overestimated in unplanted areas. This is consistent with how other Ecopath population densities are estimated.

Mediation

Ecosim mediation functions can simulate the influence of a functional group or species on the strength of predator–prey interactions between a different pair of species. The consumption rate (*Q*) of prey (*i*) by predator (*j*) is defined in Ecosim as follows:

$$Q = \left(\frac{a_{ij}}{A_{ij}}\right) \cdot \frac{v_{ij} \cdot B_i}{(2V_{ij} + \frac{a_{ij}}{A_{ij}} \cdot P_j)} \cdot P_j \qquad \text{Equation (3)}$$

where a_{ij} is the rate of effective search for *i* by *j*, A_{ij} is the search area in which *j* forages for *i*, v_{ij} is the flow rate of biomass (B_i) between pools that are vulnerable or invulnerable to predation, and P_j is the abundance of *j* in A_{ij}. A mediation function influences a_{ij}, A_{ij}, and (or) v_{ij} according to a user-defined function. An increased v_{ij} makes *i* subject to greater top-down control and increasing a_{ij} makes *j* a more efficient consumer of *i*. Input mediation multipliers range from zero to one and are rescaled by Ecosim to equal one when the biomass of the mediating group is at its initial baseline density.

Investigators included two sets of mediation functions: non-aquaculture related interactions previously published for the Central Puget Sound model (Harvey et al. 2012b, Harvey 2014, Plummer et al. 2013), and those based on an empirical study of the effects of geoduck culture on macrobenthic communities in South Puget Sound (Table 1; McDonald et al. 2015). Following Plummer et al. (2013), increasing eelgrass biomass was allowed to positively mediate v_{ij} values for the prey of juvenile salmon (i.e., greater top-down control as eelgrass aggregates prey); negatively mediate v_{ij} values for juvenile salmon and young of the year crab (i.e., more bottom-up control as eelgrass increases and provides refuge from nearshore predators); and positively mediate the a_{ij} value for juvenile Pacific herring (greater juvenile herring productivity as eelgrass increases and provides spawning substrate). Harvey et al. (2012a) described a behavioral mediation effect where resident and overwintering bald eagles (the mediating groups) harass nearshore diving and herbivorous seabirds, which causes them to expend more energy to avoid eagle predation while foraging. That is, the variables A_{ij} (of the nearshore diving and herbivorous seabirds) and v_{ij} (of their prey), which relate foraging ability, were modeled as a decreasing function of increasing eagle biomass.

The geoduck aquaculture mediation functions are primarily based on observed numerical responses of benthic invertebrates to anti-predator structure (partially buried PVC tubes with net covers) placed on plots with outplanted geoducks over their first two years (Table 1). Functional groups thought to gain refuge from the anti-predator structure, and that exhibited higher biomass densities inside geoduck plots with anti-predator structure, had mediation functions wherein vulnerability to

Table 1. Mediation effects specific to geoduck culture in Puget Sound (McDonald et al. 2015) and added to the central Puget Sound EwE model. Sign (+ or -) in the EwE Group column indicates the effect of geoduck culture on the functional group, as observed by McDonald et al. (2015). The superscript numbers 1 and 3 associated with the mediation parameter indicate whether the mediation function is based on the effect of anti-predation structure in the first stanza of culture (years 1 and 2) or due to harvest disturbance in the third stanza (years 6 or 7). Mediation parameters correspond to an increase (+) or decrease (-) in the vulnerability (v_{ij}) of the prey ($_i$) or search rate ($_{aij}$) on the predator ($_j$).

Species/group *(McDonald et al. 2015)*	**EwE group**	**Mediation parameter**
Starry flounder (*Platichthys stellatus*) Sand sole (*Psettichthys melanostictus*)	Small mouth flatfishes (-)	$-\alpha_{\text{surf perch, small mouth flatfishes}}^{(1)}$ $-\alpha_{\text{shrimp, small mouth flatfishes}}^{(1)}$ $-\alpha_{\text{YOY crab, small mouth flatfishes}}^{(1)}$ $-\alpha_{\text{other grazers, small mouth flatfishes}}^{(1)}$ $-\alpha_{\text{small crabs, small mouth flatfishes}}^{(1)}$ $-\alpha_{\text{small mouth flatfishes, barnacles}}^{(1)}$ $-\alpha_{\text{soft infauna, small mouth flatfishes}}^{(1)}$ $-\alpha_{\text{deposite feeders, small mouth flatfishes}}^{(1)}$
Speckled sanddab (*Citharichthys stigmaeus*)	Piscivorous flatfishes (-)	$-\alpha_{\text{surf perch, piscivorous flatfishes}}^{(1)}$ $-\alpha_{\text{demersal fishes, piscivorous flatfishes}}^{(1)}$ $-\alpha_{\text{shrimp, piscivorous flatfishes}}^{(1)}$ $-\alpha_{\text{other grazers, piscivorous flatfishes}}^{(1)}$ $-\alpha_{\text{small crabs, piscivorous flatfishes}}^{(1)}$ $-\alpha_{\text{barnacles, piscivorous flatfishes}}^{(1)}$ $+\alpha_{\text{soft infauna, piscivorous flatfishes}}^{(1)}$
Saddleback gunnel (*Pholis ornate*) Pinpoint gunnel (*Apodichthys flavidus*) Crescent gunnel (*Pholis laeta*) Bay pipefish (*Syngnathus leptorhynchus*) Snake prickelback (*Lumpenus sagittal*) Tubesnout (*Aulorhynchus flavidus*)	Demersal fishes (+)	$-\upsilon_{\text{demersal fishes, sea lions}}^{(1)}$ $-\upsilon_{\text{demersal fishes, gulls}}^{(1)}$ $-\upsilon_{\text{demersal fishes, resident birds}}^{(1)}$ $-\upsilon_{\text{demersal fishes, migratory birds}}^{(1)}$ $-\upsilon_{\text{demersal fishes, great blue herons}}^{(1)}$ $-\upsilon_{\text{demersal fishes, migratory eagles}}^{(1)}$ $-\upsilon_{\text{demersal fishes, resident eagles}}^{(1)}$ $-\upsilon_{\text{demersal fishes, juvenile wild salmon}}^{(1)}$ $-\upsilon_{\text{demersal fishes, juvenile hatchery salmon}}^{(1)}$ $-\upsilon_{\text{demersal fishes, piscivorous flatfish}}^{(1)}$
Shiner surf perch (*Cymatogaster aggregate*)	Surfperch (+)	$-\upsilon_{\text{surfperch, resident birds}}^{(1)}$ $-\upsilon_{\text{surfperch, migratory birds}}^{(1)}$ $-\upsilon_{\text{surfperch, great blue herons}}^{(1)}$ $-\upsilon_{\text{surfperch, migratory eagles}}^{(1)}$ $-\upsilon_{\text{surfperch, resident eagles}}^{(1)}$ $-\upsilon_{\text{surfperch, juvenile wild salmon}}^{(1)}$ $-\upsilon_{\text{surfperch, juvenile hatchery salmon}}^{(1)}$ $-\upsilon_{\text{surfperch, piscivorous flatfish}}^{(1)}$ $-\upsilon_{\text{surfperch, small mouth flatfishes}}^{(1)}$ $-\upsilon_{\text{surfperch, demersal fishes}}^{(1)}$ $+\upsilon_{\text{surfperch, demersal fishes}}^{(1)}$ $-\upsilon_{\text{surfperch, YOY crab}}^{(1)}$
Red rock crab (*Cancer productus*)	Red rock crab (+)	$-\upsilon_{\text{red rock crab, gulls}}^{(1)}$ $-\upsilon_{\text{red rock crab, resident birds}}^{(1)}$ $-\upsilon_{\text{red rock crab, demersal fishes}}^{(1)}$ $+\upsilon_{\text{red rock crab, demersal fishes}}^{(1)}$ $-\upsilon_{\text{red rock crab, octopus}}^{(1)}$ $-\upsilon_{\text{red rock crab, sea stars}}^{(1)}$ $+\upsilon_{\text{red rock crab, sea stars}}^{(1)}$

continued next page

Table 1. · continued from previous page

Pacific moon snail (*Euspira lewisii*)	Predatory gastropods (-)	$-\alpha_{\text{urchins, predatory gastropods}}^{(1)}$ $-\alpha_{\text{other grazers, predatory gastropods}}^{(1)}$ $-\alpha_{\text{mussels, predatory gastropods}}^{(1)}$ $-\alpha_{\text{barnacles, predatory gastropods}}^{(1)}$ $\alpha_{\text{large infaunal bivalves, predatory gastropods}}^{(1)}$ $-\upsilon_{\text{small infaunal bivalves, predatory gastropods}}^{(3)}$ $+\upsilon_{\text{small infaunal bivalves, predatory gastropods}}^{(3)}$ $-\alpha_{\text{suspension feeders, predatory gastropods}}^{(1)}$ $-\alpha_{\text{tunicates, predatory gastropods}}^{(1)}$
Heart cockle (*Clinocardium nuttallii*)	Large infaunal bivalves (+ (1) /- (3))	$-\upsilon_{\text{large infaunal bivalves, gulls}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, gulls}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, nearshore birds}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, nearshore birds}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, migratory shorebirds}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, migratory shorebirds}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, surf perch}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, surf perch}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, piscivorous flatfishes}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, piscivorous flatfishes}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, small mouth flatfishes}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, small mouth flatfishes}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, demersal fishes}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, demersal fishes}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, octopus}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, octopus}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, YOY crab}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, YOY crab}}^{(3)}$
Heart cockle (*Clinocardium nuttallii*)	Large infaunal bivalves (+ (1) /- (3))	$-\upsilon_{\text{large infaunal bivalves, red rock crab}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, red rock crab}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, sea stars}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, sea stars}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, small crabs}}^{(1)}$ $-\upsilon_{\text{large infaunal bivalves, small crabs}}^{(3)}$ $+\upsilon_{\text{large infaunal bivalves, small crabs}}^{(3)}$ $-\upsilon_{\text{large infaunal bivalves, predatory gastropods}}^{(1)}$ $+\upsilon_{\text{large infaunal bivalves, predatory gastropods}}^{(3)}$
Corophium amphipods	Small crustaceans (- (1) /+ (3))	$-\alpha_{\text{bacteria, small crustaceans}}^{(1)}$ $+\upsilon_{\text{bacteria, small crustaceans}}^{(3)}$ $+\alpha_{\text{phytoplankton, small crustaceans}}^{(1)}$ $+\upsilon_{\text{phytoplankton, small crustaceans}}^{(3)}$ $+\alpha_{\text{benthic microalgae, small crustaceans}}^{(1)}$ $+\upsilon_{\text{benthic microalgae, small crustaceans}}^{(3)}$ $+\alpha_{\text{benthic macroalgae, small crustaceans}}^{(1)}$ $+\upsilon_{\text{benthic macroalgae, small crustaceans}}^{(3)}$ $+\alpha_{\text{eelgrass, small crustaceans}}^{(1)}$ $+\upsilon_{\text{eelgrass, small crustaceans}}^{(3)}$ $+\alpha_{\text{algal/plant matter, small crustaceans}}^{(1)}$ $+\upsilon_{\text{algal/plant matter, small crustaceans}}^{(3)}$ $+\alpha_{\text{detritus, small crustaceans}}^{(1)}$ $+\upsilon_{\text{detritus, small crustaceans}}^{(3)}$

predation (vij) decreased as a function of increasing geoduck culture (Table 1). If a prey and its predator species both had higher biomass densities inside geoduck anti-predator structure, two separate positive and negative mediation functions on the predation vulnerability of the prey species were added, as investigators could not determine how the predator–prey dynamics would play out (e.g., demersal fish prey upon surf perch and both groups had higher biomasses inside geoduck farms; Table 1). For groups that showed lower biomass densities inside geoduck plots and that were thought to be excluded (e.g., flatfishes and predatory gastropods, Table 1), their search rates (aij) were set to decrease as a function of increasing cultured geoduck biomass (Table 1). That is, they became less efficient at finding prey. These geoduck mediation effects were only applied to predator–prey functional groups found in intertidal habitats where geoduck farms are likely to be sited.

McDonald et al. (2015) found anti-predatory structure on geoduck plots to have an exclusionary effect on flatfishes and predatory gastropods (moon snail), and an attraction effect on demersal fishes (e.g., gunnels, shiner perch), small crabs, sea stars, and red rock crabs (Table 1). The small crustaceans and large infaunal bivalve groups were unique in that they had relationships to multiple geoduck stanzas (i.e., the youngest geoduck stanza associated with anti-predator structure and the oldest stanza subject to harvest). Small crustacean biomass density (based on *Corophium* amphipods) decreased in geoduck plots with anti-predator structure and was assumed to be excluded from the plots (their search rate aij decreased; Table 1). During the geoduck harvest stage, small crustacean biomass densities increased and predator refuge was assumed (their vulnerability vij decreased; Table 1). Large infaunal bivalve biomass (based on the heart cockle) increased in geoduck anti-predator structure (i.e., predator refuge; their vulnerability vij decreased) and decreased during the final, harvest stage of cultured geoducks (i.e., habitat exclusion; their search rate a_{ij} decreased; Table 1).

In the absence of empirical data on the shape and strength of these functions, the shape of all mediation functions was set to a hyperbolic function, as this is the most conservative approach (Harvey et al. 2014); the function was defined as follows:

$$\frac{M_{min}+(M_{max}+M_{min})}{1+k\cdot B} \qquad \text{Equation (4)}$$

where the endpoints are defined by M_{max} (Ecosim: Y_{zero}) and M_{min} (Ecosim: Y_{end}) and the curve has a gradient of *k* (Ecosim: Y_{base}). The values for each parameter were set to 2, 0, and 1, respectively, for all functional groups with the exception of small crustaceans. The small crustaceans group comprises mysid shrimps, cumaceans, benthic amphipods, and benthic isopods. Because benthic amphipods are directly targeted by a cultured geoduck mediation effect (Table 1), but make up only one third of the small crustaceans group as defined by Harvey et al. (2012a), investigators made the functional curve for this mediation effect more conservative while keeping the same hyperbolic trend by setting *k* to 1.5.

Analysis

The analysis consisted of two phases. The first phase entailed estimating the ecological carrying capacity for cultured geoducks in Central Puget Sound and assessing the presence of ecological thresholds related to increasing geoduck aquaculture. The second phase involved identifying trophic and non-trophic effects of geoduck culture on individual functional groups. Ecological carrying capacity is the biomass of cultured geoducks that can be supported by the existing levels of phytoplankton production (as defined by Harvey et al. 2012a) before the food web becomes unbalanced. The food web was deemed "unbalanced" when the ecotrophic efficiency of phytoplankton exceeded a value of 1 (as calculated by the mass-balance algorithm described in Equation 1); this phenomenon occurs when phytoplankton grazing mortality exceeds total productivity (Byron et al. 2011b, Jiang and Gibbs 2005).

Ecological carrying capacity was calculated by incrementally increasing the cultured geoduck biomass and associated landings until reaching the ecological carrying capacity threshold. Cultured geoduck biomass and landings were increased proportional to the base model values.

Changes in ecosystem attributes were calculated by using four established indices: the Ecosystem Reorganization Index, the Shannon Diversity Index, Mean Trophic Level (MTL), and Mixed Trophic Impact (MTI; Libralato et al. 2006, Samhouri et al. 2010). The attributes describe the capacity of an ecosystem to absorb perturbations while retaining essential structure and function, and they quantify the ecosystem impact of individual functional groups. The ecosystem reorganization index approximates ecosystem resilience (Folke et al. 2004) by measuring the extent to which perturbations cause changes in the relative biomass of individual functional groups ($B_{t,i}$) (Samhouri et al. 2009):

$$R = -\left[\sum_i \left|\frac{B_{t_2,i}-B_{t_1,i}}{\sum_i B_{t_1,i}}\right| - \left|\frac{\sum_i B_{t_2,i}-\sum_i B_{t_1,i}}{\sum_i B_{t_1,i}}\right|\right]\cdot 100 \qquad \text{Equation (5)}$$

A value of *R* farther from zero indicates lower resilience, implying the aggregate biomass and the individual functional groups respond differently in magnitude and direction to a pressure. This is a relative index, with zero as the lower bound (unstressed) and an unlimited upper bound (stressed) dependent on changes in biomass. Shannon Diversity Index and a biomass-weighted MTL of the food web was used as additional indicators of how changes in cultured geoduck biomass might affect overall food web structure. Lower species diversity generally indicates a more stressed ecosystem as species dominance increases and functional redundancy decreases (Odum 1985). Lower MTL indicates shorter food chains and a more stressed food web due to reduced energy flow at higher trophic levels or greater sensitivity of predators to stress or both (Odum 1985). The MTI (m_{ij}) quantifies the direct and indirect impacts of (impacting) group *i* on (impacted) group *j* across all trophic pathways that link the two groups, as calculated in Ecopath with Ecosim software. The index does not include connections via mediation functions and thus does not represent non-trophic interactions. The cumulative MTI (ε_i) was calculated to determine the net influence of each functional group on the food web following Libralato et al. (2006):

$$\varepsilon_i = \sqrt{\textstyle\sum_{j \neq i}^{n} m_{ij}^2} \qquad \text{Equation (6)}$$

The trophic and non-trophic effects of adding cultured geoduck to Central Puget Sound were evaluated by creating three versions of the model: (1) current (low) level of cultured geoducks (base model), (2) 120% cultured geoduck biomass but no geoduck mediation functions (i.e., trophic effects only), and (3) 120% cultured geoduck biomass with geoduck mediation functions (i.e., trophic and non-trophic effects). To perturb the food web, cultured geoduck biomass and associated landings were increased by 120% in 50 years. A 120% increase represented a realistic level of increase in geoduck aquaculture and was a large enough perturbation to allow examination of changes across multiple trophic levels, habitats, and life histories (e.g., birds, pelagic and demersal fishes, and invertebrates). Functional group biomass predictions from the base model (low cultured geoduck biomass) were compared with those from the model with 120% cultured geoduck biomass and no geoduck mediation effects (trophic effects only), as well as the model with 120% cultured geoduck biomass with geoduck mediation functions (trophic and non-trophic effects) to determine the possible ecological impacts of expanding geoduck aquaculture. Investigators calculated the percent change in relative biomass of each functional group in year 50. They then ran the 50-year simulations with individual mediation functions turned off to determine their specific effects on the target functional group as well as their impact on other trophically linked functional groups in the food web. Finally, they ran simulations with only individual mediation functions turned on for demersal fishes and small crustaceans to determine their influence throughout the food web. These functional groups represent important prey for a large portion of the food web and are likely to have disproportionate effects on food web dynamics.

RESULTS

A 120% increase in cultured geoduck biomass had a limited impact on phytoplankton biomass and measures of ecological resilience. The current cultured geoduck standing stock is approximately 0.1% of the estimated ecological carrying capacity in Central Puget Sound (5,928 t km^{-2}). At this threshold, the ecotrophic efficiency of phytoplankton exceeded a value of one owing to grazing mortality exceeding total phytoplankton productivity. As cultured geoduck biomass approached 120% over its initial level, the Ecosystem Reorganization Index diverged from zero by a small amount, indicating a slight reduction in stability; the Mean Trophic Level slightly increased, indicating increased stability; and the Shannon Diversity index remained constant (Table 2). The MTI was very low for cultured geoduck (ranking in the bottom 10 of all 79 functional groups) (Appendix Table 4, p. 31).

The addition of cultured geoducks into the Central Puget Sound food web without any mediation functions had very little impact on the simulated biomasses of other food web members (Appendix Table 5, p. 32). That is, after increasing the geoduck biomass by 120% over 50 years, the direct trophic effect of geoduck as a grazer on phytoplankton and as prey resource to other species was nearly negligible. The biomass densities of two geoduck predator groups, sea stars and age 4+ Dungeness crab, increased by 2% while all other food web members varied by less than 1% (Appendix Table 5). The low MTI values for cultured geoduck further support these results (Appendix Table 4).

In contrast, the addition of cultured geoduck mediation functions had a notable impact on the food web (Figure 2, Appendix Table 5). The biomass of food web members that were linked to geoduck culture through mediation functions changed considerably, with the biomass densities of some members increasing and decreasing by more than 20% (e.g., surf perches, small crabs, predatory gastropods, and small mouth flatfishes; Figure 2). In addition, changes in the biomass of food web members

Table 2. Ecosystem attributes measured in response to increased geoduck biomass in the Central Puget Sound food web. Attributes reflect system conditions at the end of 50-year simulations.

	Percent increase in geoduck biomass (tons per square kilometer)							**Unstressed state**
Attribute	20%	70%	80%	90%	100%	110%	120%	
Ecosystem Reorganization Index	0.65	2.34	2.68	3.01	3.34	3.65	3.97	Close to 0
Shannon Diversity Index	3.23	3.23	3.23	3.23	3.23	3.23	3.23	High
Change in Mean Trophic Level relative to base	0.02	0.05	0.05	0.06	0.06	0.06	0.06	High MTL

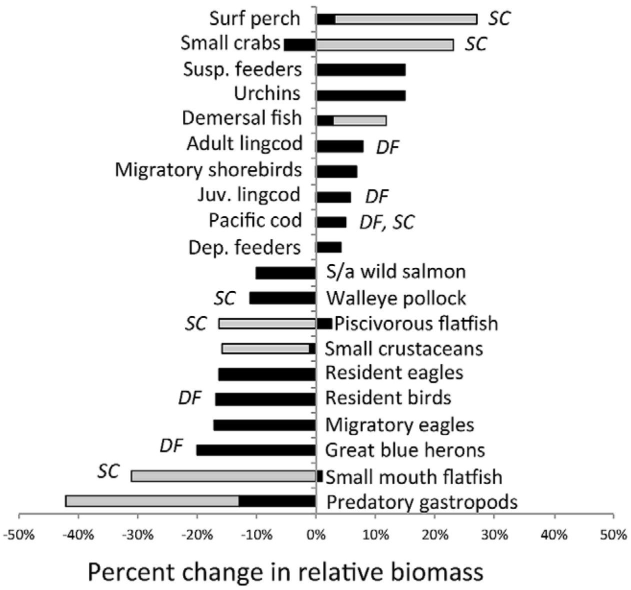

Figure 2. Functional groups with the greatest change in relative biomass between initial conditions and a simulated 120% increase in geoduck biomass over 50 years. Change in biomass resulting from targeted mediation effects (gray) or trophic connections to groups targeted by mediation effects (black) are indicated. For all but 'small crabs', effects are additive. DF (demersal fish) and SC (small crustaceans) denote if those groups are one of their top three prey (as defined by Ecosim). Relative changes in biomass for all food web members is in Appendix Table 5.

directly linked to geoduck culture propagated through the food web, contributing to additional changes in other members' biomass (Figure 2 and Appendix Table 5).

In total, the biomasses of 9 of the 10 functional groups with cultured geoduck mediation functions changed substantially and were among the top 20 groups demonstrating the greatest change in biomass (Figure 2). Red rock crab was the one exception, which showed <1% change in biomass and had a negative trend despite a positive mediation function (Appendix Table 5). Small crab biomass increased as a direct effect of the targeted mediation function and decreased without it (Figure 2). Geoduck mediation functions linked to demersal fishes and small crustaceans had substantial effects on the food web (Figure 3), supported by the high cumulative MTI values for demersal fishes and small crustaceans (ranked 11th and 25th of 79 functional groups; Appendix Table 4). For example, the cultured geoduck–demersal fish mediation function resulted in decreases in herons (-23%) and resident birds (-17%), and increases in Pacific cod (+7%) and harbor seals (+7%; Figure 3). The cultured geoduck–small crustacean mediation functions resulted in reductions in the biomasses of juvenile wild salmon (-7%) and juvenile hatchery salmon (-4%).

DISCUSSION

Food web models focused on evaluating the ecological effects of aquaculture have largely neglected non-trophic effects. This study's analysis demonstrates the importance of including non-trophic interactions when evaluating the ecological effects of shellfish aquaculture. Accounting for trophic and non-trophic interactions demonstrated that the central Puget Sound food web can support an increase in geoduck aquaculture with limited changes in individual species' biomass and ecosystem resilience at a basin scale. Also, several food web members were identified that may be substantially affected by increased geoduck culture. In contrast, models with only trophic effects of cultured geoduck predicted negligible changes in biomass for food web members due to geoduck aquaculture.

Habitat modification and facilitation are the predominant ecological effects of geoduck aquaculture in a highly productive system such as Central Puget Sound. The trophic impacts of cultured geoducks as both grazers and prey were not influential at the system level. Cultured geoducks did not substantially reduce the availability of phytoplankton for other species, as demonstrated by the small impact on ecological carrying capacity. In addition, geoduck predators (moon snails, starfish, flatfishes, red rock crab, and sea birds) are all generalists to varying degrees and showed limited change in biomass in response to increased geoduck aquaculture. However, the impact of anti-predator structure (PVC tubes and nets) placed on geoduck plots had a larger influence on the surrounding food web by providing predation refuge or by changing foraging opportunities. In turn, these effects propagated throughout the food web. The ecological effects of aquaculture structure and habitat modification have been observed for other bivalve species in a range of systems (reviewed in Coen et al. 2011). Pacific oyster on-bottom culture may reduce eelgrass densities, blade size, and growth rates (Dumbauld et al. 2009, Tallis et al. 2009), and mudflat graveling for clam cultivation may alter benthic community composition (Simenstad and Fresh 1995, Thom et al. 1994). This study suggests that efforts to understand the ecological effects of shellfish aquaculture in productive systems should go beyond modeling the direct trophic effects of bivalves and incorporate non-trophic information when possible. In addition, empirical research is required to determine the functional form and strength of these non-trophic interactions to better determine their influence on the surrounding community (Harvey 2014).

Food web members sensitive to changes in increased geoduck aquaculture represent various habitats, trophic levels, and life histories, and are candidate indicators for environmental impacts of increased bivalve aquaculture (e.g., Samhouri et al. 2009). Notably, these species were only sensitive to changes in cultured geoduck with the inclusion of non-trophic mediation effects. Some of these food web members (birds, salmon, benthic fishes) are already represented in existing and suggested indicator lists of ecosystem health for Puget Sound (Harvey et al. 2014, Kershner et al. 2011, Puget Sound Partnership 2013), which is partly due to the existence of ongoing monitoring pro-

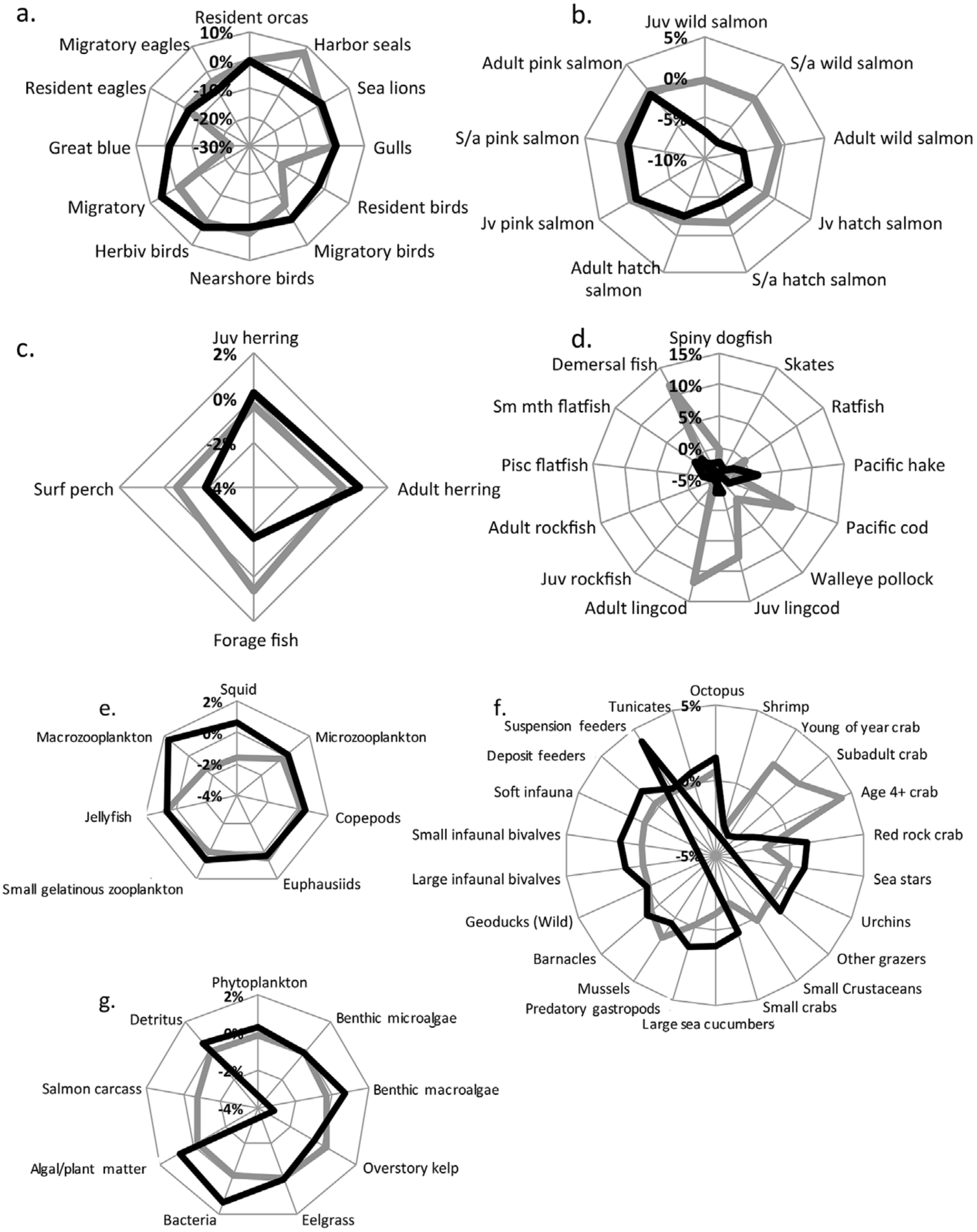

Figure 3. Percent change in relative biomass due to the addition of individual geoduck mediation effects (see Table 2 for details) on demersal fish (gray lines) and small crustaceans (black). Food web groups are divided according to: (a) marine mammals and birds, (b) salmon, (c) pelagic vertebrates, (d) benthic vertebrates, (e) pelagic invertebrates, (f) benthic invertebrates, and (g) primary producers, microbial, and detrital groups.

grams. Other species sensitive to geoduck culture (nearshore demersal fishes, small crustaceans, and flatfishes) are less consistently sampled in the region but may also prove informative as indicators. Our indicators of ecosystem structure and function (MTL, Shannon Biodiversity Index, Ecosystem Reorganization Index, and MTI) did not show conclusive trends, implying the effects of geoduck culture may be more influential at the species versus the system level. Additional diet, life history, and aquaculture interaction data for nearshore demersal fishes, small crustaceans, and various bird groups would improve the model and further refine the list of candidate ecosystem indicators for geoduck aquaculture.

The demersal fish and small crustacean functional groups were sensitive to increased cultured geoduck biomass and subsequently influenced biomass changes throughout the food web. The species' substantial bottom-up influence is due to the aggregation of multiple key prey species into single functional groups and their multiple trophic connections across the food web. The demersal fish community (e.g., poachers, eelpouts, and sculpins) is one of the most diverse and abundant in Puget Sound; however, relatively little is known of their biomass, diet, and life history (Harvey et al. 2012a, Reum and Essington 2008). In the model, demersal fishes benefit from predator refuge provided by the anti-predation structure on geoduck farms, allowing their population to increase while other predator populations (e.g., seabirds) decrease owing to lack of prey availability. Small crustaceans are one of the most important functional groups in the system, supporting the majority of bird groups, fish groups, and certain invertebrates (e.g., shrimps, octopuses, age 0+ Dungeness crabs, sea stars) (Harvey et al. 2012a). This group is one of seven functional groups that constitute 68% of the total biomass in the food web (Harvey et al. 2012a). The small crustaceans experienced a net decrease in biomass as cultured geoduck biomass increased, which was due to a negative interaction with anti-predation structure associated with cultured geoducks (although they responded positively to the harvest stage) and potentially due to an increase in predation (e.g., by surf perches and small crabs). Obtaining additional biomass, diet, and life history data and creating species-specific functional groups for demersal fishes and small crustaceans would clarify the trophic linkages responding directly to changes in cultured geoduck biomass.

The substantial decrease of most bird groups in the model is important to note, as these are important ecologically, culturally, and socio-economically. A decrease in eagle populations as cultured geoducks increase should benefit other bird groups through release from predation (Harvey et al. 2012b). However, the biomass of other birds decrease, implying bottom-up control in that they have reduced access to key prey (e.g., demersal fishes and small crustaceans) because of the predator refuge provided by anti-predator nets on geoduck farms. Migratory shore birds (biomass increase) do not primarily prey upon demersal fishes and small crustaceans and are likely benefiting from a release of eagle predation while not suffering prey depletion. Limited empirical studies have shown both negative and positive interactions between bivalve aquaculture and marine birds in other systems (Coen et al. 2011, Connolly and Colwell 2005, Kelly et al. 1996, Zydelis et al. 2009), suggesting that some interactions are likely. Further empirical study is required to understand the relationship between shellfish aquaculture and birds and validate these results.

Mediation functions in Ecosim are an important tool for incorporating non-trophic interactions into food web models and can help improve the incorporation of these data in supporting ecosystem-based approaches to aquaculture production. Although mediation functions can help incorporate habitat-specific patterns in the model, they are not equivalent to spatially-explicit models (e.g., Atlantis or Ecospace; Fulton et al. 2004a, Fulton et al. 2004b, Walters et al. 2010) and are unable to address such issues as the spatial scale of influence of geoduck farms and local community effects. For instance, shifts in the biomass of the subtidal walleye pollock and Pacific cod in response to increased cultured geoduck are most likely due to the model assumptions that demersal fishes and small crustaceans are basin-wide, continuous populations. Spatial resolution can enhance model performance (Fulton et al. 2003, Fulton et al. 2004c, Gruss et al. 2014) but may also increase uncertainty in model predictions owing to limited habitat data. Incorporating mediation functions into spatial versions of EwE (i.e., Ecospace) offers a promising area of future research as it could enable evaluation of spatially-explicit aquaculture development scenarios.

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APPENDIX

Additional Details on Methods and Results

Appendix Table 1. Functional groups in the Ecopath with Ecosim (EwE) model with major representatives.

Functional group	**Common name**	**Scientific classification**
Harbor seals	Harbor seal	*Phoca vitulina*
Sea lions	California sea lion	*Zalophus californianus*
	Steller sea lion	*Eumetopias jubatus*
Gulls	Various gulls	*Larus* spp.
Resident diving birds	Various cormorants	*Phalacrocorax* spp.
	Pigeon guillemot	*Cepphus columba*
Migratory diving birds	Western grebe	*Aechmophorus occidentalis*
	Various loons	*Gavia* spp.
	Common murre	*Uria aalga*
Nearshore diving birds	Various scoters	*Melanitta* spp.
	Various goldeneyes	*Bucephala* spp.
Herbivorous birds	Dabbling ducks	*Anas* spp.
	Various geese	*Branta* spp.
Migratory shorebirds	Dunlin	*Calidris alpina*
Great blue herons	Great blue herons	*Ardea herodias*
Raptors	Bald eagle	*Haliaeetus leucocephalus*
Wild salmon	Chum salmon	*Oncorhynchus keta*
	Chinook salmon	*O. tshawytscha*
	Coho salmon	*O. kisutch*
Hatchery salmon	Chum salmon	*O. keta*
	Chinook salmon	*O. tshawytscha*
	Coho salmon	*O. kisutch*
Pink salmon	Pink salmon	*O. gorbuscha*
Pacific herring	Pacific herring	*Clupea pallasii*
Forage fishes	Surf smelt	*Hypomesus pretiosus*
	Pacific sand lance	*Ammodytes hexapterus*
Surfperches	Shiner perch	*Cymatogaster aggregata*
	Striped seaperch	*Embiotoca lateralis*
Spiny dogfish	Spiny dogfish	*Squalus acanthias*
Skates	Longnose skate	*Raja rhina*
	Big skate	*R. binoculata*
Ratfish	Whitespotted ratfish	*Hydrolagus colliei*
Pacific hake	Pacific hake	*Merluccius productus*
Pacific cod	Pacific cod	*Gadus macrocephalus*
Walleye pollock	Walleye pollock	*Theragra chalcogramma*
Lingcod	Lingcod	*Ophiodon elongatus*
Rockfishes	Copper rockfish	*Sebastes caurinus*
	Quillback rockfish	*S. maliger*
Piscivorous flatfishes	Pacific sanddab	*Citharichthys sordidus*
Small-mouthed flatfishes	English sole	*Parophrys vetulus*
	Rock sole	*Lepidopsetta bilineata*
Demersal fishes	Various poachers	Family Agonidae
	Various eelpouts	*Lycodes* spp.
	Various small sculpins	Family Cottidae
Squid	Opalescent (market) squid	*Loligo opalescens*
Octopuses	Red octopus	*Octopus rubescens*
	Giant Pacific octopus	*Enteroctopus dofleini*
Shrimp	Pandalid shrimp	Family Pandalidae
	Sand shrimp	*Crangon* spp.
Cancer crab	Dungeness crab	*Cancer magister*
Red rock crab	Red rock crab	*Cancer productus*

Appendix Table 1 · continued

Functional group	**Common name**	**Scientific classification**
Sea stars	Sunflower star	*Pycnopodia helianthoides*
	Pink sea star	*Pisaster brevispinis*
Sea urchins	Green sea urchin	*Strongylocentrotus droebachiensis*
	Red sea urchin	*S. franciscanus*
Other grazers	Various snails	Class Gastropoda
	Various chitons	Class Polyplacophora
Small crustaceans	Various amphipods	Suborders Gammaridea, Corophiidea
	Various mysids	Family Mysidae
Small crabs	Various crabs	Infraorders Brachyura, Anomura
Large sea cucumbers	California sea cucumber	*Parastichopus californicus*
Predatory gastropods	Moon snail	*Euspira lewisii*
	Hairy triton	*Fusitriton oregonensis*
Mussels	Blue mussel	*Mytilus edulis*
Barnacles	Various barnacles	Suborder Balanomorpha
Geoduck	Geoduck	*Panopea abrupta*
Cultured geoduck	Geoduck	*Panopea generosa*
Large infaunal bivalves	Butter clam	*Saxidomus gigantea*
	Horse clam	*Tresus capax*
	Native littleneck clam	*Leukoma staminea*
	Manila clam	*Venerupis philippinarum*
Small infaunal bivalves	Purple *Transennella*	*Transennella tantilla*
	Amethyst gemclam	*Gemma gemma*
	Charlotte macoma	*Macoma carlottensis*
	Baltic macoma	*Macoma balthica*
Soft infauna	Polychaetes	Class Polychaeta
Deposit feeders	Brittle star	*Amphiodia urtica*
	Various sea cucumbers	Class Holothuroidea
Suspension feeders	Various sponges	Phylum Porifera
	Various bryozoans	Phylum Bryozoa
	Sea pen	*Ptilosarcus gurneyi*
Tunicates	Various sea squirts	Class Ascidiacea
Bacteria	Various bacteria	
Microzooplankton	Various microzooplankton	
Copepods	Various copepods	Order Calanoida
Euphausiids	Pacific krill	*Euphausia pacifica*
Small gelatinous zooplankton	Various small jellyfish, ctenophores, and other soft plankton	
Jellyfishes	Lion's mane jelly	*Cyanea capillata*
	Moon jelly	*Aurelia labiata*
	Fried egg jelly	*Phacellophora camtschatica*
Macrozooplankton	Various planktonic shrimp, amphipods, and larval crustaceans	
Phytoplankton	Various diatoms, dinoflagellates and phytoflagellates	
Benthic microalgae	Various benthic diatoms	
Benthic macroalgae	Various understory algal species	
Overstory kelp	Bull kelp	*Nereocystis luetkeanus*
Eelgrass	Native eelgrass	*Zostera marina*
Detritus	Not available	
Plant/algal material	Not available	
Salmon carcasses	Not available	*Oncorhynchus* spp.

Appendix Table 2. Parameters for new functional groups in the central Puget Sound EwE model, including trophic level (TL), biomass (B: metric tons per square kilometer), production to biomass ratio (P/B: per year), ecotrophic efficiency (EE) and production to consumption ratio (P/Q). Values in bold were calculated by the mass-balancing routine in the Ecopath model.

Group	TL	B	P/B	Q/B	EE	P/Q
Migratory shorebirds	3.660	0.039E-03[1]	0.370[2]	456.400[3]	0.222	0.005
Great blue heron	4.453	0.025E-01[4]	0.390[5]	72.310[6]	0.222	0.005
Red rock crab	3.110	1.859	1.100[7]	3.666[8]	0.900[8]	0.300
Small crustaceans	2.044	20.143	3.410[9]	25.000[8]	0.900[8]	0.136
Small crabs	2.283	15.921	0.820[10]	2.730[8]	0.800[10]	0.300
Cultured geoduck(yr1-2)	2.025	0.195[11]	0.143[12]	3.977[8]	0.434	0.036
Cultured geoduck(yr3-5)	2.025	3.542[11]	0.080[12]	1.849[8]	0.044	0.043
Cultured geoduck(yr6-7)	2.025	2.870[11]	1.000[12]	1.357[8]	0.043	0.737
Large infaunal bivalves	2.050	74.860[13]	1.010[8]	3.367[8]	0.118	0.300
Small infaunal bivalves	2.050	54.680[14]	2.059[8]	6.863[8]	0.476	0.300

[1] *Dalsgaard et al. 1998, Evenson and Buchanan 1997, Macwhirter et al. 2002*

[2] *Macwhirter et al. 2002, Warnock and Gill 1996*

[3] *Brennan 1990, Hunt 2000, Warnock and Gill 1996*

[4] *Eissinger 2007*

[5] *Butler 1997*

[6] *Butler 1995*

[7] *Parker 2002*

[8] *Harvey et al. 2012*

[9] *McLusky and McIntyre 1988*

[10] *Aydin et al. 2007*

[11] *Bradbury and Tagart 2000, Calderon-Aguilera et al. 2010, Hoffmann et al. 2000; Washington Department of Fish & Wildlife, personal communication*

[12] *J Gibbons, Taylor Shellfish, personal communication; B Phipps, Seattle Shellfish, personal communication*

[13] *Dethier 2012*

[14] *Partridge et al. 2005*

Appendix Table 3. Revised diet matrix for functional groups in the central Puget Sound model. Each column represents the diet proportions of a consumer and sums to 1. Asterisk (*) < 0.001. Please see *https://wsg.washington.edu/Ferriss-Appendix-Table-3*

Appendix Table 4. Cumulative Mixed Trophic Impact (MTI) of each functional group in order from highest to lowest impact.

Functional Group	Cumulative MTI	Rank
Phytoplankton	1.46	1
Resident eagles	1.31	2
Detritus	1.29	3
Other grazers	1.12	4
Spiny dogfish	1.05	5
Large infaunal bivalves	1.04	6
Copepods	0.94	7
Migratory eagles	0.91	8
Soft infauna	0.87	9
Ratfish	0.85	10
Demersal fishes	0.85	11
Small mouth flatfishes	0.83	12
Gulls	0.81	13
Microzooplankton	0.73	14
Harbor seals	0.71	15
Shrimp	0.68	16
Euphausiids	0.61	17
Small infaunal bivalves	0.59	18
Predatory gastropods	0.59	19
Bentich macroalgae	0.59	20
Red rock crab	0.59	21
Benthic microalgae	0.59	22
Mussels	0.57	23
Macrozooplankton	0.57	24
Small Crustaceans	0.56	25
Bacteria	0.54	26
Sea lions	0.53	27
Subadult crab	0.52	28
Adult lingcod	0.51	29
Surf perch	0.49	30
Juvenile herring	0.46	31
Subadult wild salmon	0.38	32
Walleye pollock	0.37	33
Herbivorous birds	0.37	34
Piscivorous flatfishes	0.35	35
Migratory birds	0.34	36
Salmon carcass	0.32	37
Adult hatch salmon	0.32	38
Nearshore birds	0.31	39
Subadult hatchery salmon	0.30	40
Forage fishes	0.30	41
Jellyfishes	0.27	42
Suspension feeders	0.25	43
Adult herring	0.24	44
Octopus	0.22	45
Sea stars	0.21	46
Small gelatenous zooplankton	0.20	47
Small crabs	0.20	48
Adult wild salmon	0.18	49
Squid	0.17	50
Urchins	0.17	51
Barnacles	0.16	52
Algal/plant matter	0.16	53
Pacific hake	0.15	54
Deposite feeders	0.14	55
YOY crab	0.13	56
Resident birds	0.12	57
Resident orcas	0.10	58
Eelgrass	0.10	59
Juvenile lingcod	0.09	60
Juvenile rockfishes	0.08	61
Pacific cod	0.08	62
Skates	0.08	63
Adult rockfishes	0.06	64
Overstory kelp	0.05	65
Juvenile hatchery salmon	0.03	66
Age 4+ crab	0.02	67
Great blue herons	0.02	68
Adult pink salmon	0.02	69
Juvenile wild salmon	0.02	70
Geoducks (Wild)	0.02	71
Large sea cucumbers	0.01	72
Juvenile pink salmon	0.00	73
Geoduck_yr3-5(cultured)	0.00	74
Tunicates	0.00	75
Geoduck_yr1-2(cultured)	0.00	76
Geoduck_yr6-7(cultured)	0.00	77
Migratory shorebirds	0.00	78
Subadult pink salmon	0.00	79

Appendix Table 5. Predicted relative biomass after cultured geoducks are increased by 120% over 50 years in the central Puget Sound with and without geoduck mediation functions. Biomass is relative to the base model (e.g., a value of 1 is equivalent to no change).

	Relative Biomass		
Functional Group	With Geoduck Mediation	No Geoduck Mediation	% Difference
Marine Mammals			
Resident orcas	1.000	0.993	-0.70%
Harbor seals	0.999	1.010	1.07%
Sea lions	1.000	0.950	-5.00%
Birds			
Gulls	0.999	0.990	-0.85%
Resident birds	0.998	0.829	-16.92%
Migratory birds	0.999	0.952	-4.72%
Nearshore birds	1.001	0.950	-5.06%
Herbivorous birds	1.000	1.032	3.21%
Migratory shorebirds	0.997	1.066	6.87%
Great blue herons	0.998	0.798	-19.97%
Resident eagles	1.000	0.836	-16.42%
Migratory eagles	1.006	0.836	-17.02%
Salmon			
Juvenile wild salmon	1.000	0.910	-8.94%
Subadult wild salmon	0.999	0.898	-10.17%
Adult wild salmon	1.000	0.923	-7.70%
Juvenile hatch salmon	1.000	0.938	-6.20%
Subadult hatch salmon	0.999	0.925	-7.46%
Adult hatch salmon	1.001	0.949	-5.26%
Juvenile pink salmon	1.000	0.992	-0.73%
Subadult pink salmon	0.999	0.988	-1.10%
Adult pink salmon	1.001	0.997	-0.39%
Pelagic fish			
Juvenile herring	1.000	1.003	0.29%
Adult herring	1.000	1.009	0.93%
Forage fishes	0.998	0.962	-3.61%
Surf perches	0.999	1.268	26.95%
Pacific hake	0.999	1.008	0.87%
Demersal fishes			
Spiny dogfish	0.999	0.935	-6.39%
Skates	1.000	0.966	-3.40%
Ratfish	1.000	0.993	-0.63%
Pacific cod	0.999	1.049	4.96%
Walleye pollock	1.000	0.890	-10.98%
Juvenile lingcod	1.001	1.058	5.64%
Adult lingcod	1.001	1.080	7.82%
Juvenile rockfishes	1.000	0.975	-2.54%
Adult rockfishes	1.000	0.976	-2.40%
Piscivorous flatfishes	1.001	0.864	-13.69%
Small mouth flatfishes	1.000	0.701	-29.91%
Demersal fishes	0.999	1.116	11.68%
Demersal invertebrates			
Octopus	1.000	0.959	-4.11%
Shrimp	1.000	0.980	-1.95%
YOY crab	1.000	0.964	-3.62%
Subadult crab	1.000	0.970	-2.94%
Age 4+ crab	1.000	1.035	3.46%
Red rock crab	1.002	1.006	0.43%
Sea stars	1.010	0.995	-1.55%
Urchins	0.999	1.147	14.81%
Other grazers	1.000	1.017	1.65%
Small Crustaceans(new)	1.000	0.841	-15.85%
Small crabs	1.000	1.178	17.79%
Large sea cucumbers	0.999	1.009	1.04%
Predatory gastropods	1.000	0.580	-41.99%
Mussels	0.999	0.963	-3.65%
Barnacles	0.999	0.998	-0.15%
Geoducks (Wild)	1.000	1.003	0.30%
Geoduck_yr1-2(cultured)	1.919	2.201	28.22%
Geoduck_yr3-5(cultured)	3.043	2.200	-84.34%
Geoduck_yr6-7(cultured)	2.200	2.200	0.00%
Large infaunal bivalves	1.000	1.039	3.95%
Small infaunal bivalves	1.000	0.987	-1.31%
Soft infauna	1.000	1.033	3.33%
Deposit feeders	1.000	1.041	4.12%
Suspension feeders	0.999	1.148	14.92%

Appendix Table 5 · continued

	Relative Biomass		
Functional Group	With Geoduck Mediation	No Geoduck Mediation	% Difference
Pelagic invertebrates			
Squid	0.999	0.990	-0.91%
Tunicates	0.999	1.030	3.09%
Microzooplankton	1.000	1.005	0.52%
Copepods	0.999	1.000	0.10%
Euphausiids	0.999	1.004	0.50%
Small gelatinous zooplankton	1.000	1.007	0.71%
Jellyfish	0.999	1.002	0.37%
Macrozooplankton	0.999	1.001	0.19%
Primary producers			
Phytoplankton	1.000	1.004	0.41%
Benthic microalgae	1.000	0.979	-2.08%
Benthic macroalgae	1.000	0.982	-1.86%
Overstory kelp	1.000	0.958	-4.17%
Eelgrass	1.000	0.998	-0.19%
Microbial and detrital			
Bacteria	1.000	1.004	0.42%
Algal/plant matter	1.000	0.974	-2.62%
Salmon carcass	1.000	0.927	-7.29%
Detritus	1.000	1.004	0.41%

Qualitative Network Models in Support of Ecosystem Approaches to Aquaculture Production: Potential Applications to Management and Climate Change

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ABSTRACT

Predicting the effects of aquaculture development in coastal ecosystems remains challenging, and tools that account for complex ecological interactions are needed to support ecosystem approaches to aquaculture. In this study, investigators used qualitative network models (QNMs) to examine the potential community effects of increasing bivalve aquaculture in South Puget Sound and Willapa Bay, Washington. QNMs are formalized conceptual models that require only a qualitative understanding of how variables composing a system interact (that is, the sign of interactions: +, –, and 0) and are, therefore, well suited to data-limited systems. The versatility of the approach was demonstrated by examining different sets of scenarios for each system. For South Puget Sound, community-wide responses to scenarios in which bivalve cultivation effort increased for three different bivalve species (Manila clam *Venerupis philippinarum*, Pacific oyster *Crassostrea* gigas, and geoduck *Panopea generosa*) were examined. Further evaluations addressed community-wide responses to the removal of benthic bivalve predators, a future increase in nutrient loadings, and combinations of these scenarios acting simultaneously. The scenarios enabled identification of potential tradeoffs between increased aquaculture and shifts in the abundance of community members and assessment of the possible effects of different management actions. For Willapa Bay, the investigators evaluated the potential implications of different hypothesized OA effects on the main cultivated species (Pacific oyster, Manila clam) as well as the community as a whole. In addition, identified key interactions that influence the sign outcome of community responses to press perturbations were identified, highlighting potential points for management intervention and linkages deserving of more focused quantitative study. QNMs are mathematically robust and highly flexible but remain underutilized. They may serve as valuable tools for supporting ecosystem approaches to aquaculture.

INTRODUCTION

Shellfish aquaculture production has increased rapidly worldwide and supplies protein to meet growing human demands as well as jobs and income that benefit coastal economies (National Research Council 2010). In some regions of Washington State, shellfish aquaculture has taken place for more than a century, but the industry is evolving in terms of growout methods and the variety of species cultivated. As the industry expands, the conversion of coastal habitat to shellfish farms has raised interest in understanding the potential ecological effects, positive and negative, on coastal ecosystems.

At the same time, coastal ecosystems are increasingly under pressure owing to a variety of issues including shoreline development, reduced water quality, overfishing, and climate change. Because cultured shellfish are integrated within and dependent upon healthy coastal ecosystems, shifts in one or a few components of the food web may have consequences for shellfish production. To better understand the effects of aquaculture on the environment and environmental changes on aquaculture, modeling approaches are needed that account for the complex network of ecological interactions that influence system behavior.

Quantitative food web models offer one framework for modeling complex systems and can help facilitate ecosystem approaches to aquaculture production. These models can facilitate a more holistic perspective on management decisions by capturing the response of the community to different perturbation scenarios (Byron et al. 2011, Jiang and Gibbs 2005). Although significant headway has been made in developing quantitative food web models for Central Puget Sound (Ferriss et al. 2015, Harvey et al. 2012), in general, their parameterization requires large amounts of data and can be expensive and time-consuming (McKindsey et al. 2006, Plaganyi and Butterworth 2004). Consequently, their application to more data-poor regions of the state is challenging. In contrast, Qualitative Network Models (QNMs) and the closely related "loop analysis" are well suited for modeling data-poor systems (Puccia and Levins 1985). QNMs were first developed to facilitate the analysis of feedbacks in network models (Levins 1974, Puccia and Levins 1985) and require only a qualitative understanding of the relationships linking species and variables within a system: that is, information on only the sign of interactions between variables (+, –, or 0) are needed. The method permits the rapid assembly of hypotheses of system structure and provides qualitative predictions of the response of community members to a sustained change, or press perturbation (Bender et al. 1984), in any system variable(s).

In QNMs, the predicted responses are qualitative, and therefore imprecise, but this can be considered advantageous because it de-emphasizes precise measurements of model parameters, which in practice are often difficult or impossible to obtain (Dambacher et al. 2009). Instead, the model focuses effort on describing general relationships among variables, which is typically more feasible for complex ecosystems (Dambacher et al. 2009, Levins 1998). Given their versatility, QNMs have been used in a range of different ecological applications including predicting community-level effects of eutrophication (Carey et al. 2014, Lane and Levins 1977), habitat disturbance (Dambacher et al. 2007), fishing (Metcalf 2010, Ortiz and Wolff 2002), species invasions and eradications (Castillo et al. 2000, Raymond et al. 2011), and assessing the effects of climate change on ecosystems (Dambacher et al. 2010, Melbourne-Thomas et al. 2013). However, QNMs have seen only limited application in the context of aquaculture (e.g., Whitlatch and Osman 1994).

In this study, QNMs were built that correspond with South Puget Sound and Willapa Bay (Figure 1). This enabled the investigators to summarize system knowledge and use the models to explore the potential effects of changes in aquaculture or the food web on cultured species and the community as a whole. For the South Puget Sound QNM, three different types of scenarios were examined: First, potential community-wide responses to increased aquaculture were examined, with the goal being to identify potential tradeoffs between bivalve species and the abundance of other community members. Second, investigators examined whether reducing benthic bivalve predators in the system (for instance, through targeted fisheries or manual removal) might improve bivalve production. Last, given predictions that nitrogen inputs are likely to increase in South Puget Sound (Ahmed et al. 2014, Roberts et al. 2014), scenarios of increased nutrient concentrations on cultured bivalves and the community were evaluated.

The Willapa Bay study focused on evaluating the potential effects of changes in seawater carbonate chemistry resulting from ocean uptake of anthropogenic atmospheric CO_2 (ocean acidification, OA) on key members of the food web. Specifically, three potential OA impacts were simulated: increased primary production, reduced production of bivalves, and enhanced predation by crabs and gastropods on bivalves. Qualitative examinations were conducted on how OA impacts on individual species propagate through the community and which interactions were most influential in determining the overall impact of OA.

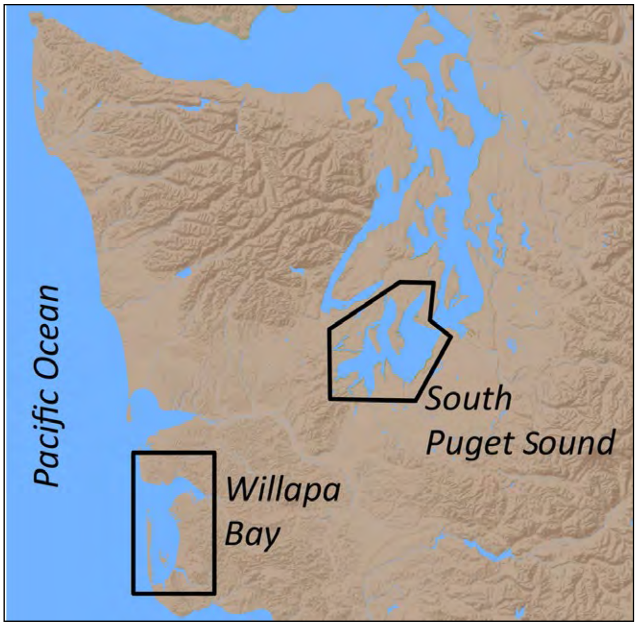

Figure 1. Location of Willapa Bay and South Puget Sound. Qualitative network models (QNMs) describing shellfish–food web interactions were developed for both regions.

MATERIALS AND METHODS

Study Site Overview

South Puget Sound

South Puget Sound is a large (449 square kilometers, 37 meters mean depth) subbasin of Puget Sound; approximately 15% of the basin is tidelands by area (Figure 1a, Burns 1985). South Puget Sound supports a diverse ecological community that includes marine mammals, migratory waterfowl, species of management and conservation concern (e.g., the eelgrass *Zostera marina*, Chinook salmon *Oncorhynchus tshawytscha*) as well as commercial, tribal, and recreational capture fisheries (e.g., Chinook salmon, Dungeness crab *Cancer magister*). Cultivation of non-native Pacific oyster *Crassostrea gigas* began in the 1920s after the collapse of native Olympia oyster *Ostrea lurida* populations. Manila clam *Venerupis philippinarum*, which may have been accidentally introduced with oysters brought from Japan, became a focus of cultivation efforts in the 1940s. Commercial culture of geoduck *Panopea generosa* was developed in the early 1990s to augment lucrative wild harvest in subtidal areas and has since increased dramatically. Recent reported shellfish aquaculture landings have approached 1,500,000 kilograms per year and consist of Pacific oyster (55%), Manila clam (23%), and geoduck (16%), with remaining landings (10%) composed of assorted non-native bivalves (blue mussel *Mytilus spp.*, European oyster *Ostrea edulis*, eastern oyster *Crassostrea virginica*, Kumamoto oyster *Crassostrea sikamea)* and native Olympia oyster (shellfish aquaculture landings statistics for 2010, Washington Department of Fish & Wildlife).

Willapa Bay

Willapa Bay is the largest estuary on the outer Washington coast (260 km^2) and has extensive tidelands (greater than 50% of the bay by area) that have supported commercial shellfish aquaculture for more than a century (Feldman et al. 2000). The estuary is an important region for cultivating the non-native Pacific oyster and supplies ~10% of all oysters consumed domestically (Ruesink et al. 2006). In addition, the introduced Manila clam is also intensively cultivated. Apart from cultivated shellfish, the estuary also supports a wild fishery for Dungeness crab and provides habitat to species of management and conservation concern including threatened fishes (Chinook salmon, green sturgeon *Acipenser medirostris*) and migratory waterfowl (black brant *Branta bernicla nigricans*). During the spring and summer months, northerly winds result in the upwelling of nutrient-rich waters along the open coast which, in turn, promote high rates of primary production and dense standing stocks of phytoplankton that circulate into the estuary, supporting secondary production (Banas et al. 2007, Hickey and Banas 2003).

Like many other estuaries, levels of partial pressure CO$_2$ (pCO$_2$) in Willapa Bay range widely, from 300 to 4,000 microatmospheres (µatm; for reference, current atmospheric pCO$_2$ is ~400 µatm), and vary spatially, with the highest values occurring up-estuary and in association with low-salinity waters (Ruesink et al. 2015). Carbonate chemistry dynamics in Willapa Bay are more variable than open ocean systems and are strongly influenced by freshwater inputs, rates of photosynthesis, and processes that influence the abundance and remineralization of organic material (Ruesink et al. 2015). However, marine carbonate chemistry conditions in Willapa Bay are also partly influenced by atmospheric pCO$_2$ levels, as are conditions in adjoining coastal waters (Feely et al. 2008). Consequently, pCO$_2$ levels in Willapa Bay are likely to increase over the long term (years to decades) with increasing atmospheric pCO$_2$, assuming that watershed processes and community metabolism also remain stable over time (Borges and Abril 2011, Duarte et al. 2013).

Qualitative Network Models: Background

QNMs are a special type of graph — known as a digraph — that consist of variables and linkages or, equivalently, nodes and edges (Puccia and Levins 1985). The linkages in the graph correspond to a matrix of interactions that, in ecology, typically represent trophic interactions. However, linkages can also represent other ecological interactions such as competition and facilitation or interactions between species or any other type of variable (e.g., abiotic, social, economic). The analysis of QNMs draws upon graph theory and matrix algebra and is based specifically on analysis of the community matrix (Levins 1974, Puccia and Levins 1985).

A central premise of the approach is that the per capita change in a species or the level of some non-species variable can be described as a continuous function of the other variables in the system. The dynamics of n interacting variables can be represented as a set of ordinary differential equations, where for each variable x (I = 1, 2, ..., n):

$$\frac{dx_i}{dt} = f_i(x_1, x_2, \ldots, x_n;\ c_1, c_2, \ldots, c_n)$$

That is, the growth rate of variable x_i is a function of the levels of some or all variables in the system, and usually itself, and a set of growth parameters *c*. In the case of species variables, their *c* parameters may correspond with birth, death, or immigration rates. The interaction coefficient a_{ij} measures the direct effect of a small change in the level of variable *j* on the growth rate of variable *i*, and is defined as the partial derivative of f_i with respect to x_i (Bender et al. 1984):

$$a_{ij} = \partial f_i / \partial x_j$$

Although the effects of x_j on x_i may not necessarily be linear, the approach assumes that the dynamics of each variable can be adequately approximated by a linearization near equilibrium levels (Stone and Roberts 1991). The $i \times j$ matrix containing the a_{ij} elements is the community interaction matrix **A**. The negative inverse of **A** can be used to estimate the long-term effects of a press perturbation, which is defined as a sustained shift in the magnitude of a species' growth parameter (Bender et al. 1984). However, for natural ecosystems, precise quantitative specification of **A** is rarely possible (Levins 1998).

Instead, under a qualitative approach, only the signs of the aij terms are needed. In traditional "loop analysis," sign specification of **A** alone can provide qualitative predictions of press perturbation impacts (Puccia and Levins 1985), but even in relatively simple systems, multiple feedbacks can result in qualitative predictions with high sign indeterminacy (Dambacher et al. 2003). By using a simulation framework, both parameter uncertainty (i.e., the magnitude of aij) and potential structural uncertainty (i.e., the presence or absence of links) can be incorporated into predictions of community outcomes to a given press perturbation (Melbourne-Thomas et al. 2012, Raymond et al. 2011). As used in the context of QNMs, structural uncertainty refers to instances when it is unclear if a linkage is present or absent, but if it does occur the sign of the link is assumed known (Raymond et al. 2011). The simulation procedure proceeds as follows: (1) a simulated community interaction matrix (**A***) is generated by retaining all certain linkages and the inclusion of uncertain linkages is determined by sampling from a binomial distribution; (2) interaction coefficients (aij) for all links are then sampled from uniform distribution spanning two orders of magnitude (0.01 to 1.0); (3) the simulated community interaction matrix (**A***) is tested against stability criteria (Melbourne-Thomas et al. 2012) and, if the matrix is stable, the negative inverse of **A*** is calculated to obtain the predicted response of the community to a given press perturbation. The procedure is repeated many times (10^4) to obtain distributions of the community outcomes due to a given press perturbation. Further extensions of the simulation approach exist that permit additional filtering of **A*** to only those matrices that also predict community responses in agreement with experimental or observational evidence (Melbourne-Thomas et al. 2012, Raymond et al. 2011).

Model Development

This study sought to build QNMs that described the major ecological interactions likely to influence the dynamics of cultured species and the communities they are embedded within. To do so, a literature review of relevant ecological studies conducted in South Puget Sound and Willapa Bay was conducted and supplemented with studies from other estuaries in the Northeast Pacific. In addition, shellfish growers and researchers with expertise in either system were consulted to identify key cultured bivalves species, their main predators and competitors, and other species or functional groups that, in turn, influence their respective dynamics. Interactions thought to influence the dynamics of variables within the system were identified. Some interactions were considered uncertain, reflecting uncertainty in model structure. Variables included in each QNM are depicted as nodes (Figures 2 and 3) and interactions corresponding with the linkages are described for South Puget Sound and Willapa Bay (Appendix Tables 1 and 2, respectively; p. 52). Further details of model structure are provided for each system as follows.

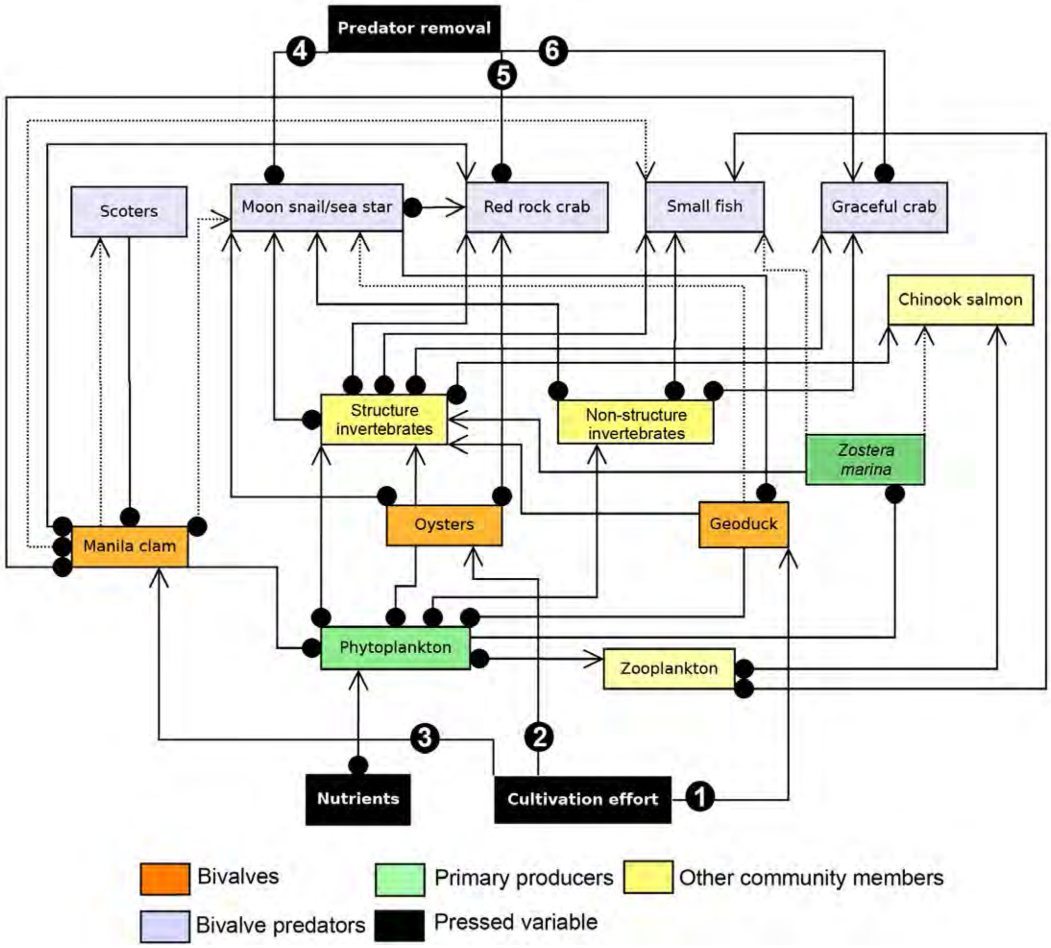

Figure 2. Qualitative interaction network of bivalve aquaculture in South Puget Sound, Washington. Links that terminate with an arrowhead indicate a positive effect; those that terminate with a filled circle indicate a negative effect. Links with both an arrow and a solid circle indicate a predator–prey relationship. All community members have a limiting self-interaction (negative), but for clarity these are not shown. Dashed lines indicate uncertain linkages. Detailed descriptions of the relationships (unnumbered) between nodes are provided in Appendix Table 1. Links labeled 1–6 are included in the model based on the scenario under consideration (see Table 1).

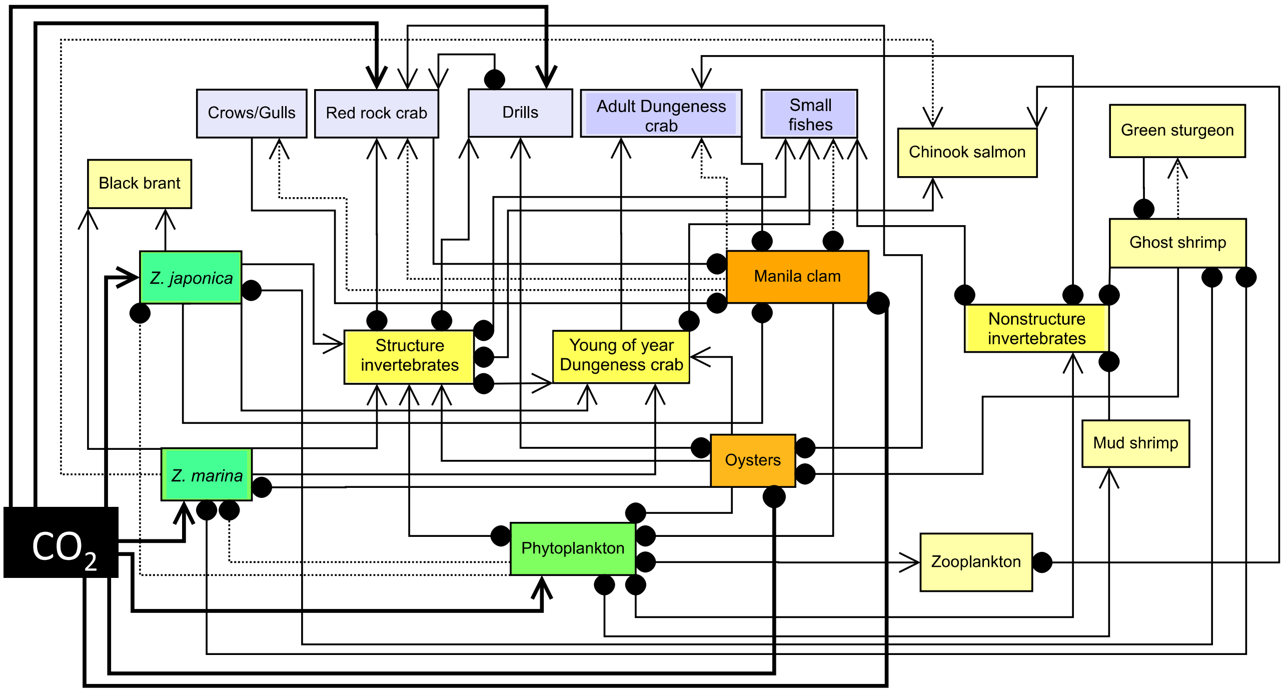

Figure 3. Qualitative interaction network of Willapa Bay, Washington. Lines terminated with arrowheads indicate a positive influence; those terminated with a filled circle indicate a negative influence. Links with both an arrowhead and a solid circle indicate predator–prey relationships. Dashed lines indicate uncertain interactions. Interactions between CO_2 and community members that correspond to different ocean acidification scenarios are in bold. All community members have a limiting (negative) self-interaction, but for clarity these are not shown. Detailed descriptions of the relationships between nodes are provided in Appendix Table 2. See Figure 2 for color legend.

South Puget Sound

For South Puget Sound, the investigators sought to evaluate the potential effects of aquaculture, bivalve predator control, and increased nutrient loadings. Therefore, additional nodes and linkages were included in the QNM to allow simulation of these perturbation scenarios (described under Perturbation Scenarios, p. 40). To simplify the model and reduce the number of nodes, functionally similar species were grouped (Puccia and Levins 1985). For instance, the nodes “small fishes,” “zooplankton,” and “phytoplankton” represent taxonomically diverse groups, but the ecological function of constituent species was assumed to be similar. In addition, small-bodied benthic invertebrates were grouped into one of two classes: those that associate with structurally complex habitats (e.g., biogenic structure such as eelgrass meadows and oyster beds, as well as growout gear associated with oyster and geoduck cultivation) and those that prefer mud or unstructured habitat (e.g., Ferraro and Cole 2007). The former and latter were referred to as “structure invertebrates” and “non-structure invertebrates,” respectively. Although benthic invertebrate community structure may differ among types of complex habitats in South Puget Sound, a simplifying assumption was made that these species play similar functional roles and could be utilized by similar predator assemblages in the absence of detailed information on invertebrate community structure across habitat types.

Willapa Bay

For Willapa Bay, the focus of the modeling was on the potential impacts of OA. Like that used for South Puget Sound, the Willapa Bay QNM also included the main species of cultivated shellfish (Manila clam and Pacific oyster) and ecologically relevant competitors, predators, prey, and other functionally dependent species to capture community interactions. The species included in the model and the nature of their interactions were also informed by a literature review and consultation with shellfish growers and scientists with expertise on the ecology of Willapa Bay. Functionally similar taxa were again grouped to simplify the model in a manner similar to South Puget Sound (e.g., the aggregate variables included “small fishes,” “zooplankton,” and “phytoplankton”), and small-bodied benthic invertebrates were divided into two functional groups reflecting association with structurally complex (eelgrass beds and oyster beds) and unstructured (mud) habitats (Ferraro and Cole 2007).

Both models are “minimal realistic” in that they include enough detail to capture the interplay of direct and indirect interactions that influence aquaculture and community-wide dynamics but also minimize the number of variables to aid interpretability and reduce prediction uncertainty (Fulton et al. 2003).

Perturbation Scenarios

South Puget Sound

Three main types of perturbation scenarios were considered: (1) increase in bivalve aquaculture, (2) decrease in bivalve predation rates through predator removal, and (3) increase in nutrient loads. To implement the scenarios, the nodes "Cultivation effort" and "Predator removal" were added to the community QNM (Figure 2) and linkages extending from these nodes to community member nodes were added depending on the specific perturbation scenario (Table 1). For example, to evaluate potential community-wide responses to increased geoduck cultivation, a positive link was added to the model, extending from "Cultivation effort" to "Geoduck" (the linkage labeled "1" in Figure 2). The node "Cultivation effort" corresponds to the effort placed by growers into expanding the area over which bivalve cultivation occurs. The remaining labeled linkages (2 through 6) were excluded from the model. The "Cultivation effort" node was then pressed in the simulation, and the response of the community was calculated. Similarly, community responses to increases in Pacific oyster or Manila clam culture were simulated by adding linkages labeled 2 or 3, respectively, to the model, excluding all other labeled linkages, and pressing "Cultivation effort" (Table 1).

In South Puget Sound, anti-predator exclusion technologies (e.g., mesh netting, bag-on-rack or bag-on-bottom methods, protective polyvinyl chloride (PVC) tube sections) are already used extensively on Pacific oyster, Manila clam, and geoduck plots (McDonald et al. 2015, Simenstad and Fresh 1995, Toba et al. 1992); however, predation loss remains an issue. As an added measure, predators could be culled. In practice, this might be achieved by manually removing predators on culture plots or initiating a targeted fishery on predators. The effects of removing predators on the community were evaluated by adding the node "Predator removal," which corresponds to the level of effort applied to bivalve predator removal. The study specifically examined the community-wide effects of removing four common benthic invertebrate predators that were represented by three different nodes in the model: red rock crab *Cancer productus*, graceful crab *Cancer gracilis*, and the moon snail/sea star complex (Figure 2), which is characterized by moon snails (*Euspira lewisii*) and sea stars (sunflower sea star *Pycnopodia helianthoides*, pink sea star *Pisaster brevispinus*, ochre sea star *Pisaster ochraceus*, mottled sea star *Evasterias troscheli*). Negative linkages extending from "Predator removal" to each benthic predator node were added to the model to simulate reductions in predator densities (Table 1).

In the third scenario, an increase in nutrient loadings was considered. In South Puget Sound, nitrogen levels are likely to increase over the next several decades as human populations in the surrounding watersheds grow. In addition, circulation patterns on the Washington coast may shift in response to anthropogenic climate change, resulting in the delivery of additional marine-derived nitrogen relative to present-day conditions (Ahmed et al. 2014, Mackas and Harrison 1997). The effects of a potential future increase in nutrient loadings on the community were evaluated by pressing the node "Nutrients" (Figure 1, Table 1).

In addition to the three main types of perturbation scenarios, the investigators also examined community-wide outcomes when scenarios were combined (Table 1). The goal was to identify how scenario combinations might reinforce or counteract the predicted outcome of community members relative to the individual scenarios.

Table 1. Summary of model scenarios evaluated for the South Puget Sound QNM. For each scenario, the pressed node is indicated (i.e., press variable(s)). Link numbers correspond to labeled links in Figure 2, and the sign of the relationship between the pressed node and community members is denoted. Pressed nodes are as follows: CE, cultivation effort; PR, predator removal; NU, nutrients.

Scenario code	Press	Links added geoduck variable(s)	Pacific oyster	Manila clam	Moon snail/ sea stars	Red rock crab	Graceful crab
A1	CE	1 (+)					
A2	CE		2 (+)				
A3	CE			3 (+)			
A4	CE	1 (+)	2 (+)	3 (+)			
B1	PR				4 (–)		
B2	PR					5 (–)	
B3	PR						6 (–)
B4	PR				4 (–)	5 (–)	6 (–)
C1	NU						
D1	CE, PR	1 (+)	2 (+)	3 (+)	4 (–)	5 (–)	6 (–)
D2	CE, NU	1 (+)	2 (+)	3 (+)			
D3	PR, NU				4 (–)	5 (–)	6 (–)
D4	CE, PR, NU	1 (+)	2 (+)	3 (+)	4 (–)	5 (–)	6 (–)

Willapa Bay

The biological processes that OA can influence at the individual and population levels are likely diverse but are only partially understood. Given this state of knowledge, the intention of this study was not to evaluate all OA effects but rather explore the potential role of community interactions in mediating and propagating three commonly discussed OA impacts, as follows:

- OA will stimulate primary production. Increased pCO_2 can cause a fertilization effect that elevates photosynthetic rates, leading to higher growth rates among phytoplankon and eelgrass (Koch et al. 2013, Kroeker et al. 2013, Palacios and Zimmerman 2007, Thom 1996, Zimmerman et al. 1997).
- OA will decrease bivalve production. OA may reduce bivalve production directly by reducing larval survival or adversely affecting variables that influence survival like individual growth, development, and calcification rates (Barton et al. 2012, Kurihara et al. 2007, Timmins-Schiffman et al. 2012, Waldbusser et al. 2013). These effects in turn may reduce the density or viability of natural or outplanted sets (Barton et al. 2012).
- OA will alter predator–prey interaction strengths. OA may cause declines in bivalve shell strength, thickness, or size, resulting in higher vulnerability to predators, thereby strengthening predation interactions (Kroeker et al. 2014, Sanford et al. 2014).

The qualitative network model included the variable “CO_2,” which represents carbonate chemistry conditions (Figure 3). It was linked to species in a manner that corresponded to different hypothesized OA effects (Figure 3). First, model scenarios were examined in which the effect of CO_2 was linked to individual functional groups or species of primary producers (phytoplankton; the eelgrasses *Z. marina* and *Z. japonica*) and another in which CO_2 affected all primary producers simultaneously (Table 2). Next, the effect of CO_2 was linked to individual bivalve species (Pacific oyster, Manila clam) and to both species simultaneously (Table 1). Finally, the potential for enhanced predatory interactions was tested at two points in the model: the predation linkages between red rock crab (predator) and Pacific oysters (prey) and between drills (predatory gastropods) and Pacific oysters (prey). To simulate enhanced predation due to OA, positive interactions extending from CO_2 to the predator and negative interactions extending from CO_2 to the prey were added (Dambacher and Ramos-Jiliberto 2007). Model scenarios were examined where enhanced interactions were considered individually and in combination (Table 1). In addition to the three main scenarios, the study evaluated community responses in scenarios that included pair-wise combinations of the three hypotheses as well as a scenario that included all hypothesized OA effects acting simultaneously (Table 1).

Simulations

A simulation approach was used to estimate the level of sign determinacy in the predicted response of community members to the different press scenarios (Melbourne-Thomas et al. 2012, Raymond et al. 2011). The following simulation protocol was used: (1) a community matrix configuration was first generated by sampling uncertain links from a binomial distribution, (2) the interaction strengths of the community matrix were then drawn from a uniform distribution that spanned two orders of magnitude (0.01 to 1), and all negative self-effect interaction coefficients were drawn from a uniform distribution spanning 0.25 to 1 (Raymond et al. 2011), and (3) the community matrix was checked against system stability criteria (Melbourne-Thomas et al. 2012) and, if stable, the responses of the community to the press perturbation were calculated. If unstable, the community matrix was discarded and a new community matrix was drawn and the simulation procedure was run again. For each scenario, the sign responses from 104 stable community matrices were obtained.

Table 2. Summary of the OA scenarios examined using QNMs of ecological interactions in Willapa Bay, Washington. For each scenario, the qualitative relationship (sign) linking the variable CO_2 to the respective community member is listed in the table column.

		Primary producers			Bivalves		Enhanced drill–oyster		Enhanced red rock crab–	
					predator interaction				oyster predator interaction	
Scenario	Scenario code	Phyto	*Z. marina*	*Z. japonica*	Pacific oyster	Manila clam	Drills	Pacific oyster	Red rock crab	Pacific oyster
Primary	A1	(+)								
producer	A2		(+)							
	A3			(+)						
	A4		(+)	(+)						
	A5	(+)	(+)	(+)						
Bivalves	B1				(–)					
	B2					(–)				
	B3				(–)	(–)				
Enhanced	C1						(+)	(–)		
predation	C2								(+)	(–)
	C3						(+)	(–)	(+)	(–)
Combinations	D1 (A5+B3)	(+)	(+)	(+)	(–)	(–)				
	D2 (A5+C3)	(+)	(+)	(+)			(+)	(–)	(+)	(–)
	D3 (B3+C3)				(–)	(–)	(+)	(–)	(+)	(–)
	D4 (A5+B3+C3)	(+)	(+)	(+)	(–)	(–)	(+)	(–)	(+)	(–)

Linkage Influence

In addition to yielding predictions, QNMs can also be analyzed to gain insight into which linkages principally influence the sign outcome of community members to a given press scenario (Melbourne-Thomas et al. 2012). To illustrate the method, an assessment was made of the linkage influence on Willapa Bay community responses to the press scenario in which all hypothesized OA linkages were included (scenario D4, Table 2).

To simplify analysis, no structural uncertainty in the model was assumed (Raymond et al. 2011). That is, all linkages, including those noted as uncertain, were retained in the network. Next, 1,500 community matrices were simulated and their associated press perturbation response to OA calculated. For each community member, investigators fit a multivariate adaptive regression splines (MARS) model in which the simulated interaction coefficient parameters were treated as predictor variables and the sign outcomes of species to the press perturbation were the response variables. MARS are a nonparametric statistical method that can fit nonlinear functions and higher-order interactions (Friedman 1991, Hastie et al. 2009). The method is well suited for analyzing large datasets and combines the strengths of regression trees and spline fitting by replacing the step functions normally associated with regression trees with piecewise linear basis functions (Hastie et al. 2009). In an earlier study, Boosted Regression Trees (BRT) models were introduced as tools for evaluating linkage influence on node responses in QNMs (Melbourne-Thomas et al. 2012). The BRT approach also permits estimation of nonlinear responses and higher-order interactions and, in some cases, performance based on predictive ability is comparable with or slightly better than MARS (Elith et al. 2006, Stohlgren et al. 2010). However, the MARS algorithm enabled variable selection based on deviance reduction criteria, which was useful for identifying subsets of key linkages. Furthermore, in preliminary comparisons, MARS was computationally faster than BRT, which was valuable given the intended number of models to fit.

The MARS models were fit assuming a binomial response error model following Leathwick et al. (2005). Variables were retained if they reduced the residual squared error of the model by 0.01 or more. For all fitted models, the percentage of explained deviance associated with each retained predictor (i.e., the predictor's relative importance) was calculated (Milborrow 2014). Cluster analyses were performed on the relative importance values to identify both linkages that influenced similar community members and community members that were influenced by similar linkages; dendrograms were calculated based on the Bray–Curtis dissimilarity coefficient and the complete linkage clustering method (Legendre and Legendre 1998). All statistical analyses were performed using the statistical software package "R" version 3.0.3 (R Development Core Team 2014). MARS models were estimated using the library "earth" version 4.0.0 (Milborrow 2014) and dendrograms were calculated using the library "vegan" version 2.0-10 (Oksanen et al. 2013).

RESULTS

South Puget Sound

Cultivation Effort

Increased cultivation effort, when applied to individual bivalve species (scenarios A1 through A3), resulted in positive responses to the bivalve species directly affected. Sign determinacy, which corresponds to the level of consistency in the simulated sign responses, was greater than 70% in all scenarios (Figure 4). For most other community members, sign determinacy was lower (less than 70%) but some trends were apparent. Phytoplankton responded negatively and *Z. marina* responded positively across scenarios, and the bivalve predator red rock crab increased as well (Figure 4). Consistent trends in other community members toward negative (zooplankton, non-structure invertebrates) and positive responses (nutrients) were also observed (Figure 4).

In contrast, when cultivation effort was applied to all three bivalve species simultaneously (scenario A4), each bivalve species responded positively but sign determinacy decreased relative to the individual press scenarios for Manila clam and Pacific oyster relative to their individual press scenarios (Figure 4). Additionally, the sign responses for nutrients, phytoplankton, zooplankton, non-structure invertebrates, *Z. marina*, and red rock crab were similar to those under the individual scenarios, but for these community members sign determinacy increased, exceeding 70% (Figure 4).

Predator Removal

In the individual predator removal scenarios (B1 to B3), each targeted predator decreased (Figure 4). However, the responses of cultured bivalve populations to the different predator removal scenarios varied. Removing moon snails/sea stars (B1) increased geoduck and Pacific oyster populations, while removing red rock crabs (B2) increased Manila clams but decreased geoduck populations. Removing graceful crab (B3) also increased Manila clams, but Pacific oyster populations decreased. Responses of the remaining community members also differed as well between scenarios, with no consistent trends in sign responses among primary producers, bivalve predators, or other community members (Figure 4).

In the scenario in which all three predators were removed simultaneously (scenario B4), the sign responses of the predators were negative, sign determinacy was low, and among the cultured bivalves, only Manila clam population numbers showed a positive response with high sign determinacy (Figure 4). Primary producers, nutrients, and zooplankton responded in the same manner as when cultivation effort was increased on all three species simultaneously (Figure 4).

Nutrients

For primary producers, increased nutrients resulted in a positive response in phytoplankton and negative response in *Z. marina* (scenario C1), which was the opposite of the pattern observed in the cultivation effort and predator control scenarios

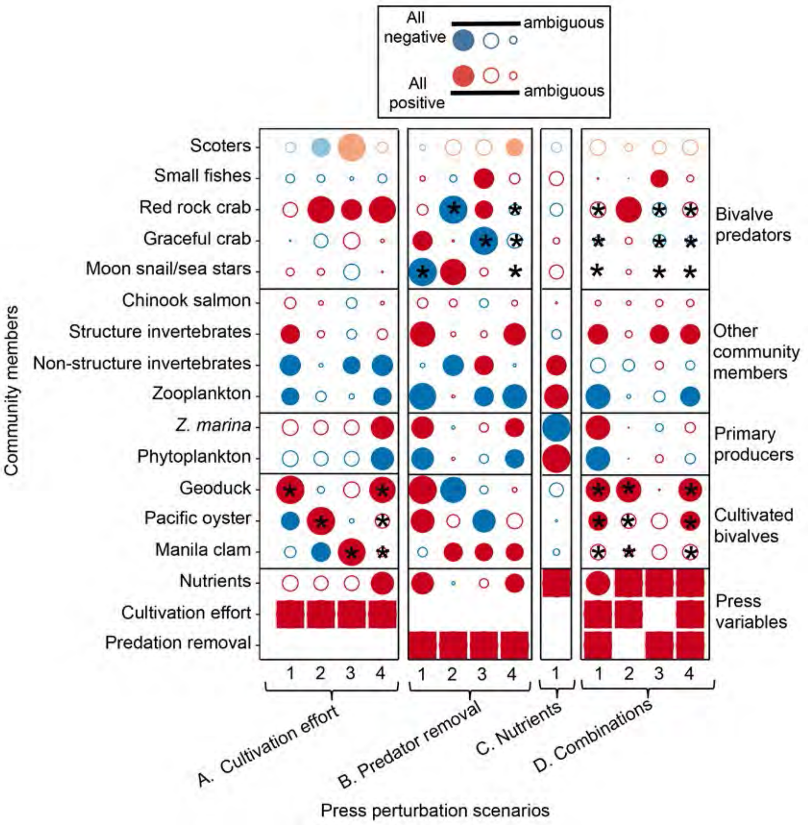

Figure 4. Simulated community responses to increased bivalve cultivation effort, benthic predator removals, and nutrients inputs in South Puget Sound, Washington. Scenario letter and number codes correspond to scenario descriptions provided in Table 1. Nodes pressed in each scenario are indicated by a solid square. The relative size of the red and blue circles scale with the level of consistency of the simulated sign response of community members. For added reference, solid circles indicate sign consistency greater than 70%; open circles indicate less than or equal to 70%. Red and blue symbol colors correspond to net positive and negative responses, respectively. Light red and light blue symbols indicate instances where greater than 25% of the simulated responses were 0 (symbol scale is based on the non-zero predicted sign responses). For each scenario, community members directly linked to the pressed variable(s) are noted by an asterisk overlying their respective responses.

(Figure 4). Further, increased nutrients resulted in a predicted increase in phytoplankton and non-structure invertebrates (Figure 4). Responses for all the remaining community members, including the bivalves and bivalve predators, had low sign determinacy (Figure 4).

Scenario Combinations

In scenario D1, cultivation effort and predator removals for all three bivalves were pressed. Overall, the sign responses of all bivalves, primary producers, nutrients, and zooplankton were similar to both separate scenarios (A4 and B4), though variation in sign determinacy was apparent for a few community members (e.g., red rock crab, structure invertebrates, Pacific oyster, manila clam; Figure 4).

With increased nutrients and cultivation effort (scenario D2), most community members exhibited responses with low sign determinacy; only geoduck and red rock crab (both positive responses) showed high sign determinacy. Similarly, sign determinacy was predominately low for community members when nutrients and predator removal were increased (D3). In that case, positive responses in small fishes and structure invertebrates had high sign determinacy.

Last, simultaneous increases in cultivation effort, predator removal, and nutrients (scenario D4) resulted in positive responses in all three bivalves, though sign determinacy was high for only geoduck and Pacific oyster (Figure 4). As for the remaining community members, only two exhibited responses with high sign determinacy — structure invertebrates and zooplankton — which responded positively and negatively, respectively (Figure 4).

Willapa Bay

Community Responses Across OA Scenarios

In general, the level of sign determinacy exceeded 70% for 21–57% of the community members regardless of the OA scenario (Figure 5). For several community members, including phytoplankton, Manila clam, mud shrimp, and crows/gulls, sign determinacy was high across most OA scenarios and the sign of the response was also consistent (Figure 5). In contrast, sign determinacy was low in the responses of other community members (e.g., small fishes, non-structure invertebrates, adult Dungeness crab) regardless of the OA scenario (Figure 5).

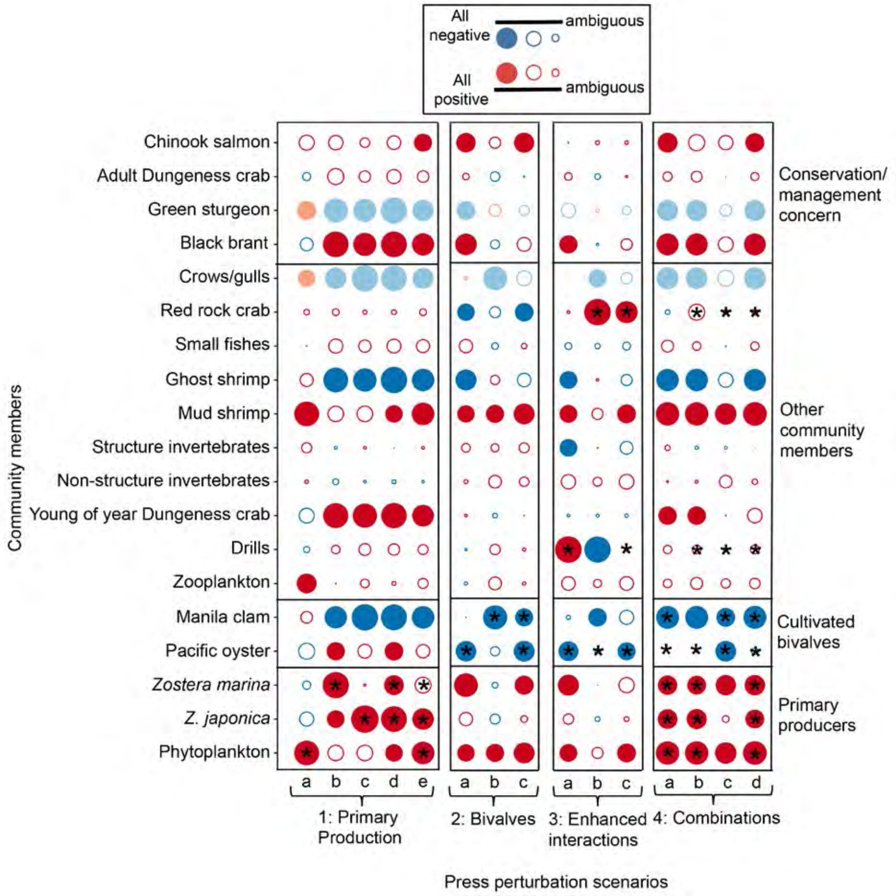

Figure 5. Simulated community responses to increased CO_2 in Willapa Bay, Washington. Scenario letter and number codes correspond to scenario descriptions provided in Table 2. The relative size of the circle symbols scale with the level of consistency of the simulated sign response of community members. For added reference, closed circles indicate sign consistency greater than 70%; open circles indicate less than or equal to 70%. Red and blue symbol colors correspond to net positive and negative responses, respectively. Light red and light blue symbols indicate instances where greater than 25% of the simulated responses were 0 (symbol scale is based on the non-zero predicted sign responses). For each scenario, community members directly linked to CO_2 are noted by an asterisk overlying their respective responses.

Primary Producers

In the primary producer scenarios in which phytoplankton, *Z. marina*, and *Z. japonica* were linked directly with CO_2 (scenarios 1a–c), the linked species exhibited positive responses in their respective scenarios (Figure 5). However, in scenario 1b, the direct effect of CO_2 on *Z. marina* was also associated with an increase in *Z. japonica* via indirect pathways. Similarly, in scenario 1d where more than one community member was linked to CO_2, indirect pathways resulted in a positive response in phytoplankton when direct effects of CO_2 were included for *Z. japonica* and *Z. marina*. In contrast, direct linkages with CO_2 did not correspond to high sign determinacy in the response of *Z. marina* when all three primary producers were linked to CO_2 (scenario 1e, Figure 5).

Among species of conservation or management concern, results were mixed. The response of the herbivorous black brant was consistently positive in all scenarios that included direct linkages between CO_2 and either eelgrass species (Figure 5). However, the response of the threatened green sturgeon was negative but unclear owing to model structural uncertainty (Figure 5). While the response of adult Dungeness crab was ambiguous across all primary producer scenarios, the response of Chinook salmon was unambiguous and positive only when direct positive CO_2 effects were included for all primary producers (scenario 1e, Figure 5). Ghost shrimp, which can destabilize the substrate and smother oysters with sediments, responded negatively in all scenarios in which eelgrass increased, as did crows/gulls though uncertainty due to model structure was high (Figure 5).

Bivalves

In the bivalve scenarios (2a–c), direct negative linkages between CO_2 and Pacific oyster and Manila clam were associated with reductions in both species (Figure 5). However, in terms of indirect effects, the number of community members affected by reductions in both species differed. A decrease in Pacific oyster (scenario 2a) was associated with likely changes in eight community members, while responses were more ambiguous in the Manila clam scenario (2b), with likely change predicted in only three members (Figure 5). Across bivalve scenarios, all showed a positive increase in phytoplankton, while increases in the eelgrass *Z. marina* were observed only in Pacific oyster scenarios (2a and c, Figure 5). Several of the remaining community members also differed in level of sign determinacy between the three bivalve scenarios (Figure 5).

Enhanced Predation Interaction

Overall, the response of the community to enhanced predation on Pacific oyster differed depending on which predation interaction was enhanced. Increased predation by drills (3a) resulted in community responses similar to those observed in bivalve scenario 2a, except for a negative response in structure invertebrates, a positive response in drills, and higher ambiguity in the response of Chinook salmon, green sturgeon, and red rock crab (Figure 5). In contrast, the community response to enhanced red rock crab predation differed substantially; relative to scenario 2a, the responses of several community members increased in ambiguity, including the response of Pacific oyster, and negative responses were predicted for drills, Manila clam, and crows/gulls (Figure 2). When both predatory interactions (drills–Pacific oyster and red rock crab–Pacific oyster) were enhanced (scenario 3c), ambiguity increased further in the response of drills relative to scenario 3b, where only the drill–Pacific oyster interaction was enhanced, and ambiguity in the sign response of most community members remained high (Figure 2).

In general, community responses in scenarios that included linkage combinations from the three different sets of OA hypotheses were relatively consistent when direct linkages from CO_2 to the three primary producers were included (scenarios 4a, b, and d, Figure 2). Only the level of ambiguity in the responses of Chinook salmon and young of year Dungeness crab differed among the scenarios (Figure 2). Conversely, negative direct effects on bivalves and enhanced predation by red rock crab and drills on Pacific oyster yielded community responses with higher levels of ambiguity relative to the other scenarios (scenario 4c, Figure 2). Furthermore, in all scenarios, sign ambiguity in the outcomes of Pacific oyster, drills, and red rock crab remained high even though each species was connected to CO_2 through direct linkages (Figure 2).

Linkage Influence

Linkages with interaction strengths that were associated with the sign response of community members to OA were determined (Table 3). For all species, the proportion of positive responses from the simulated community interaction matrices ranged from 0.19 to 0.93, with an average of value of 0.56 (Table 3). The proportion of deviance in the sign responses of community members explained by the MARS models was variable, ranging from 7% to 42% (Table 3). Of the 7 direct linkages between CO_2 and various community members in the OA scenario, 1 to 4 linkages (average: 2.8) were included as important predictors of sign responses; of the 70 non-CO_2 linkages, between 4 and 14 linkages (average: 8.7) were also included as predictors (Table 3).

Linkages between CO_2 and phytoplankton, *Z. japonica*, *Z. marina*, Pacific oyster, and Manila clam were important to varying degrees in predicting sign responses in 16, 13, 11, 9, and 6 community members, respectively (Figure 6). However, direct linkages between CO_2 and red rock crab and drills were not important in modeling variance in the response of any community member.

Table 3. Summary of multivariate adaptive regression splines (MARS) models predicting the sign response of Willapa Bay community members where community interaction coefficients are predictor variables. For each MARS model, the count of OA and non-OA linkages included in the fitted model are noted. The total potential number of OA and non-OA linkages are 6 and 70, respectively. Asterisks (*) denote species with direct linkages to CO_2 in the press scenario.

Community member	Proportion (–)	% deviance explained	OA linkages	Non-OA linkages
*Zostera japonica**	0.28	42	2	9
Ghost shrimp	0.81	35	3	9
Green sturgeon	0.81	35	3	9
Black brant	0.19	31	4	10
Pacific oyster*	0.66	30	4	13
Young of year Dungeness crab	0.27	29	4	12
Crows/gulls	0.84	24	4	6
Manila clam*	0.84	24	4	6
*Z. marina**	0.19	25	3	4
Zooplankton	0.45	23	2	11
Non-structure invertebrates	0.39	20	1	9
Structure invertebrates	0.38	19	2	14
Small fishes	0.37	17	4	12
Adult Dungeness crab	0.46	17	4	10
Drills*	0.28	15	4	9
Mud shrimp	0.07	12	1	5
Phytoplankton*	0.07	12	1	5
Red rock crab*	0.78	12	2	9
Chinook salmon	0.11	7	3	4

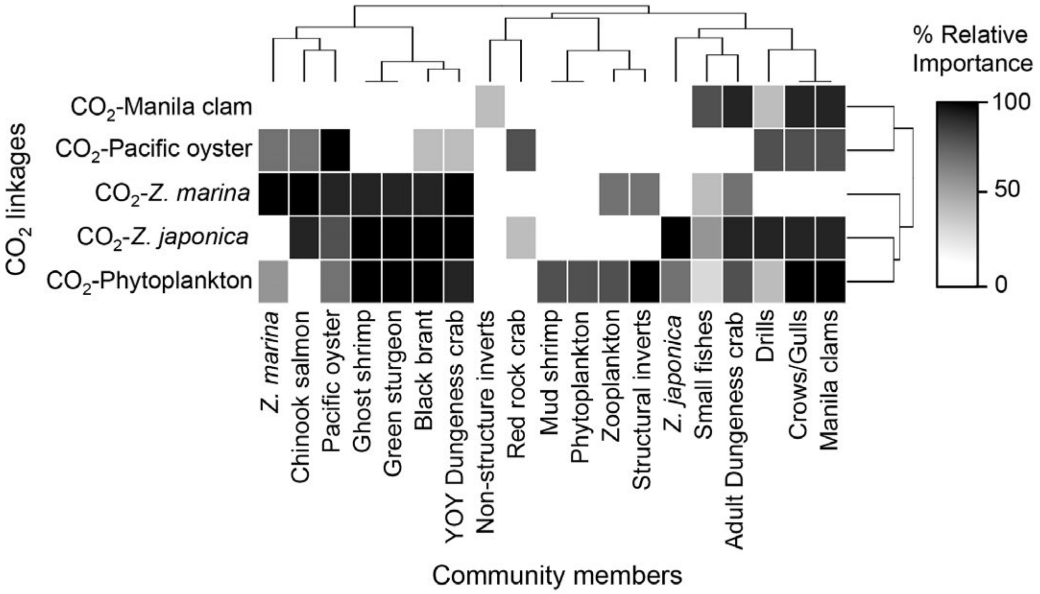

Figure 6. Relative importance of the interaction strength of direct CO_2 linkages to the sign response of community members to a CO_2 press perturbation. Community members and linkages are both ordered according to similarity.

Partial dependency plots were examined for Pacific oyster, which had an ambiguous sign response to the press scenario (63% of responses were negative, Table 3). In total, 14 linkages were included in the Pacific oyster MARS model, but for brevity and illustrative purposes, partial dependency plots were presented for the five most important linkages in terms of deviance reduction; these linkages account for ~70% of the explained deviance and include three linkages to CO_2 and two non-CO_2 linkages (Figure 7a–f). As expected, the probability of a negative response in Pacific oyster increased with the strength of the negative CO_2–Pacific oyster interaction (Figure 7a). However, the probability decreased as interaction strength between CO_2 and *Z. marina* and *Z. japonica* increased (Figure 7b–c). Among the non-CO_2 interactions, negative responses were more likely when the negative *Z. marina*–ghost shrimp and ghost shrimp–Pacific oyster interactions were weak, and less likely when the negative phytoplankton–*Z. marina* interaction was weak (Figure 7d–e).

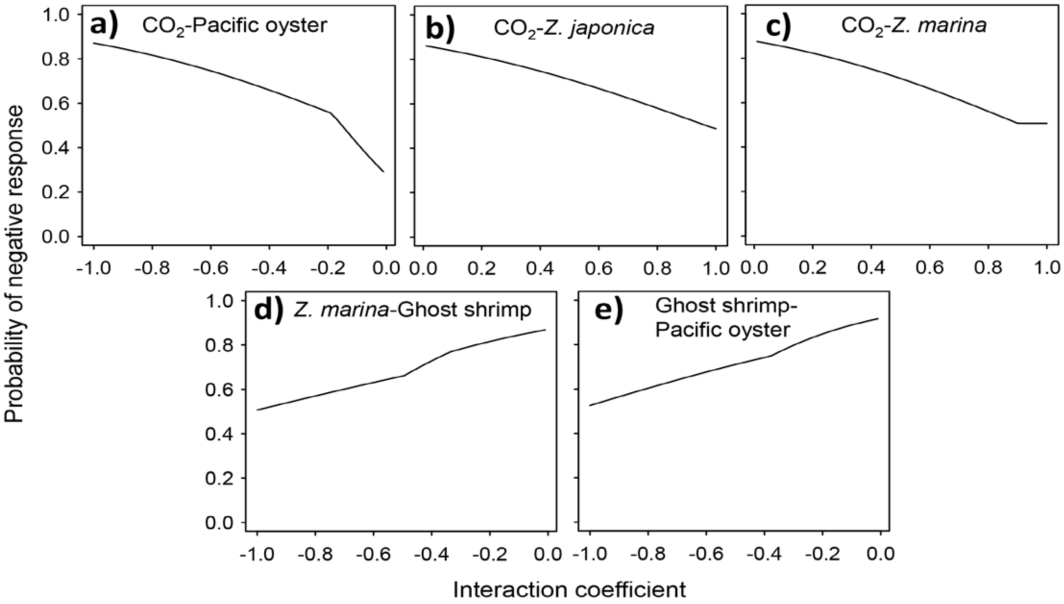

Figure 7. Partial dependence plots of the sign response of Pacific oyster to the five most important interaction coefficients in the community matrix.

DISCUSSION

As shown for South Puget Sound and Willapa Bay, specifying network structure alone can allow qualitative prediction and help identify outcomes that may be counterintuitive or potential tradeoffs resulting from a particular management or environmental change scenario. For instance, in the individual scenarios for bivalve cultivation effort, tradeoffs between different bivalve species were predicted: cultivation effort applied to geoduck alone increased geoduck, but led to decreases in Pacific oyster. Similarly, cultivation effort applied only to Pacific oyster increased Pacific oyster, but led to a decrease in Manila clam. Such patterns are likely due in part to indirect pathways involving the predator red rock crab, wherein an increase in one bivalve results in higher abundances of red rock crab, which increases predation on other bivalve prey. Tradeoffs were also evident in removal scenarios of individual predators, which had opposing effects on different bivalve species: removing red rock crab decreased geoduck and increased Manila clam, while removing graceful crab decreased Pacific oyster and increased Manila clam. Because QNMs integrate direct effects, indirect effects, and feedbacks, they can help identify tradeoffs arising from complex ecological interactions that might otherwise be difficult to anticipate (Levins 1998).

A key benefit of QNMs is that they allow rapid assessment of many scenarios and can help screen management actions that may yield ambiguous or problematic outcomes (Carey et al. 2014, Dambacher et al. 2009). For example, increased cultivation effort in the South Puget Sound QNM did not always ensure increased bivalve production. In scenarios where cultivation effort was applied to only one species of bivalve, the species responded positively and with high sign determinacy. However, under the multispecies press scenario, sign determinacy of the response of two of the three bivalves (Pacific oyster and Manila clam) decreased relative to the individual species press scenarios. Combining cultivation effort with predator removals or increased nutrients also resulted in ambiguous responses in some bivalves. The reduced sign determinacy is due to increases in the number of countervailing feedbacks; that is, the number of pathways conveying negative effects increased relative to the number conveying positive effects (Dambacher et al. 2003). Sign determinacy could be improved with quantitative information on interaction strengths, but this may be impractical to obtain (Dambacher et al. 2003, Puccia and Levins 1985). From a precautionary perspective, analyzing a variety of development and management scenarios can offer insight into conditions that lead to increased outcome uncertainty and into where action should proceed with caution (Carey et al. 2014).

For complicated QNMs, statistical analyses of associations between the simulated interaction coefficients and the predicted response of species provide a simple approach for revealing key linkages and the manner in which they influence the likelihood of negative or positive outcomes. For instance, under the scenario in which all hypothesized OA effects occur in Willapa Bay (scenario 4d), the sign response of Pacific oyster was ambiguous, but analysis of the simulated responses using MARS showed that the sign depended on the magnitude of a subset of network interactions. Unsurprisingly, the probability of observing a negative response in Pacific oyster increased with the negative interaction strength between CO_2 and Pacific oyster. However, the remaining linkages indicate that the effect of CO_2 on eelgrasses is transmitted to Pacific oyster indirectly through a linkage to ghost shrimp. Ghost shrimp negatively influence Pacific oysters and a decline in ghost shrimp due to an increase in eelgrass (via their negative interaction) decreases the probability of observing a negative response in Pacific oyster. Whether the interaction pathway is able to counteract the direct negative effects of OA on Pacific oysters will require additional study and highlights an area on which to focus future research. In the same vein, systematic assessment of linkage influence for all community members can highlight important community-wide interactions. Among the hypothesized direct CO_2 effects in Willapa Bay, the CO_2–phytoplankton interaction was retained as a significant predictor for a majority of community members (84%), while the CO_2–Manila clam linkage influenced the fewest (31%). Such information can help identify research priorities when considering the community as a whole.

The scenarios examined for South Puget Sound aquaculture reflect a small subset of potential applications, and the models could easily be tailored to address other aquaculture management issues including pest eradication, invasive species, disease, and climate variability. In addition, changes in policy that influence aquaculture permitting practices could also be evaluated using the QNM. For instance, it was assumed that aquaculture would not expand into eelgrass habitats in South Puget Sound, in accordance with current regulations. A policy change allowing aquaculture expansion into eelgrass could be simulated by adding negative linkages to *Z. marina* from the bivalve species that are cultivated at the same tidal depths where *Z. marina* occurs (e.g., Pacific oyster and geoduck; Ruesink and Rowell 2012, Tallis et al. 2009, Wagner et al. 2012). In the network corresponding to such a policy change, an increase in either bivalve species would have a negative effect on *Z. marina*. More generally, the network could be expanded further to include social and economic variables (e.g., demand, profit, jobs, recreational opportunities, scenic quality) to examine social–ecological tradeoffs in support of more holistic management approaches (Cranford et al. 2012, Dambacher et al. 2009, Soto et al. 2008).

Similar to other modeling approaches, QNMs have important limitations. First, a key assumption underpinning the method is that system variables are at or near equilibrium or closely tracking moving equilibrium conditions (Puccia and Levins 1985). In marine ecosystems, frequent disturbances (e.g., climate variability, pollution, fishing) may make this assumption unrealistic (Dambacher et al. 2009). However, the assumption is also routinely used in quantitative community and food web models (Bender et al. 1984, Yodzis 1998) and, if the system exhibits sustained bounded motion, the issue can be addressed by considering predicted responses within the context of an appropriately long time scale (Dambacher et al. 2009, Puccia and Levins 1985).

Second, the model assumes that the partial derivatives of system variables are adequately approximated by linear functions near equilibrium conditions. Strong nonlinearity may result from the system transitioning across a threshold, whereby links may be created or broken, or reversed in sign. Such thresholds would require the consideration of multiple networks corresponding to different states of the system (Dambacher and Ramos-Jiliberto 2007). Last, like all ecosystem models, simplifying assumptions were made regarding how species were aggregated. In general, an effort was made to aggregate sets of species into variables that would likely possess similar linkages and therefore respond similarly to system perturbations (Puccia and Levins 1985). The necessity of lumping variables in speciose ecosystems and the associated caveats of doing so are understood well in both qualitative and quantitative ecosystem modeling arenas (Fulton et al. 2003, Metcalf et al. 2008), and the final models reflected study efforts to simplify these estuarine food webs to improve interpretability.

Although quantitative models are helpful for understanding and predicting the effects of aquaculture, they are difficult to parameterize in systems with limited data. Qualitative models offer an alternative method, requiring as a minimum only basic knowledge of the natural history of key species composing a system (Levins 1998). QNMs provide imprecise predictions, but this can be viewed as advantageous because emphasis is moved away from the precise measurement of parameters (which may be costly and difficult or impossible to do) and towards understanding the main processes and community interactions that influence the dynamics of the complete system (Dambacher et al. 2009, Puccia and Levins 1985). Ecosystem approaches to aquaculture require modeling methods that can synthesize systems-level processes. QNMs are flexible, highly robust, and effective frameworks for organizing diverse types of information, and they should be of considerable value to resource managers and growers alike.

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Reum JCP, Ferriss BE, McDonald PS, Farrell DM, Harvey CJ, Klinger T, Levin PS (2015) Evaluating community impacts of ocean acidification using qualitative network models. Marine Ecology Progress Series 536:11-24. doi: 10.3354/meps11417.

Reum JCP, McDonald PS, Ferriss BE, Farrell DM, Harvey CJ, Levin PS (2015) Qualitative network models in support of ecosystem approaches to bivalve aquaculture. ICES Journal of Marine Science. doi: 10.1093/icesjms/fsv119.

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APPENDIX

Linkages in Aquaculture–Environment Interactions in South Puget Sound and Willapa Bay

Appendix Table 1. Description of linkages in the South Puget Sound model of aquaculture–environment interactions and primary references where available. Linkage types denoted by an asterisk indicate uncertain interactions (represented as dashed lines in Figure 2). For brevity, only the predator linkage is listed when the predator effect and prey effect in a predator–prey relationship are either both certain or uncertain.

Effect of	Effect on	Type	Comments and references
Scoters	Manila clam	Predator–prey (predator)	Scoters prey on manila clams, reducing clam densities (DeFrancesco and Murray 2010, Lewis et al. 2007).
Manila clam	Scoters	Predator–prey (prey)*	Unclear to what extent scoter populations are driven by Manila clam aquaculture (Žydelis et al. 2006).
Small fishes	Manila clam	Predator–prey*	Unclear if clam loss due to siphon nipping from flatfish and sculpin occurs; unknown if cultured manila clams are important to small fish populations (e.g., Armstrong et al. 1995, Meyer and Byers 2005, Peterson and Quammen 1982).
Small fishes	Structure invertebrates	Predator–prey	Benthic invertebrates are important prey for English sole (Buechner et al. 1981), sculpin (Armstrong et al. 1995, Dinnel et al. 1990, Williams 1994), shiner perch (Troiano et al. 2013).
Small fishes	Non-structure invertebrates	Predator–prey	Benthic invertebrates are important prey for English sole (Buechner et al. 1981), sculpin (Armstrong et al. 1995, Dinnel et al. 1990, Williams 1994), shiner perch (Troiano et al. 2013).
Graceful crab	Manila clam	Predator–prey (predator)	Manila clam loss due to predation by graceful crab (DeFrancesco and Murray 2010).
Graceful crab	Structure invertebrates	Predator–prey	Graceful crab are likely generalist predators, similar to red rock crab (Knudsen 1964). Common in areas with and without aquaculture structure (Brown and Thuesen 2011, McDonald et al. 2015).
Graceful crab	Non-structure invertebrates	Predator–prey	Graceful crab are likely generalist predators, similar to red rock crab (Knudsen 1964). Common in areas with and without aquaculture structure (Brown and Thuesen 2011, McDonald et al. 2015).
Red rock crab	Structure invertebrates	Predator–prey	Generalist predator, may occur in mud habitats (Knudsen 1964, Robles et al. 1989).
Red rock crab	Pacific oyster	Predator–prey	Red rock crab prey on Pacific oyster (Grason and Miner 2012) and prefer oyster bed habitat (Holsman et al. 2006).
Red rock crab	Moon snail/sea stars	Predator–prey	Moon snail and sea stars are preyed upon by red rock crab (PS McDonald, Univ. Washington School of Aquatic and Fisheries Sciences, Seattle, personal communication).
Red rock crab	Manila clam	Predator–prey	Red rock crab prey on cultured Manila clams (Anderson et al. 1982, Boulding and Hay 1984, Chew 1989, Toba et al. 1992).
Chinook salmon	Structure invertebrates	Predator–prey	Predator to benthic invertebrates (Buechner et al. 1981).
Chinook salmon	Zooplankton	Predator–prey	Zooplankton common in Chinook salmon diet (e.g., Duffy et al. 2010, Troiano et al. 2013).
Moon snail/sea stars	Manila clam	Predator–prey*	Known predator of clams (Kozloff 1983, Toba et al. 1992), but unclear if moon snail significantly reduces commercial Manila clam productivity (Cook and Bendell-Young 2010).
Moon snail/sea stars	Structure Invertebrates	Predator–prey	Generalist predators of small sedentary invertebrates (Kozloff 1983).
Moon snail/sea stars	Nonstructure invertebrates	Predator–prey	Moon snail and sea stars both feed on bivalves and other infaunal invertebrates (Kozloff 1983).
Moon snail/sea stars	Pacific oyster	Predator–prey	Known predator of Pacific oyster (DeFrancesco and Murray 2010).
Moon snail/sea stars	Geoduck	Predator–prey (predator)	Sea stars prey on geoduck (Mauzey et al. 1968, Sloan and Robinson 1983, Van Veldhuizen and Phillips 1978), though moon snail predation has not been directly observed (Straus et al. 2013).
Geoduck	Moon snail/sea stars	Predator–prey (prey)*	Unclear if cultured geoduck are important to moon snail/sea star productivity.

Appendix Table 1 • continued

Pacific oyster	Structure invertebrates	Positive	Pacific oyster addition increases epibenthic invertebrate abundance (Dumbauld et al. 2001).
Pacific oyster	Phytoplankton	Negative	Pacific oysters are filter feeders (Wheat and Ruesink 2013).
Zostera marina	Structure invertebrates	Positive	Increase in plant density likely to increase benthic invertebrate abundance (e.g., Attrill et al. 2000).
Z. marina	Chinook salmon	Positive*	*Z. marina* serves as a refuge for Chinook salmon (Semmens 2008), but Chinook salmon are also found in other habitats and associations with *Z. marina* do not appear to be tied to foraging (Dumbauld et al. 2015, Hosack et al. 2006).
Z. marina	Small fish	Positive*	Increase in eelgrass may potentially increase small fish abundance (e.g., Kelly et al. 2008), but is uncertain.
Geoduck	Structure invertebrates	Positive	Increased invertebrate abundance with geoduck farm structures (McDonald et al. 2015).
Geoduck	Phytoplankton	Negative	Geoduck filter feed, consume phytoplankton (Goodwin and Pease 1989).
Structure invertebrates	Phytoplankton	Predator–prey	Structure invertebrates include deposit and filter feeders.
Non-structure invertebrates	Phytoplankton	Predator–prey	Non-structure invertebrates include deposit and filter feeders.
Zooplankton	Phytoplankton	Predator–prey	Zooplankton include filter feeders (Harvey et al. 2012).
Phytoplankton	*Z. marina*	Predator–prey	*Z. marina* likely light-limited at lower end of its distribution (Britton-Simmons et al. 2010, Thom and Albright 1990, Thom et al. 2008).

Appendix Table 2. Details of the interactions within Willapa Bay as depicted in Figure 3. Interactions denoted by asterisks under Type indicate those that are poorly understood (represented by dashed lines in Figure 2). For brevity only the predator linkage is listed when the predator effect and prey effect in a predator–prey relationship are either both certain or uncertain. If certainty differs, predator and prey linkages are noted separately.

Effect of	Effect on	Type	Comments and references
Crows/Gulls	Manila clam	Predator–prey (predator)	Important predators of manila clams in Willapa Bay (DeFrancesco and Murray 2010).
Manila clam	Crows/Gulls	Predator–prey (prey)*	Unknown if manila clam are important to crow/gull population productivity.
Small fishes	Manila clam	Predator–prey*	Potential manila clam loss due to siphon nipping (e.g., by flatfish and sculpin) (Armstrong et al. 1995, Meyer and Byers 2005, Peterson and Quammen 1982, Williams 1994); unknown if important mortality source to Willapa Bay clams.
Small fishes	Non-structure invertebrates	Predator–prey	Benthic invertebrates are important prey for English sole (Buechner et al. 1981), sculpin (Armstrong et al. 1995, Dinnel et al. 1990, Williams 1994), and shiner perch (Troiano et al. 2013).
Small fishes	Structure invertebrates	Predator–prey	Benthic invertebrates are important prey for English sole (Buechner et al. 1981), sculpin (Armstrong et al. 1995, Dinnel et al. 1990, Williams 1994), and shiner perch (Troiano et al. 2013).
Small fishes	Young of year (YOY) Dungeness crab	Predator–prey	Common diet item in sculpin (Armstrong et al. 1995).
Adult Dungeness crab	Manila clam	Predator–prey (predator)	Known predator of Manila clams (e.g., Smith 1996).
Manila clam	Adult Dungeness crab	Predator–prey (prey)*	Unknown if Dungeness crab abundance depends on Manila clams.
Adult Dungeness crab	Non-structure invertebrates	Predator–prey	Dungeness prey on bivalves, small crustaceans (Stevens et al. 1982); prefer mud habitat over oyster and eelgrass (Holsman et al. 2006).
YOY Dungeness crab	Adult Dungeness crab	Positive	YOY Dungeness crab prefer structured habitats over mud (Armstrong et al. 1995 Dumbauld et al. 1993, Eggleston and Armstrong 1994, McMillan et al. 1995) and recruit into the adult population.
Red rock crab	Pacific oyster	Predator–prey	Significant predator–prey interaction (Garson and Miner 2012); red rock crab prefer oyster bed habitat (Holsman et al. 2006).
Red rock crab	Drills	Predator–prey	Significant predator–prey interaction (Garson and Minter 2012).
Red rock crab	Structure invertebrates	Predator–prey	Potential invertebrate prey (Knudsen 1964, Robles et al. 1989); red rock crab prefer structured habitats (Holsman et al. 2006).
Red rock crab	Manila clam	Predator–prey (predator)	Red rock crab prey on cultured Manila clams (Anderson et al. 1982, Boulding and Hay 1984, Chew 1989, Toba et al. 1992).
Manila clam	Red rock crab	Predator–prey (prey)*	Unknown if red rock abundance depends on Manila clams.
Chinook salmon	Zooplankton	Predator–prey	Zooplankton are prey to Chinook salmon in Willapa Bay (Troiano et al. 2013).
Chinook salmon	Structure invertebrates	Predator–prey	Chinook salmon prey on benthic invertebrates (Buechner et al. 1981) that occur in structured habitats (oyster beds, eelgrass; Hosack et al. 2006).
Green sturgeon	Ghost shrimp	Predator–prey (predator)	Predator exclusion experiments indicate green sturgeon can locally impact shrimp densities (Dumbauld et al. 2008).
Ghost shrimp	Green sturgeon	Predator–prey (prey)*	Unknown if ghost shrimp influence green sturgeon abundance.
Z. japonica	Manila Clam	Negative	*Z. japonica* reduces early survival (Ruesink et al. 2014) and growth in Manila clam (Patten 2014, Tsai et al. 2010).
Manila clam	Phytoplankton	Negative	Manila clam are filter feeders.
Z. japonica	Structure invertebrates	Positive	Increased plant density likely increases invertebrate abundance (e.g., Attrill et al. 2000).
Structure invertebrates	Phytoplankton	Predator–prey	Filter feeding invertebrates common in structured habitats (Ferraro and Cole 2007, Hosack et al. 2006).
Pacific oyster	*Z. marina*	Negative	Competition for space (Tallis et al. 2009, Wagner et al. 2012).
Pacific oyster	Structure invertebrates	Positive	Oysters increases epibenthic invertebrate abundance (Dumbauld et al. 2001).
Pacific oyster	Drills	Predator–prey	Drills prey on Pacific oyster (Buhle and Ruesink 2009).

Appendix Table 2 · continued

Drills	Structure invertebrates	Predator–prey	The drill *Urosalpinx cinerea* preys on barnacles, sedentary invertebrates (Kozloff 1983).
Pacific oyster	YOY Dungeness crab	Positive	Habitat and predator refuge (Armstrong et al. 1995, Dumbauld et al. 1993, Eggleston and Armstrong 1995, Fernandez et al. 1993).
Z. marina	YOY Dungeness crab	Positive	Positive association between eelgrass density and Dungeness crab, especially in spring (e.g., McMillan et al. 1995).
Pacific oyster	Phytoplankton	Predator–prey	Modeling evidence (Banas et al. 2007) and field studies (Wheat and Ruesink 2013) indicate drawdown control of phytoplankton.
YOY Dungeness crab	Structure invertebrates	Predator–prey	YOY Dungeness crab feed on benthic invertebrates (Iribarne et al. 1995).
Ghost shrimp	Pacific oyster	Negative	Ghost shrimp destabilize substrate and smother Pacific oysters with sediments (Dumbauld et al. 2006, Feldman et al. 2000).
Ghost shrimp	Non-structure invertebrates	Negative	Decreases sedentary benthic organisms, filter feeders (Posey 1986).
Z. marina	Structure invertebrates	Positive	Increase in plant density likely increases invertebrate abundances (e.g., Attrill et al. 2000) .
Z. marina	Chinook salmon	Positive*	Chinook salmon may have an affinity for *Z. marina* because of prey availability and predator refuge (Semmens 2008), but trawl survey data show no relationship between Chinook salmon abundance and eelgrass (Hosack et al. 2006).
Ghost shrimp	*Z. marina*	Negative	Ghost shrimp may bury seeds, smother eelgrass seedlings (Dumbauld and Wyllie-Echeverria 2003, Harrison 1987)
Ghost shrimp	*Z. japonica*	Negative	Ghost shrimp may bury seeds, smother eelgrass seedlings (Dumbauld and Wyllie-Echeverria 2003, Harrison 1987)
Z. marina	Ghost shrimp	Negative	Eelgrass roots may inhibit burrowing (Harrison 1987).
Z. japonica	Ghost shrimp	Negative	Eelgrass roots may inhibit burrowing (Harrison 1987).
Z. japonica	Structure invertebrates	Positive	Provides habitat for invertebrates (Posey 1988); increase in plant density may increase invertebrate abundance (e.g., Attrill et al. 2000).
Z. japonica	YOY Dungeness crab	Positive	Positive association between eelgrass density and YOY Dungeness crab, especially in spring (McMillan et al. 1995).
Z. japonica	Brant	Positive	Important prey item (Baldwin and Lovvorn 1994a, b); eelgrass area positivity correlated with Brant abundance (Ganter 2000, Wilson and Atkinson 1995).
Z. marina	Brant	Positive	Important prey item (Baldwin and Lovvorn 1994a, b); eelgrass area positivity correlated with Brant abundance (Ganter 2000; Wilson and Atkinson 1995).
Ghost shrimp	Green sturgeon	Predator–prey (prey)*	Unknown if prey abundance is limiting green sturgeon populations (Dumbauld et al. 2008).
Ghost shrimp	Non-structure invertebrates	Negative	Decreases sedentary benthic organisms, filter feeders (Posey 1986).
Mud shrimp	Phytoplankton	Predator–prey	Feeds on phytoplankton, can potentially reduce standing stock (Griffen et al. 2004).
Mud shrimp	Non-structure invertebrates	Negative	Reduction in sedentary invertebrates (Posey et al. 1991).
Phytoplankton	*Z. marina*	Negative	Lower subtidal distribution may be light limited, but results are ambiguous (Thom et al. 2008).
Phytoplankton	*Z. japonica*	Negative	Lower distribution potentially light limited but unresolved (Britton-Simmons et al. 2010, Kaldy 2006).
Phytoplankton	Zooplankton	Predator–prey	Zooplankton are important grazers (e.g., Calbet and Landry 2004).

APPENDIX LITERATURE CITED

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An Oceanographic Circulation Model for South Puget Sound

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ABSTRACT

A new, high-resolution (200 meter) circulation model for South Puget Sound was developed, both to illuminate water connectivity and residence-time patterns with application to South Puget Sound shellfish aquaculture and, as a pilot effort, to construct efficient methods for nesting high-resolution submodels within the model framework run by the University of Washington Coastal Modeling Group. A six-week simulation of late summer, low riverflow conditions (1 August to 15 October 2006) was performed, nested within a previously published full-year simulation of Puget Sound and the adjacent coastal ocean. Comparisons with tide-gauge records from Seattle and Tacoma show that the South Puget Sound model inherits the tidal-height performance of the larger model with almost no further modification. Modeled surface and bottom temperatures in the South Puget Sound domain show good agreement with Washington Department of Ecology monitoring data at 10 stations, although salinity stratification is likely biased by the omission of small, local freshwater sources in the model.

In the model, virtual particles (148,320 total) were released at the surface in each grid cell within South Puget Sound every six hours for the first 14 days of the model run and tracked in three dimensions. In general, the surface particles dispersed across South Puget Sound in a few days, with a mean motion toward the deep central channels and Main Basin from each of the fringing inlets. Results also suggest a strong gradient in residence time from the central, deep channels to the small, western inlets, creating a potential for localized effects on water quality that a bulk analysis would not resolve. A map of "drawdown time" — the time required for cultured shellfish to reduce the standing stock of phytoplankton by 50%, given their inlet-scale densities — was estimated and compared with the map of residence time. Results suggest that Henderson Inlet, Eld Inlet, Totten Inlet, Hammersley Inlet, Oakland Bay, and upper Case Inlet have combinations of long residence time and high densities of aquacultured filter-feeders such that aquaculture operations there may potentially control local phytoplankton concentrations. This is strong motivation to further investigate both the possible downstream effects on other consumers of phytoplankton and the possible role of aquaculture in mitigating eutrophication in western South Puget Sound.

INTRODUCTION

Recent advances have occurred in an effort to build a multi-scale biological–chemical–physical model of Puget Sound and its adjacent coastal waters that can link both local and large-scale stressors (e.g., land-use pressure, climate change, ocean acidification) to their impact on habitat for wild and cultured aquatic species. A high level of spatial detail is required if such a model is to inform management, policy, and site-specific concerns. This phase of work has focused on spatial resolution and developing tools to smoothly nest high-resolution submodels within the regional models run by the UW Coastal Modeling Group (CMG), using South Puget Sound as a test case.* The South Puget Sound work has been coordinated with a parallel effort through the Washington Ocean Acidification Center to add short-term forecasting ability and carbon chemistry to the CMG Cascadia model (Davis et al. 2014, Giddings et al. 2014, Siedlecki et al. 2015). Together, these efforts point the way toward an operational oxygen/pH early-warning system for Puget Sound and its surrounding waters.

This class of oceanographic model has many other potential applications; for example, analysis of pollution and sewage dispersal, larval supply and population connectivity, and the issue that has been focused on in this pilot effort: coupling between benthic grazers (such as cultured shellfish) and their phytoplankton diet. In general, benthic filter feeders in shallow estuaries limit and are limited by phytoplankton in the water column. The balance among local phytoplankton production, hydrodynamic import and export, shellfish consumption rates, and consumption and recycling by other grazers like zooplankton controls the carrying capacity of the system for shellfish production. In systems near their carrying capacity, food competition can arise both among filter feeders and between them and other biota. For example, previous work (Banas et al. 2007) sponsored by Washington Sea Grant demonstrated that in Willapa Bay, cultured shellfish and other benthic grazers appear to control phytoplankton concentrations. Consequently, aquaculture in Willapa Bay may be nearing a point of diminishing returns, where adding one more oyster increases total oyster productivity but decreases the average oyster's food intake. In systems where the leading concern is not undersupply but rather oversupply of phytoplankton (i.e., systems vulnerable to eutrophication†, such as some South Puget Sound inlets), the same calculation of phytoplankton drawdown in relation to supply indicates the potential for aquaculture to mitigate water quality concerns. This section describes the South Puget Sound model setup; comparisons with tide-gauge, temperature, and salinity data; an analysis of residence time and connectivity in South Puget Sound in late summer; and finally, an exploratory analysis of phytoplankton drawdown potential.

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* *http://faculty.washington.edu/pmacc/cmg/cmg.html*

† Overgrowth of phytoplankton, often associated with water quality problems such as low oxygen.

MATERIALS AND METHODS

The Model

The South Puget Sound model was implemented in ROMS (Regional Ocean Modeling System; Haidvogel et al. 2000), an oceanographic community standard for hydrodynamic and biophysical modeling. The South Puget Sound model is one-way nested within the Puget Sound and adjacent coast model of Sutherland et al. (2011) — the "Salish" model — which is in turn nested within the global Navy Coastal Ocean Model (Barron et al. 2006, 2007). The Salish model has variable horizontal resolution, generally 300–1,000 meters across Puget Sound and the southern Salish Sea; the nested South Puget Sound model has a constant resolution of 200 meters as far north as Tacoma Narrows, expanding to 800 meters in a transition region in southern Main Basin. The model has 30 vertical levels, which use terrain-following coordinates. Output is saved hourly.

This pilot study conducted a six-week simulation of late summer, low riverflow conditions, August 1–October 15, 2006, driven by the full year 2006 simulation described by Sutherland et al. (2011). Tidal and water-property signals were passed from the Salish model to the South Puget Sound model through an open boundary near Seattle (Figure 1). The South Puget Sound model additionally received direct input from the Duwamish, Puyallup, Nisqually, and Deschutes rivers (Banas et al. 2014, Sutherland et al. 2011), and heat fluxes and wind stress from the the MM5 atmospheric model (Mass et al. 2003), following the methods described by Sutherland et al. (2011). The South Puget Sound model includes wetting and drying of the intertidal zone, unlike the Salish model, which has a minimum water depth of 4 meters. Bathymetry is interpolated from the Finlayson (2005) digital elevation model.

RESULTS AND DISCUSSION

Comparison with Observations

Modeled and observed tidal heights at Seattle and Tacoma were compared (Table 1) for both the Salish model and the South Puget Sound model nested within it. As described by Sutherland et al. (2011), the amplitudes of the semi-diurnal tides (the M2, S2 constituents) are biased low by approximately 25%, an error which likely resulted from under-resolved topographic mixing or a bias in the resonance characteristics (i.e., interaction between incoming and reflected, outgoing waves, or amphidromic structure) of the modeled Puget Sound/Strait of Georgia system as a whole. For purposes of this study, the significant result is that the South Puget Sound model inherits the tidal-height performance of the Salish model with almost no further modification.

Comparisons between the South Puget Sound model and in situ surface and bottom temperature and salinity were performed at Washington Department of Ecology stations that were regularly occupied ($n \geq 9$) in 2006 (Figure 2; see Sutherland et al. 2011 for a much more extensive comparison between the Salish model and hydrographic data). Within the six-week study period, there were 17 observations across these stations. Modeled surface and bottom temperatures ($n = 34$) show good agreement with these observations ($r^2 = 0.56$, mean bias 0.24°C, ratio of standard deviation 1.07). This is indirect evidence that the balance of local heating (which is presumably accentuated in both model and reality by the extensive shallows in the region) and flushing toward deeper water is approximately correct in the model. Modeled salinities ($n = 33$, omitting one bad value) are significantly correlated with the observations (r^2

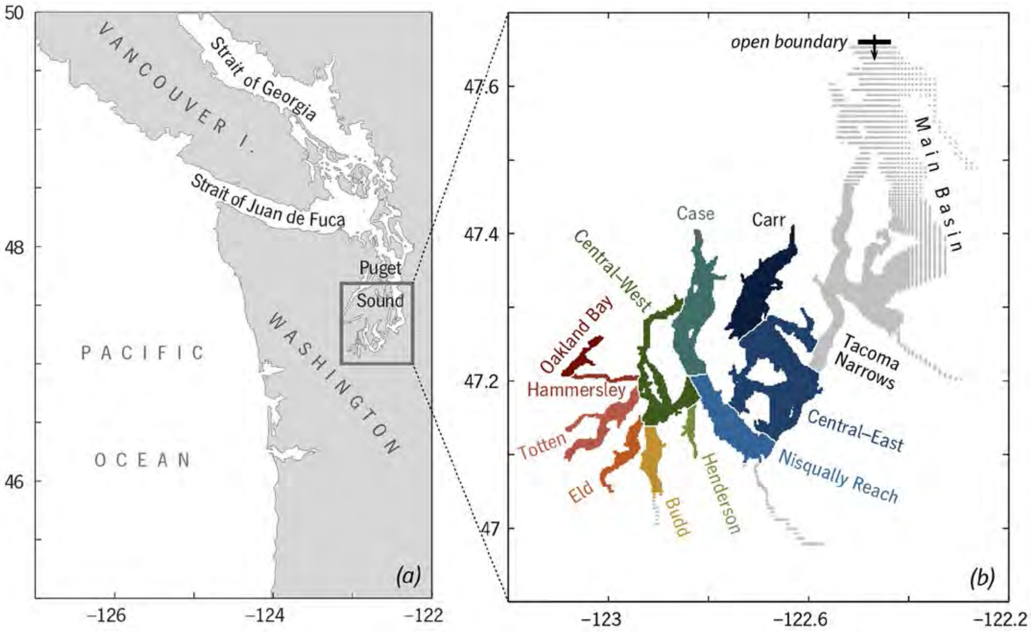


Figure 1. (A) Extent of the Sutherland et al. (2011) "Salish" model domain. (B) South Puget Sound model domain, showing the open boundary where signals from the Salish model are passed in and definitions of the inlets used in the connectivity analysis.

Table 1. Comparison between modeled and observed phase and amplitude of the three leading tidal constituents for the Sutherland et al. (2011) Salish model, and the new South Puget Sound model nested within it. Results are reported for tide gauges at Seattle and Tacoma. An amplitude ratio of 1 and phase difference of 0 would indicate perfect performance.

	M2		S2		K1	
	Amplitude ratio	**Phase difference**	**Amplitude ratio**	**Phase difference**	**Amplitude ratio**	**Phase difference**
Salish model						
Seattle	0.76	11.8	0.76	10.2	1.02	-3.6
Tacoma	0.76	11.5	0.77	9.5	1.02	-3.5
South Puget Sound model						
Seattle	0.75	22.6	0.77	24.1	1.03	1.2
Tacoma	0.73	24.2	0.76	24.5	1.02	2.4

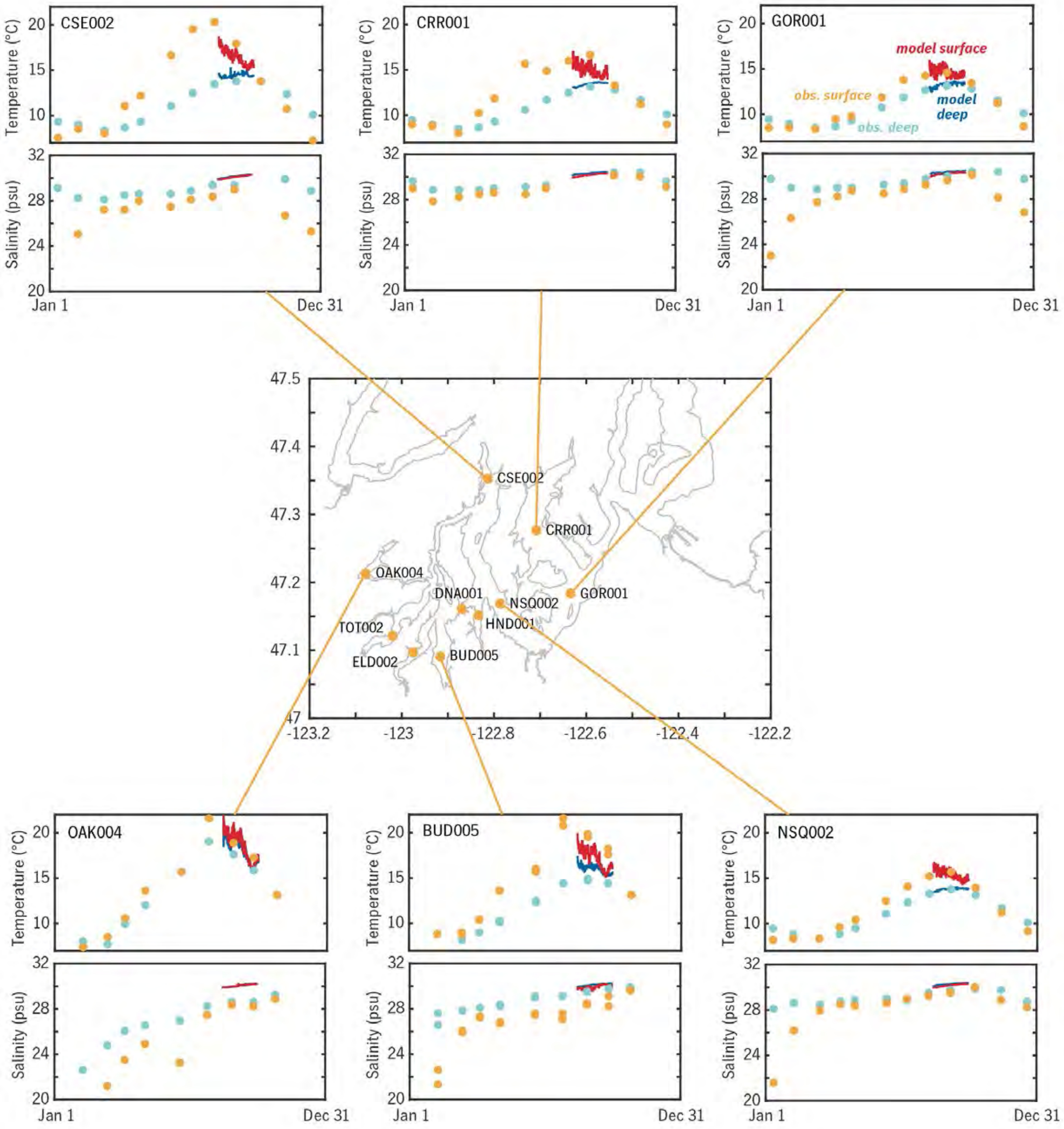


Figure 2. Annual cycles of surface and bottom salinity and temperature at six Washington Department of Ecology monitoring stations in South Puget Sound, Jan–Dec 2006. The locations of these and four other stations included in the statistical model validation described in the text are shown in the central panel. Approximately monthly in situ observations are shown in orange (surface) and light blue (bottom); hourly time series from the six-week South Puget Sound model run is shown in red (surface) and dark blue (bottom).

= 0.51) but biased somewhat high (mean bias 0.9 psu (practical salinity units)) and with reduced variance (ratio of standard deviations 0.24). The bias is comparable with that reported by Sutherland et al. (2011) for the Salish model, and thus is probably inherited directly from that model. Although not the only cause, one cause of the reduced variance is under-stratification in the South Puget Sound model (Figure 2; note that this phenomenon is on a different time and space scale from the more general stratification validation presented by Sutherland et al. 2011). Since tidal amplitude and thus tidal mixing is biased low, not high, the lack of local salinity stratification may reflect the omission of small freshwater inputs other than the four major rivers mentioned previously.

Estimating the effect of bias in stratification and tidal amplitude on overall residence time is not straightforward. To the extent that South Puget Sound is flushed by baroclinic, river-driven mechanisms, both the low stratification and low tidal amplitude suggest an upward bias in estimated flushing rate. In terms of flushing due to tidal dispersion, the low tidal amplitude suggests a downward bias. To the extent that it is flushed by wind-driven circulation, both of these measures may be irrelevant to flushing rate. Progress on this front — better diagnosis of model biases, and resolution of them — will require analysis of a full seasonal cycle and detailed comparisons with process studies such as Edwards et al. (2007).

Residence Time and Connectivity

As in other Pacific Northwest estuaries (Hickey and Banas 2003), late summer is the low riverflow season in Puget Sound, during which river-driven estuarine circulation is expected to be at a minimum and residence time, particularly for surface waters, to be longer than at other times of year. Note that wind-driven circulation may complicate this picture in some inlets (Edwards et al. 2007), as an analysis across a full annual cycle could elucidate in future work.

Virtual particles were released in the model at the surface in each grid cell within South Puget Sound (colored areas, Figure 1) every six hours for the first 14 days of the model run to uniformly sample a full spring–neap cycle (2,643 release locations, 148,320 particles total). Particles were tracked in three dimensions, taking vertical mixing into account, following the methods described in Banas et al. (2014). In general, particles dispersed across South Puget Sound in a few days (illustrated for a few representative inlets, Figure 3). The mean motion of surface particles is seaward, toward the deep central channels and Main Basin, from each of the fringing inlets. This is consistent with the typical structure of tidally averaged estuarine circulation (inflow at depth, outflow at the surface), although the particle-tracking experiment described here may obscure more complex transient or localized patterns, such as wind-driven reversals or flow structures with more than two layers.

This detailed particle experiment allows mapping which subregions of South Puget Sound are “downstream” of others, and includes time lags between them (Figure 4; see Banas et al. 2014 for a comparable analysis on a larger spatial scale). In general, eastern South Puget Sound is downstream of western South Puget Sound, with the “Central–West” inlet (Figure 1) forming a natural dividing line. Note that much of the overall volume–flux pattern simply reflects the relative volumes or surface areas of the “from” and “to” inlets. Although most of the flux from Case Inlet is found in eastern South Puget Sound (or beyond, in Main Basin) after approximately one week, because of its relative size, a measurable fraction of Case Inlet water is found in the small western inlets (Budd, Eld, Totten, Hammersley/Oakland Bay) also after about one week. Budd, Eld, and Totten inlets exchange non-negligible amounts of surface water on the same timescale.

The time required for the median particle to exit the source basin is one convenient measure of the residence time. Note that this metric is scale-dependent, and so residence times for each inlet individually are different from the residence time of particles from each inlet in South Puget Sound as a whole (Figure 5, Table 2). Since this analysis resolves the exit pathways of surface water only, the overall residence time of South Puget Sound as a whole (14 days) is somewhat lower than that calculated by Sutherland et al. (2011) for water at all depths. The median surface-water particle in each inlet is found to exit its inlet in less than one week, although the median particle from each of the fringing inlets is still found somewhere in South Puget Sound after two weeks. Water from Oakland Bay dispersed from South Puget Sound too slowly to calculate a median residence time from this six-week model run.

Overall, these results suggest that while tidal flushing of South Puget Sound is quite efficient on average, the gradient in residence time from the central, deep channels to the small, western inlets is quite strong, potentially creating localized effects on water quality that a bulk analysis would not resolve. This pattern is motivation for returning to the initial question that promped this study: Where in South Puget Sound might cultured shellfish significantly affect phytoplankton biomass?

Phytoplankton-Drawdown Potential

In general, the balance of (i) local phytoplankton production, (ii) hydrodynamic import and export, and (iii) filter feeder consumption rates controls the carrying capacity of a shallow estuary for filter-feeder production (Cloern 1982, Dame and Prins 1988, Peterson and Black 1987). The same balance determines the potential for benthic filter feeders to act as a brake on eutrophication. In systems near their carrying capacity, food competition can arise both among filter feeders and between them and other biota. The balance of (i), (ii), and (iii) needs to be considered across a range of scales — from the system down to individual mudflats — and so it is not straightforward to determine a priori whether a given aquaculture region is near its carrying capacity or capable of causing “downstream” effects on other ecosystem components, whether positive or negative, via depletion of phytoplankton.

The full balance of (i), (ii), and (iii) cannot be assessed using the present version of the model, but an upper bound can be placed on the potential for local benthic control of phytoplank-

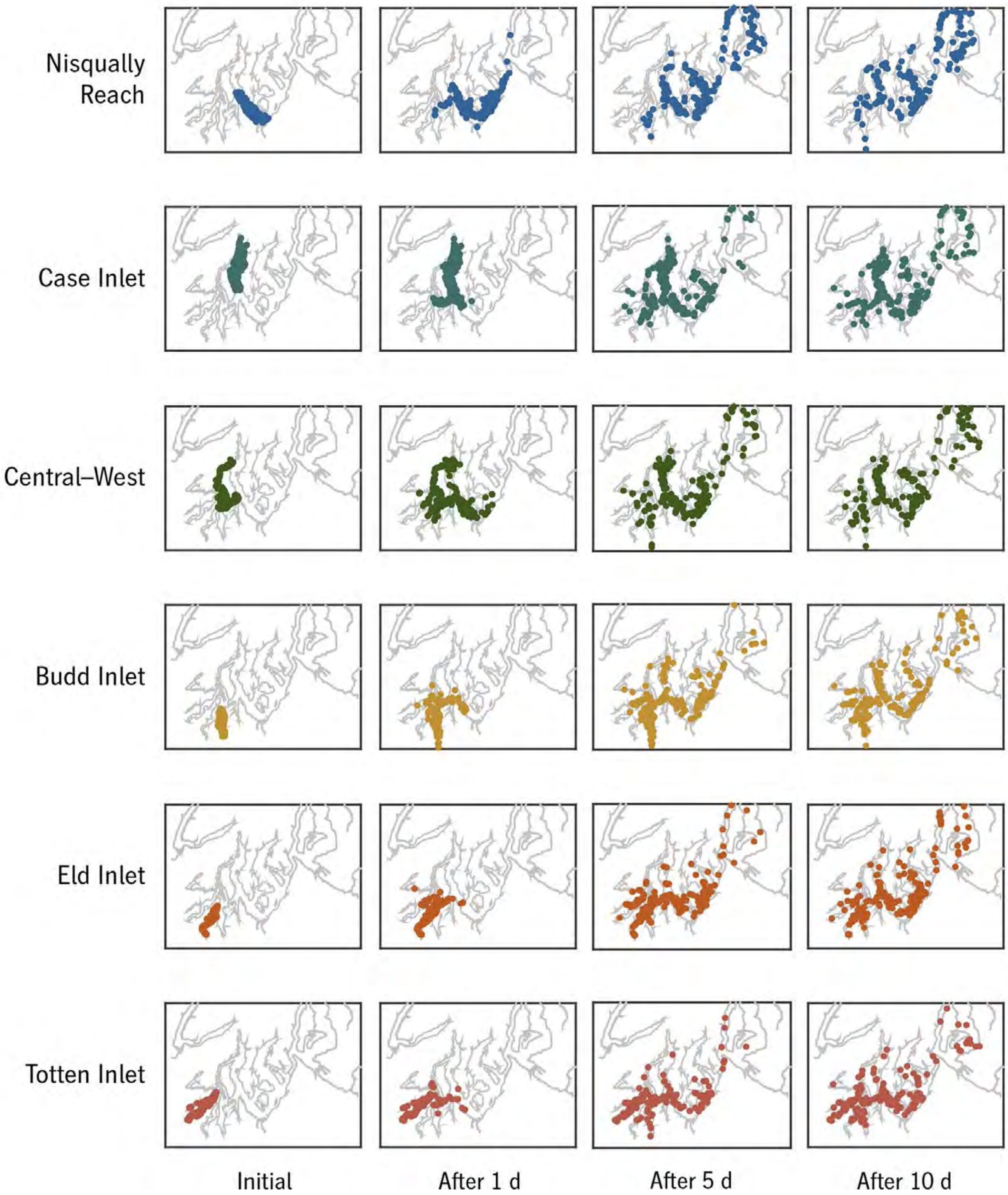
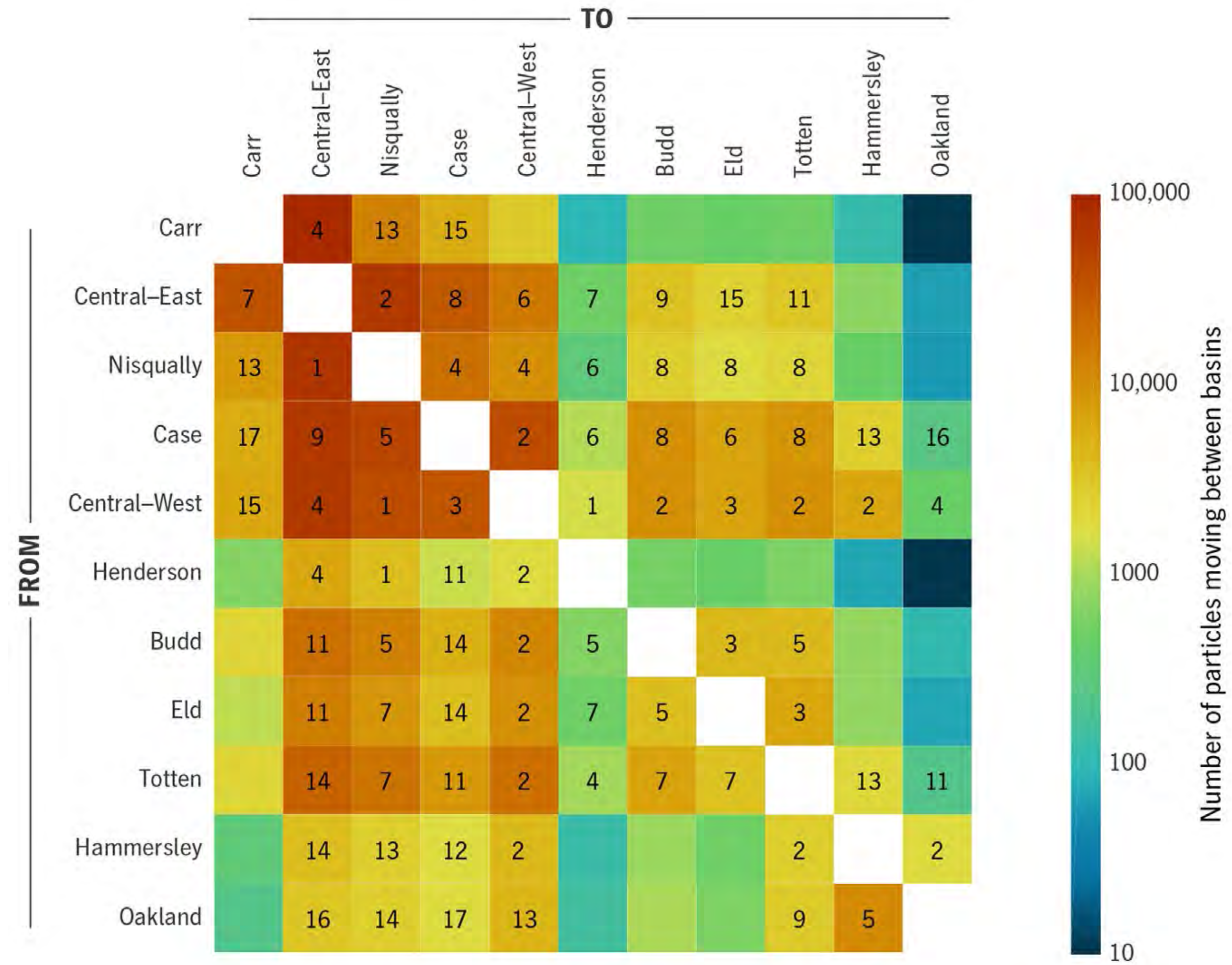


Figure 3. Dispersion of model particles released at the surface in each of six inlets initially and after 1, 5, and 10 days of transport.



	Carr	Central–East	Nisqually	Case	Central–West	Henderson	Budd	Eld	Totten	Hammersley	Oakland
Carr		4	13	15							
Central–East	7		2	8	6	7	9	15	11		
Nisqually	13	1		4	4	6	8	8	8		
Case	17	9	5		2	6	8	6	8	13	16
Central–West	15	4	1	3		1	2	3	2	2	4
Henderson		4	1	11	2						
Budd		11	5	14	2	5		3	5		
Eld		11	7	14	2	7	5		3		
Totten		14	7	11	2	4	7	7		13	11
Hammersley		14	13	12	2				2		2
Oakland		16	14	17	13				9	5	

Figure 4. Connectivity among South Puget Sound inlets. Color indicates the total number of model particles found to move from one inlet to another (note the logarithmic scale). Superimposed numbers indicate the time lag of the peak transport for each connection. Small-volume connections equivalent to <5% of the particles leaving the "from" basin and entering the "to" basin are unlabeled.

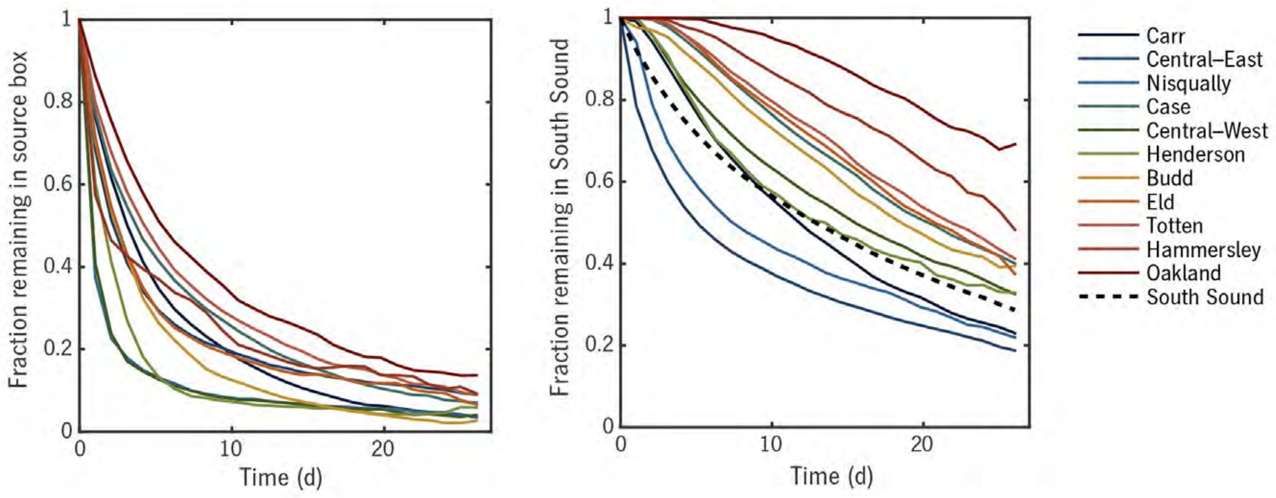


Figure 5. Fraction of model particles remaining in each source basin and in South Puget Sound as a whole over time. Time is measured as days since particles were released; releases occurred at six-hour intervals for 14 days and have been conflated. The time at which each curve crosses 0.5 is the median residence time.

Table 2. Median residence time in the source inlets and in South Puget Sound as a whole for surface water in each inlet shown in Figure 1.

	Median residence time in source inlet (days)	Median residence time in South Puget Sound (days)
Carr Inlet	4	13
Central–East	3	5
Nisqually Reach	1	8
Case Inlet	4	21
Central–West	1	16
Henderson Inlet	2	14
Budd Inlet	3	19
Eld Inlet	3	21
Totten Inlet	5	22
Hammersley Inlet	2	26
Oakland Bay	6	>28
Total	—	14

ton standing stock at each location in the model domain. A benthic clearance rate (in m^3 of overlying water per m of benthic area per s, or m s^{-1}) was estimated for each inlet shown in Figure 1, based on multiplying cultured shellfish density (g_{dry} km^{-2}) by shellfish clearance rate (L $hr^{-1} g_{dry}^{-1}$) and the proportion of total South Puget Sound cultivated area. Clearance rates were then summed across species to obtain the total water filtered by region (see Appendix for additional details, p. 67). The depletion of an initial phytoplankton concentration P by benthic grazers with clearance rate α follows:

$$\frac{1}{P}\frac{dP}{dt} = \frac{\alpha}{h}$$

where h is water depth (See Banas et al. 2007, Lucas et al. 1999 for a fuller treatment). This describes an exponential decay whose timescale can be written as follows:

$$T_{drawdown} = \frac{\ln 2}{86400}\frac{1}{f_{shallow}}\frac{h_{graz}}{\alpha}$$

Here, $f_{shallow}$ denotes the fraction of time a given model particle spends in shallow water overlying benthic grazers, and h_{graz} the typical water-column depth experienced during those conditions (this study assumed two meters). The leading coefficients make $T_{drawdown}$ an estimate of the time, in hours, required for this type of intermittent benthic grazing to reduce an initial phytoplankton population by half. It is thus directly comparable with the median residence time calculated previously, denoted T_{res}. If $T_{drawdown} >> T_{res}$, then the circulation is likely to flush phytoplankton from the area too fast for benthic grazers to have much effect, and the likely balance for the phytoplankton budget is between growth and advection. If $T_{drawdown} << T_{res}$, then it is possible for benthic grazing to constitute the dominant loss term, and the phytoplankton budget is likely a balance between local growth and local pelagic and benthic grazing.

$T_{drawdown}$ was calculated for each model particle and averaged results were calculated by release location. Maps of $T_{drawdown}$ and T_{res} are shown in Figure 6. To emphasize the (very approximate) threshold where $T_{drawdown}$ and T_{res} balance, values of $T_{drawdown}$ longer than T_{res} are blanked out (gray). As a simple sensitivity test, $T_{drawdown}$ was also calculated using uniform high (10^{-4} m s^{-1}) and low (10^{-5} m s^{-1}) values for α in place of the spatially explicit best guess described previously.

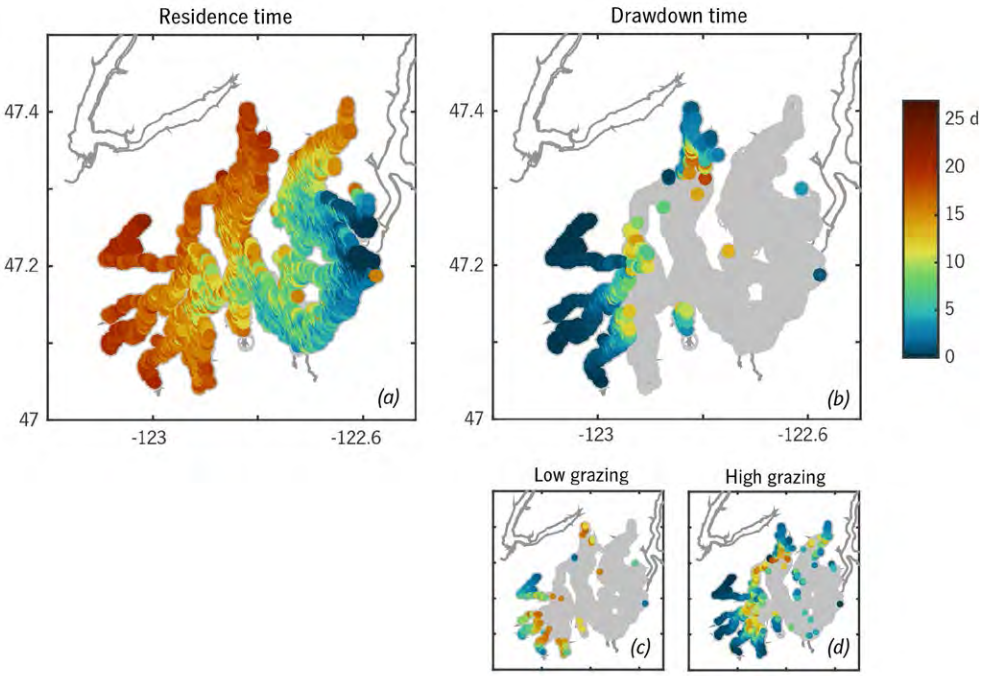


Figure 6. Timescales of (a) flushing by physical processes and (b) depletion of phytoplankton by aquacultured benthic grazers. Areas with drawdown time longer than residence time are shown in gray. (c,d) Comparison cases in which drawdown time was estimated using spatially uniform high and low estimates of benthic clearance rate.

The criterion for potential local control by benthic grazers is met in Henderson, Eld, Totten, Hammersley, and upper Case inlets, and Oakland Bay. This is strong motivation for further study, via both observations and modeling, of coupling between phytoplankton, cultured shellfish, and water quality in these systems.

CONCLUSION

This project served as a pilot study for incorporating high-resolution submodels and intertidal processes into the regional hindcast/forecast model under development by the UW Coastal Modeling Group. The potential for model forecasts on scales relevant to aquaculture operations in South Puget Sound is high. This project also served as a first, approximate mapping of the areas of strong potential interaction between aquaculture and total phytoplankton production. Based on the preceding results, one might hypothesize that the small inlets of western South Puget Sound experience noticeable food competition between cultured bivalves and other consumers of phytoplankton. One might also hypothesize that these inlets are at noticeably lower risk of eutrophication than they would be in the absence of shellfish aquaculture. Methodologically, the results indicate that future modeling of biogeochemistry and water quality in South Puget Sound needs to take the benthic grazer population into account, much as Banas et al. (2007) found was true for Willapa Bay.

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Appendix

FILTERING CAPACITY CALCULATIONS

Bridget E Ferris, Washington Sea Grant

An estimate of the filtering capacity of cultured bivalves in South Puget Sound was calculated. Specifically, the study focused on the filtering capacity of the three species that account for the vast majority of harvest in the region: Pacific oyster (*Crassostrea gigas*), Manila clam (*Venerupis philippinarum*), and geoduck (*Panopea generosa*). An overview is provided of the procedure followed by additional detailed information on specific calculations in the following subsections.

Overview

Filtering capacity was estimated by multiplying bivalve density (g_{dry} km^{-2}) and clearance rate estimates (L hr^{-1} $g_{dry}{}^{-1}$) with the proportion of area cultivated in each Washington Department of Fish & Wildlife (WDFW) aquaculture district in South Puget Sound and then normalizing to obtain a species- and district-specific weighted filtering capacity (L hr^{-1} $g_{dry}{}^{-1}$). Filtering capacity was then summed across species to obtain the total water filtered within each WDFW aquaculture district. Filtration rate (L hr^{-1} $g_{dry}{}^{-1}$) was converted to m s^{-1} (the equivalent of m^{3} s m^{-2} or volume (m^{3}) of water filtered per second by a given density (m^{2}) of bivalves (Banas et al. 2007)) to facilitate calculation of potential phytoplankton drawdown in the South Puget Sound circulation model (Table 1).

Density Calculations

Standing stock density (kg km^{-2}) was calculated based on harvest biomass and age of harvest, following Banas et al. (2007). For each bivalve species, density was estimated following the formula:

$$Density = \frac{1}{Cultivated\ area} \cdot \frac{Annual\ harvest}{Fraction\ of\ standing\ stock\ harvest\ annually}$$

Species-specific, estimates of aquaculture harvest for each WDFW aquaculture district in South Puget Sound in 2010 were obtained from WDFW and summarized by the Pacific Shellfish Institute (Table 2). For each species, published estimates of age of harvest (Table 3) were used, and an equal ratio of plots at each year within the planting/harvesting cycle was assumed. For example, a Manila clam is harvested at age 3, and thus the standing stock would equal $1/3m+2/3m+m$ where m is the density of a mature harvestable bed. In this scenario, harvest equals half the standing stock. Here, linear growth (Banas et al. 2007) and no temporal trend in planting or harvesting were assumed (PS McDonald, Univ. Washington School of Aquatic and Fishery Sciences, T King, Washington Sea Grant, personal communication).

Appendix Table 1. Filtering capacity of cultured Pacific oyster, Manila clam, and geoduck in Washington Department of Fish & Wildlife (WDFW) aquaculture districts in South Puget Sound.

	Filtering capacity			
District	**L h^{-1} km^{-2}**	**L h^{-1} m^{-2}**	**L s^{-1} m^{-2}**	**m3 s^{-1} m^{-2}**
41A	0.00E+00	0.00E+00	0.00E+00	0.00E+00
41B	1.77E+05	1.77E-01	2.95E-03	2.95E-06
41C	7.21E+05	7.21E-01	1.20E-02	1.20E-05
41D	5.03E+06	5.03E+00	8.38E-02	8.38E-05
41E	8.96E+06	8.96E+00	1.49E-01	1.49E-04
41F	2.34E+06	2.34E+00	3.90E-02	3.90E-05
41G	1.24E+07	1.24E+01	2.06E-01	2.06E-04
41H	1.50E+07	1.50E+01	2.50E-01	2.50E-04
41J	2.27E+06	2.27E+00	3.79E-02	3.79E-05
41K	1.96E+06	1.96E+00	3.27E-02	3.27E-05
41L	0.00E+00	0.00E+00	0.00E+00	0.00E+00

Appendix Table 2. Summary of 2011 cultivated area (km^2) and 2010 landings (lbs) for the three main species cultivated in South Puget Sound within WDFW aquaculture districts.

	Species					
	Pacific oyster		**Manila clam**		**Geoduck**	
District	Area	Landings	Area	Landings	Area	Landings
41A	NA	0	NA	0	NA	0
41B	0.29	0	0.431	0	46.895	0
41C	0.142	436	0.21	4,080	2.532	375,881
41D	1.018	27,606	1.069	146,756	0.862	120,655
41E	2.61	265,779	1.136	102,884	2.745	213,090
41F	2.187	223,086	1.772	2,814	14.677	165,359
41G	2.333	5,378	2.29	956,504	0.202	3,664
41H	6.652	415,032	6.676	2,356,049	1.63	147,729
41J	1.155	97,921	1.047	9,835	6.864	273,055
41K	0.384	10,036	0.403	0	0.199	2,873

Appendix Table 3. Summary of age (years) and size (either shell length (mm) or biomass (lbs)) at harvest by species.

Species	Harvest size	Harvest age (yr)	Source
Pacific oyster	100 mm	2	Kobayashi et al. 1997, Ruesink et al. 2006
Manila clam	50 mm	2–3	Ruesink et al. 2006, Toba et al. 2005
Geoduck	2 lbs	6	National Research Council 2010; Teri King, Washington Sea Grant, and P Sean McDonald, Univ. Washington School of Aquatic and Fishery Sciences, personal communication

Standing stock was converted from wet weight, W_{wet} (g), to dry weight, W_{dry}, using a relationship established for Pacific oysters (Kobayashi et al. 1997), as dry weight is a better predictor of filtration rate:

$$W_{dry} = 0.225W_{wet} - 0.193$$

Standing stock dry weight (in kg) was divided by cultivated area (km^2) to determine standing stock density (kg km^{-2}) within each WDFW aquaculture district (Table 1). Then the proportion of total cultivated area by species in South Puget Sound represented in each aquaculture district was calculated and these species-specific proportions were used to weight the final filtering capacity estimate across each district. Using this method to estimate standing stock density produces a minimum estimate, which is due to underreported landings and using the maximum potential value for cultivated area to calculate densities.

Clearance Rates

Filtration rate estimates based on published relationships to body size are available for geoduck, Pacific oyster and Manila clam (Table 4). Body size for these calculations was assumed to be same as average size at harvest. Geoduck clearance rate estimates were obtained from Davis (2010), measured from intertidal geoducks in Hood Canal, Puget Sound. These clearance rates were converted to $L\ h^{-1}\ g_{dry}^{-1}$. Geoduck weight was converted from wet to dry using Pacific oyster conversion equations (Kobayashi et al. 1997).

Appendix Table 4. Clearance rate calculations for Pacific oyster, Manila clam, and geoduck. W is expressed in g.

Species	Size	W_{wet}	W_{dry}	$L\ hr^{-1}\ indiv^{-1}$	$L\ hr^{-1}\ g_{wet}^{-1}$	$L\ hr^{-1}\ g_{dry}^{-1}$	Source
Pacific oyster	100 mm, 2.4 g_{dry}	11.52	2.4	3	0.260	1.250	Kobayashi et al. 1997, Ruesink et al. 2006
Manila clam	50 mm, 3.9 gwet	18.19	3.9	1	0.060	0.260	Ruesink et al. 2006, Solidoro et al. 2003
Geoduck	980 gwet		220.3	3	0.003	0.014	Davis 2014

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Geographic Information System Approaches and Spatial Datasets Relevant to Shellfish Aquaculture Siting in Washington State

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ABSTRACT

Decisions on aquaculture siting increasingly require a spatial understanding of the physical, ecological, and social attributes of the coastal environment. Geographic information systems (GIS) offer an important framework for organizing spatial databases and performing spatial analyses. This section provides both an overview of a framework for applying GIS to evaluate spatial decisions regarding aquaculture siting and an inventory of key spatial datasets relevant to shellfish aquaculture in Washington State.

INTRODUCTION

In Washington State, shellfish aquaculture is culturally significant and economically important to coastal communities, and interest exists in further expanding the industry. However, shellfish aquaculture is often just one of several competing uses for the coastal environment, and local communities and governments may be confronted with complex questions regarding where and to what spatial extent aquaculture should be permitted (Frankic and Hershner 2003, Ross et al. 2013). At the same time, aquaculture expansion is also increasingly considered in relation to efforts to maintain the ecological integrity of coastal ecosystems and protect species of conservation or management concern (NRC 2010, Soto et al. 2008). Converting coastal habitat to aquaculture may bring some ecological benefits: for instance, possible reductions in phytoplankton, which may lower risk of hypoxic conditions (Dame 2011, Prins et al. 1998). However, this may potentially come at the exclusion of other uses or the loss of benthic habitats with significant ecological functions (Coen et al. 2011, NRC 2010).

Decisions on aquaculture siting increasingly require a spatial understanding of the physical, ecological, and social attributes of the coastal environment (Kapetsky et al. 2010, Ross et al. 2009). Poor site selection can result in decreased production, adverse ecosystem impacts, low economic performance, and conflict between growers and neighbors or the public (Kapetsky et al. 2010, Spencer 2008). However, compiling and analyzing data layers that correspond to criteria related to site feasibility can help growers and managers identify tradeoffs between potential production at a given site and ecological or social constraints. Geographic Information Systems (GIS) have emerged as an important tool for performing such analyses and have seen increased use in aquaculture spatial planning and site selection (Kapetsky et al. 2010, Nath et al. 2000, Ross et al. 2013). GIS offers a platform for organizing and assembling databases relevant to aquaculture siting and facilitates spatial analyses and map rendering, which can offer a powerful visual tools for supporting the decision-making process (Ross et al. 2009). To date, GIS has predominantly been applied to spatial planning issues related to finfish net pen or cage placement in coastal waters, but applications to shellfish aquaculture are growing (see review in Kapetsky et al. 2010).

In Washington State, shellfish growers and managers could potentially benefit from the application of GIS tools to the issue of site selection. To help stimulate and guide research efforts, this study provides both an overview of GIS approaches to evaluating spatial decisions regarding aquaculture siting and an inventory of key spatial datasets relevant to shellfish aquaculture in Washington State.

GIS USE IN AQUACULTURE PLANNING

Although spatial decision making can be approached in a number of ways, Nath et al. (2000) notes that the application of GIS for decision support in aquaculture planning ideally consists of seven phases: (1) identifying project objectives, (2) formulating specifications, (3) developing the analytical framework, (4) locating data sources, (5) organizing and manipulating data for input, (6) analyzing data and verifying outcomes, and (7) evaluating output (Figure 1). The scheme has met support elsewhere in the literature (Dempster and Sanchez-Jerez 2008, Kapetsky et al. 2010) and should be considered an iterative process. This study summarizes each phase with respect to the specific issue of identifying areas suitable for shellfish aquaculture.

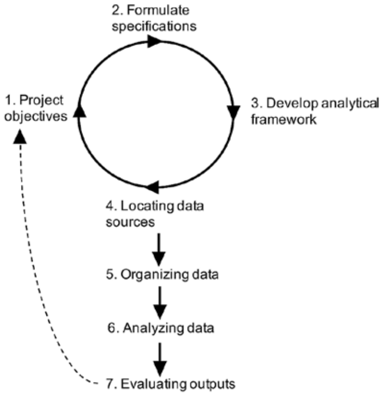

Figure 1. Overview of seven phases for applying geographic information systems in aquaculture planning decision support (adapted from Nath et al. 2000).

1. Identify Project Objectives

The first phase consists of conceptualizing the issue to be addressed with GIS. Articulating clear objectives and project goals will have bearing on the ultimate utility of the analysis to end users and, therefore, requires active participation and close involvement of multiple stakeholder groups (Soto et al. 2008). Until recently, this stage received limited treatment in the GIS literature, but it is among the most critical steps; carefully articulating the decision to be supported by GIS can yield time and cost savings by avoiding preparation of data layers that may go unused. In terms of the general issue of aquaculture siting, objectives will vary. For instance, the objective may be to identify sites optimal for aquaculture based primarily on production potential (Arnold et al. 2000, Buitrago et al. 2005). Alternatively, more comprehensive spatial planning efforts may view aquaculture as just one of several competing uses within the larger coastal environment (Ross et al. 2013). The goal may then include simultaneously optimizing the siting of aquaculture operations and zones for other industries and uses (Arnold et al. 2000, Hamouda et al. 2004, Klein et al. 2009).

2. Formulate Specifications

Once an overall understanding of project objectives has been developed, it may be helpful to develop a list of more functional specifications related to each objective. For instance, the project may require that the final GIS be interactive so that end users can explore alternate scenarios on their own (e.g., Alexander et al. 2012, Quan et al. 2001).

3. Develop Analytical Framework

Developing the analytical framework primarily concerns how project objectives identified in earlier steps will be met. In aquaculture siting studies, Multi-Criteria Evaluation (MCE) methods are often applied and generally entail calculating a habitat suitability index for aquaculture from parameters corresponding to several criteria (Longdill et al. 2008, Pérez et al. 2005, Radiarta et al. 2008, Silva et al. 2011, Simms 2002, Vincenzi et al. 2006; Figure 2). Various methods used are currently available for arriving at a habitat suitability index (Kapetsky and Aguilar-Manjarrez 2007, Malczewski 2006); this study highlights three of the most commonly used methods.

Additive Weighting

Under the simplest approach, data layers are first identified that correspond to criteria that enhance or detract from the level of suitability. Layers consisting of ordinal or continuous variables are then standardized to a common range (usually between 0 and 1). If each layer is assumed to contribute equally towards determining the value of a site for aquaculture, the values of the overlapping layers are summed, yielding a map conveying a suitability index (Malczewiski 1999). However, in most cases, criteria may be unequal in terms of importance. In these instances, criteria can be assigned relative weights, usually based on expert opinion. A habitat suitability index based on the weighted sum of overlapping layers is then calculated (e.g., Arnold et al. 2000, Buitrago et al. 2005).

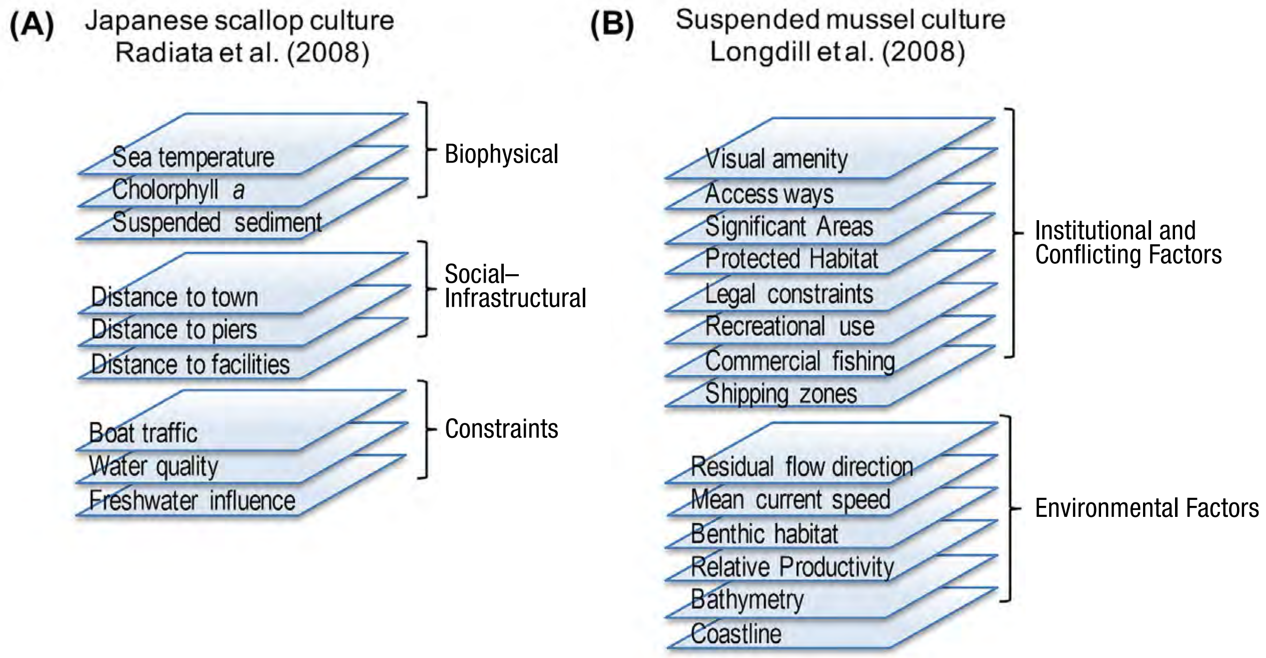

Figure 2. (A) Spatial layers corresponding to criteria included in an overlay analysis of habitat suitability for Japanese scallop *Mizuhopecten yessoensis* (Radiarta et al. 2008). Criteria were categorized into three submodels: biophysical, social-infrastructural, and constraints. (B) A set of criteria considered in the placement of mussel rafts in a coastal region in New Zealand (Longdill et al. 2008). Criteria were assigned to two categories: institutional and conflicting factors and environmental factors.

Parameter-Specific Suitability Functions

An elaboration of the additive weighting approach involves estimating parameter-specific suitability functions (PSSF), whereby each parameter is expressed in terms of a suitability index, usually defined on an arbitrary scale between 0 and 1 (0 corresponds to non-suitable conditions, 1 the most suitable conditions). PSSF may take linear or non-linear forms. As a nonlinear example, the PSSF of environmental variables (e.g., temperature, salinity) may be roughly bell-shaped, where the maximum value of 1 occurs at the physiological or survival optimum, and 0 occurs at values at extremes to both sides of the optimum (Vincenzi et al. 2006). Next, for each parameter, a new layer reflecting suitability based on the PSSF is calculated and the layer is assigned a weight reflecting its relative importance to overall habitat suitability. Rather than taking a weighted sum of the suitability layers to arrive at the habitat suitability index, a weighted geometric mean is instead calculated (Longdill et al. 2008, Vincenzi et al. 2006). The geometric mean implies that if a site is unsuitable with respect to one parameter (i.e., the PSSF value is 0), the overall habitat suitability index of the site is 0 regardless of the PSSF values of the other parameters (Vincenzi et al. 2006).

Analytic Hierarchy Process

The Analytic Hierarchy Process (AHP) was originally developed by Saaty (1980) and has been increasingly applied in spatial multi-criteria decision-making contexts (Malczewski 2006), including aquaculture siting (Radiarta et al. 2008). The method is based on the pairwise comparison of parameters included in the siting decision. All parameters are ranked against each other on a common continuous scale, and ratios conveying the level of importance of one parameter over another are obtained. The "importance ratios" are then organized into a matrix and cross-checked for consistency in rank order, and the weight of importance of each parameter is derived from the principal eigenvector of the matrix. A habitat suitability index can then be obtained by multiplying the normalized weights with the scaled value for each parameter and summing across all parameters (Malczewski 1999).

The approach also readily accommodates hierarchical criteria structures. For instance, site placement may depend on two broad criteria, economics and environment, which in turn may consist of several subcriteria corresponding to specific parameters. Under the AHP method, pairwise comparisons are performed separately at each level of the hierarchy. That is, the importance of economics relative to environment is specified, and similar pairwise comparisons are performed among subcriteria (parameters) within each criterion. The relative weights calculated for parameters within each criterion are then multiplied by the relative weights calculated for the respective broad criteria to form a vector of composite weights that represent the importance of parameters with respect to habitat suitability. Habitat suitability is then calculated by multiplying composite weights with the scaled parameter values and summing across all parameters as described previously (Radiarta et al. 2008). In general, the AHP method can be applied to criteria hierarchies consisting of any number of levels and can help reduce the conceptual complexity of the decision-making processes because only two parameters are considered at any give time (Malczewski 1999).

Considerations for Layer Weighting

All three approaches require subjective decisions regarding the weighting of layers, and how this is done requires careful thought based on the project objectives. Criteria weightings can be based on expert knowledge from a few individuals or information from the literature (e.g., Arnold et al. 2000, Vincenzi et al. 2006). Alternatively, many shellfish and aquaculture experts can be surveyed and asked to weight the relative importance of criteria for shellfish aquaculture siting (e.g., Buitrago et al. 2005, Longdill et al. 2008, Radiarta et al. 2008). As a third approach, a group decision-making process could be employed whereby stakeholders with an interest in the end product are brought together with the goal of reaching consensus on a weighting scheme (Malczewski 1999). If consensus is not possible, maps of habitat suitability corresponding to different stakeholder weighting schemes could be generated and compared for similarities to highlight regions of agreement (Malczewski 1999).

4. Identifying Data Sources

After an analytical framework has been developed, the data needed for the overall analysis must be identified (Nath et al. 2000). Ultimately, the criteria considered in any given analysis, and the manner in which they are grouped, will vary depending on the specific goals of the analysis, the types of aquaculture considered (species, cultivation method), interregional differences in regulatory constraints, and physical, ecological, and social conditions. For instance, in a habitat suitability evaluation for siting Japanese scallop culture, nine subcriteria were identified and organized into three broad criteria: biophysical, social-infrastructural, and constraints (Figure 2a; Radiarta et al. 2008). In contrast, an analysis of habitat suitability for mussel raft placement categorized 14 subcriteria into two broad criteria: institutional and conflicting factors and environmental factors (Figure 2b; Longdill et al. 2008). Although studies vary, some general patterns are apparent, with subcriteria typically falling into five broad criteria: physical, production, ecological, economic, and social considerations.

If data required for the analysis are lacking, project objectives should be revised or reevaluated altogether (Figure 1). If data resources have been identified and are sufficient for the analysis, they must then be sourced. In general, collecting primary (field) data is costly and time consuming (Nath et al. 2000). Instead, data are usually acquired through various secondary sources. For this study, investigators provided an overview of key datasets that may be relevant to aquaculture siting decisions in Washington State, their sources, and additional information on their spatial coverage, constraints, and limitations later in this section. The data search may uncover data gaps and issues that may require revising initial project objectives, specifications, or the analytical framework. Five broad data criteria are described as follows.

Physical

Physical criteria typically reflect constraints on the geographic extent of physically adequate habitat for a given type of cultivated species. The favorability of a habitat depends on the level of overlap between the physical requirements of the species and the physical properties of an area of interest. For example, water depth, exposure to air, temperature, siltation, substrate type, currents, and wave action may limit the feasibility of aquaculture at a given location (McKindsey et al. 2006, Spencer 2008). Similarly, physical properties that include chemical variables (e.g., salinity, concentrations of pollutants or dissolved oxygen) may influence survival and the potential geographic extent of aquaculture (McKindsey et al. 2006, Spencer 2008). In general, physical criteria provide a coarse indication of potential areas suitable for aquaculture production from which more specific site selections can be made for actual development (Ross et al. 2013).

Production

Production criteria correspond to harvest potential at a given site and include variables that influence shellfish growth rates. For filter feeding shellfish, growth is strongly influenced by food concentrations (phytoplankton, particulate organic matter), temperature, and stocking densities (Dame and Prins 1997), though other environmental variables (salinity, dissolved oxygen concentrations) can also be important (Spencer 2008). While spatial estimates of growth potential are possible using available modeling tools (e.g., Ferreira et al. 2007, Grant et al. 2007), they generally require considerable site-specific hydrodynamic, biological, and environmental information (McKindsey et al. 2006). If such data are available, site-specific estimates of growth potential can be estimated and included in siting decisions (Silva et al. 2011). However, in most regions, only some parameters relevant to growth potential are available. As a practical alternative, the variables themselves (e.g., temperature, food concentration, salinity) can be considered indicators of production potential and used as criteria for site suitability (Longdill et al. 2008, Radiarta et al. 2008, Vincenzi et al. 2006).

Ecological

Ecological siting criteria generally aim to minimize unacceptable ecological impacts including changes to ecological processes, services, species, populations, or communities in the environment (McKindsey et al. 2006, Ross et al. 2013). However, in practice, criteria will depend on society, which must choose specific components or processes of interest and identify limits for acceptable change (Byron et al. 2011, McKindsey et al. 2006), and this may be controversial (Lackey 2001). Specific criteria examples may include avoidance of ecologically important habitats (e.g., eelgrass beds) or areas where endangered or threatened species occur.

Economic

Economic criteria relate to investment demand or potential costs associated with sites. For instance, whether a site is near the base of operation, requires cost-prohibitive modifications (e.g., substrate graveling), or is in proximity to piers or land-based facilities may factor into the economic viability of a site (Spencer 2008). Other factors, including some biological variables, may also detract from a site. For instance, abundant predators, competitors, or disease may lead to high loss rates that may be costly to combat (Spencer 2008). Similarly, placement of farms in regions with frequent toxic algae blooms may reduce the availability of harvestable product that meets health standards.

Social

Social criteria include a potentially wide range of considerations. Criteria may include legal constraints on aquaculture development: for instance, coastal zoning plans at the city, county, and state level may expressly prohibit aquaculture development in some areas. Further, marine parks, protected habitats, tribal lands, designated shipping lanes, and military property may also restrict aquaculture (Kapetsky and Aguilar-Manjarrez 2007). A common goal in siting is to minimize potential impacts on other users of the coastal environment (Gilliland and Laffoley 2008). Therefore, criteria related to other activities may also be desirable. For instance, tourism, capture fisheries, and recreation (e.g., fishing, clamming, wind surfing, kayaking, sailing) are some of the activities that may conflict with aquaculture (Longdill et al. 2008, Perez et al. 2003, Silva et al. 2011). In addition, placement decisions may include social considerations such as proximity to public parks and the potential visibility of farms and their perceived impact on the scenic quality of coastlines (Outeiro and Villasante 2013, Radiarta et al. 2008).

5. Organizing and Manipulating Data

Once the data have been identified and collected, they should be organized into a database for use in the target GIS (Nath et al. 2000). This phase includes verifying data quality, consolidating and reformatting data and, in some cases, creating derived data layers. For instance, layers depicting wind-wave height and period can be derived using formulas that require spatial information on fetch distance and maximum wind speed and direction (Tallis et al. 2013). Alternatively, interpolation methods may be required to derive continuous spatial layers from point data obtained at discrete sampling stations (e.g., chlorophyll *a*, dissolved oxygen, or toxic algae concentrations; Vincenzi et al. 2006).

6. Analyzing Data

This phase includes generating habitat suitability maps and may entail performing overlay analyses based on multi-criteria evaluation methods selected in earlier planning phases (Hossain et. al 2009, Nath et. al 2000). Ultimately, details of the analysis will depend on the goals of the research.

7. Evaluating Outputs

The last phase involves evaluating the outputs of the analysis and ideally should involve end users, subject matter specialists, and the GIS analyst (Nath et al. 2000). Activities may include more detailed examination of individual project components together with any potential estimates of uncertainty or underlying assumptions (Ross et al. 2013). Initial project objectives should be compared with the outputs and updated, and the seven-step procedure reinitiated if necessary (Figure 1).

OVERVIEW OF WASHINGTON STATE DATA

To facilitate the potential use of GIS in aquaculture planning and site selection in Washington State waters, this study identified data layers and spatial products that might be useful in analyzing habitat suitability. In so doing, general data availability and needs were assessed to provide a starting point for those wishing to pursue aquaculture-relevant spatial analyses. Those setting out to perform spatial analysis should always directly examine the datasets they intend to use and judge for themselves their value and quality given the analysis goals. The study investigators primarily focused on identifying datasets with spatial coverage in Willapa Bay and South Puget Sound, as these areas are major regions of aquaculture development. However, they also noted when data coverage extended to other marine waters of the state. Extant data layers have been grouped under five themes: (1) current aquaculture and (2) physical, (3) production, (4) ecological, and (5) social considerations. General descriptions of each category are provided below and data sources are summarized in the appendix (p. 82).

1. Current Aquaculture

Considering the current spatial extent of aquaculture is important for identifying potential new sites. Currently, the best aquaculture siting data consist of point data for certified harvest locations (Figure 3), which are usually matched with additional site identification information (site tax parcel, state beach, or DNR-managed subtidal geoduck tract identification codes). The total area permitted for cultivation is noted for each location, but this may be significantly larger than the area actively cultivated because shellfish growers often leave some portion of the area fallow.

Aquaculture landings data are also available from the Washington Department of Fish & Wildlife (WDFW). Statistics are generated on a quarterly basis and landings are aggregated by WDFW shellfish harvest regions.

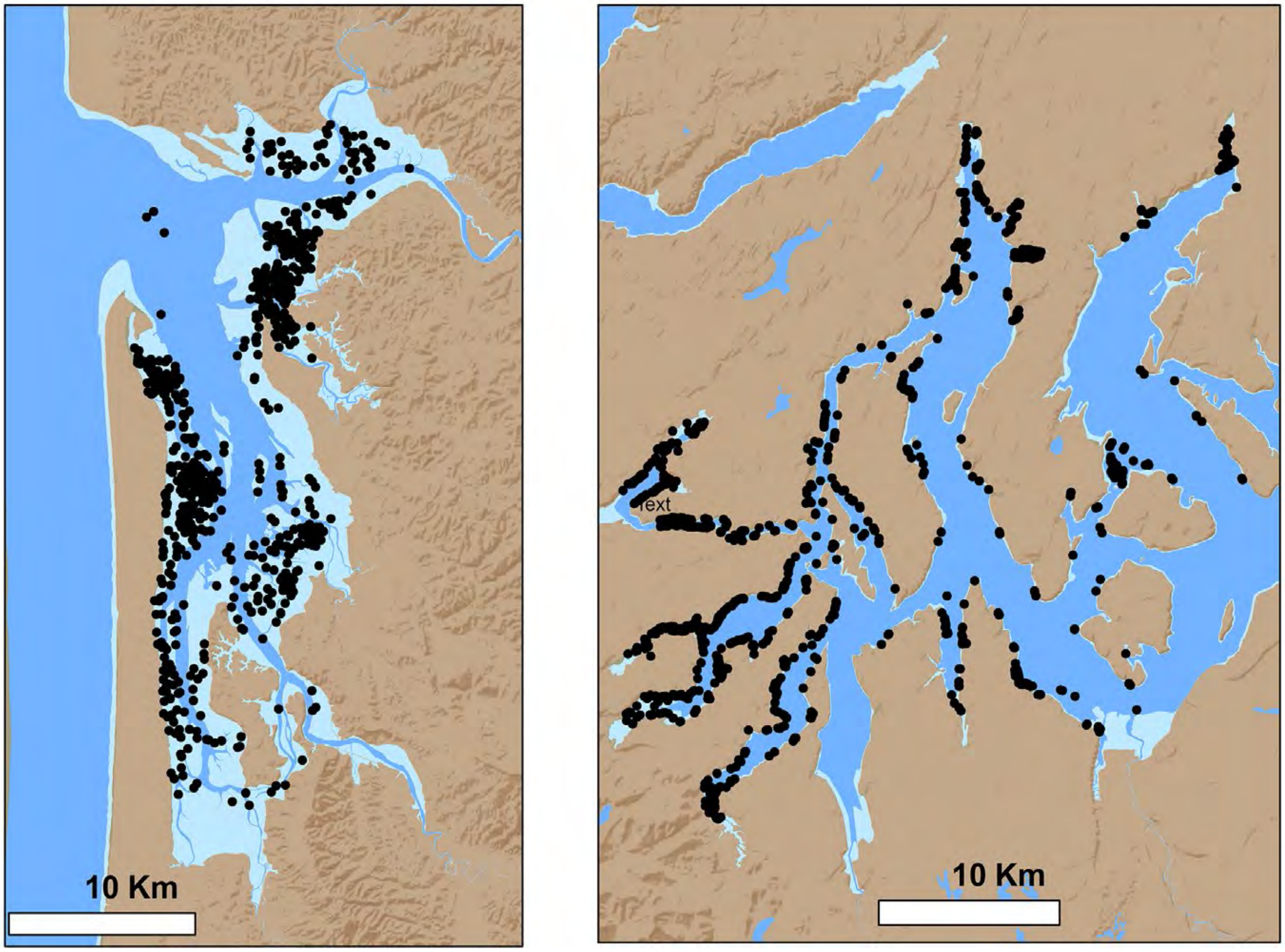

Figure 3. Locations of plots permitted for commercial shellfish harvest for Willapa Bay (left) and South Puget Sound (right); data from 2010, Washington State Department of Health.

2. Physical

Physical considerations include data layers that may inform whether a site is physically amenable to shellfish aquaculture, as well as layers that describe:

- bathymetry (via Digital Elevation Models (DEMs)),
- the presence of maritime infrastructure (e.g., over-water structures, ferry terminals, shipping lanes),
- boat traffic (e.g., shipping lanes), and
- regions with possible water quality issues (e.g., hazardous waste sites, state cleanup sites, proximity to combined sewage overflow and high stormwater outfalls; see appendix, p. 82).

Currently, publicly available DEMs from the U.S. Geological Survey and National Oceanic and Atmospheric Administration (NOAA) vary in terms of accuracy and resolution with regard to the intertidal depth band. For Puget Sound, Finlayson (2005) developed a continuous DEM that has been useful for a wide range of applications including oceanographic modeling. This DEM was derived from high resolution LIDAR and multibeam SONAR wherever these data were available. However, depth anomalies are apparent in some intertidal regions of the DEM (for example Totten Inlet in South Puget Sound). A layer identifying the source of data for each DEM value is available, which facilitates DEM updating with more accurate data if appropriate. Therefore, users should verify the data source for their study area if using this DEM for analysis in the intertidal band. For Willapa Bay, a field-verified, high resolution (5 square meters) DEM has been developed (B Dumbauld, United States Department of Agriculture, personal communication).

Aquaculture siting can also benefit from information on water properties such as temperature, salinity, and dissolved oxygen. Currently, the Washington State Department of Ecology (Ecology) samples marine water column properties on a monthly basis at stations located in Willapa Bay, Grays Harbor, and Puget Sound (5, 3, and 28 stations, respectively). Stations are generally located in waters deeper than 5 meters and separated by several kilometers. The utility of these data for inferring water conditions, particularly temperature and oxygen, in the nearshore may therefore be limited. For salinity, proxies such as distance to known freshwater point sources may be more useful (e.g., Radiarta et al. 2008) but may remain difficult to directly interpret. High-resolution oceanographic models may eventually offer an alternative resource for inferring nearshore water properties, but the current class of available oceanographic models of Puget Sound and the outer coast require additional development and testing against observations at the relevant and fine scale (e.g., Banas et al. 2007, Khangaonkar et al. 2012, Sutherland et al. 2011), though progress is being made (see Banas and Wei, p. 59, this report).

3. Production

As noted under the physical considerations, some data are limited to sampling stations that may not be informative about conditions in shallower, inshore waters (appendix, p. 82). In addition, data on potential food concentrations (based on phytoplankton standing stock densities) available from some of these same sampling stations have similar limitations. Although data on phytoplankton standing stock and production are meager, remote sensing methods may offer a promising avenue for characterizing fine-scale, spatiotemporal productivity patterns in Puget Sound and the outer coastal estuaries (see Box 1, Chlorophyll *a* Remote Sensing).

4. Ecological

Layers under this theme identify critical habitats (e.g., pocket estuaries, wetlands) and flora and fauna that are protected, threatened, or potentially sensitive to habitat loss or alteration. This information may be useful for identifying ecological tradeoffs or potential legal limitations when considering farm siting. The largest database on priority habitats and species is maintained by the WDFW (appendix, p. 82). The database includes layers corresponding to the general distribution of ecologically important or endangered taxa such as birds, marine mammals, fishes (e.g., salmon, forage fishes, pelagic fishes, bottom fishes) and the habitat types with which they associate. These layers do not represent exhaustive inventories and should be interpreted accordingly.

Eelgrass beds (*Zostera* spp.) form an ecologically important habitat type and can potentially be disturbed by shellfish aquaculture (Dumbauld et al. 2009). Information on the distribution of eelgrass in Washington State varies in quality and resolution. Specific resources include a relational database available through the Encyclopedia of Puget Sound that classifies coastline segments according to habitat type (Dethier 1990) and notes the presence of eelgrass (Dethier 2014). Coastline segments range in length from 18 to 38,337 meters and the dataset covers all Washington State coastlines. The National Land Cover Database offers a second resource on the potential distribution of eelgrass. In that dataset, satellite-based land imagery with a resolution of 30 square meters has been classified into different habitat types, and includes a "submerged aquatic vegetation" classification. The accuracy of the layer for depicting the extent of eelgrass has not been ground truthed. The current database (published 2011) corresponds to land cover patterns in 2006 and will not reflect any recent expansions or contractions in eelgrass habitat. In addition, the layer does not distinguish between native *Z. marina* and the non-native *Z. japonica*, which differ in terms of potential interaction with aquaculture (summarized in Reum et al. 2015).

Box 1

Chlorophyll a Remote Sensing

Phytoplankton is the main food source for filter-feeding shellfishes and directly influences individual growth rates and optimal farm stocking densities (Ferreira et al. 2007, Grant 1996). Therefore, shellfish production estimates are strongly dependent on ambient phytoplankton concentrations and, if farms are situated in low-productivity waters, harvests may fall short of levels required for economic viability. As a result, phytoplankton availability is an important criterion in siting decisions (Kapetsky and Aguilar-Manjarrez 2007). For some systems, there are hydrodynamic models that incorporate spatially resolved nutrient–phytoplankton–zooplankton dynamics, which can aid in estimating spatial primary production patterns (e.g., Grant et al. 2008). However, such models are data intensive, costly, and time-demanding. As a practical alternative, products derived from satellite-based remote-sensing technologies can offer estimates of synoptic surface chlorophyll a (Chl *a*) concentrations, which are correlated with phytoplankton biomass and can thus help inform siting decisions (Longdill et al. 2008, Radiarta et al. 2008).

In Puget Sound, efforts are currently underway to convert measurements of ocean color from a variety of sensors to estimates of surface Chl *a* concentrations (Sackmann 2014). Calibration of the estimates is facilitated by using in situ Chl *a* fluorescence measurements obtained from instrumentation installed onboard the passenger ferry *Victoria Clipper IV*. The satellite products provide surface Chl *a* concentration estimates at resolutions of 250 to 500 meters, and because images are taken at regular time intervals, seasonal or interannual variation in productivity can be examined. In the South Puget Sound subbasin, preliminary surface Chl *a* estimates reveal that the highest and most persistent concentrations generally occur in the smaller bays and inlets (Box 1 figure). While suspended sediment and bottom reflectance may impart considerable uncertainty into Chl *a* estimates for shallow areas, the relative patterns between deeper subbasins are more robust and suggest that seasonal variation in Chl *a* is minor relative to spatial variability (Box 1 figure).

Cloud cover and spurious measures of reflectance (due to confusion of land and water pixels) can also affect the accuracy of estimates from this method. However, further model refinement using in situ validation can help reduce prediction errors. Future research directions could include development of a real-time image processing workflow and a framework for disseminating results to shellfish growers and other end users.

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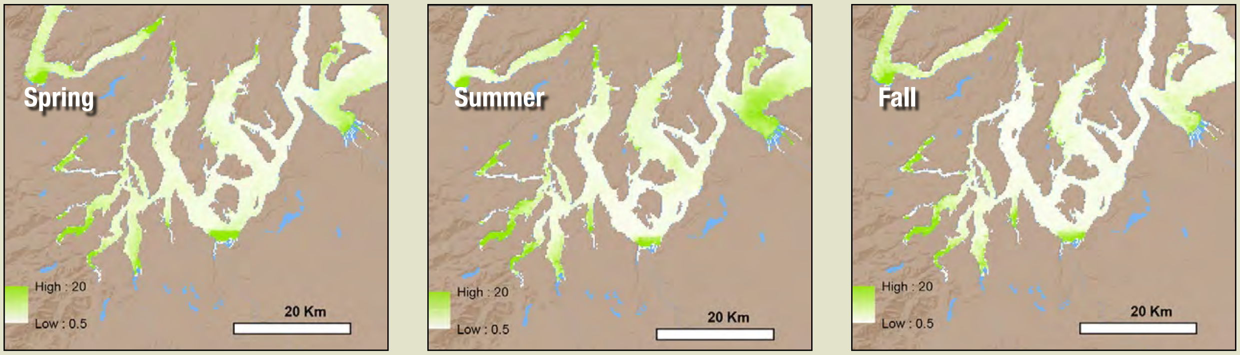


Estimates of South Puget Sound surface chlorophyll a concentrations (milligrams per cubic meter) during spring, summer, and fall 2013 based on remote sensing.

5. Social

Layers under this theme may help identify areas that possibly conflict with other uses or which have already been zoned for other regulated uses. Social considerations may include areas with potential legal constraints: for instance, public parks, tribal lands, military areas, marine protected areas, and oyster reserves. In addition, municipality- and county-level marine shoreline management plans may further impose constraints on farm placement.

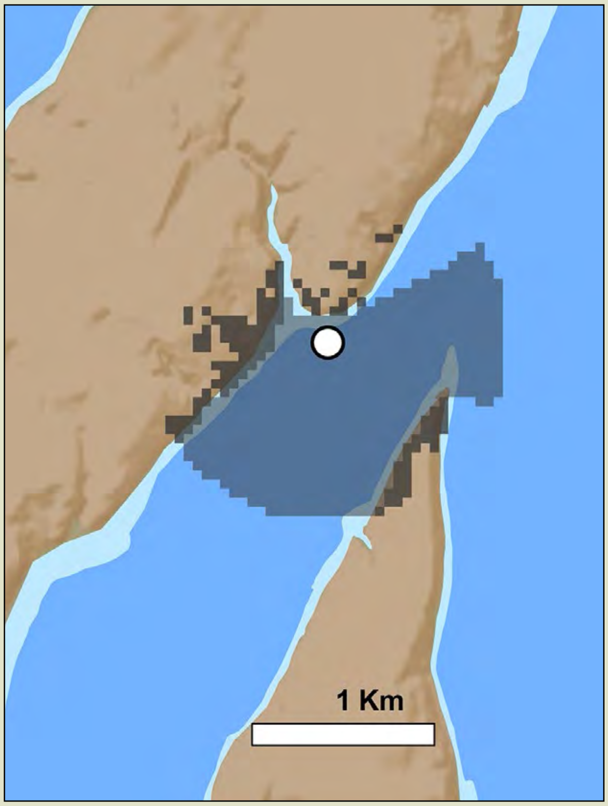
Further, there may be interest in avoiding sites near high densities of people or public parks to reduce the potential for poaching. Spatial layers of human density based on national census data are available as well as layers depicting public lands in Washington State. Alternatively, if shellfish farms are perceived as altering the aesthetic quality of coastlines, low visibility may be an important siting criteria. To help address this, The Natural Capital Project has developed the “Scenic Quality” tool, which estimates the visibility of geographic features (Tallis et al. 2013; Box 2, Viewshed). This tool can be used to generate data layers that relate the visibility of potential new farms to private homes, public parks, or any other location of interest, and it could easily be applied within a farm siting analysis.

Box 2

Viewshed

Potential social criteria relevant to aquaculture siting analyses include the level of visibility of proposed farms within viewsheds. Overall, the visibility of structures associated with farms in a viewshed depends on several factors including elevation (both of the viewer and farm), the level of exposure in relation to tide height, and the spatial extent and height of natural and man-made structures in the line of sight (e.g., trees, buildings). GIS-based tools are available that enable analysis of viewsheds given different scenarios of aquaculture development (Outeiro and Villasante 2013, Puniwai et al. 2014), and they can generate layers that could be incorporated into spatial multi-criteria decision support frameworks for farm siting (Malczewski 1999).

Widely used GIS software packages such as ArcGIS, QGIS, and GRASS facilitate viewshed analyses through built-in tools or plugins. The Scenic Quality tool developed by The Natural Capital Project (Tallis et al. 2013) is designed especially for analyzing viewsheds in the marine nearshore environment. The package contains built-in raster layers for population density and elevation, but these may have insufficient spatial resolution, depending on the goals and spatial scale of the intended analysis. However, analysts can easily supply their own elevation and population density data layers and include additional layers to account for trees, buildings, or other features that may obstruct views. The output from the Scenic Quality tool includes a raster layer that classifies the visual quality of the analysis region (from no visual impact to very high visual impact) and provides additional summary metrics. To help illustrate the approach, the Scenic Quality tool was used to estimate the area over which a hypothetical farm could be viewed at a location in South Puget Sound (Box 2 figure). In this example analysis, the Finlayson (2005) DEM was modified to reflect forested areas using a surrogate canopy raster created using the National Land Cover Database. Areas within the DEM with more than 50% tree coverage were assigned an additional elevation of 20 meters. Populations residing in forested areas (greater than 50% tree coverage) were assumed to not have water views; they were consequently removed from the population density data layer. The area from which structure at the hypothetical farm may be visible at low-tide during daylight hours was depicted (Box 2 figure).



An example calculation of the area from which a proposed farm structure (open circle symbol) may be visible using the Scenic Quality tool (Tallis et al. 2013). The dark shading surrounding open symbol corresponds to areas from which the site may be visible.

Currently, several challenges exist for implementing viewshed analyses related to intertidal aquaculture in Washington State and Puget Sound in particular. Foremost, high- resolution digital elevation data of the intertidal is critical, and data of sufficient accuracy are not available in many regions. This is especially important because structure visibility will vary with tidal exposure and such calculations will be sensitive to relatively small inaccuracies in bathymetry. Next, visibility will depend on the type of farm structure, and the analysis requires the subjective weighting of visibility. The visibility of a newly planted geoduck plot with anti-predator netting likely differs from that of an on-bottom oyster bed. Distinguishing differences in visibility between the farms is possible using the Scenic Quality tool, though this requires a subjective choice in the relative difference in visibility “weight.” This could potentially be approached by assigning weights based on input from multiple stakeholder groups. Finally, estimates of visibility would benefit from more extensive cataloguing of use patterns in coastal recreational areas to better estimate the number of persons with line of sight of proposed new sites.

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CONCLUSIONS

In the last two decades, GIS has emerged as an important tool for supporting aquaculture siting decisions but remains underutilized in Washington State. GIS can help develop a useful framework for organizing spatial data resources and a powerful platform for performing analyses that can inform the decision-making process (Arnold et al. 2000, Longdill et al. 2008, Radiarta et al. 2008). Currently, various data resources could feed directly into an assessment of aquaculture feasibility, which might help inform long-term planning. In addition, tools are available that can generate data layers particularly relevant to issues in Washington State such as visibility, and potential data sources that, with further refinement and support, may offer valuable information for shellfish growers (e.g., remotely sensed sea surface estimates of Chl *a*). The dataset inventory provided by this study is not exhaustive, and other available data may be valuable depending on the question at hand. County-maintained GIS repositories and geospatial data clearinghouses for Washington State data are good starting points (several are indicated herein), and many also provide other suggested resources. Not all data may be maintained by the respective agencies and more current records may be available from the data originator.

GIS Repositories and Geospatial Data Clearinghouses for Washington State

Washington State Department of Natural Resources GIS Data Center: This site features layers related to natural resources such as aquatic, habitat, climatology, geology, forest practices, hydrography, and natural heritage.
http://www.dnr.wa.gov/programs-and-services/geology-and-earth-resources

Ecology's spatial dataset: This includes data related to air and water quality, public beaches, tribal lands, and pollution sources.
http://www.ecy.wa.gov/services/gis/data/data.htm

Washington State Geospatial Data Archive: This is maintained by The Map Collection & Cartographic Information Services, University of Washington (UW) Libraries, and also contains selected non-Washington geospatial datasets created by students and researchers at the UW. Some datasets are restricted to persons affiliated with the UW, though many are public domain datasets.
http://wagda.lib.washington.edu

Washington State Department of Health: This agency has data pertinent to commercial and recreational shellfish harvesting including recreational shellfish beaches and closed beaches.
https://fortress.wa.gov/doh/eh/maps/OSWPViewer/index.html

NOAA's Digital Coast site: This site includes data on physical and oceanographic variables, elevation, marine habitats and species, climatology, and marine planning data such as usage, jurisdictions, and boundaries.
http://coast.noaa.gov/dataregistry/search/collection

Washington Marine Spatial Planning Data Catalog: The catalog primarily contains datasets relevant to activities and physical and environmental variables on the outer Washington coast.
http://www.msp.wa.gov

Washington State Geospatial Portal: This portal links users to GIS data layers and other geospatial information and products produced and maintained by state agencies such as the WDFW.
http://geography.wa.gov/data-products-services/data/data-catalog

Washington State Department of Transportation (WSDoT) GeoData Distribution Catalog: This catalog includes data layers produced by WSDOT related to transportation routes (including ferry routes).
http://www.wsdot.wa.gov/Mapsdata/GeoDataCatalog/default.htm

Encyclopedia of Puget Sound: This online encyclopedia is a growing compilation of data related to Puget Sound with some real-time data available via their online viewer.
http://www.eopugetsound.org/maps

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Appendix

DATA LAYERS RELEVANT TO AQUACULTURE SITING IN WASHINGTON STATE

Apendix Table 1. Description of extant data layers that may be relevant to aquaculture siting issues in Washington State. In addition, some useful tools for calculating relevant data layers are indicated. Data were categorized according to five themes: current aquaculture areas and landings, and physical, production, ecological, and social constraints. Abbreviations: DNR, Washington Department of Natural Resources; DOH, Washington State Department of Health; Ecology, Washington State Department of Ecology; NOAA, National Oceanic and Atmospheric Administration; OFM, Office of Financial Management; PSI, Pacific Shellfish Institute; RCO, Washington State Recreation and Conservation Office; USDOI, U.S. Department of Interior; USFWS, U.S. Fish and Wildlife Service; USGS, U.S. Geological Survey; WDFW, Washington Department of Fisheries & Wildlife; WSDOT; Washington State Department of Transportation.

Variable	Dataset description	Data source	Spatial coverage
	CURRENT AQUACULTURE AREAS AND LANDINGS		
Commercial shellfish harvest locations	These are point data for certified harvest locations with descriptive data that includes area permitted for cultivation and the species. Sites corresponding to privately owned tax parcels are assumed to reflect commercial aquaculture locations.	DOH; available upon request	State-wide
Shellfish landings, aggregated to commercial shellfish growing regions	Tabulated landings aggregated to commercial shellfish growing areas. Landings available by species and on a quarterly basis. This is possibly an incomplete picture of total landings owing to underreporting.	WDFW; available upon request	State-wide
	PHYSICAL		
Intertidal habitat (aquatic land parcels)	The aquatic land parcel data layer indicates the spatial extent of intertidal and subtidal habitats. The layer contains ownership information and physical and legal characteristics for each aquatic parcel.	DNR; *https://fortress.wa.gov/dnr/adminsa/gisdata/datadownload/state_aqparcel.zip*	State-wide
Digital elevation model	The most comprehensive digital elevation model (DEM) for the inland waters of Washington is Finlayson (2005). The data layer synthesizes numerous DEMs, with varying spatial resolution to yield a continuous surface spanning Puget Sound and surrounding watersheds. Measurement of intertidal depths is challenging in general, and depth accuracy of the DEM may be low in regions (e.g., anomalies are present in intertidal habitats in Totten Inlet, South Puget Sound).	Finlayson (2005)	Puget Sound, Straight of Juan de fuca
Digital elevation model	Relevant sources include: The USGS National Elevation dataset; NOAA's Digital Coast site; Olympic Natural Resource Center DEM mosaic (combines data from NOAA, USDA and USGS).	*http://ned.usgs.gov/* *http://www.coast.noaa.gov/* *http://www.onrc.washington.edu/clearinghouse*	Willapa Bay
Presence of overwater structures	Overwater structures in marine waters of Washington State: location and footprint of overwater structures such as docks, bridges, floats, structural support fill, and other structures such as floating homes.	DNR; *https://fortress.wa.gov/dnr/adminsa/gisdata/datadownload/wa_overwater_marine.zip*	State-wide
Ferry terminals	This layer depicts the locations of ferry terminals in Washington State. Only ferry terminals that are directly adjacent to a Washington State highway routes are available.	DOT; *ftp://ftp.wsdot.wa.gov/gis/GeoDataDistribution/Maps/24k/DOT_Cartog/ferrytermspubpriv.zip*	State-wide
Shipping lanes	Marine Cadastre/Navigation and Marine Transportation. Online at http://marinecadastre.gov.	NOAA; *ftp://ftp.csc.noaa.gov/pub/MSP/ShippingFairwaysLanesandZones.zip*	State-wide
Ferry routes	Routes of vessels providing scheduled, public car ferry service in the waters of Washington State are depicted as linear features. Known private, provincial, tribal, and passenger-only ferry services are also shown.	WSDOT; *ftp://ftp.wsdot.wa.gov/gis/GeoDataDistribution/Maps/24k/DOT_Cartog/ferry.zip*	State-wide

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Appendix Table 1 · continued

Variable	Dataset description	Data source	Spatial coverage
	PHYSICAL continued		
Hazardous waste sites, state cleanup sites, superfund sites	Facilities database (updated every Sunday)	Ecology; *ftp://www.ecy.wa.gov/gis_a/enviro/FacilitySite.gdb.zip*	State-wide
Proximity to combined sewage overflow (CSO) and highway stormwater outfalls	Point locations of CSO and outfalls	People for Puget Sound (via WDFW)	Puget Sound counties
Water properties (temperature, salinity, oxygen)	Ecology, marine monitoring stations	Ecology; *http://www.ecy.wa.gov/programs/eap/mar_wat/pdf/stationinfo.html*	Willapa Bay, Grays Harbor, Puget Sound
Shellfish biotoxin closure zones	Biotoxin Closure Zones: this dataset defines areas of marine waters that are managed for shellfish biotoxin closures.	DOH; *ftp://ftp.doh.wa.gov/geodata/layers/closurezones.zip*	State-wide
	PRODUCTION		
Water properties (temperature, salinity, oxygen)	Ecology, marine monitoring stations	Ecology; *http://www.ecy.wa.gov/programs/eap/mar_wat/pdf/stationinfo.html*	Willapa Bay, Grays Harbor, Puget Sound
Chlorophyll *a*	Ecology, marine monitoring stations	Ecology; *http://www.ecy.wa.gov/programs/eap/mar_wat/pdf/stationinfo.html*	Willapa Bay, Grays Harbor, Puget Sound
	ECOLOGY		
Protected habitats and species	Protected Habitats and Species Generalized Digital Data. Generalized distribution of ecologically important or endangered taxa: birds, marine mammals, fishes (salmon, forage fishes, pelagic fishes, bottom fishes), or the habitat type with which they are associated. Information on specific locations of some fish and wildlife species is considered sensitive and such data are removed from non-sensitive layers that might be of sufficient resolution to reveal these locations. More detailed analysis may require field investigations and additional assistance may be needed in interpreting and applying information from the database, depending on the species and area being considered.	WDFW; *http://wdfw.wa.gov/mapping/phs/*	State-wide
Summer and winter bird survey data	Puget Sound Assessment and Monitoring Program (PSAMP) Geodatabase. Winter and summer bird survey data for select species.	WDFW; *available upon request*	Puget Sound
Eelgrass	This layer provides shore type descriptions, physical attributes, and related species lists (including eelgrass species) that align spatially with classifications adapted from the Washington State ShoreZone Inventory linear shoreline data. More information is available at *http://www.eopugetsound.org/habitats/shore-types*.	DNR; *https://erma.noaa.gov/northwest/erma.html#/x=-120.95568&y=46.09146&z=7&layers=16+7942+1276+11371+1284*	State-wide
Submerged aquatic vegetation	U.S. Geological Survey, 20140331, NLCD 2006 Land Cover (2011 Edition): U.S. Geological Survey, Sioux Falls, SD. These data are compiled from the National Land Coverage Database. Submerged aquatic vegetation may be useful as a surrogate for an eelgrass location layer for certain locations. This layer has not been ground truthed for accuracy. More information is available at *http://www.mrlc.gov*.	USDOI, USGS; *http://viewer.nationalmap.gov/basic/*	State-wide
Proximity to pocket estuaries	Point locations of pocket estuaries	NOAA; *https://erma.noaa.gov/northwest/erma.html#/x=-120.95568&y=46.09146&z=7&layers=16+7942+1276+11371+1284*	unknown
Proximity to wetlands	National Wetlands Inventory	USFWS; *http://www.fws.gov/wetlands/index.html*	State-wide
Impaired or threatened water bodies	2012 Water Quality Assessment: Washington areas reported to the Environmental Protection Agency as impaired water under the Clean Water Act.	Ecology; *ftp://www.ecy.wa.gov/gis_a/environment/303d12.gdb.zip*	State-wide

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Appendix Table 1 • *continued from previous page*

Variable	Dataset description	Data source	Spatial coverage
	SOCIAL		
Tribal Lands	Tribal lands: this dataset describes Native American ceded tribal lands in Washington State.	Ecology; *ftp://www.ecy.wa.gov/gis_a/boundaries/tribal.zip*	State-wide
Military/naval waters	Military and naval waters are indicated in the Aquatic Land Parcel layer. More generally, the layer contains ownership information and physical and legal characteristics of Washington State's aquatic land ownership records. For example, this layer may be used to define military or naval waters.	DNR; *https://fortress.wa.gov/dnr/adminsa/gisdata/datadownload/state_aqparcel.zip*	State-wide
Protected areas (wildlife refuge, state/county parks)	Public Lands Inventory database	Ecology; *ftp://www.ecy.wa.gov/gis_a/boundaries/tribal.zip*	State-wide
Marine Protected Areas Inventory	The Marine Protected Areas Inventory (MPA Inventory): geospatial database designed to catalog and classify marine protected areas within U.S. waters.	NOAA; *http://marineprotectedareas.noaa.gov/pdf/helpful-resources/inventory/mpa_inventory_2013_public_gdb.zip*	State-wide
Marinas/Boat launches and moorages	Motorized boat launches of Washington State: this dataset contains geographic point data for motorized boat launches found to be open to the public in Washington State at the time of the field inventory (1997). Moorage facilities of Washington State: this dataset is based on a comprehensive field inventory of large boat facilities conducted by the Washington State Recreation and Conservation Office in 2000.	RCO; *http://www.rco.wa.gov/data/RCOBoatFacilities.gdb.zip*	State-wide
Oyster reserves	Aquatic Land Parcel contains ownership information and physical and legal characteristics of Washington State's aquatic land ownership records: this layer is used to show oyster reserves.	DNR; *https://fortress.wa.gov/dnr/adminsa/gisdata/datadownload/state_aqparcel.zip*	State-wide
Scenic quality	The Scenic Quality model employs viewshed analysis to estimate the visibility of new nearshore or offshore features. The model generates maps that can identify the visual footprint of offshore development plans and highlight coastal areas more likely to be directly affected by additions to the seascape. Requires data layer on density of people. Can also be used to evaluate scenic quality from the vantage of public parks and beaches.	*Natural Capital Project, Scenic Quality Tool v3.01;* *program available at: http://naturalcapitalproject.org/InVEST.html*	N/A
Human densities	GRUMP - Global Rural-Urban Mapping Project. Population data estimates are provided for 1990, 1995, and 2000, and projected (in 2004, when GPWv3 was released) to 2005, 2010, and 2015.These globally available population data do not account for seasonal or daily users in an area.	*http://sedac.ciesin.columbia.edu/data/collection/gpw-v3*	State-wide

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Appendix D

CONFLUENCE
ENVIRONMENTAL COMPANY

To: Margaret Barrette, Pacific Coast Shellfish Growers Association
cc: Jesse DeNike, Plauché & Carr

From: Phil Bloch, Marlene Meaders, and Chris Cziesla

Philip Bloch Chris Cziesla

Date: July 29, 2016

Re: Eelgrass and Shellfish Aquaculture – Review of Relevant Scientific Literature on Ecological Functions and Interactions

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This memorandum summarizes scientific literature and observations regarding ecological function at sites containing shellfish aquaculture. As the summaries below note, the introduction of shellfish aquaculture to intertidal habitats has numerous potential positive and negative interactions. Some of these interactions are short-term associated with specific aquaculture activities while others are long-term. However, shellfish aquaculture, when implemented with appropriate best management practices and minimization measures, appears to have minimal individual and cumulative effects to ecological function, particularly when the ecological benefits associated with shellfish aquaculture and the landscape context are considered in addition to the potential suppression or loss of intertidal habitats in areas where aquaculture occurs.

This review of ecological functions evaluates the potential negative and positive interactions of shellfish aquaculture with estuarine habitats through the various potential mechanisms that aquaculture interacts with estuarine habitats.

Ecological Impact Assessment

Negative Interactions of Shellfish Aquaculture

While the placement of gear or activities associated with shellfish aquaculture can result in a disturbance, this change does not necessarily equate to a negative impact. The interactions that can potentially result in a disturbance to the surrounding environment include: (1) shellfish aquaculture gear, (2) working practices, and (3) cumulative effects with other stressors. This information primarily focuses on kelp and native eelgrass also known as submerged aquatic vegetation (SAV). Intermediate levels of disturbance can be associated with increased diversity and species richness (e.g., Connell 1978) because competitively dominant species are unable to exclude other species, a concept that has been observed in a wide variety of ecosystems including marine reefs (e.g., Rogers 1993) and eelgrass beds (Reusch 2006). Dumbauld et al. (2009) indicated that it is important to not only consider disturbance in terms of a degradation from baseline functions, but also how disturbance can influence the resilience of the system to withstand or recover from additional disturbances. This concept of resiliency is also explored in this section in relation to eelgrass habitat and shellfish aquaculture.

Shellfish Aquaculture Gear

Equipment associated with the culture of shellfish (e.g., nets, racks, bags) can lead to shading, which may affect the spatial extent and density of SAV in the immediate vicinity of shellfish gear. The type and concentration of equipment influence the level of this effect. For example, Everett et al. (1995) found that oyster racks could lead to a total loss of eelgrass directly under the racks. Comparatively, Rumrill and Poulton (2004) determined that the spatial extent of an eelgrass bed and shoot density were negatively influenced when oyster longline culture was closely spaced (1.5 ft to 2.5 ft) but showed no significant effects compared to control sites when spacing occurred at 5-ft and 10-ft spaces between longlines.

Predator exclusion netting used in Manila clam and geoduck clam aquaculture can be detrimental to SAV. Predator exclusion netting is generally not placed over shellfish in areas with eelgrass. However, in at least one example in Washington State (Fisk Bar, Samish Bay), eelgrass recruited into a geoduck clam culture area after the placement of aquaculture gear stabilized the sediment and protected the new eelgrass shoots from erosive wave energy (see the discussion below in positive interactions of shellfish aquaculture). During the grow-out portion of the culture cycle, predator exclusion nets were placed on the bed and Horwith (2013) reported a total loss of eelgrass under predator exclusion netting due to shading effects from *Ulva* sp. growth on the net surface. It should be noted that the eelgrass was shown to recover within 2 years of net removal (Horwith, pers. comm., 2014). Under such a scenario, the functions of eelgrass may have been temporarily offset by the development of a macroalgal community, as described by Powers et al. (2007). Predator exclusion nets or culture gear often gets colonized with macroalgae (Figure 1), which was quantified by Powers et al. (2007) as providing comparable ecosystem functions (e.g., nursery habitat, epibiota biomass) as seagrass beds. However, for the time that they are in place, the netting can negatively impact SAV directly underneath the predator exclusion nets.

Figure 1 Macroalgae Colonizing Oyster Lines in Blue Heron Bay

Source: Dewey, pers. comm., 2015

Other effects of shellfish aquaculture gear to SAV include the potential to abrade, scour, or desiccate the plants, although the overall effects can be both positive and negative. For example, Wisehart et al. (2007) explored the concept that, while shellfish can break eelgrass blades through abrasion or harvesting techniques, the reduction in density can release individuals from intraspecific light competition and result in increased growth rates near the aquaculture plots. Similarly, Wisehart et al. (2007) reported that scouring or dredging can result in higher eelgrass seedling density and seed production in the disturbed areas from a mechanical dredge harvest. Alternatively, eelgrass blades can desiccate on shellfish or aquaculture gear, which can eventually lead to a reduction in shoot size (Wisehart et al. 2007, Tallis et al. 2009). These tradeoffs are explored in more detail below.

Working Practices

Shellfish harvest can cause localized and temporary increases in suspended sediments, physical damage and/or removal of SAV, as well as changes to other metrics (e.g., biomass, seed germination, growth). The two practices that likely generate the most suspended sediment are mechanical harvest of oysters and geoduck clam harvest. Shellfish aquaculture typically occurs in areas with sand or gravel substrate, which are substrate sizes that have high settling velocities. For example, Mercaldo-Allen and

Goldberg (2011) reported that suspended sediments may take 30 minutes to 24 hours to resettle in areas typical of oyster and clam aquaculture operations. Suspended sediment effects of shellfish harvest are generally short-lived and recovery is rapid (Short and Walton 1992, Liu and Pearce 2015). Additionally, shellfish culture often occurs in shallow estuarine embayments where freshwater runoff, currents, and wind waves can lead to naturally high background levels of suspended sediments. Therefore, pulse disturbances of suspended sediment by shellfish harvesting often fall within baseline measurements and the natural variability of the system.

Tallis et al (2009) compared eelgrass densities in areas that were harvested: (1) by hand in ground culture, (2) using a mechanical dredge harvester, or (3) by hand on longlines. Although there was a 70% reduction in eelgrass productivity at all aquaculture sites when averaged together, Tallis et al. (2009) pointed out that effects to eelgrass from aquaculture occurred in both directions (positive and negative), and the magnitude of effects observed were dependent on the site and type of harvest method. For example, mechanical dredge harvesting had the greatest level of impact and longline and ground culture had the lowest. While the authors concluded that oyster aquaculture taking place within an eelgrass bed will affect eelgrass, they also indicated that there are opportunities for decreased impacts with tailored culture methods and timing.

Wisehart et al. (2007) also examined the effects of different aquaculture techniques on eelgrass biomass, density, and growth rates in Willapa Bay. As discussed above, the authors reported that shellfish aquaculture may facilitate increased growth rates due to a reduction in intraspecific competition by surrounding plants (e.g., increased light availability), increased seed supply and germination, and a more open seed dispersal setting. In addition, while oysters grown on longlines caused some minor reduction in eelgrass density and cover, the highest eelgrass growth rates occurred at the longline culture and reference sites. These areas also had the greatest eelgrass biomass, density, and percent cover. The study reported statistically significant site and culture type interactions for most variables, suggesting that site-specific conditions may be just as influential as aquaculture techniques in determining eelgrass parameters.

Boat access can also result in potential negative impacts to eelgrass shoots. Ruesink et al. (2012) conducted experimental treatments in Willapa Bay where they imposed two disturbance types: shoot damage and shoot removal. For the most part, the extent of damage from boat propellers would result in taking off the ends of the shoots (i.e., shoot damage), but not removing the entire shoot. Regrowth for eelgrass that is only damaged on the surface requires branching of the plant to replace the lost biomass. Growth rates of eelgrass affected by shoot damage for less than 4 weeks recovered within 2 months following a single cutting event when the rhizome was still rooted. There would be no long-term reduction in eelgrass density for this type of action.

Potential longer term impacts were calculated based on an accumulation of shoot removal over a year or more (e.g., consistent access routes). If the shoot is removed, the removal area can be repopulated by rhizome extension from shoots at the edge (asexual reproduction) or germination of seeds (sexual reproduction). Ruesink et al. (2012) reported that recovery of eelgrass after complete shoot removal (6.6 x 6.6 ft gaps) could occur after 2 years. Based on this rate of recovery, a conservative estimate of a propeller scar width of 3 ft could replace the lost biomass in approximately 0.9 year. If regrowth occurs at a rate faster than removal, it can be assumed that there would be no significant loss in biomass from this type of activity unless an area is not allowed to regrow through continuous disturbance.

Cumulative Effects with Other Stressors

Boström et al. (2006) conducted a meta-analysis of the scientific literature associated with plant-animal interactions in seagrass landscapes. According to this analysis, the authors indicated that, "The growth and recruitment dynamics of seagrasses as well as man-made and/or natural disturbances create complex spatial configurations of seagrass over broad (metres to kilometres) spatial scales. Hence, it is important to identify mechanisms maintaining and/or threatening the diversity-promoting function of seagrass meadows and to understand their effects on benthic populations and communities." It is well recognized that there are a variety of natural and anthropogenic stressors on aquatic environments (Dennison 1987, Fonseca and Bell 1998, Shaughnessy et al. 2004, Boese et al. 2005, Mumford 2007, Thom et al. 2011, Stevens and Lacy 2012).

A few studies address landscape-scale changes to eelgrass relative to shellfish aquaculture. The most comprehensive analysis of factors that drive the changes at a landscape-scale was conducted by Dumbauld and McCoy (2015). This study modeled eelgrass (*Z. marina*) density in Willapa Bay and compared a number of predictors, including: (1) distance to estuary mouth, (2) distance to channel, (3) salinity, (4) elevation, (5) cumulative wave stress, and (6) shellfish aquaculture. The amount of eelgrass cover within oyster aquaculture beds was slightly lower than the model predicted, but the impact directly associated with aquaculture represented less than 1.5% of the total predicted eelgrass cover in any year. Eelgrass is generally present and intermingles with oysters on all aquaculture beds at the tidal elevations where eelgrass is found in Willapa Bay, which suggests that current oyster aquaculture practices do not substantial reduce and may even enhance the presence of eelgrass at the estuarine landscape scale. The observed reductions in eelgrass cover in Willapa Bay are within the range of existing natural disturbances to the system (e.g., winter storms) that eelgrass is inherently adapted to. Furthermore, when the functional value of eelgrass and shellfish are combined, and the landscape matrix of habitats are considered, it is possible that a broader ecosystem perspective would find benefits from the presence of aquaculture (Dumbauld and McCoy 2015).

Aside from the overall low amount of impact to eelgrass at the landscape-scale, the Dumbauld and McCoy (2015) work also indicated that the harvest method was a significant predictor in explaining eelgrass reduction. For example, mechanically harvested beds had a significantly lower amount of eelgrass compared to beds harvested by hand or with a mixed harvest technique (similar to the results reported by Tallis et al. 2009). Comparatively, the type of aquaculture (e.g., longline, seed bed, fattening ground) was not a significant contributor to the variation of eelgrass predicted versus actually observed. The authors suggested that, overall, aquaculture resulted in a minor change to eelgrass at the landscape-scale because the effect of culture was variable enough at smaller spatial scales as to eliminate a significant effect at the landscape-scale.

As stated throughout this report, the landscape-scale is very important to consider when trying to protect for mobile species, such as fish and crabs. This sentiment was stated within Semmens (2008), where the author indicated that, "From a management perspective, it may therefore be tempting to downplay the importance of fine-scale benthic habitats in favor of larger-scale estuarine features such as deep tidal channels and salinity gradients for smolt-sized fish." Although it is important to understand small-scale effects in order to effectively manage potential effects to eelgrass, it is the landscape-scale that determines how species will use the habitat.

Eelgrass Resilience

Holling (1973) defined resilience as "a measure of the ability of these systems to absorb change of state variables, driving variables, and parameters, and still persist." In a study of evaluating the effects of disturbances to eelgrass, Reusch (2006) notes that eelgrass shows "remarkable resilience" towards small scale physical gap disturbance. Native eelgrass exhibits a stable and possibly increasing trend in distribution and abundance in areas like Willapa and Humboldt bays where oysters have been actively farmed for over 100 years and are currently used by commercial growers (Barrett 1963, Tallis et al. 2009, Dumbauld et al. 2011). Therefore, it appears that there is resilience of eelgrass to the level of shellfish aquaculture activities in these estuaries. It is notable that shellfish aquaculture in both Willapa Bay and Humboldt Bay represent approximately 20% or more of the surface area within the bay.

Positive Interactions of Shellfish Aquaculture

Many effects of shellfish aquaculture can be considered a positive interaction with SAV and the surrounding habitat. According to Forrest et al. (2009), "the acceptability of aquaculture operations or new developments should recognize the full range of effects, since adverse impacts may be compensated to some extent by the nominally 'positive' effects of cultivation." There are a number of effects described below that can potentially result in beneficial changes to the surrounding environment.

Sediment Stabilization and Eelgrass Colonization

Shellfish have been labeled "ecosystem engineers" because of the ecological roles that they play in coastal habitat processes (Jones et al. 1994, Lenihan 1999). For example, the presence of shellfish can protect shorelines from erosion by stabilizing sediments and dampening waves (Meyer et al. 1997, Scyphers et al. 2011, Spalding et al. 2014). This same function provided by shellfish can benefit eelgrass. Eelgrass has been known to expand into areas after sediments are stabilized. There are numerous examples along the West Coast where eelgrass expanded into shellfish aquaculture plots. The information for most of these examples is primarily anecdotal, with notable exceptions (e.g., Ward et al. 2003), and the cause has not been directly linked to the aquaculture operation. Overall, current shellfish aquaculture practices have not been associated with significant loss in the spatial extent of eelgrass and in some cases can be linked to expansion of eelgrass at a landscape-scale.

Potential recruitment of eelgrass into shellfish aquaculture plots is driven by three main mechanisms. First, by providing a larger boundary layer and slowing water current speed, shellfish may increase recruitment of floating seeds as they travel singly or within detached reproductive shoots. Retention of seedlings could also be facilitated by the structure shellfish gear provide, although the density and type of gear can impede seed dispersal (see Tallis et al. 2009). Seed dispersal is typically limited outside of an eelgrass bed; approximately 80% of seeds travel within 33 ft (10 m) of parent plants (Orth et al. 1994, Ruckelshaus 1996). Therefore, this effect is only important when eelgrass beds are nearby. Second, by filtering seawater and increasing sediment organic content, bivalves provide superior conditions for seed germination. Eelgrass seed germination is dependent on burial depth, with the highest germination occurring at the anaerobic/aerobic interface (Bigley 1981). Seeds buried below this depth have very low germination and are essentially lost from the population. Shellfish may act to bury and fertilize seeds at a depth that is appropriate for germination. Third, shellfish may increase the survival of seedlings, which have very high mortality rates, by increasing light levels, nutrients, and protecting against erosion and herbivory (Orth et al. 1994, Ruckelshaus 1996). Note that filter-feeding effects to light levels are discussed in more detail below. Reusch (2006) characterizes Z. marina as a continual recruitment species where seedlings are continuously being introduced into existing beds in addition to establishing into canopy gaps and adjacent areas.

A case study that highlights the potential for eelgrass to colonize into an area previously devoid of eelgrass is a sand bar (Fisk Bar) in the center of Samish Bay, Washington. Prior to geoduck aquaculture on Fisk Bar, seeds from the surrounding eelgrass beds would occasionally result in ephemeral shoots on the sand bar that would get eroded during winter storms. In 2002, geoduck nursery tubes (6-inch-diameter polyvinyl chloride [PVC] tubes) for planting geoduck seed were placed to establish the first geoduck crop on Fisk Bar (Figure 2). Shortly after nursery tubes were placed, eelgrass began to fill in and establish a dense bed around the tubes (Dewey, pers. comm., 2015). For this first crop of geoduck, individual net caps were placed on each tube. When the tubes were removed 2 years after seeding, eelgrass was established well enough that it remained and thrived on the sand bar.

In 2008, when the geoducks were harvested, eelgrass was significantly reduced but not eliminated. After this first harvest event, nursery tubes were reinstalled, seeded with geoducks, and the entire tube field was covered by predator exclusion nets. As described above, Horwith (2013) reported a total loss of eelgrass in areas where the predator exclusion netting was placed. The eelgrass loss was attributed to shading effects from *Ulva* sp. growth on the net surface. Recovery of eelgrass began 1 year after removal of tubes and nets. In July 2014, Dr. Horwith (pers. comm., 2014) indicated that, "there is no longer any significant difference in eelgrass coverage or density between the farmed and unfarmed areas" (see Figure 2). Overall, the Fisk Bar eelgrass bed appears to be resilient and thriving in an area where it could not previously establish.

Fisk Bar, Samish Bay, 2002: Planting Geoduck Seed with Culture Tubes and Individual Net Caps. No eelgrass bed present prior to planting activities.

Fisk Bar, Samish Bay, 2014: Eelgrass Bed prior to Harvest Activities

Figure 2 **Eelgrass at Fisk Bar, Samish Bay, between 2002 and 2014**

Source: Dewey (pers. comm., 2015)

Increased Water Clarity and Light Penetration

Shellfish aquaculture can result in a reduction in turbidity due to removal of phytoplankton and particulate organic matter through filtration (Peterson and Heck 2001, Newell and Koch 2004, Cranford et al. 2011). By consuming phytoplankton and particulate organic matter, shellfish increase the amount of light reaching the sediment surface that is available for photosynthesis (Dame et al. 1984, Koch and Beer 1996, Newell 2004, Newell and Koch 2004). Improvements to water clarity and light penetration can improve habitat conditions that promote the growth of SAV and other aquatic vegetation. There is an optimum range for feeding efficiency, identified as suspended solid concentrations between 5 mg/L and 25 mg/L based on oysters in Chesapeake Bay, Maryland (Cerco and Noel 2007). Therefore, this benefit can be limited when suspended sediment concentrations are outside of this range.

The removal of nutrients (especially nitrogen) through filtration can also benefit SAV growth by reducing epiphytes and macroalgae (Figure 3). Epiphytes (primarily diatoms) can form thick layers on eelgrass blades. This is a natural process, and important in the food chain because this layer of epiphytes is grazed by aquatic invertebrates (van Montfrans et al. 1984, Nelson and Waaland 1997). However, overproduction of epiphytes is a result of nutrient water column pollution (Williams and Ruckelshaus 1993, Hauxwell et al. 2001, Nielsen et al. 2004). Shellfish aquaculture can provide mitigation of these conditions through water filtration and control of nutrients that promote the growth of epiphytes.

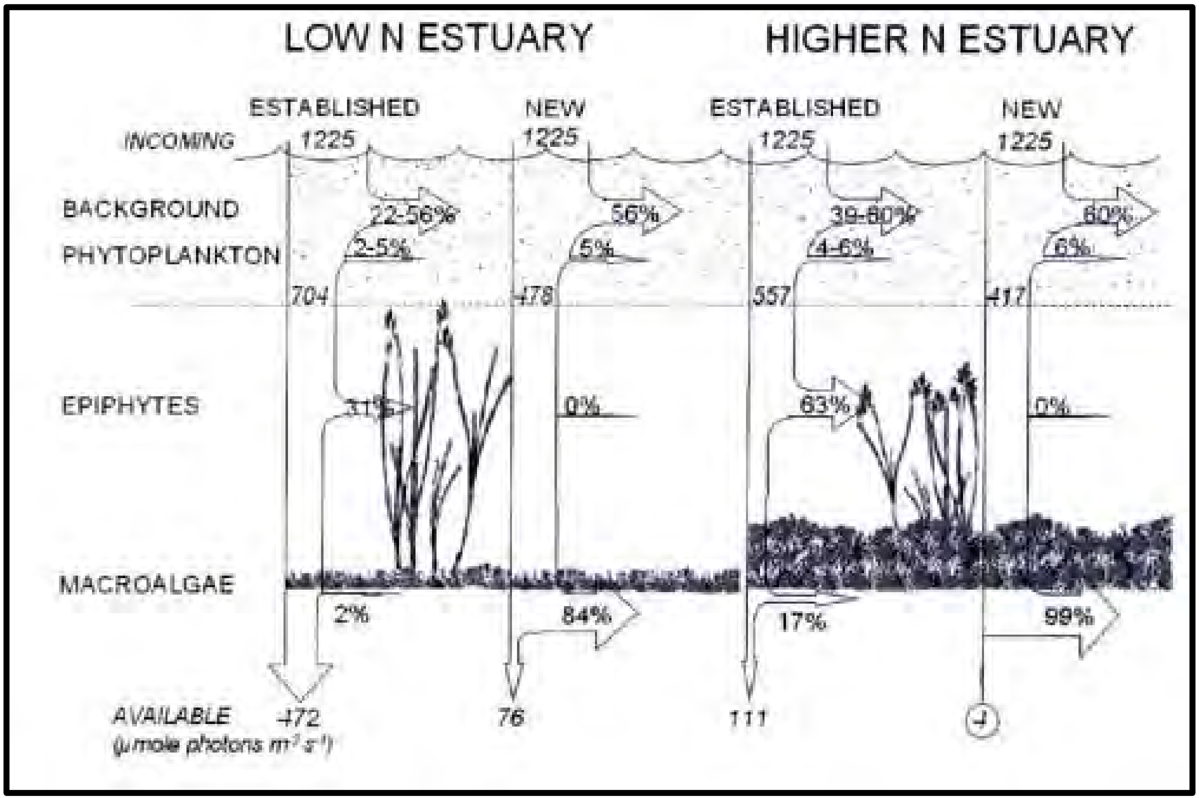

Figure 3 **Illustration of Nitrogen Concentration Effects to Eelgrass**

Source: Hauxwell et al. (2001)

Another service potentially provided by shellfish related to epiphytes was explored by Peterson and Heck (2001). The authors observed a significantly reduced epiphytic load on seagrass leaves when mussels were present. Spaces between shells of adjacent mussels were thought to provide a predation refuge for epiphytic grazers (e.g., small gastropods and amphipods). Increased densities of epiphytic grazers could then lead to an increased amount of grazing, which consequently might lead to an increase in leaf light absorption. This study also noted that the mussels themselves may potentially reduce epiphytic loads by consuming the epiphyte propagules before recruitment to the leaves. Although likely a benefit to eelgrass, the shellfish would need to be in the eelgrass bed to provide this service for epiphytic grazers.

Macroalgae does not colonize eelgrass shoots in the way that epiphytes do, but changes in the amounts of nutrients in the aquatic environment can shift the competitive balance between aquatic vegetation species, allowing plants that can respond quickly to nutrients to dominate (Taylor et al. 1995, Schramm and Nienhuis 1996, Taylor et al. 2001, Cardoso et al. 2004, Nielsen et al. 2004, Mumford 2007, Smetacek and Zingone 2013). The mechanism for loss of eelgrass and other SAV is likely related to a combination of light competition (Nienhuis 1996), smothering by macroalgal blooms (den Hartog and Phillips 2000), and competition for nutrients (Nienhuis 1996).

Improvements to Dissolved Oxygen and Nutrient Sequestration

Phytoplankton, as primary producers, create oxygen through photosynthesis. The mechanism by which dissolved oxygen (DO) is reduced is a result of a phytoplankton bloom produced by an increase in nitrogen in the system. As Albertson et al. (2002) indicated, south Puget Sound is sensitive to nitrogen addition generating phytoplankton blooms. When excess plankton die from not being consumed, bacteria consume the plankton and have a secondary bloom as a result, which consumes oxygen and results in low DO concentrations. This process is also known as the biochemical oxygen demand (BOD) of the system.

Washington Department of Ecology created a model of south and central Puget Sound, Washington (Ahmed et al. 2014) to identify how much nutrient sources are contributing to low DO concentrations. The model predicted minimum DO concentrations under natural conditions (left map of Figure 4), which was then used to evaluate whether water quality standards (right map of Figure 4) were being violated. Figure 4 shows that DO falls below the applicable criterion throughout most of south and central Puget Sound. Ahmed et al. (2014) also predicted that reducing the internal human nutrient load would decrease the magnitude and extent of DO depletion in south and central Puget Sound. According to recent modeling by Banas and Cheng (2015), the authors hypothesized that "these inlets [Henderson, Eld, Totten, Hammersley, and upper Case inlets] are at noticeably lower risk of eutrophication than they would be in the absence of shellfish aquaculture."

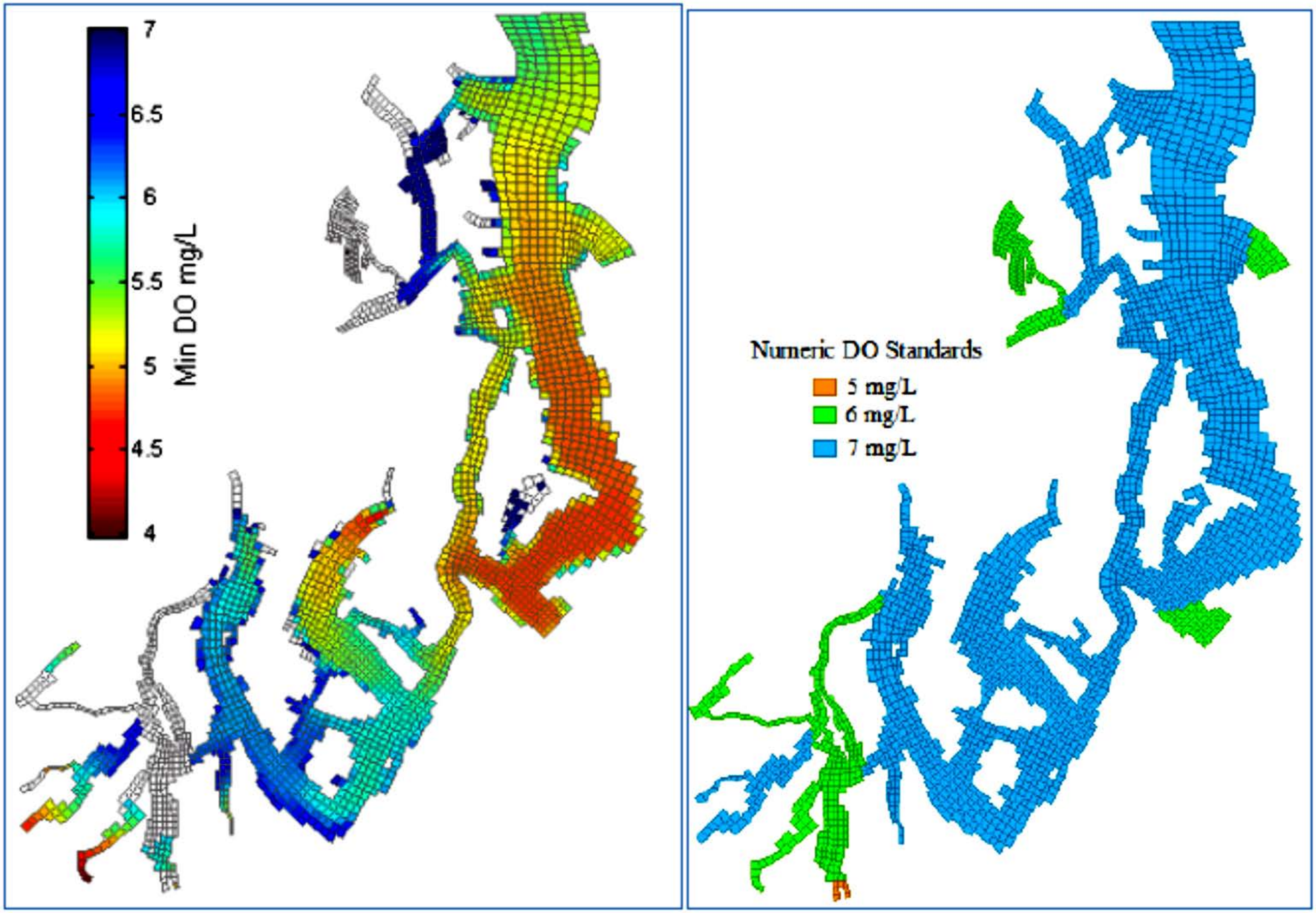

Figure 4 **Minimum Dissolved Oxygen below the Numeric Standard under Natural Conditions**

Source: Ahmed et al. 2014

An emerging body of literature is indicating that shellfish aquaculture, or the presence of a dense bivalve community, may provide some control of human nutrient loading to water bodies (Newell 2004, Shumway et al. 2003, Newell et al. 2005, Burkholder and Shumway 2011, Kellogg et al. 2013, Banas and Cheng 2015, Bricker et al. 2015). Bivalves remove more nutrients from the water column than they input as biodeposits, which can have a net benefit to water quality. As bivalves filter organic matter from the water column, they assimilate nitrogen and phosphorus into their shells and tissue (Figure 5).

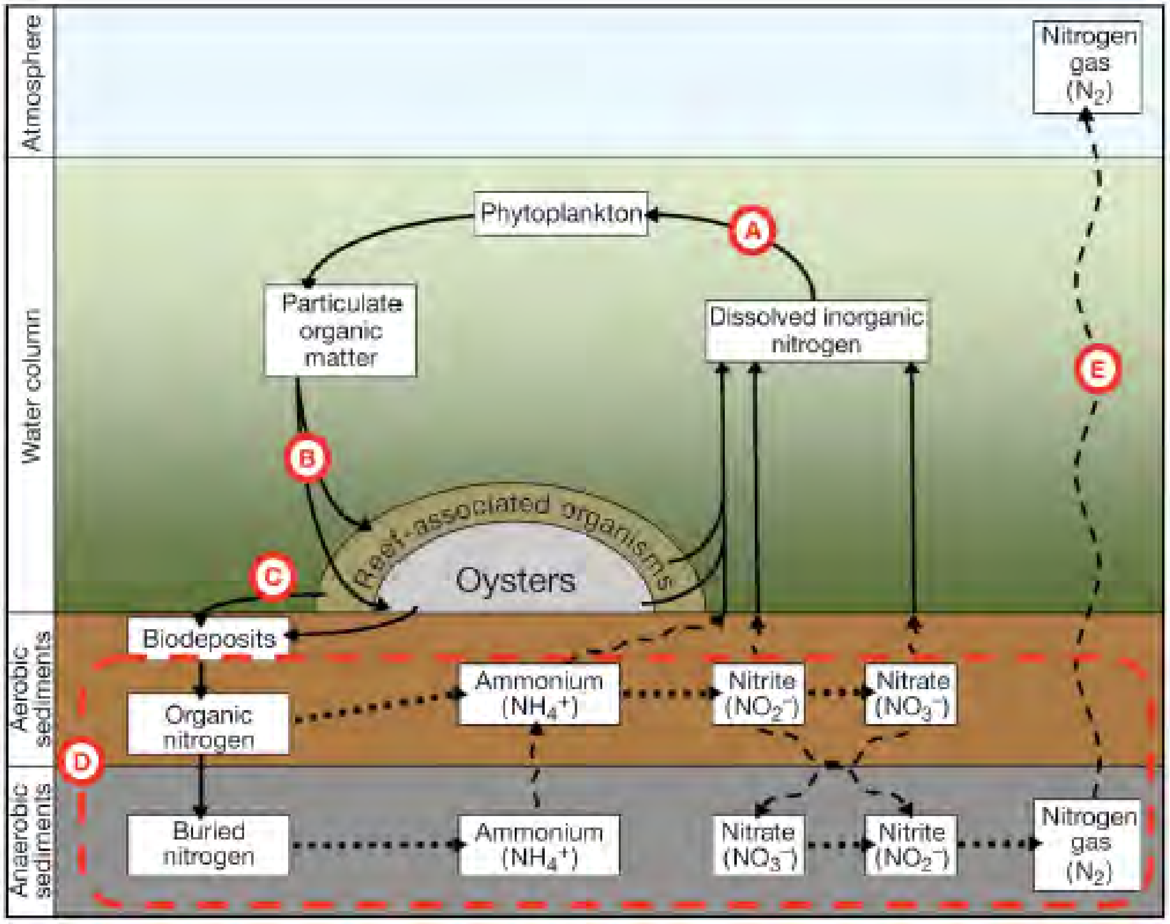

Figure 5 **Nitrogen Pathways Associated with Oysters and Reef-Associated Organisms**

Notes: Phytoplankton use dissolved inorganic nitrogen for their growth (A), oysters and reef-associated organisms filter phytoplankton and other particulate organic matter from the water column (B), some of the associated nitrogen is incorporated into organisms and some is deposited on the surface of the sediments (C), and, given the right conditions, a portion of the nitrogen in these biodeposits is transformed into nitrogen gas (D) which diffuses out of the sediments back to the atmosphere where it is no longer available to phytoplankton for growth (diagram adapted from Newell et al. 2005).

Source: Kellogg et al. (2013)

When shellfish are harvested, the sequestered nutrients are permanently removed from the system, also known as bioextraction. According to Newell (2004), bioextraction is one of the only methods available that removes nutrients after they have entered a system, which can then make that system more resilient to nutrient loading and ultimately decreases in DO. Similarly, bivalve filter-feeding also serves an important role in improving water quality conditions through benthic-pelagic coupling, which is when biodeposits become incorporated into aerobic surficial sediments, and microbially mediated processes facilitate nitrification-denitrification coupling to permanently remove sediment-associated nitrogen as nitrogen gas (also shown on Figure 5).

The amount of benefit to water quality is dependent on species-specific filtration rates. A recent effort to calculate filtering capacity within south Puget Sound (Ferriss 2015) compiled clearance rates for Pacific oyster, Manila clam, and geoduck (Table 1). According to Banas and Cheng (2015), the potential for local control by shellfish was shown to be possible in Henderson, Eld, Totten, Hammersley, and upper Case inlets, and Oakland Bay. Therefore, shellfish filtration in estuaries could have an influence on local water quality parameters, even if small compared to the inputs into the system.

Table 1 Clearance Rate Calculations for Pacific Oyster, Manila Clam, and Geoduck.

Species	Indiv. W_{wet} (g)	L hr^{-1} $indiv^{-1}$	L hr^{-1} W_{wet}^{-1}	Source
Pacific oyster	11.52	3	0.260	Kobayashi et al. 1997, Ruesink et al. 2006
Manila clam	18.19	1	0.060	Ruesink et al. 2006, Solidoro et al. 2003
Geoduck	980	3	0.003	Davis 2010
Source: Banas and Cheng 2015				

An example of the potential benefits offered by shellfish filtration and nutrient sequestration is provided by Kellogg et al. (2013), who partially quantified the removal of nutrients from the water column at a subtidal oyster reef restoration site compared to an adjacent control site in the Choptank River within Chesapeake Bay, Maryland. The authors indicated that denitrification rates at the oyster reef in August were "among the highest ever recorded for an aquatic system." In addition, a significant portion (47% and 48% of total standing stock) of the available nitrogen and phosphorus were sequestered in the shells of live oysters and mussels. An ancillary benefit of the shellfish reef structure, which is also true for shellfish aquaculture gear and shellfish, was that the structure and faunal composition provided ample microhabitats for communities of nitrifying microbes. One of the conclusions by Kellogg et al. (2013) was that oyster reef restoration could be considered a "safety net" to reduce additional downstream impacts to water quality. Because shellfish aquaculture provides many of the same benefits, with the added benefit of the total removal of nutrients at harvest, commercial shellfish aquaculture can be considered a net benefit to water quality ecosystem functions.

Sediment Enrichment

The biodeposits created through bivalve filter feeding contribute to organic materials in the sediment surface, as described above. When organic materials accumulate, the amount of DO needed to break down the material can be exceeded and lead to anoxic conditions in the sediment (Nizzoli et al. 2005). Effects are variable, depending on the type of culture. For example, suspended culture results in only the transfer of organic material to sediment compared to more mixing for ground culture. Effects are expected to be localized, and the extent is influenced by the density of shellfish relative to water circulation (Callier et al. 2006, Dumbauld et al. 2009, Konrad 2013).

Eelgrass can derive nutrients from both the sediments and the water column. The interstitial water (or sediment porewater) contains relatively higher concentrations of dissolved inorganic and organic nutrients than the water column, and eelgrass obtains most macronutrients from sediments. Sediment reservoirs of nutrients can become depleted when biogeochemical regeneration rates cannot meet plant demands (Short 1987). However, in the course of removing water column particulates, shellfish also alter sediment characteristics positively by moving carbon and nutrients from the water column to the benthos. Although studies related to sediment "fertilization" from bivalve deposition have shown enhanced eelgrass growth along the East Coast (e.g., Peterson and Heck 1999), similar studies in the Pacific Northwest appear to show no effect on eelgrass growth (Wagner et al. 2012, Ruesink and Rowell 2012, Wheat and Ruesink 2013). Studies in the Pacific Northwest indicate that eelgrass is not generally nutrient limited or that sediment porewater nutrients are naturally high.

Reduced Desiccation

As the tide recedes, shellfish retain seawater as they shut down filter feeding to wait for the returning tide. The water that is retained in the mantle cavity is expelled prior to the tide returning, creating a spray of water that is released into the surrounding environment. One of the species that can expel a significant amount of water is the geoduck (Figure 6). Water retention and release from other shellfish species may act in a similar fashion. Although this is likely a minor ecosystem function, it potentially reduces desiccation pressure when eelgrass is exposed during a low tide.

Shellfish gear can also result in the creation of micro-habitat around the structures, which can also retain water and provide opportunities for eelgrass growth. For example, at an existing longline aquaculture operation in Humboldt Bay, California, there is scouring around the PVC posts and eelgrass is growing within the depressions. Based on the surrounding habitat, the presence of culture is providing a slight change in elevation that allows eelgrass to persist. While sediment changes shift seasonally, especially at higher elevations, existing data suggests that sediment changes are minor, especially in relation to the natural sediment dynamics that drive the system (Forrest and Creese 2006, Forrest et al. 2009, Osborne 2015).

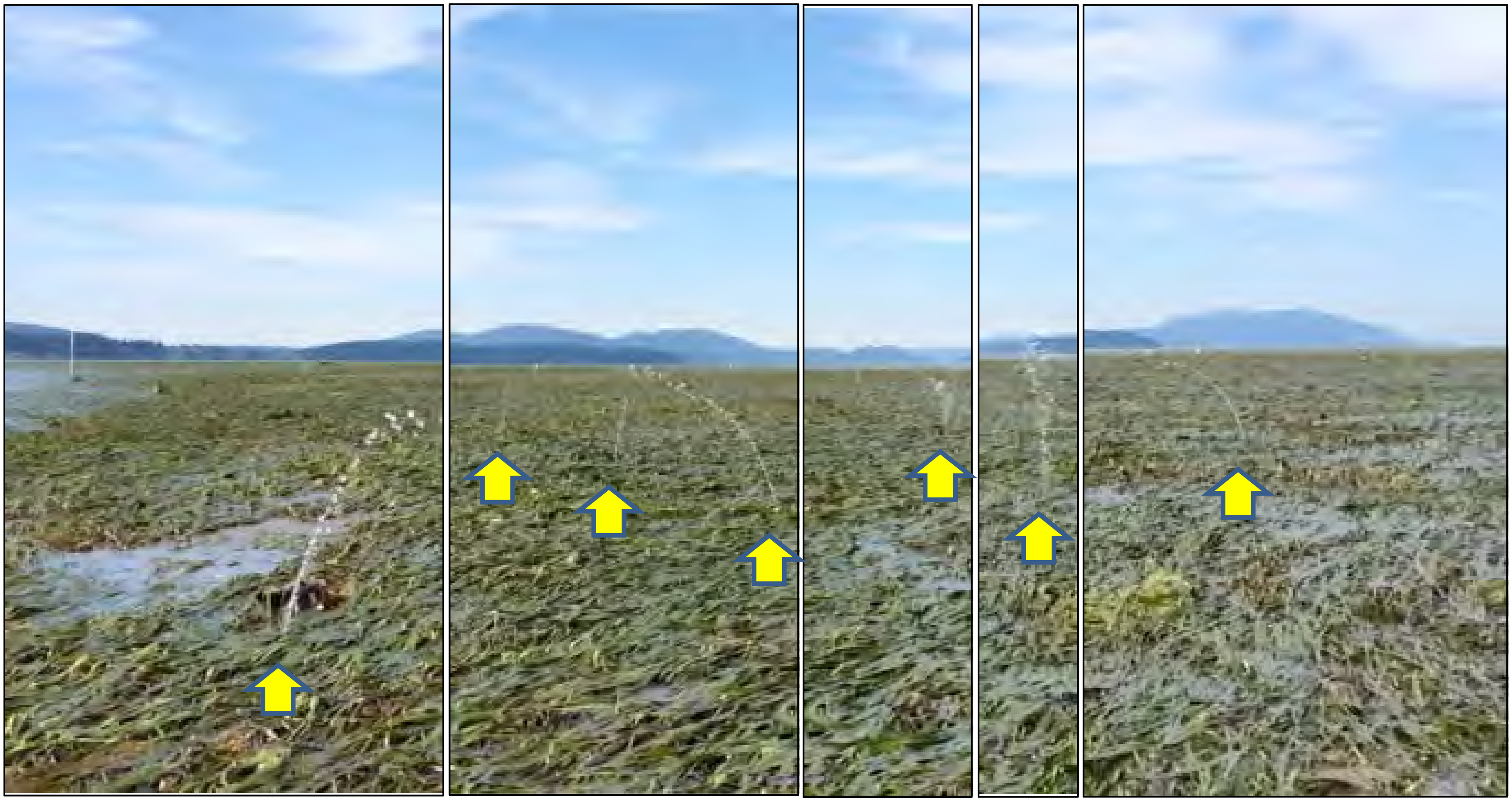

Figure 6 Potential Ecosystem Service (Reduced Desiccation) Provided by Geoduck Aquaculture to Native Eelgrass

Notes: Arrows indicate geoduck expelling water onto the adjacent eelgrass bed.

Source: Dewey (pers. comm., 2015)

Support of the Food Web

Eelgrass and kelp (i.e., SAV) are common perennial aquatic plants that create three-dimensional habitat structure and form extensive intertidal and subtidal beds in estuaries and coastal areas. These beds are an important component of coastal ecosystems because they stabilize coastal sediments, provide direct and indirect food sources for marine species, and provide nursery habitat for many marine species (Phillips 1984, Short et al. 2000, DNR n.d.). Because eelgrass and kelp are autotrophs, which means that they produce complex organic compounds (e.g., carbon-based solid) from simple substances present in the surrounding environment (e.g., light and nutrients), many ecosystem functions of plants cannot be replaced by higher trophic organisms, such as shellfish. That said, many of the cultured shellfish crops, with the exception of triploid oysters, spawn several times before reaching harvest size. These events produce billions of planktonic larvae, the vast majority of which are consumed by predators.

In addition, structured habitat (both eelgrass and shellfish aquaculture plots) can result in a higher abundance of prey organisms. Hosack (2003a) reported that important fish prey, such as harpacticoid copepods, exhibited an inverse trend with higher densities in both dense eelgrass and oyster habitats. These observations parallel those of Ferraro and Cole (2007, 2011, 2012), from oyster bottom culture in Yaquina Bay, Oregon, Willapa Bay, Washington, and Grays Harbor, Washington. The authors reported similar species abundance and richness in benthic macrofaunal communities between native eelgrass and oyster habitat in the three areas studied. Both eelgrass and oyster habitats had significantly more prey resources than mudflat or sandy habitats. This serves to illustrate the relative importance of eelgrass and shellfish-rich habitat in coastal estuaries as refugia and a source of prey for foraging nekton and other marine life.

A recent study by Dumbauld et al. (2015) studied whether intertidal oyster aquaculture in Willapa Bay effects the distribution and feeding ecology of juvenile salmonids. The study identified no significant differences in the density of juvenile salmonids caught in the four habitat types analyzed (undisturbed open mudflat, seagrass, channel habitats, and oyster aquaculture), and few significant associations with the prey items that the fish consumed. The majority of salmon found over low intertidal habitats were not dependent on structured habitat (e.g., eelgrass or oyster aquaculture) for prey items. Chum salmon was the possible exception, which is typically a smaller fish during estuarine residency. The final conclusion by Dumbauld et al. (2015) was that: "Permanent or 'press' disturbances like diking marshes, dredging and filling shallower estuarine habitats and even hardening shorelines would be expected to have significant impacts for other stocks and life history variants with smaller juveniles that utilize upper intertidal areas (Fresh 2006; Bottom et al. 2009), but our research suggests that short term 'pulse' disturbances like aquaculture which alter the benthic substrate in lower intertidal areas used primarily by larger juvenile salmon outmigrants may pose a less significant threat to maintaining resilience of these fish populations."

Finally, McDonald et al. (2015), included observations of fish species and groups associated with PVC tubes and nets, including flatfish, demersal fish, and surfperch (discussed in more detail below). The results of the McDonald et al. (2015) study were incorporated into a recent model for central Puget Sound that looked at the effects of geoduck aquaculture on food web ecology (or predator-prey interactions). This effort reported that a 120% increase in the current level of geoduck culture would result in substantial increases in biomass densities of surfperch, nearshore demersal fish, and small crabs, and decreases in great blue herons, bald eagles, seabirds, flatfish, and certain invertebrates (e.g., predatory gastropods and small crustaceans) (Ferriss et al. 2015). The results were based on the mediating function in the Ecopath with Ecosim (EwE) model, which to a certain extent forced the entire food web to use the resources associated with the geoduck farms rather than more broadly. When this was not the case (e.g., without the mediating function), there was no change in the food web ecology associated with central Puget Sound. Based on a recent conversation with the authors (McDonald, Pers. Com. 2015), it was understood that the Ferris et al. (2015) paper was intended as a way to identify new research priorities and potential pathways of effect, and was not intended to be used as a way to predict impacts from an increase in gear associated with geoduck aquaculture operations. The model represents a model reality which is useful for evaluating general patterns and to identify sensitivities of resources to ecosystem changes, however the model is not and should not be used as predictive, nor should values be considered to be absolute (McDonald, pers. com. 2015). Due to assumptions implicit in the model outputs, further field observations will be needed to validate modelled predictions associated with increasing geoduck culture are being realized and to further validate the model.

Preikshot et al. (2015) developed a similar EwE model, but for south Puget Sound with parameters extracted from observations during the period 1970 to 2012, and not using the mediation functions in the same manner as Ferriss et al. (2015). This parameterized model by Preikshot et al. (2015) was used to evaluate the potential effects of growth in shellfish aquaculture on other aspects of the food web. The model forecasted various scenarios through 2054, and the scenarios that included a 10-fold expansion of shellfish aquaculture (oysters, mussels and geoduck clams) was unlikely to significantly influence the biomass of other species. While such ecosystem models can identify biomasses of ecosystem components, they are not an effective mechanism for identifying the mechanism for biomass change and the cause of that change may be due to processes outside of the modelled area (e.g., salmon life stages that occur beyond the modelled area). That said, the model identified few negative feedbacks associated with bivalve aquaculture. Overall, the study suggested that shellfish aquaculture, as presently configured and even with a significant expansion of culture activities, is benign or beneficial to most species.

Habitat Structure (Density-Dependent)

A significant benefit offered by shellfish aquaculture is the ability to provide nursery habitats that create transitional zones between mudflats and SAV habitat. One of the most comprehensive analyses of the attributes relevant to identifying the role of nursery habitat was performed by Heck et al. (2003). The authors conducted a meta-analysis of more than 200 papers that compared seagrass beds to other habitats, and examined the data using the attributes suggested by Beck et al. (2001) for defining the ecological processes operating in nursery habitats, including: density, growth, survival, and migration to adult habitat. The results indicated that few significant differences existed between the relevant attributes when seagrass meadows were compared to other structured habitats, such as oyster reefs, cobble reefs, or macroalgal beds. The most important determinant of nursery value was the presence of structure rather than the type of structure.

What does appear to be an important determinant in terms of the quality of habitat provided is density and diversity. Optimal foraging/movement and fitness strategies depend on a mosaic of different habitats, and edges or transitional zones between two habitat types often represent areas with increased biological diversity (Holt et al. 1983, Orth et al. 1984, Boström et al. 2006). For example, several species of fish are found in higher densities in patchy eelgrass beds versus continuous dense beds (Orth et al. 1984). Holt et al. (1983) suggested that some species of fish require open feeding areas and refuge areas in the same location, and that patchy vegetation with a high percentage of edges may support higher densities of mobile foraging species.

The observations of edge effect are partially supported by a recent study in Humboldt Bay, California, by Pinnix et al. (2013). The study used acoustic transmitters that were surgically implanted into out-migrating coho salmon (*Oncorhynchus kisutch*) smolts. During their residency in Humboldt Bay, coho smolts primarily used deep channels and channel margins. They were detected near floating eelgrass mats adjacent to the channels, but not over eelgrass beds. The results from this study potentially emphasize the importance of edge habitat and the need for structural heterogeneity during salmonid residency and migration through the estuary.

In terms of prey resources, similar to that reported above, Tanner (2005) found epifauna, such as tanaids and gammaridean amphipods (i.e., typical salmonid prey items), exhibited significantly higher abundances at sand/seagrass edges versus seagrass bed interiors. Similarly, Hirst and Attrill (2008) determined that eelgrass patches, regardless of size or number of plants, were found to support a higher level of biodiversity than surrounding sand habitats. Thus, it could be argued that modest displacement of eelgrass resulting in some patchiness may be neutral or beneficial for certain species, such as salmonids, provided that an abundance of eelgrass was present in the surrounding environment to ensure that none of the other ecological functions provided by eelgrass were significantly reduced.

Ancillary Benefits

In addition to direct beneficial interactions between shellfish aquaculture and eelgrass, the presence of aquaculture within an embayment or watershed may provide indirect benefits to SAV through a variety of mechanisms. The aquaculture industry is inherently reliant on the maintenance of good water quality conditions to ensure the safety and survival of their product. Because of this incentive, there are numerous examples of actions taken by aquaculture companies and their supporters that result in improvements to water quality and/or the prevention of anthropogenic activities threatening water quality and habitat function in areas were aquaculture occurs (Dewey et al. 2011).

Examples of some ancillary benefits of the shellfish aquaculture industry include:

- Working with local jurisdictions and regulators to identify and eliminate point and non-point source pollution, including agricultural, industrial, and municipal discharges.
- Participating and providing input on regulatory updates to ensure that high water quality standards are included in local, state, and federal policies.

- Lobbying state and federal legislatures for improvements to water quality and developing water quality standards (e.g., shellfish industry contribution to the enactment of the Clean Water Act in 1972).
- Maintaining ownership or leases of large aquatic areas and upland, thereby eliminating the risk of environmentally deleterious uses.
- Participating in and collecting water quality samples as part of monitoring programs with federal and state agencies (e.g., National Shellfish Sanitation Program) to track water quality trends and identify areas targeted for improvement. These efforts have directly resulted in numerous areas now being determined suitable for shellfish harvesting and have provided data for other target areas with opportunities for improvement.
- Donating to local and state organizations to improve water quality conditions within the estuaries that shellfish aquaculture occurs.
- Organizing and participating in beach cleanup events that collect marine debris from both shoreline development and shellfish aquaculture operations.
- Actively engaging in efforts to quickly remediate and clean up oil spills and other hazardous waste sites to protect water quality and the health of shellfish.
- Encouraging shellfish gardening through sponsored seed and gear sales (e.g., Taylor Shellfish annual events in Washington State). Shellfish gardening encourages land owners to learn about the importance of maintaining properly functioning septic systems, controlling pet and domestic animal wastes, and the fate of herbicides and pesticides from lawns and gardens.

CONCLUSIONS

This technical report provided a review of the relevant literature, as it pertains to interactions between shellfish aquaculture and the surrounding environment. Overall, commercial shellfish aquaculture is a highly regulated activity within federal, state, and local jurisdictions. As indicated by numerous independent researchers and agencies, a well-managed shellfish aquaculture farm can result in minimal individual and cumulative adverse impacts to ecological functions, and for some parameters (e.g., water quality) can result in net positive benefits to the surrounding environment. Shellfish aquaculture can work within the resiliency of the system because of the avoidance, conservation, and minimization measures developed, and advancing technologies that allow for a reduction of human presence within a farm.

Although shellfish aquaculture does not provide identical functions as eelgrass, there are similarities in terms of the organisms it supports and services that it provides. Some of the most pertinent examples include enhanced water quality and clarity, nutrient cycling, habitat structure, and prey resources that can support an elevated community of fish and wildlife.

Through a thorough exploration of the literature we believe that properly implement aquaculture activities have minimal, or even beneficial, individual and cumulative environmental impacts and are consistent with a finding of no net loss of ecological function in the estuarine habitats where aquaculture occurs. Shellfish aquaculture is a temporary pulse disturbance (compared to a long-term press disturbance such as a bulkhead). Although there may be short term effects, on the landscape scale these effects are distributed both spatially and temporally.

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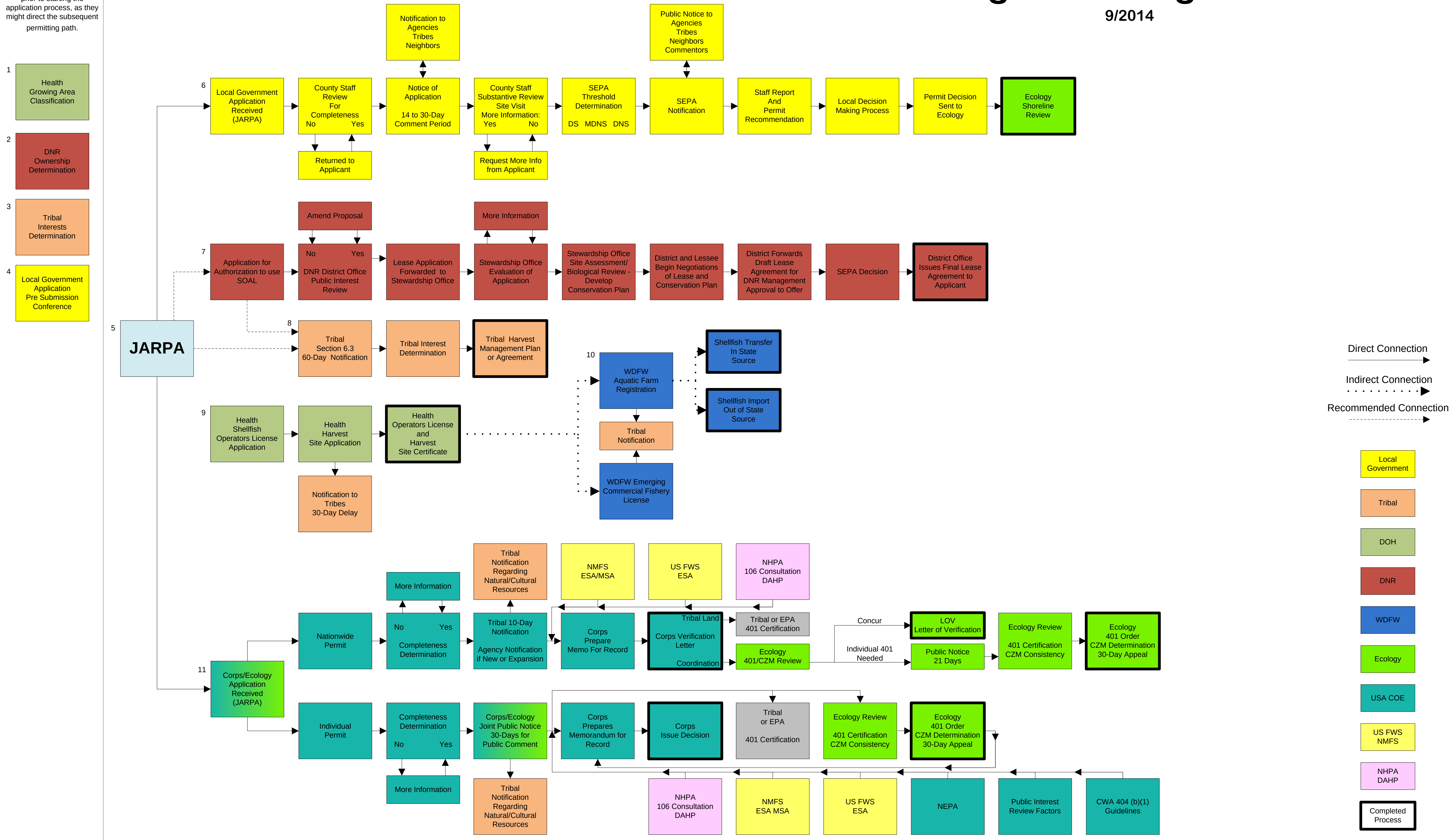
Appendix E

Existing Permitting Processes

9/2014

Early Inquiries:

These are items to check prior to starting the application process, as they might direct the subsequent permitting path.



1 Health Growing Area Classification

2 DNR Ownership Determination

3 Tribal Interests Determination

4 Local Government Application Pre Submission Conference

5 JARPA

6 Local Government Application Received (JARPA) → County Staff Review For Completeness (No → Returned to Applicant; Yes) → Notice of Application 14 to 30-Day Comment Period (↔ Notification to Agencies Tribes Neighbors) → County Staff Substantive Review Site Visit More Information: Yes (→ Request More Info from Applicant) No → SEPA Threshold Determination DS MDNS DNS → SEPA Notification (↔ Public Notice to Agencies Tribes Neighbors Commentors) → Staff Report And Permit Recommendation → Local Decision Making Process → Permit Decision Sent to Ecology → Ecology Shoreline Review

7 Application for Authorization to use SOAL → DNR District Office Public Interest Review (No → Amend Proposal; Yes) → Lease Application Forwarded to Stewardship Office → Stewardship Office Evaluation of Application (↔ More Information) → Stewardship Office Site Assessment/ Biological Review - Develop Conservation Plan → District and Lessee Begin Negotiations of Lease and Conservation Plan → District Forwards Draft Lease Agreement for DNR Management Approval to Offer → SEPA Decision → District Office Issues Final Lease Agreement to Applicant

8 Tribal Section 6.3 60-Day Notification → Tribal Interest Determination → Tribal Harvest Management Plan or Agreement

9 Health Shellfish Operators License Application → Health Harvest Site Application (→ Notification to Tribes 30-Day Delay) → Health Operators License and Harvest Site Certificate

10 WDFW Aquatic Farm Registration → Shellfish Transfer In State Source; Shellfish Import Out of State Source; Tribal Notification; WDFW Emerging Commercial Fishery License

11 Corps/Ecology Application Received (JARPA)

Nationwide Permit → More Information; No Yes Completeness Determination → Tribal 10-Day Notification Agency Notification if New or Expansion (→ Tribal Notification Regarding Natural/Cultural Resources) → Corps Prepare Memo For Record (← NMFS ESA/MSA; US FWS ESA; NHPA 106 Consultation DAHP) → Tribal Land Corps Verification Letter Coordination → Tribal or EPA 401 Certification; Ecology 401/CZM Review → Concur → LOV Letter of Verification; Individual 401 Needed → Public Notice 21 Days → Ecology Review 401 Certification CZM Consistency → Ecology 401 Order CZM Determination 30-Day Appeal

Individual Permit → Completeness Determination No Yes (↔ More Information) → Corps/Ecology Joint Public Notice 30-Days for Public Comment (→ Tribal Notification Regarding Natural/Cultural Resources) → Corps Prepares Memorandum for Record → Corps Issue Decision; Tribal or EPA 401 Certification; Ecology Review 401 Certification CZM Consistency → Ecology 401 Order CZM Determination 30-Day Appeal (← NHPA 106 Consultation DAHP; NMFS ESA MSA; US FWS ESA; NEPA; Public Interest Review Factors; CWA 404 (b)(1) Guidelines)

Direct Connection

Indirect Connection

Recommended Connection

🟨 Local Government

🟧 Tribal

🟩 DOH

🟥 DNR

🟦 WDFW

🟩 Ecology

🟦 USA COE

🟨 US FWS NMFS

🟪 NHPA DAHP

☐ Completed Process

Supplemental Narrative for Existing Permitting Processes Flowchart
09/2014

This document is meant to be used as a supplement to the flowchart of 'Existing Permitting Processes developed by the Shellfish Interagency Permitting Team. Top level numbers 1-8 correspond with numbered boxes on the flowchart. Ancillary information follows the flowchart's arrows radiating from boxes 1-8.

It is recommended that the steps in numbers 1-4 be completed prior to making formal application with local, State, or Federal agencies below to minimize possible delays later in the permitting process.

1. **Health Growing Area Classification:** contact Department of Health (DOH) to determine if growing area is classified:
   http://www.doh.wa.gov/CommunityandEnvironment/Shellfish/GrowingAreas.aspx
   Classifications include:
   A = approved
   CA = conditionally approved
   R = restricted
   P = prohibited
   U = unclassified; if unclassified you can only request classification if you are a licensed shellfish company or intend to be licensed (DOH Shellfish License)

2. **Department of Natural Resources (DNR) Ownership Determination:** determine if land is privately owned or owned by the state:
   a. To determine if **State Owned Aquatic Lands (SOAL)**, call DNR's Aquatic Program: 360-902-1100 or via: http://www.dnr.wa.gov/BusinessPermits/Topics/ShellfishAquaticLeasing/Pages/aqr_aquatic_land_leasing.aspx
      - If SOAL, you will need to obtain a lease from DNR (see step 7 for more details)
      - If not SOAL, and your project is subtidal, you will need to determine if there is Bush Act/Callow Act Reversionary Interest (79.135.010). DNR will assist with determination.

   b. For **private ownership**, you will need signed documentation from owner

3. **Tribal Interests Determination:** if the land parcel is not part of the Settlement Agreement[1], you must determine if there are Tribal Interests by submitting a 6.3 form to the appropriate tribe.
   a. To determine if the land parcel is part of the Settlement Agreement contact the Northwest Indian Fisheries Commission (NWIFC) staff shellfish contact. If not

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[1] Settlement Agreement: in 2007 Puget Sound commercial shellfish growers and 17 treaty Indian tribes in western Washington reached a landmark agreement that addressed treaty shellfish harvest rights, preserved the health of the shellfish industry and provided greater shellfish harvest opportunities for everyone in the state: http://nwifc.org/about-us/shellfish/commercial-shellfish-growers-settlement/

part of the Settlement Agreement a 6.3 form must be submitted to the appropriate Tribe(s). The NWIFC staff member can help determine the appropriate Tribe(s) to contact:
http://nwifc.org/about-us/staff-directory/
http://nwifc.org/about-us/shellfish/downloads/
http://nwifc.org/w/wp-content/uploads/downloads/2012/01/Section-6.3-Form.pdf

   b. Note that there are multiple times in the permit process that the tribes are notified. For instance, when you go through the DOH process the tribes will be notified and a 6.3 form provided. However **it is the applicant's responsibility to fill out the 6.3 form and submit directly to the Tribes**. It is advisable to start this process early to be sure other permits not held up later.
   c. 6.3 process outlined in detail in #8 below.

4. **Local Government Application Pre-Submission Conference:** the local government review process can be the most detailed and time consuming part of an aquaculture application. A pre-submission conference will explain the review process and help ensure that the applicant understands what information must be included in the application. For some local governments this meeting is required and for some it is optional.
   a. A fee may be required dependent on type of meeting and County
   b. The pre-submission conference will give you information about what needs to go into the JARPA

With adequate support, the Shellfish Interagency Permitting Team (SIP) could facilitate these initial steps with all necessary entities to promote early coordination.

5. **JARPA:** fill out a Joint Aquatic Resource Permit Application (JARPA): http://www.epermitting.wa.gov/site/alias__resourcecenter/jarpa_jarpa_form/9984/jarpa_form.aspx
   a. Please refer to *JARPA Instruction B: For Shellfish Aquaculture* for information about how best to fill out this form (link TBD).
   b. Use the *Aquaculture JARPA Supplemental Drawing Checklist* to be sure your maps and drawings contain all required information (link TBD).
   c. Applicant is required to submit an original **signed** JARPA to each permitting entity that accepts JARPA individually to initiate permitting process (see below for more details by entity). Please ensure content is the same for each JARPA submitted to each permitting entity.

6. **Local Government Application Received**: outlined here is the general process for local government permit review. These steps will vary amongst local governments. It is highly recommended to have a pre-submission conference prior to submitting your JARPA to make sure all required information is included in your application and to understand the local process.
   a. Submit JARPA to local government (if local government requires a permit) to initiate their review
   b. Pay application fee (varies by county)

c. **County Staff Review For Completeness** (varies by local government): for some local governments, this initial review simply confirms that all necessary paperwork is included in application. In others a thorough review of content is done at this point.
    i. If application is not complete it is **Returned to Applicant**.
    ii. When application is complete, the county will send out a notice of application to interested parties.

d. **Notice of Application, Comment Period:** the notice of application is sent out as a solicitation for comments. This triggers a **14 to 30-Day Comment Period** (local government has the discretion to set the length of the comment period from 14 to 30 days):
    i. **Notification to Agencies/Tribes/Neighbors** (per code); notice of application is sent to interested parties as a solicitation for comments only.
        1. Note that this is NOT a start of application process for state/federal agency permits, it is simply a request for comments to the County; applications must be submitted directly to other agencies (as outlined below) to begin those permitting processes.
        2. In general, neighbors receive general project descriptions while agencies receive more detailed information.
        3. At this time, the notification of the project is also sent to internal county agencies
        4. Some Counties may require signage with information about the project to be posted at the site at this point.

e. **County Staff Substantive Review, Site Visit:** the County conducts a thorough substantive review of the content of the application which usually includes a site visit. Timelines for review are in code and best attempts made to stay within them but may vary dependent on complexity of project and comments received from interested parties.

Site visit could include all regulating entities.

    i. The County may **Request More Information from Applicant.** A back and forth process may occur during which supplemental information is gathered and the project may be modified.
    ii. Concerns of county and interested parties raised during Comment Period, are addressed during this process.
    iii. Every jurisdiction has requirement for timely response, but timelines may vary and deviations may be allowed.
        1. Example: Pierce and Thurston have 30 days allowed for substantive review but if more information is required a request for information is sent to applicant. In Pierce the applicant has 360 days to reply. In Thurston the applicant has 180 days to reply (which can be extended if needed). Once information is provided by the applicant, the County has 14 days to review (which may

        result in another request for information, extending the timeline further).

f. **SEPA Threshold Determination**: project modifications that may be made during substantive review lead to this final determination.
    i. **DS** = Determination of Significance
        1. Will require an Environmental Impact Statement (EIS)
    ii. **MDNS** = Mitigated Determination of Non-Significance
        1. Mitigating Conditions: MDNS means there are specific SEPA mitigation measures (conditions) that do not exist in code applied to the project (and indicated via notes to applicant)
        2. Project may also have a list of conditions that bring it into compliance with County Code;
    iii. **DNS** = Determination of Non-Significance
        1. Approval requirements will only be those via County code (code compliant). May include descriptions of how project meets the codes by listing out what will be done (described in notes to applicant)
g. **SEPA Notification**: applicant/agencies/tribes/neighbors notified of threshold determination.
    i. **Public Notice to Agencies/Tribes/Neighbors**
        1. Neighbors may only be notified if requested (i.e. Pierce), or all neighbors within a certain distance of the project may be notified.
        2. Note that the threshold determination can be appealed but will be consolidated with any appeals of permit issuance further along in the process.
h. **Staff Report and Shoreline Permit Recommendation**
    i. Staff report lists proposal, applicable policies and regulations, and County interpretation; will include Conditional Use Permit (CUP) and/or Shoreline Substantial Development Permit (SSDP) findings
    ii. Recommendation
        1. Approval, Approval with Conditions, or Denial
        2. To what entity the recommendation is made varies by County (i.e. Thurston makes recommendation to Hearing Examiner)
i. **Local Decision Making Process:** varies by County. This is where appeals can be made (which can substantially affect the timing of final decisions) and additional conditions may be added.
j. **Shoreline Permit Decision Sent to Ecology:** varies by County and may be appealed
    i. Approved, Approved with Conditions, or Denied
    ii. After Hearing Examiner or Shoreline Administrator issues decision, it is sent to Ecology
k. **Ecology Shoreline Review:**
    i. Varies by permit type.
        1. For SSDPs Ecology simply receives and files the decision, and notifies applicant of filing.
        2. For CUPs Ecology has authority to Approve, Approve with Conditions, or Deny

Appeals processes may occur at the local and state level and can affect timing and outcome of permit process. Please see supplemental flowchart (in preparation) for detailed examples of local and state appeals processes

7. Submit JARPA to DNR District Office as the **Application for Authorization to use SOAL** with $25 application fee: http://www.epermitting.wa.gov/site/alias__ResourceCenter/2489/jarpa_contacts.aspx
    a. If project area is not part of Settlement Agreement (see #3 above) and you have not already done so, notify the appropriate Tribe and submit a 6.3 form. You must have a **written** Tribal Agreement or Management Plan in place for DNR to issue a lease (see #8 below).
    b. **DNR District Office Public Interest Review:** application received and reviewed for conformance with statutory requirements ("4+1 directives": encourage, foster, ensure, utilize; (+1) generate revenue; RCW 79.105.030): http://www.dnr.wa.gov/BusinessPermits/Topics/ShellfishAquaticLeasing/Pages/Home.aspx
        i. If no, or not consistent with goals (i.e. use conflict) project needs to be amended **(Amend Proposal)** or is declined
        ii. If yes (or after project appropriately amended), the **Lease Application Forwarded to Stewardship Office** by the district for evaluation
    a. **Stewardship Office Evaluation of Application:** if needed **More Information** may be requested. Work with Stewardship Office to accomplish the next steps:
        i. **Stewardship Office Site Assessment/Biological Review—Develop Conservation Plan:**
            i. Site visit

Site visit could occur at same time as County site visit.

            ii. Stewardship specialist makes recommendations and puts together site summary (assessment)
            iii. Stewardship office sends recommendations and site assessment to District
        ii. **District and Lessee Begin Negotiations of Lease and Conservation Plan**: draft lease with recommendations for conservation and decision documents
        iii. **District Forwards Draft Lease Agreement of DNR Management Approval to Offer:** must have compliance with all other permits (if you don't then DNR won't make offer)
        iv. **SEPA Decision:** if no SEPA at County level (i.e. no County permit required) then DNR will be SEPA lead and issue the SEPA threshold determination (similar to 5f. above)
        v. **District Office Issues Final Lease Agreement to Applicant**

8. **Tribal Section 6.3 60-Day Notification**: required if any work will occur that affects wild shellfish if parcel not part of Settlement Agreement (see #3 above)
    a. Contact appropriate Tribe(s) and submit 6.3 Form. The NWIFC shellfish staff member can help determine the appropriate Tribe(s) to contact:
    http://nwifc.org/about-us/staff-directory/
    http://nwifc.org/about-us/shellfish/downloads/
    http://nwifc.org/w/wp-content/uploads/downloads/2012/01/Section-6.3-Form.pdf
    b. Once 6.3 Form submitted to Tribe(s) you must wait 60 days before undertaking any activity on the parcel (i.e. harvest or enhancement). It is strongly recommended that you have Tribal Agreement or Harvest Management Plan (or written no interest determination) in place before doing so.
    c. **Tribal Interest Determination**: Tribe(s) determines if they have interest in area (to determine if they have Tribal Treaty Rights) by surveying area.
        1. If determined that there is a natural bed that the Tribe(s) has interest in, technically the Tribe(s) and applicant have 30 days to negotiate and develop a Harvest Management Plan/Agreement. If disagreement on survey results the 30 days may be extended.
        2. If Tribe(s) determines no interest you will need this determination in writing. Thus, a Tribal Agreement may simply be a written indication from the Tribe(s) that they are not interested in the parcel. If Tribe(s) determines no shellfish beds then no Harvest Management Plan needed.
    d. **Tribal Harvest Management Plan or Agreement** with Tribe(s).

9. Submit the Department of Health (DOH) **Shellfish Operators License Application and/or Harvest Site Application** to obtain Operators License and Harvest Site Certification. These licenses and certifications are required to harvest or sell a commercial quantity of shellfish. Applications and additional information may be found at:
    http://www.doh.wa.gov/CommunityandEnvironment/Shellfish/CommercialShellfish/ApplyforaLicense.aspx
    a. **Shellfish Operators License**: required for any commercial shellfish activity. There are three types of licenses:
        1. Harvester License—can harvest and sell to another licensed dealer in state only
        2. Shell Stock Shipper—can harvest, sell to restaurants, retail, inter/intra state and international
        3. Shucker/Packer—can shuck shellfish and can sell to same as #2
    b. **Harvest Site Application**: DOH Harvest Site Certification is required for each site/parcel that is farmed (must have Operators License to apply)
        i. Once received, DOH sends notification to Tribes and posts on website for Tribes (thus, if you haven't already notified Tribes in steps above, the Tribes will contact grower individually here); DOH sends applicant 6.3 form and lets applicant know to send to tribes (if you have already done this at step #3 or #8 above, you do not have to do it again).

        - ii. As a courtesy to Tribes and to allow time for the step #8 process, DOH waits 30 days from receipt of application to issue the **Harvester Site Certificate**. The Certificate will list all sites/parcels on which a company is licensed to harvest.

10. Washington Department of Fish and Wildlife (**WDFW**): there are four different permits issued by WDFW that you may need:
    - a. **Aquatic Farm Registration (AFR):** required for commercially growing and selling shellfish
        - i. Before grower obtains AFR they must have DOH Harvest Site Certificate.
        - ii. WDFW will supply Tribe(s) copies of AFR (causes no delay on issuance of AFR).
    - b. **Emerging Commercial Fishery License (ECF)**: required for harvesting wild shellfish (i.e. clearing standing stock)
        - i. WDFW will notify the Tribe(s) of the application for ECF License.
        - ii. Before obtaining the ECF grower must have DOH Harvest Site Certificate.
    - c. **Shellfish Import Permit**: required for importing live shellfish into WA state waters
    - d. **Shellfish Transfer Permit**: required for transport of shellfish within WA state waters

11. Submit JARPA to Corps and Ecology (**Corps/Ecology Application Received**). Corps determines if project qualifies (meets terms & conditions) for Nationwide Permit or if an Individual Permit is required.
    - a. **Nationwide Permit (NWP)**
        - i. **Completeness Determination:** Corps determines if application complete (refer to Pre-Construction Notification requirements for NWP48). If yes, move on. If no, Corps requests for more information with 30 days for the applicant to respond or application cancelled.
        - ii. **Tribal 10-Day Notification/Agency Notification:**
            1. Agency notification (DOE, DNR, EPA, NOAA, USFWS) for new or expansion projects.
            2. **Tribal Notification Regarding Natural/Cultural Resources[2]:**
                - a. Tribal Natural Resource Dept: has 10 days to provide comments and if not received Corps moves on. Tribe can request additional 15 day extension. Tribes may provide comments on project related to habitat and treaty entrusted Usual and Accustomed (U&A) areas[3]. Any comments must be addressed by the Corps prior to issuing a permit.

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[2] Note that this notification is not the same as #8 (Tribal Section 6.3 60-Day Notification).

[3] Fifteen western Washington tribes each have a “usual and accustomed” harvest area (U&A) that reflects the historical region in which finfish, shellfish, and other natural resources were collected. All tidelands in Puget Sound are within the usual and accustomed harvest areas of one or more tribe. The fifteen tribes with U&A are: Jamestown S’Klallam, Lower Elwha Klallam, Lummi, Makah, Muckleshoot, Nisqually, Nooksack, Port Gamble S’Klallam, Puyallup, Skokomish, Squaxin Island, Suquamish, Swinomish, Tulalip, and Upper Skagit.

        b. Tribal Cultural Resource Dept: provides information on potential to affect (or no potential to affect) historic properties. If potential to affect (determined by Corps) then consultation occurs (see 11.a.iii.3. below).

iii. **Memo for Record** (this is the rationale for the decision); all of the items below (1-3) must be completed before the Memo for Record is finalized. Time associated with completion varies depending on level of effects.
    1. **NMFS ESA/MSA** consultations (ESA section 7 and MSA for Essential Fish Habitat) must be completed. Consultations are initiated by Corps. Individual consultations may not be necessary if project meets conditions of programmatic consultation or Corps determines “no effect”.
    2. **USFWS ESA** section 7 consultation must be completed. Consultation is initiated by Corps. Individual consultation may not be necessary if project meets conditions of programmatic consultation or Corps determines “no effect”.
    3. **NHPA 106 Consultation DAHP and Tribal**: Corps reviews project for 106 compliance, which includes consultation with State Historic Preservation Officer (SHPO) and tribe(s) when there is a potential to effect historic properties. Tribal consultation on natural and/or cultural resources must occur if comments received during 11.a.ii.2.b. above.

iv. **Corps Verification Letter**[4]: when the Memo for Record is completed then verification of NWP coverage issued by Corps to the applicant and copy sent to Ecology or EPA dependent on project location[5] for verification that project meets their conditions of NWP.

v. **Ecology 401/CZM Review**:
    1. If Ecology **concurs** that the project meets the state 401 conditions for the NWP, then Ecology **Letter of Verification (LOV)** is issued to applicant.
    2. If the project does not meet the state 401 conditions for the NWP, then an **individual 401** Certification review process will be triggered.
        a. Ecology issues **Public Notice** for 21 days.
        b. **Ecology Review/401 Certification/CZM Consistency**
            i. SEPA determination from lead agency will be necessary unless 401 is the only state/local permit required[6].
            ii. CZM Consistency Determination: Ecology will review the Applicant’s CZM Consistency Statement to determine if the project is consistent with the 6

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[4] Note that the Corps Verification Letter is different that the Letter of Verification (LOV) issued by Ecology.

[5] If the project is on state or federal land the copy is sent to Ecology. If on tribal land the copy is sent to EPA. Some tribes have their own 401 WQ standards so if project on their land the copy would be sent directly to respective tribe.

[6] 401 SEPA Categorical Exemption : WAC 197-11-800(9) Water Quality Certifications.

                Enforceable Policies of the Coastal Zone Management Program[7].
                    1. If consistent, then a 'Consistency Determination' is issued. Issued separate from 401 but review occurs concurrently.
                    2. If not consistent, Ecology will issue a Letter of Objection. After determination there is a 30 day appeal process.
                iii. Project reviewed to determine there is reasonable assurance that state water quality standards will be met.
                    1. If yes, then Ecology issues a **401 Order** (401 Certification) to applicant that may have conditions. This decision has a 30 day appeal period.
                    2. If no, then project denied. Applicant may modify project and reapply.
        vi. **EPA or Tribal 401 Certification:** only occurs when project on tribal land.
    b. **Individual Permit (IP)**
        i. **Completeness Determination** (application review): Determine if application complete (33 CFR, 325.1(d)). If yes move on. If no, Corps requests for more information with 30 days to respond or application cancelled.
        ii. Corps and Ecology send out **Joint Public Notice** with 30 days for public comment.
            1. **Tribal Notification Regarding Natural/Cultural[8].**
            2. Issuance of the Joint Public Notices starts Ecology's statutory one year review (401 Trigger):
                a. **Ecology Review:** this outcome informs Corps Memo for Record (see #3 below)
                    i. **401 Certification**: Project is reviewed to determine there is reasonable assurance that state water quality standards will be met.
                        1. If yes, then **401 Order** issued to applicant. SEPA determination must be completed for Ecology to issue the 401 certification.
                        2. If no, then project denied. Applicant may modify project and reapply.
                        3. Ecology decision becomes part of Corps permit.
                    ii. **CZM Consistency Determination:** Ecology will review the Applicant's CZM Consistency Statement

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[7] The 6 Enforceable Policies of the Washington State Coastal Zone Management Program are: (1) the Shoreline Management Act; (2) the Clean Water Act; (3) the Clean Air Act; (4) the State Environmental Policy Act; (5) the Energy Facility Site Evaluation Council Law; and (6) the Ocean Resources Management Act.

[8] Note that this notification is not the same as #8 (Tribal Section 6.3 60-Day Notification).

            to determine if the project is consistent with the 6 Enforceable Policies of the Coastal Zone Management Program.
                1. If consistent, then a 'Consistency Determination' is issued. Issued separate from 401 but review occurs concurrently.
                2. If not consistent, Ecology will issue a Letter of Objection. After determination there is a 30 day appeal process.
        3. **EPA or Tribal 401 Certification:** only occurs when project on tribal land.
    iii. **Corps Prepares Memo for Record:** All things below (1-7) must be completed first:
        1. Corps Process: additional information may be required to complete alternatives analysis, public interest review, address any treaty rights issues, and/or address any public/agency comments received. Determination will be made whether mitigation is required. If so, mitigation plan must be submitted and approved.
        2. **NHPA 106 Consultation DAHP and Tribal**: Corps reviews project for 106 compliance, which includes consultation with State Historic Preservation Officer (SHPO) and tribe(s) when there is a potential to effect historic properties. Corps will take into consideration comments received and coordinate with Tribes appropriately (see 11.b.ii.1. above).
        3. **NMFS ESA/MSA** consultations (ESA section 7 and MSA for Essential Fish Habitat) must be completed. Consultations are initiated by Corps.
        4. **USFWS ESA** section 7 consultation must be completed. Consultation is initiated by Corps.
        5. **NEPA** compliance for major federal actions per Appendix B to Part 325-NEPA Implementation Procedures for the Regulatory Program
        6. **Public Interest** Determination must discuss whether the project is (or is not) contrary to the public interest per 33 CFR 320.4(a)(1).
        7. **CWA 404(b)(1) Guidelines** compliance (40 CFR part 230) for evaluating discharges of dredged or fill material into waters of the U.S. Evaluation of compliance with the 404(b)(1) Guidelines is not required for Section 10 activities only.

Acronyms & Abbreviations

AFR – Aquatic Farm Registration
Corps – Army Corps of Engineers
CUP – Conditional Use Permit
CZM – Coastal Zone Management
DAHP – Department of Archaeology and Historic Preservation
DNR – Washington Department of Natural Resources
DOE – Washington Department of Ecology
DOH – Washington Department of Health
ECF – Emerging Commercial Fishery License
EIS – Environmental Impact Statement
EPA – Environmental Protection Agency
HE – Hearing Examiner
JARPA – Joint Aquatic Resource Permit Application
LOV – Ecology Letter of Verification
NHPA – National Historic Preservation Act
NWIFC – Northwest Indian Fisheries Commission
PCN – Preconstruction Notification
SOAL – State Owned Aquatic Lands
SSDP – Shoreline Substantial Development Permit
U&A – usual and accustomed area
WDFW – Washington Department of Fish and Wildlife