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EFFECTS OF GEODUCK (PANOPEA GENEROSA GOLDS, 1850) AQUACULTURE GEAR ON RESIDENT AND TRANSIENT MACROFAUNA COMMUNITIES OF PUGET SOUND, WASHINGTON

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ABSTRACT In Washington state, commercial culture of geoducks (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. Structures associated with this nascent aquaculture method are examined to determine whether they affect patterns of use by resident and transient macrofauna. Results are summarized from regular surveys of aquaculture operations and reference beaches in 2009 to 2011 at three sites during three phases of culture: (1) pregear (+geoducks, –structures), (2) gear present (+geoducks, +structures), and (3) postgear (+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 and homogeneity of multivariate dispersion tests. Scuba and shoreline transect surveys were used to examine habitat use by transient fish and macroinvertebrates. Analysis of similarity and complementary nonmetric multidimensional scaling were used to compare differences between species functional groups and habitat type during 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 greater at culture plots than reference areas when aquaculture structures were in place. Community composition differed (analysis of similarity) between culture and reference plots during the gear-present phase, but did not persist to the next farming stage (postgear). Habitat complexity associated with shellfish aquaculture may attract some structure-associated transient species observed infrequently on reference beaches, and may displace other species that typically occur in areas lacking epibenthic structure. This study provides a first look at the effects of multiple phases of geoduck farming on macrofauna, and has important implications for the management of a rapidly expanding sector of the aquaculture industry.

KEY WORDS: aquaculture effects, benthic community, geoduck, habitat provision, macrofauna, press disturbance, structural complexity, geoduck, Panopea generosa

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 by 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 in the disturbed area (e.g., Wolfsen 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, altering species diversity dramatically. 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 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 altered 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 and Gust (2003) observed significantly greater densities of predatory sea stars (*Coscinasterias muricata*) associated with longline mussel farms in New Zealand compared with 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 geoducks (*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., pers. comm. September 13, 2007) and has rapidly developed into an important industry in Washington state and British Columbia, with estimated annual production valued at US$21.4 million (FAO 2012). Culture practices involve large-scale out-planting of hatchery-reared juvenile clams to intertidal habitats, and installation of PVC tubes and netting to exclude predators and increase early survival. Juvenile clams (shell length, 10–20 mm) are placed in tubes (diameter, 10–15 cm) 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 after clams reach a size refuge from predators, generally 1–2 y after planting. Clams are harvested after an additional 3–5-y grow-out period (see VanBlaricom et al. [2015] for details).

Although 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 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 current study were to assess differences in the abundance and diversity of resident and transient macrofauna at sites with (culture) and without (reference) geoduck aquaculture during 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 postlarval 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 to each other (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 after structures were added. If any differences in resident or transient macrofauna communities were detected when habitat complexity was increased (i.e., when aquaculture gear was present), it was hypothesized that these changes would not persist after gear was removed and the disturbance associated with structure addition was ameliorated.

**MATERIALS AND METHODS**

**Study Sites**

Work described here was done in South Puget Sound, Washington, a subbasin 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 subbasin is shallow (mean depth, 37 m) and characterized by extensive littoral mud and sandflats (674 km²) that constitute more than 15% of the total area (Burns 1985). 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 the south shore of the northeastern portion of Totten Inlet (Fig. 2). None of these sites had been used previously for geoduck aquaculture, which afforded the opportunity to examine the resident and transient macrofauna community prior to the initiation of aquaculture operations (pregear) and the early phases of culture, including the addition of aquaculture structure (gear present) and subsequent removal approximately 2 y later (postgear).

**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 to 2011 at culture plots and adjacent reference areas. Ten randomly distributed core samples (diameter, 5 cm; depth, 10 cm; surface area, 19.6 cm²; volume, 196 cm³) were collected in culture plots and adjacent reference areas. In addition, 10 larger excavation samples (diameter, 29 cm; depth, 20 cm; surface area, 660.5 cm²; volume, 13.2 L) were taken on each sampling date occurring prior to deployment of protective PVC tubes and nets (pregear), and after removal of the structures (postgear). 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 sampled adequately (see VanBlaricom et al. [2015]). Moreover, small cores are used frequently 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, and the sample was mixed so that

**TABLE 1.**

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

<table>
<thead>
<tr>
<th>Site/status</th>
<th>Description</th>
<th>Biota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stratford site: gear placed June 2009; gear removed April 2011 5,100-m² farm, 2,500-m² plots</td>
<td>The site is on the east shore of Case Inlet (47°19′10.86″ N, 122°47′38.56″ W). It has a sandy substrate (grain size, ~500 μm), with a moderate slope from +0.61 m to −0.61 m MLLW. The reference area is 150 m to the south on private property.</td>
<td>Horse clams and cockles are present; sand dollars, patchy.</td>
</tr>
<tr>
<td>Rogers site: gear placed November 2008; gear removed April 2011 5,100-m² farm, 2,500-m² plots</td>
<td>The site is on the east shore of Case Inlet (47°14′53.13″ N, 122°49′37.38″ W). The substrate is sandy to muddy sand (grain size, ~250–500 μm). The beach is steeper and narrower than other sites. Green algae are abundant, and freshwater seepage occurs. The reference area is 150 m to the south on private property.</td>
<td>Horse clams and cockles are present; graceful crab is abundant; sand dollars, patchy.</td>
</tr>
<tr>
<td>Fisher site: gear placed June 2009 to July 2009; 90% of gear removed April 2011 2,500-m² farm, 2,500-m² plots</td>
<td>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). The substrate is muddy sand (grain size, ~250 μm). The reference area is 75 m to the east on private property.</td>
<td>Horse clams are present; crabs, sea stars, and moon snails are abundant.</td>
</tr>
</tbody>
</table>
individual samples and the calculated centroid of the sample. Calculate the average distance in multivariate space between dispersion uses a Bray–Curtis distance matrix of species data to further characterization of contrasts of core data between dispersion (HMD [Anderson 2006]) were also conducted for set at \( \alpha = 0.05 \).

In addition to the community analyses, generalized linear mixed models (GLMMs [McCullagh & Nelder 1989]) were used, 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 used to compare models formally, including the interaction term as part of a “frequentist” hypothesis testing approach. Regression coefficients and their 95% confidence intervals were calculated for each model.

Survey of Transient Fish and Macroinvertebrates

To investigate transient fish and macroinvertebrate assemblages at the three study sites, scuba surveys were conducted during daytime high tides (3–4.25 m above MLLW) from 2009 to 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 2,500-m² habitat spaces: a culture plot with active geoduck farming and a nearby reference area (the same reference area used 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.

All observed fish and macroinvertebrates larger than 60 mm were identified and enumerated to species or genus, and observations of size (estimated total length for fish, and diameter, carapace width or length for sea stars, crabs, and other benthic invertebrates), water column position, behavior, and associated substrate type (sand, gravel, tubes –netting, tubes –netting) were recorded. Observed species were assembled into 10 functional groups: sea stars, moon snails, hermit crabs, crabs (Brachyura), other benthic invertebrates, flatfishes, sculpins, other demersal fishes, other nearshore fishes, and sea perch (Table 2). Numbers
of organisms were converted to raw density values to offset the different transect lengths. Species that occurred in less than 5% 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 to September period. Three phases of the aquaculture cycle were represented in the data set: pregear (in 2009, prior to any aquaculture operations –geoducks, –structure), gear present (in 2010, during active geoduck aquaculture operation, aquaculture structure and postgear (in 2011, protective tubes and nets were removed). Although the 2010 to 2011 data represent periods in which geoducks remained during grow-out (in 2010, during active geoduck aquaculture operation, aquaculture structure and postgear (in 2011, protective tubes and nets were removed), farming occurred at culture plots only; however, the 2009 data indicated that aquaculture was active, farming occurred at culture plots only; and postgear (in 2011, protective tubes and nets were removed but geoducks remained during grow-out [+geoducks, –gear]).

Analyses of similarity (ANOSIMs [Clarke 1993]) were conducted 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). An $R$ value of zero indicates no differences (McCune et al. 2002).

Visual representations of species abundance in different habitat types and during aquaculture phases were explored using nonmetric 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 nonnormal data sets of species abundance (McCune et al. 2002). Nonmetric multidimensional scaling was conducted on a Bray–Curtis dissimilarity matrix of the untransformed, raw density data, and 1,000 iterations were performed to ensure convergence with minimal stress. Stress significance was tested
using a Monte Carlo randomization approach. Linear correlation of the functional groups and NMDS axis scores were used to calculate variable weights. Significant functional groups were determined with permutation tests and were 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 and Currie [2001]). The Shannon index was used to compare differences in diversity between plots for each aquaculture phase. This measure is commonly used in ecological studies; it combines aspects of species richness and relative abundance to produce a value typically from 0–3.5 (Shannon 1948, Shannon & Weaver 1949). A higher index value indicates greater 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 earlier, observations were made of salmon smolts in the vicinity of aquaculture operations. Pilot observations by divers and snorkelers indicated that smolts at the study sites were not sampled effectively by those methods, possibly because observers altered fish behavior. Moreover, salmon smolts—in particular, 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 to July) to coincide with out-migration of chum, Pink (Oncorhynchus gorbuscha), and coho (Oncorhynchus 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 in 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 (total length) 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 with a Beaufort scale score of 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 the greatest densities typically occurred during July to September (Fig. 3). Total taxa density in core samples showed substantial site-specific variation, with no consistent pattern of greater 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/m² and 278.6 ± 115.7/m², 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.

In total, 68 taxa from 63 sampling events were collected and identified. Results of the PerMANOVAs 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 nonsignificant 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).

Figure 3. (A–C) Density 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.
Permutational analysis of variance results for multivariate abundance data for all resident macroinfaunal taxa in core samples.

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<th>Site</th>
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<th>MS</th>
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<th>P value</th>
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<td>0.092</td>
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<td>5.729</td>
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</table>

Models included month of sampling (Month), plot type (culture plot or reference area; Plot), phase of culture (pregear, gear present, postgear; Phase), and the interaction of plot type and phase. * Significant results. Significance was set at α = 0.05.

Within each site, HMD values for the community data from the pregear 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 greater at the Rogers and Fisher sites (Table 4). During the postgear phase, values for culture plots and reference areas were less (relative to the previous phase) and not significantly different at Rogers and Fisher (P = 0.335 and P = 0.436, respectively). At Stratford, the postgear 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 affected negatively by geoducks and aquaculture gear, as indicated by a significant plot type × phase interaction (GLMM chi square, 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 square = 22.89, df = 2, P < 0.001) and Orbiniidae (chi square = 109.17, df = 2, P < 0.001). Abundance of the amphipod *Americorhiphus salmonis* (chi square = 174.23, df = 2, P < 0.001) and polychaete Family Hesionidae (chi square = 341.18, df = 2, P < 0.001) were reduced by the presence of aquaculture gear. Multivariate dispersion, a measure of β 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 as defined by Bray–Curtis compositional dissimilarity. * Significant results. Significance was set at α = 0.05.

**Surveys of Transient Fish and Macroinvertebrates**

The presence of aquaculture gear affects the 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 pregear or postgear (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). Tests of ANOSIM between aquaculture phases (Table 5) resulted in a statistically significant difference when comparing the pregear versus gear-present phases and gear-present versus postgear phases for culture plots (R = 0.156, P = 0.040; R = 0.164, P = 0.003, respectively). There was also a significant difference between gear-present and postgear 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 used to aid in visualization of differences between habitats within sites and across phases of aquaculture operations. The NMDS plots also recovered after gear was removed, and the cumacean *Cumella vulgaris* (chi square = 199.16, df = 2, P < 0.001) and polychaete Families Glyceridae (chi square = 94.75, df = 2, P < 0.001) and Opheliidae (chi square = 105.31, df = 2, P < 0.001) increased when gear was present, and recovered to pregear levels after gear was removed. The bivalve genus *Rocheфорia* (chi square = 6.99, df = 2, P = 0.030), nemertean genus *Mircura* (chi square = 0.52, df = 2, P = 0.772), and polychaete Family Capitellidae (chi square = 4.83, df = 2, P = 0.089) showed no response to geoduck aquaculture activities.
confirmed the assumption that the three sites were functionally similar for purposes of analyzing transient macrofauna communities during April to September. The NMDS ordination of the reference plot data shows some intermixing 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 associated consistently 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, whereas sculpin and flatfish correlated highly and were more often associated with reference areas. Two additional NMDS ordination plots represent comparisons of the pregear and gear-present phases (Fig. 8), and the gear-present and postgear phases (Fig. 9).

Survey data for the culture plots when PVC tubes and nets were present were more widely dispersed in ordination space compared with the pregear phase (Fig. 8). Differences in assemblages between pregear 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, although communities associated with culture plots were represented by a variety of functional groups when nets and tubes were in place...
Differences among culture plots and reference areas were examined with Welch tests comparing the transient fish and macroinvertebrate community assemblage in geoduck culture plots and reference areas across three phases of aquaculture operations: pregear, gear present, and postgear.

<table>
<thead>
<tr>
<th>Test groups</th>
<th>ANOSIM R</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregear reference area vs. culture plot</td>
<td>-0.0501</td>
<td>0.761</td>
</tr>
<tr>
<td>Gear-present reference area vs. culture plot</td>
<td>0.0808*</td>
<td>0.035*</td>
</tr>
<tr>
<td>Postgear reference area vs. culture plot</td>
<td>-0.0254</td>
<td>0.789</td>
</tr>
<tr>
<td>Pregear vs. gear-present reference area</td>
<td>0.1176</td>
<td>0.093</td>
</tr>
<tr>
<td>Pregear vs. gear-present culture plot</td>
<td>0.1557*</td>
<td>0.040*</td>
</tr>
<tr>
<td>Pregear vs. postgear reference area</td>
<td>-0.0268</td>
<td>0.600</td>
</tr>
<tr>
<td>Pregear vs. postgear culture plot</td>
<td>-0.0851</td>
<td>0.842</td>
</tr>
<tr>
<td>Gear present vs. postgear reference area</td>
<td>0.0900*</td>
<td>0.029*</td>
</tr>
<tr>
<td>Gear present vs. postgear culture plot</td>
<td>0.1604*</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

A Monte Carlo permutation test with 999 iterations generated the test statistics (R). * Significant results. Significance was set at α = 0.05.

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 during 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) (Table 6). Total numbers of organisms observed at culture and reference plots were similar prior to gear deployment (pregear, 2009) and after gear removal (postgear, 2011). However, there was an overall increase in total abundance while aquaculture gear was present, and macrofauna counts were more than two times greater at culture plots compared with the reference areas (Table 5).

Supplementary Observations of Salmon Smolts

Salmon smolts, chum (Oncorhynchus keta) and Pink (Oncorhynchus gorbuscha) salmon, were rarely observed during shore-based visual surveys (total, 8%). When present, schools of salmon traveled parallel to the shoreline in less than 2 m of water. No difference in the occurrence of salmon smolts adjacent to culture plots and reference areas was observed, although evidence is anecdotal, given the low encounter rate. No discernible 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 current study suggest that structures associated with geoduck aquaculture have little influence on community composition of resident benthic macroinvertebrates (i.e., nonsignificant 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), whereas 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).

### 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: pregear, gear present, and postgear.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Plot type</th>
<th>Shannon diversity index (H')</th>
<th>t-Test results for diversity values</th>
<th>Total organisms observed (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregear</td>
<td>Reference</td>
<td>1.111</td>
<td>t = 0.703, df = 11, P = 0.496</td>
<td>530</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>1.188</td>
<td></td>
<td>628</td>
</tr>
<tr>
<td>Gear present</td>
<td>Reference</td>
<td>0.923</td>
<td>t = 0.727, df = 18, P = 0.476</td>
<td>795</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>1.021</td>
<td></td>
<td>1,692</td>
</tr>
<tr>
<td>Postgear</td>
<td>Reference</td>
<td>1.163</td>
<td>t = 0.309, df = 25, P = 0.760</td>
<td>621</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>1.207</td>
<td></td>
<td>694</td>
</tr>
</tbody>
</table>

Differences among culture plots and reference areas were examined with Welch's t-test with α = 0.05. Total abundance of all observed organisms is included.
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 the 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. [2015]). 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 current study, several infaunal taxa recovered to pregear abundance, or increased in abundance, after 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 current study. Thus, elucidating potential mechanisms responsible for differences in the response of infauna requires further study. Additional data and analytical inference would also permit more direct comparison with 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. The presence of PVC tubes and nets altered abundance and composition significantly, but not diversity, of transient macrofauna. More than 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. Analysis of similarity results demonstrated a statistically significant difference between the transient macrofaunal communities in culture plots and reference areas when aquaculture gear was present (Table 5; \( R = 0.881, P = 0.035 \)). Yet, the low \( R \) value of the test suggests minimal ecological difference between the habitats. The 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 sea perch 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). The data from the
current study 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 smaller fish and crabs were observed retreating under netting when larger animals or divers approached. Similarly, Inglis and Gust (2003) observed increased predation by sea stars at New Zealand longline mussel farms, and Tallman and Forrester (2007) identified refuge value as a major factor leading to greater 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 current study, some taxa, particularly flatfish and the snail *Lunatia lewisi*, 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 after 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 versus postgear ($R = 0.160, P = 0.003$), and the ANOSIM $R$ value for this test was the highest of all tests conducted, suggesting moderate ecological significance, which 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 pregear condition (ANOSIM $R = -0.085, P = 0.842$). These data suggest transient macrofauna communities associated with these intertidal beaches begin to recover to preaquaculture conditions within a few months of removal of the PVC tubes and nets.

Transient macrofaunal communities in reference areas were also significantly different between the gear-present and postgear 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 the current study may also be somewhat affected by removal of aquaculture structures between the gear-present and postgear 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 current study. Average diversity of transient macrofauna at culture plots when gear was present was slightly greater than at reference areas (but not significant), and diversity measures for the pregear and
postgear 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 the current one, which focuses on distinguishing between the raw abundance of species groups in different areas. Nevertheless, the results clearly contrast with previous work linking aquaculture disturbance with changes in diversity (Erbland and Ozbay [2008]; see review by Dumbauld et al. [2009]). Brown and Thuesen (2011) observed greater 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—*Cancer (Metacarcinus) gracilis*—captured in the reference area. Overall, more organisms were captured in traps set in the reference area than in the 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 (*Ammodites hexapterus*), which spawn on littoral beaches at high tidal levels (November to 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 and Penttila (2000) suggest that spawning typically occurs at 2–2.75 m above MLLW, which is well above geoduck aquaculture operations and sampling in the current study (Table 1). No other adult forage fish, such as surf smelt (*Hypomesus pretiosus*) and herring (*Clupea pallasi*), or evidence of spawning activities were observed during the study. Although these results suggest negligible effects, the opportunistic sampling may be inadequate given spatiotemporal variability in spawning behavior, and additional targeted investigation is warranted to elucidate potential broader impacts on forage fish populations.

The current 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 y) on intertidal beaches. However, it should be noted that the current 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 greater abundance of the species at aquaculture sites. Also, the current study 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 efforts and spatially explicit modeling work are required to develop an understanding of these phenomena, which are critical if this method of aquaculture continues to expand in the region. Moreover, the sampling used in the current study 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. It is recommended that alternative sampling methods, such as beach seining, be used to evaluate use of geoduck aquaculture by out-migrating smolts.

Future research should focus on the issues just described, as well as on ecosystem effects on higher trophic levels. Nevertheless, the results of this study 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 important, these data will aid regulatory authorities and resource managers in placing aquaculture-related disturbance in an appropriate context for decision making to balance the needs of stakeholders and environmental protection.

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**LITERATURE CITED**


