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Geoducks in the genus *Panopea* are the largest hiatellid clams and are unique in many ways—with a fused siphon and mantle, incompletely enclosing valves, and proportionally small adductor muscles. Geoducks occupy deep burrows in soft sediments and can be very long-lived. These and many other attributes continue to fascinate scientists around the world.

As a high-value product, fishery and aquaculture activities are increasing to keep up with demand, and geoduck-related industries are generating substantial export revenues in a number of countries worldwide. Scientific studies are necessary for sustainable fisheries and viable aquaculture, and to conserve the ecological value of these fascinating clams. In recent years, the number of publications on geoduck biology, ecology, and fisheries has increased dramatically. This special issue adds considerably to the body of literature on geoducks. Several of the new papers address basic biological topics such as reproduction, phylogenetics, disease, longevity, and larval life history characteristics, whereas others address population dynamics and growth models, population genetics, and the ecological impacts of geoduck aquaculture.

Taken together, the articles in this issue represent the most current information on *Panopea* spp. available to the scientific community and resource managers, and illustrate the breadth of approaches that, in the best cases, will result in synergies that propel the science forward. We believe the geoduck research community will benefit from the work presented here, not just as a valuable source of information, but also as a conduit for future collaborations to shed more light on the enigmatic and fascinating geoduck.

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Brent Vadopalas and Jonathan P. Davis

MATURATION, SPAWNING, AND FECUNDITY OF THE FARMED PACIFIC GEODUCK *PANOPEA GENEROSA* IN PUGET SOUND, WASHINGTON

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ABSTRACT Among the challenges facing aquaculture of native species are potential negative effects of gene flow from cultured to wild populations. Estimates of gene flow are based in large part on the capacity for gamete exchange between individuals, and make estimates of reproductive output and timing of gametogenesis in adjacent cultured and wild populations important to assess. Farmed geoducks of known age from each of five year classes and from nearby wild populations were sampled for reproductive development and other morphometric parameters in March, April, and May 2007 from three Puget Sound, Washington, locations. Results indicate that, at all three locations, cultured geoducks began to mature during year 2 and were fully mature by year 3, with males maturing earlier and at a smaller size than females. It was estimated that 50% maturation occurs at 64 mm in shell length. The gender ratio in 2–5-y-old geoducks was male biased relative to the 1:1 sex ratio observed in wild populations ($P < 0.05$), providing evidence for facultative protandric dioecy. Rates of maturation in cultured populations were synchronous with nearby wild populations. Overall, mean relative fecundity of cultured 3-, 4-, and 5-y-old clams was approximately 25% that of mean wild relative fecundity. These results suggest that reproductive interactions between cultured and wild geoducks can potentially occur through two mechanisms. First, when farmed geoducks are in proximity to wild geoduck aggregations, spawning may be synchronized, with subsequent gametic interaction occurring. Second, planktonic larvae produced from cultured populations may subsequently settle and mature to propagate with wild conspecifics. Interactions between cultured and wild conspecifics are important to assess especially in cases when domestication selection is proceeding via hatchery-based breeding and other approaches.

KEY WORDS: *Panopea generosa*, Pacific geoduck, gametogenesis, maturation, aquaculture

INTRODUCTION

Aquaculture production worldwide has increased at a rate of 8.3% since 1970 (Diana 2009)—a rate three times greater than land-based agriculture. Cultured fish and shellfish comprise the majority of seafood-based production worldwide as well (Diana 2009, Diana et al. 2013). Where intensive shellfish production has increased in the nearshore, public concern over impacts to native species and aquatic habitats has intensified (Naylor et al. 2001, Sarà 2007)). In recent years, public outcry has included concerns over potential genetic interactions between wild and cultured animals in cases where natural beds of shellfish lie in close proximity to intensively cultured locations. Genetic interactions between wild and farmed plants and animals can result in changes in the composition of wild populations and genetic structure, and changes and/or losses in overall genetic variation. Natural resource management efforts in this case shift from problems associated with competition for space with native species and invasion dynamics from nonnative species, and move toward possible disease or genetic perturbations of native wild stocks.

Reducing the alteration of naturally occurring levels of genetic diversity is central to maintaining healthy wild stocks. This issue is exacerbated when native species are considered for further domestication through genetic improvement programs that, for example, are well underway with native oysters on the U.S. East Coast and nonnative Pacific oysters on the U.S. West Coast. Genetic change to wild populations of native species under culture can occur through a number of mechanisms. Cohorts of cultured animals may exhibit low effective population

sizes, and some level of domestication selection may be associated with production in a hatchery environment (Williams & Hoffman 2009, Straus 2010, Straus et al. 2015). Interbreeding of cultured and wild organisms may have significant adverse effects on wild populations, such as decreased fitness or outbreeding depression (reviewed in Camara and Vadopalas [2009]).

Fundamental to the assessment of potential genetic impacts of cultured organisms on wild conspecifics is an understanding of reproductive capacity, including gametogenesis and spawning behavior in adults, behaviors and settlement dynamics in larvae and postlarvae, and behavior of juveniles as they relate to potential interaction with wild counterparts.

The Pacific geoduck *Panopea generosa* Gould, 1850, is native to the eastern Pacific, from southeast Alaska to northern Baja California, Mexico (Coan et al. 2000, Vadopalas et al. 2010). Commercial geoduck aquaculture commenced in the mid 1990s in Washington state as ex-vessel prices for geoducks rose dramatically to meet market demand, mainly from Asian countries. Geoducks are hatchery produced and outplanted as seed for cultivation in the low intertidal zone until harvest 6–7 y later. Density of cultured clams may be high, with up to 150,000 clams (100 t) produced per hectare in some locations that are proximate to subtidal aggregations of wild geoducks (www.pcsqa.org). In Washington state, approximately 140 ha are used exclusively for intertidal geoduck culture, with plans for significant expansion in the years ahead. Subtidal planting of cultured geoducks occurs at lower densities in British Columbia, Canada, but the habitats used for planting are generally much larger in area. If farmed clams mature during the culture cycle, interbreeding between cultured and wild geoducks may occur either directly via gamete cross-fertilization or indirectly if progeny of cultured origin settle proximate to wild populations, mature, and spawn.

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Although reproduction in marine bivalves has been described and reviewed extensively for a large number of species (e.g., *Mercenaria mercenaria* [Bricelj & Malouf 1980], *Mya arenaria* [Chung 2007], *Meretrix usoria* [Kang et al. 2007], *Coacella chinensis* [Mladineo et al. 2007], *Modiolus barbatus* [Roseberry et al. 1991], *Crassostrea gigas* [Royer et al. 2008], *Ruditapes philippinarum* [Uddin et al. 2012], among many other species), little information exists on Pacific geoduck reproductive biology in general, including accurate assessments of age at maturation. All existing information on reproductive parameters is based on ages estimated from either shell length (SL) or annuli from wild specimens, making accurate assessments difficult for this long-lived clam. Andersen (1971) found that 50% maturity occurred at a 75-mm maximum SL at an estimated age of 3 y, whereas Sloan and Robinson (1984) reported that males were fully mature at age 6 y and females at age 12 y. More recently, Campbell and Ming (2003) reported 50% maturity at 58 mm and 61 mm at two different sites, with ages estimated to be 3 y and 2 y, respectively. For the purpose of assessing genetic risks to wild from cultured geoduck populations, these available age-at-maturation estimates are problematic for two reasons. First, they are based on estimated ages; even with an optimal precision of ± 1 y, the age-at-maturation

ranges overlap ages pertinent to the question of maturation in cultured geoducks. Second, tidal elevation, location, and body size may affect age at maturation (Eversole 1989, Walker & Heffernan 1994), but these variables have not been addressed in previous work.

The goal of this study was to determine age at maturation and spawning season in farmed geoducks of known ages cultured on intertidal farms. The objectives included assessing whether geoduck maturation and spawn timing differed (1) by age, (2) among Puget Sound locales, (3) between adjacent intertidal (cultured) and subtidal (wild) aggregations, and (4) among sizes within five discreet year classes.

MATERIALS AND METHODS

Study Sites

This study was conducted in the greater Puget Sound estuary in Washington state, the geographic center of both wild populations and a growing geoduck aquaculture industry. The study sites, Hartstine Island and Totten Inlet in the south sound subbasin and Thorndyke Bay in Hood Canal (Fig. 1), were selected based on the availability of five contiguous year classes, close proximity to wild aggregations, geographically

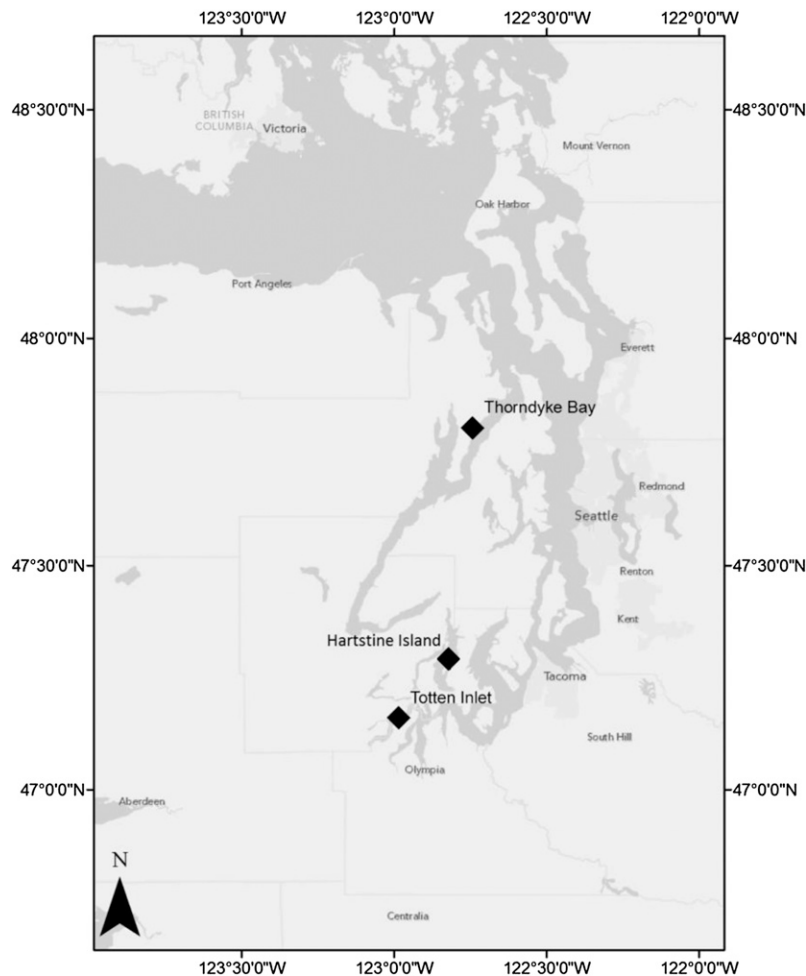


Figure 1. Map showing collection locations for farmed and wild Pacific geoduck *Panopea generosa* in Puget Sound, Washington.

varied location, and the cooperation of industry. At all three sites, surface water temperatures typically vary from approximately 7°C during winter to 16°C in summer; salinities are generally 27–30, with seasonal (e.g., spring) periods of salinities as low as ~23 (Moore et al. 2012). During the peak in spawning behavior from March to June, temperature and salinity conditions are similar for farmed intertidal populations, whereas subtidal wild populations experience cooler temperatures as a result of surface water stratification.

Sampling Design

Cultured geoduck were planted at a density of approximately 20/m² at an SL of 5–12 mm. Seed geoduck were protected from predation for the first 1–2 y using PVC pipe enclosures (45-cm-long section by 15–20-cm diameter) inserted vertically into the substrate with the top 10 cm exposed. Clam seed (2–3 per tube) were dropped onto the sediment surface, covered with mesh netting, and secured with a rubber band. After the first 12 mo, the mesh top and band were removed. After 2+ y in culture the PVC pipe sections were removed and the clams remained free-living until harvest. So, for each year class under consideration, cultured geoducks were either protected by PVC tubes (year class 1–2) or unprotected (year class 3–5).

During the same week and concurrent with the same series of daytime low tides in March, April, and May 2007, 10 individuals were collected from each of five age groups (12, 24, 36, 48, and 60 mo old, 2002–2006 y classes) at Hartstine Island, Totten Inlet, and Thorndyke Bay. Concurrently, and adjacent to farms culturing geoduck, 15 wild geoducks were also collected from subtidal beds via scuba (Table 1). Individual geoducks were collected from both intertidal and subtidal samples using standard harvest protocols. Briefly, clams were removed from the substrate using a “stinger,” a harvest device that uses pumped seawater and a 1-m section of 1.9-cm-diameter PVC pipe to fluidize the sand substrate around individual clams.

Morphometric and Histological Analyses

All geoducks were initially stored on ice after removal from substrates, returned to the laboratory (University of Washington), and processed within 24 h. Shell length, width, and live weight were recorded before removing the gonadovisceral mass; gonadovisceral weight and diameter were also recorded. A single, 3-mm-thick section from the gonadoviscera immediately posterior to the foot was subsequently removed. Tissue sections were fixed immediately in Davidson’s solution for 24 h (Shaw & Battle 1967), transferred to 70% EtOH, processed for routine paraffin histology, and stained with hematoxylin–eosin (Luna 1968). Light microscopy (Nikon E600; Nikon Inc., Melville, NY) was used to visualize each section before digitizing selected section images for each clam using a high-resolution digital camera (Nikon Coolpix 900).

Aside from gender, two forms of data were taken from histological sections. First, to gauge maturation qualitatively, following Goodwin (1976) and Ropes (1968), gonad sections were scored as inactive (0), early active (1), late active (2), ripe (3), partially spawned (4), or spent/resorbed (5). To obtain proportion mature, scores 0, 1, and 2 were combined as “immature,” and scores 3 and 4 as “mature.” For statistical G tests (described later), sections were scored as immature, ripe, and partially spawned.

TABLE 1.

Analysis of deviance table for the generalized linear model fitted to frequencies in the farmed Pacific geoduck *Panopea generosa* by age, month, and maturation stage.

| Parameter | df | Deviance | Residual df | Residual deviance | Pr (>χ) |
|----------------|-----|----------|-------------|-------------------|---------|
| Null | 161 | 617.85 | | | |
| Site | 2 | 0.20 | 159 | 617.65 | 0.9057 |
| Age | 5 | 0.19 | 154 | 617.46 | 0.9992 |
| Month | 2 | 0.28 | 152 | 617.18 | 0.8700 |
| Stage | 2 | 95.69 | 150 | 521.49 | <0.0001 |
| Site:age | 10 | 0.43 | 140 | 521.05 | 1.0000 |
| Site:month | 4 | 0.44 | 136 | 520.61 | 0.9788 |
| Age:month | 10 | 0.37 | 126 | 520.24 | 1.0000 |
| Age:stage | 10 | 414.06 | 116 | 106.18 | <0.0001 |
| Site:age:month | 20 | 1.27 | 96 | 104.91 | 1.0000 |

Model, Poisson; link, log. Terms added sequentially (first to last).

Second, to obtain quantitative maturation data, Image J (version 1.34s [Schneider et al. 2012]) was used to estimate the gonad occupation index (GOI) by calculating the proportion of each histological section occupied by gonad follicles, according to Delgado and Pérez Camacho (2003) and Quintana et al. (2011). The product of GOI and gonadovisceral weight was then used to obtain gonad weight (GW) as a proxy for relative fecundity, using the following equation: $GW = GOI \times GV$.

Statistical Analyses

Analysis of variance (ANOVA) was used to determine whether site, month, gender, age, and origin (cultured or wild) affect GOI and GW. Proportion data were arcsin-transformed before analysis; post hoc analyses were performed using Tukey’s honestly significant difference tests. Chi square tests with Yates’ continuity correction were used to test for differences in gender ratios. The proportion mature was regressed on length and age using a binomial general linear model with logit link, and regressed log GW on log SL to illustrate allometric relationships.

Log linear analysis using a general linear model with family = Poisson and link = log (G test) was used to examine the relationships among age, site, month, and maturation stage (immature, ripe, and partially spawned). The Akaike information criterion was used to simplify the model stepwise down from the saturated model. Dispersion was calculated by dividing the residual deviance by the residual degrees of freedom. General linear models with family = binomial and link = logit were used to determine age and length at maturation. All tests were performed using S-plus (Insightful) or R (R Development Core Team 2012).

RESULTS

Gender Ratios

In the wild group, the ratio of males to females did not differ significantly from 1:1 (chi square = 0.0027, $df = 1$, $P = 0.9585$). In contrast, across sites and ages, the majority of cultured geoducks within all year classes were males. The majority (73%) of age 1 (2006 year class) clams were indiscriminant in gender;

27% were immature males ($n = 24$). Within the age 2-, 3-, 4-, and 5-y-olds, 78%, 67%, 64%, and 65%, respectively, were male. Sex ratio did not differ significantly among these age classes (chi square = 6.2716, $df = 3$, $P = 0.09912$). The overall gender ratio of 2.3:1 in age 2–5-y geoducks deviated significantly from 1:1 (chi square = 28.8575, $df = 1$, $P = 0$). Two simultaneous hermaphrodites were observed compared with 480 of determinate gender (0.4%).

Maturation Stage

No interactions among site or month with maturation stage were observed in this study; as a result, interactions involving these factors were not retained in the model. The interaction of age and stage was highly significant (G test, deviance = 414.06, $df = 10$, $P < 0.001$), and was retained in the model along with all nuisance variables (Table 1). The dispersion parameter was close to unity (1.093). Age was the only factor that affected maturation stage significantly (Fig. 2). There were no significant effects of age on maturation stage with age 1 and age 2 removed from the model. Among ages 3, 4, and 5, and mixed-age wild samples overall, 2.3% were immature, 24.4% mature, and 73.3% partially spawned. These proportions were significantly different from age 1 (98.9% immature, 1.1% mature, 0% partially spawned; chi square = 382.0396, $df = 2$, $P = 0$), age 2 in March (46.7% immature, 6.6% mature, 46.7% partially spawned; chi square = 98.7539, $df = 2$, $P = 0$), and age 2 in April/

May (43.1% immature, 36.2% mature, 20.7% partially spawned; chi square = 124.0597, $df = 2$, $P = 0$). For the 2-y-olds, the proportion of mature geoducks differed significantly between March and April/May (chi square = 11.17425, $df = 2$, $P = 0.003746$). The logistic models for age and length at 50% maturation predicted 23.9 mo and 63.5 mm, respectively; predictions for male clams (22.5 mo and 58.1 mm, respectively) were significantly different from females (28.8 mo and 79.8 mm, respectively; Table 2).

Gonad Weight

Age 1 farmed geoducks exhibited only very low levels of maturation (GOL, <5%); thus, they were excluded from further analyses. Among ages 2, 3, 4, and 5 y, and mixed-age wild clams, a significant effect of both gender (ANOVA, $F = 18.493$, $df = 1$, $P < 0.0001$) and age (ANOVA, $F = 123.583$, $df = 4$, $P < 0.0001$) were noted for GW. Pairwise GW differences among groups are shown in Figure 3. The allometric relationship between SL and GW is shown in Figure 4.

DISCUSSION

Reproductive activity in geoducks starting at the known age of 2 y has been demonstrated for Puget Sound, Washington. Farmed geoducks show signs of gonadogenesis at year 1, widespread maturity during year 2, and are fully mature by

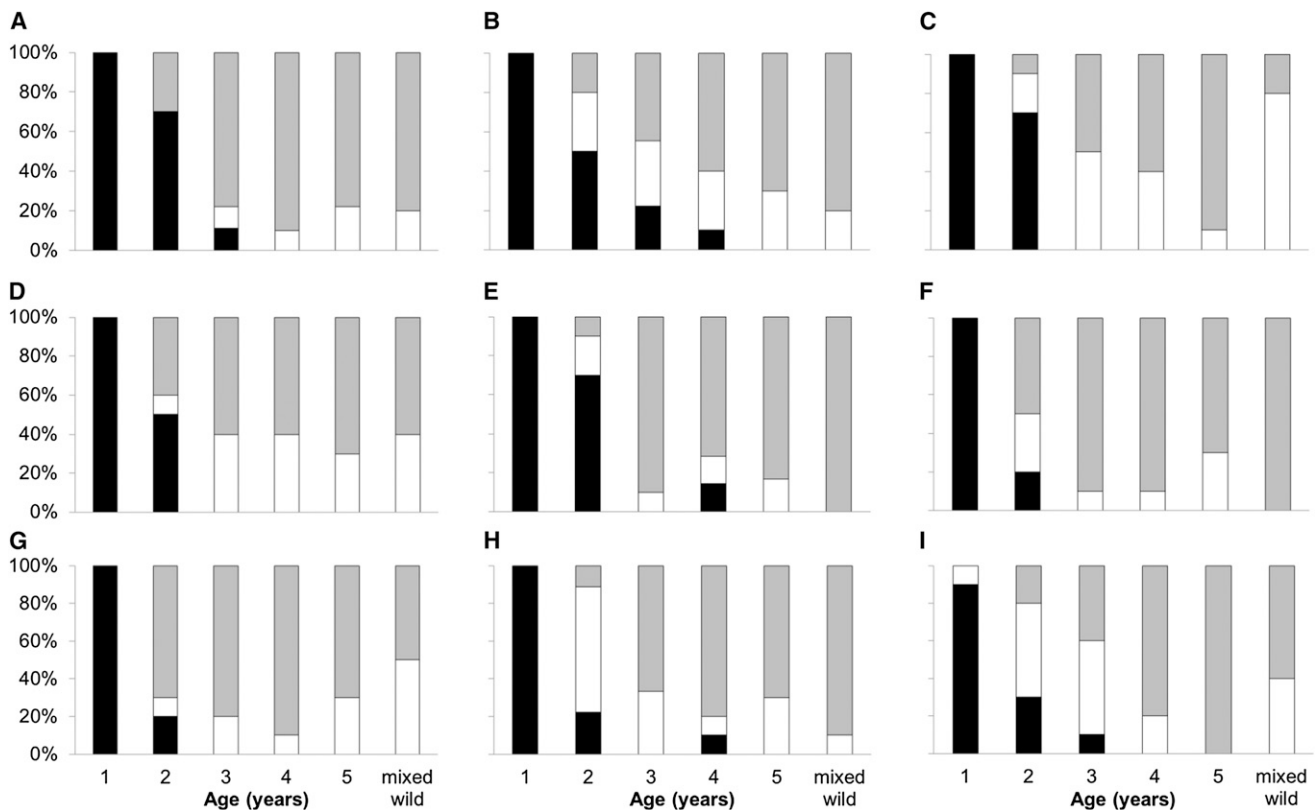


Figure 2. (A–I) Maturation proportions for the farmed and wild geoduck *Panopea generosa*. Immature, black bars; mature, white bars; gamete release, gray bars. Differences among proportions are nonsignificant among sites (by row: Thorndyke Bay, A, B, C; Hartstine Island, D, E, F; and Totten Inlet, G, H, I), months (by column: March, A, D, G; April, B, E, H; and May, C, F, I), and groups older than 2 y, including wild geoducks. Maturation proportions for 1- and 2-y-old geoducks are significantly less than other age groups (chi square = 382.0396, $df = 2$, $P = 0$; and chi square = 124.0597, $df = 2$, $P = 0$, respectively).

TABLE 2.
Age and length at 50% maturity for the farmed Pacific geoduck *Panopea generosa*.

| | Age (mo) | SE | Length (mm) | SE | n |
|--------|----------|--------|-------------|--------|-----|
| All | 23.9 | 0.7546 | 63.5 | 1.7639 | 441 |
| Male | 22.5 | 1.0043 | 58.1 | 2.2822 | 264 |
| Female | 28.8 | 2.0170 | 79.8 | 2.4864 | 109 |

Individuals of indeterminate gender (stage 0, $n = 68$) are included in "All."

year 3 (Fig. 2). In addition, male clams appear to mature earlier and at a smaller size than females. Reproduction in farmed and adjacent wild geoducks appeared synchronous temporally, and differences in maturation stage among locations and within age groups were minimal (Fig. 2). Taken together, the data suggest that wild-cultured genetic interactions have the potential to occur when geoduck farming operations are near wild populations.

These results differ from some earlier reports of geoduck age and length at maturation. In a study conducted in Hood Canal, Washington, Andersen (1971) found that wild clams were 50% mature at an SL of 75 mm, estimated to be 3 y old, which is different from the estimate of the current study (63.5 mm and 23.9 mo). In sharp contrast to the current study, Sloan and Robinson (1984) reported for wild clams in Puget Sound that males attained maturity at age 6 and females at age 12. In contrast, the results of the current study are in strong accord with Campbell and Ming (2003), who reported 50% maturity in geoducks at SLs of 58 mm and 61 mm at two different sites in British Columbia.

The skewed gender ratio among young clams is an obvious feature of the current study. Similar male-dominated sex ratios have been reported by others, including those by Andersen (1971) at 17:1, Sloan and Robinson (1984) at 9:1, and Campbell and Ming (2003) at 12:1. However, the forgoing studies did not consider age as a factor; younger clams in these studies were simply pooled by size. For Puget Sound, location does not appear to affect gender ratios, which strongly suggests that the species is characterized by protandry, as concluded by Andersen (1971), and for *Panopea zelandica* by Gribben and Creese

(2003). The low level of simultaneous hermaphroditism (0.4%) observed in the current study is similarly in accord with observations made for *Panopea generosa* in other locations (Campbell & Ming 2003).

Aside from distinct differences in size and age at 50% maturation for males and females (Table 2), maturation characteristics at the three sites in the current study were similar. The most striking differences were observed between genders; not only did females mature later than males (28.8 mo and 22.5 mo, respectively) and at a larger SL (79.8 mm and 58.1 mm, respectively), GWs were significantly greater for females than for males (Fig. 3). The majority of age 2 females remained immature, whereas immature age 2 males were in the minority (75% and 33%, respectively). Among ages 3–5 farmed and mixed-age wild geoducks, there were no significant differences in maturation stage among months or sites, indicating reproductive synchrony. Although reproductive effort is similar relative to size (Fig. 4), the significant differences in GW between farmed and wild demonstrate reduced reproductive output for the smaller farmed geoducks (Fig. 3).

These results demonstrate there is potential for proximate cultured and wild geoducks to interact genetically through two possible mechanisms. First, farmed and wild geoducks appear to be in reproductive synchrony, and released gametes can remain viable for hours (Vadopalas 1999, Vadopalas & Friedman unpubl. data), establishing the potential for cross-fertilization between wild and cultured populations. Second, reproductively active farmed populations may result in farm-derived larvae settling into wild aggregations, setting up the potential for future genetic interactions.

Increases in the number of geoduck farms or the density of culture may have consequences for the resource management of wild geoduck. For example, smaller wild populations reduced through ongoing fishing pressure (Bradbury & Tagart 2000) may be more vulnerable to genetic perturbation via interbreeding with genetically different (e.g., via reduced diversity, domestication selection) cultured stocks. The genetic diversity of seed from two Washington state geoduck hatcheries has been characterized as significantly lower in farmed than in wild populations (Straus 2010). In a separate study, an aggregate of farmed geoducks likewise exhibited less genetic diversity than a wild population (Straus et al. 2015).

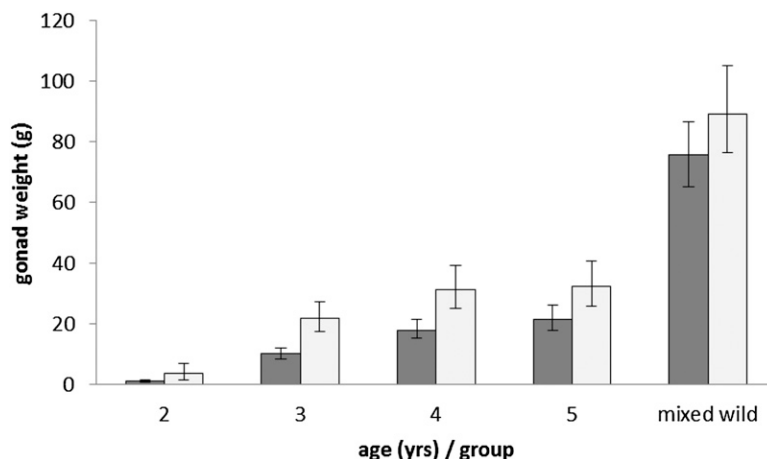


Figure 3. Male (dark bars) and female (light bars) mean ($\pm 95\%$ bias-corrected and accelerated confidence intervals) gonad weights for 2-, 3-, 4-, and 5-year-old farmed and a random sample of wild Pacific geoducks (*Panopea generosa*).

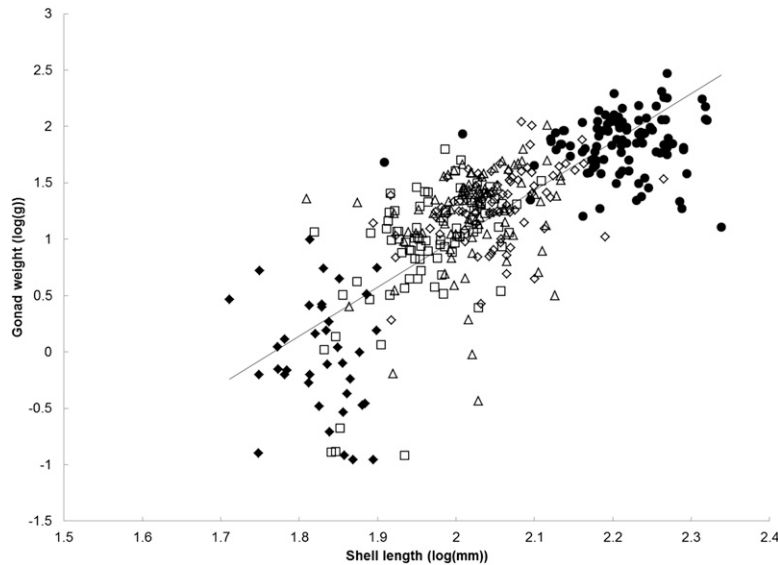


Figure 4. Relationship of gonad weight (log) to shell length (log) for 2- (solid diamonds), 3- (boxes), 4- (triangles), and 5- (open diamonds) year-old farmed and a random sample of wild (solid circles) Pacific geoducks (*Panopea generosa*). Regression equation: $y = 4.2973x - 7.5906$; $R^2 = 0.6313$.

It is evident that farmed–wild interactions may occur, but the genetic risk to wild populations is not clear. On one hand, lifetime reproductive success is probably markedly lower in farmed than in wild geoducks. Based on GW, farmed geoduck fecundity is significantly less than that of wild geoducks (Figs. 3 and 4), reducing the potential for successful breeding in the wild. More important, through removal from the breeding population via harvest, the reproductive life span of farmed geoducks is truncated to only 3–4 y—an order of magnitude less than the approximate 30-y average reproductive life span of wild geoducks (Sloan & Robinson 1984). On the other hand, the high density of farmed geoduck populations (up to 150,000 clams/ha) may greatly increase overall reproductive success compared with wild populations.

It may be prudent to consider geoduck aquaculture as a form of wild supplementation (Camara & Vadopalas 2009), with commercial hatchery practices focused on the production of genetically diverse seed. For example, to maximize genetic diversity and minimize genetic differences from wild, hatchery practices can (1) use wild broodstock exclusively, (2) maximize the effective number of breeders used in the production of hatchery seed, (3) avoid recycling of broodstock from year to year, and (4) source wild broodstock from the general areas where seed clams are subsequently planted (i.e., maintain local provenance of clams). In addition, protective measures that may assist in insulating wild clams from their farmed counterparts may include maturation control through triploidy (Vadopalas & Davis 2004). Maturation control would also enable the advancement of domestication

through intentional selection and other approaches in this commercially valuable species while reducing genetic risks to wild populations.

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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 past 20 y, 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 (*P. generosa*) parasites from three natural populations in Washington state (Totten Inlet, Thorndyke Bay, Freshwater Bay) was conducted in 2008 to 2010. Histopathology of 634 animals was used to explore trends of parasite presence and to identify potential environmental factors (site distribution, collection depth, and season) that influence parasite assemblages. Endosymbionts observed on histological examination included *Rickettsia*-like organisms (RLOs) in the ctenidia ($n = 246$); an unidentified metazoan parasite in the siphon epithelium ($n = 220$); and 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 geoducks. Assemblages of most parasites showed strong seasonal variations and site-specific distributions throughout the year. The presence of *Rickettsia*-like organisms 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, *Panopea generosa*

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 spillover (e.g., farmed to wild) and spillback 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 as a result of the increases in population density or shifts in environmental conditions within culture settings (May et al. 1981). Shellfish transport has been long thought to spread disease potentially within wild and cultured populations. Strict shellfish transportation regulations exist as important management tools to help control disease interactions and to prevent further transmission. Movements of shellfish stock or seed may pose a significant threat to native populations, especially if animals are not monitored properly 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 2008), and two diseases, *Haplosporidium nelsoni* (or multinucleated sphere unknown, or 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 United States (Alaska, Washington state, California), Canada (British Columbia), and Mexico (North Baja Pacific Coast). Geoducks are one of the most economically important commercial shellfish species harvested for export (Hoffmann et al. 2000, Bower & Blackbourn 2003). A commercial Washington state geoduck fishery initiated in 1970 became highly lucrative during 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 geoducks. 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

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within close proximity to cultured geoduck stocks. The goal of this study was (1) to explore trends of parasite presence within wild geoduck populations and (2) to 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 will assist in future research regarding the impact of these diseases on northwest populations of Pacific geoducks.

MATERIALS AND METHODS

Sample Collection and Histology

A target of 60 Pacific geoducks that ranged in size from 80–225 mm (mean \pm SD, 141 \pm 31.13 mm) were collected randomly by Washington Department of Fish and Wildlife divers at two depth strata from three natural populations in Washington state over multiple seasons during a 2-y 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. Collection depths were either shallow (10–30 ft MLLW) or deep (30–70 ft MLLW). Freshwater Bay geoducks were aggregated only in shallow depths at the time of sampling and therefore were not collected in deep water.

Animals were dissected within 24 h of harvesting. Length, width, and depth of shells were measured. 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

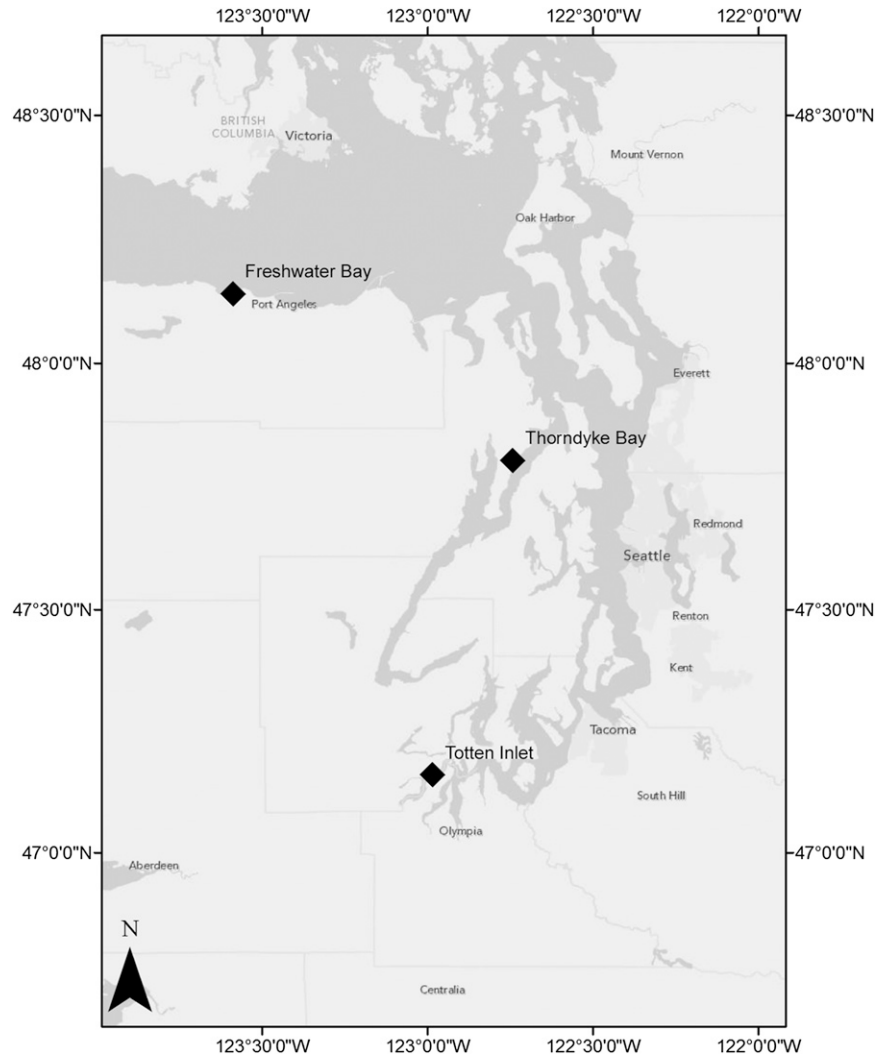


Figure 1. Geoduck sampling sites in Washington state.

and future molecular characterization. All tissue samples were preserved in Davidson's solution for 24 h and stored in 70% ethanol until processed for routine paraffin histology (Shaw & Battle 1957, Luna 1968). Deparaffinized tissue sections were stained with hematoxylin–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 (RLOs), microsporidia-like organisms (MLO), and metazoan parasites. For each category, tissue sections were assigned a semiquantitative 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); and 4, large numbers of parasites (>30). The parasite data set consisted of 634 geoducks and five 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 to February; spring, March to May; summer, June to August; fall, September to November), and site (Thorndyke Bay, Totten Inlet, Freshwater Bay).

Statistical Analysis

Generalized linear models (GLMs) were created with the binomial family distribution and the logit link function and were used 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 used for pairwise comparisons of parasite frequency according to the model of best fit. Kruskal–Wallis one-way analysis of variance (ANOVA) was 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. Generalized linear models, ANOVAs, and chi square and Tukey's honest significant difference tests were performed using R software v. 2.11.1 (R Development Core Team 2012). Post hoc analyses were performed with SigmaPlot software v. 11.0 (Systat Software, Inc.).

RESULTS

Parasite Morphology and Characterization

The most common geoduck parasites observed on histological examination included an RLO in the ctenidia (39%; Fig. 2A), an unidentified metazoan in the siphon external epithelium (35%; Fig. 2B), a *Steinhausia*-like organism (SLO) in the ovum (16%; Fig. 2C), and MLO in the intestine (16%; Fig. 2D) and siphon muscle (4%; Fig. 2E, F, Table 1). *Rickettsia*-like organisms were characterized by the presence of basophilic inclusions that stained violet with hematoxylin–eosin within the ctenidia epithelium (Fig. 2A) and were Gram negative. Inclusions were spherical and measured $13.22 \pm 0.85 \mu\text{m}$

(mean \pm SD) 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 $128.81 \pm 49.48 \mu\text{m}$ in length and $74.04 \pm 36.57 \mu\text{m}$ in width ($n = 15$; Fig. 2B). In addition, *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 MLO were observed in inflammatory lesions within the intestinal submucosa. The larger merogonic stage measured $4.89 \pm 1.16 \mu\text{m}$ ($n = 15$) and the smaller, spore-like stages measured $0.85 \pm 0.28 \mu\text{m}$ ($n = 15$) and were found in intracytoplasmic sporocysts of hemocytes (Fig. 2D). Multifocal inflammatory lesions that contained several sporocysts of an MLO were observed in the siphon musculature of some geoducks. Sporocysts measured a mean of $13.43 \pm 3.5 \mu\text{m}$ ($n = 20$) and contained 4–15 spores (mean, 6.8 ± 2.8 spores per sporocyst; $n = 20$), which measured a mean of $2.91 \pm 0.47 \mu\text{m}$ ($n = 15$; Fig. 2E). The spores stained PAS positive and were not acid fast.

Overall Parasite Prevalence and Intensity

Parasite intensity was measured using a semiquantitative score of 1–4, as described earlier (Fig. 3). Parasite prevalence varied among seasons for all parasites except for the SLO (chi square = 0.44, $df = 1$, $P > 0.05$). Prevalence for RLOs was greater in geoducks collected in the shallow depths (chi square = 4.8, $df = 1$, $P < 0.05$). Siphon MLO were observed only in shallow collection depths. Both the intestinal MLO and metazoan parasites were more prevalent at the deeper collection depths (chi square = 26.99, $df = 1$, $P < 0.001$; chi square = 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 Organisms

The most commonly encountered parasite was an RLO within ctenidial epithelia, which was observed in 39% of the sampled geoducks (Fig. 2A, Table 1). Prevalence of RLOs was greatest 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 because of 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 (parasite intensity score, 2.13 ± 0.14) and winter (parasite intensity score, 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).

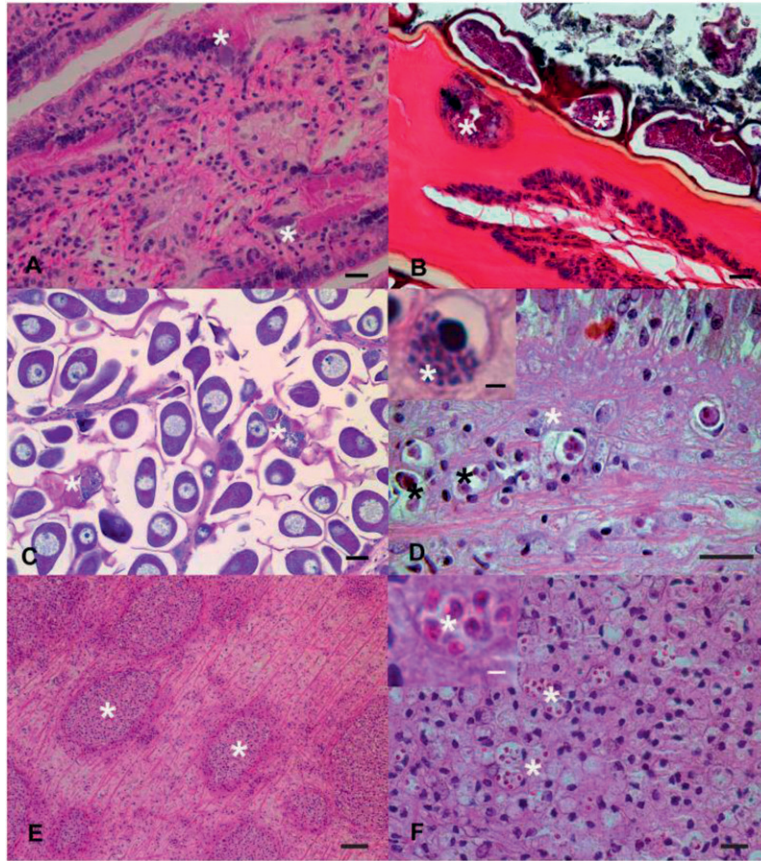


Figure 2. Commonly observed parasites in wild geoducks in Washington state. White asterisks indicate parasite presence. (A) Noted are *Rickettsia*-like inclusion bodies in geoduck ctenidia tissue (bar = 13 μm). (B) Metazoan parasites (bar = 25 μm). (C). Seen are *Steinhausia*-like microsporidians with oocytes (bar = 25 μm). (D) Microsporidia-like organism (MLO) parasites within intestinal submucosa illustrating meronts (black asterisks) and spores (white asterisks and inset image; bar = 20 μm , inset bar = 2 μm). (E) Low magnification illustrating the multifocal nature of the MLO within the siphon musculature (bar = 50 μm). (F) High magnification of siphonal MLO (bar = 8 μm , inset bar = 2 μm). Stained with hematoxylin–eosin.

Overall seasonal trends in metazoan prevalence were not determined because of 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: $H = 53.65$, $df = 2$, $P \leq 0.001$; 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 with winter and summer (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 (parasite intensity score, 3.26 ± 0.11) relative to those in animals from both Freshwater (parasite intensity score, 1.60 ± 0.26) and Thorndyke (parasite intensity score, 2.03 ± 0.14 ; $P < 0.05$) bays, which were similar to one another ($Q = 1.16$, $P > 0.05$).

Steinhausia-like Organisms

Steinhausia-like organism parasites were observed in oocytes of 16% of total geoducks sampled in this study (Fig. 2C,

Table 1). Mean prevalence (28%33%) and intensity (parasite intensity score, 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 greatest 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 Organisms

Intestinal MLO 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 (parasite intensity score, 2.46 ± 0.20) was greater than spring (parasite intensity

TABLE 1. Overall mean parasite prevalence and intensity in natural populations of Washington state *Panopea generosa*.

| Parasite | Tissue | Prevalence | | | | Overall mean intensity ± SE | Intensity† | | | |
|--|----------------------|------------------------|-------------------|-------------------|-------------------|-----------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
| | | Overall prevalence (%) | Winter (n = 89) | Spring (n = 204) | Summer (n = 161) | | Fall (n = 180) | Winter (n = 94) | Spring (n = 210) | Summer (n = 99) |
| <i>Rickettsia</i> -like organism | Gill | 39 | 4.7* | 36.1* | 44.5* | 57.8* | 1.75 ± 0.75 ^A | 1.18 ± 0.05 ^{AD} | 2.13 ± 0.14 ^{AB} | 1.60 ± 0.08 ^{AC} |
| Metazoan | Siphon epithelium | 35 | 50.0* | 24.6* | 52.0* | 32.7* | 3.05 ± 0.2 ^A | 2.19 ± 0.19 ^B | 2.94 ± 0.19 ^A | 2.54 ± 0.19 ^{AB} |
| <i>Steinhausia</i> -like organism | Oocytes | 16 | 70.7 ^a | 58.0 ^a | 14.3 ^b | 1.9 ^b | 1.14 ± 0.06 ^A | 1.24 ± 0.08 ^A | 1.10 ± 0.10 ^A | 1.50 ± 0.71 ^A |
| Microsporidia-like organism, intestine | Intestinal submucosa | 16 | 22.6 ^a | 16.2 ^a | 16.8 ^a | 15.1 ^a | 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 | 0 ^A | 2.25 ± 0.48 ^A | 3.13 ± 2.26 ^A | 2.44 ± 0.34 ^A |

* Significant differences in prevalence not determined. † Parasite intensity is based on a semiquantitative score of 0–4: 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). Significant interactions between season and site detected. Different letters indicate significant differences in prevalence (lowercase) or intensity (uppercase); alphabetical order reflects values ordered higher to lower.

score, 1.75 ± 0.16) and summer (parasite intensity score, 1.73 ± 0.15), but significantly exceeded that observed in winter, when the lowest mean infection intensity (parasite intensity score, 1.47 ± 0.19) was observed (Q = 3.33, P < 0.05).

Siphon Microsporidia-like Organisms

Siphon MLO 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; Figs. 3C and 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 MLO varied among sites. Nine percent of Totten Inlet animals and 6% of those from Thorndyke Bay were infected, whereas no MLO were observed in the siphon of Freshwater Bay geoducks (Fig. 4C, Table 2). Mean overall infection intensity was high (parasite intensity score, 2.79 ± 0.19) and was similar among seasons (H = 4.7, df = 2, P > 0.05; Fig. 3C, Table 1). Siphon muscle MLO were observed in the highest infection intensities at Totten Inlet (parasite intensity score, 2.67 ± 0.26) and Thorndyke Bay (parasite intensity score, 3.00 ± 0.30), and intensity differences were nonsignificant between the two sites (Mann-Whitney U-test, 75; P > 0.05; Table 2).

DISCUSSION

This study revealed five morphologically distinct endosymbionts of natural Pacific geoduck populations in the Pacific Northwest: an RLO in the ctenidia, an unidentified metazoan in the siphon epithelium, *Steinhausia*-like spp. in oocytes, and two other MLO within siphon muscle and intestinal submucosa. To our knowledge, this is the first report of microsporidia-like parasites, including *Steinhausia*-like parasites, in geoducks. 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 (Elston 1986, Fries & Grant 1991, Friedman et al. 2000, Bower & Blackburn 2003). The most common geoduck parasite (39%) observed in this study were RLOs. Microscopic examination revealed that RLO prevalence peaked in warmer months (fall sampling), with the greatest infection intensity observed during summer months. This finding suggests that elevated temperature may be an important driver of RLO presence in geoducks, and it 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 RLO, “*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

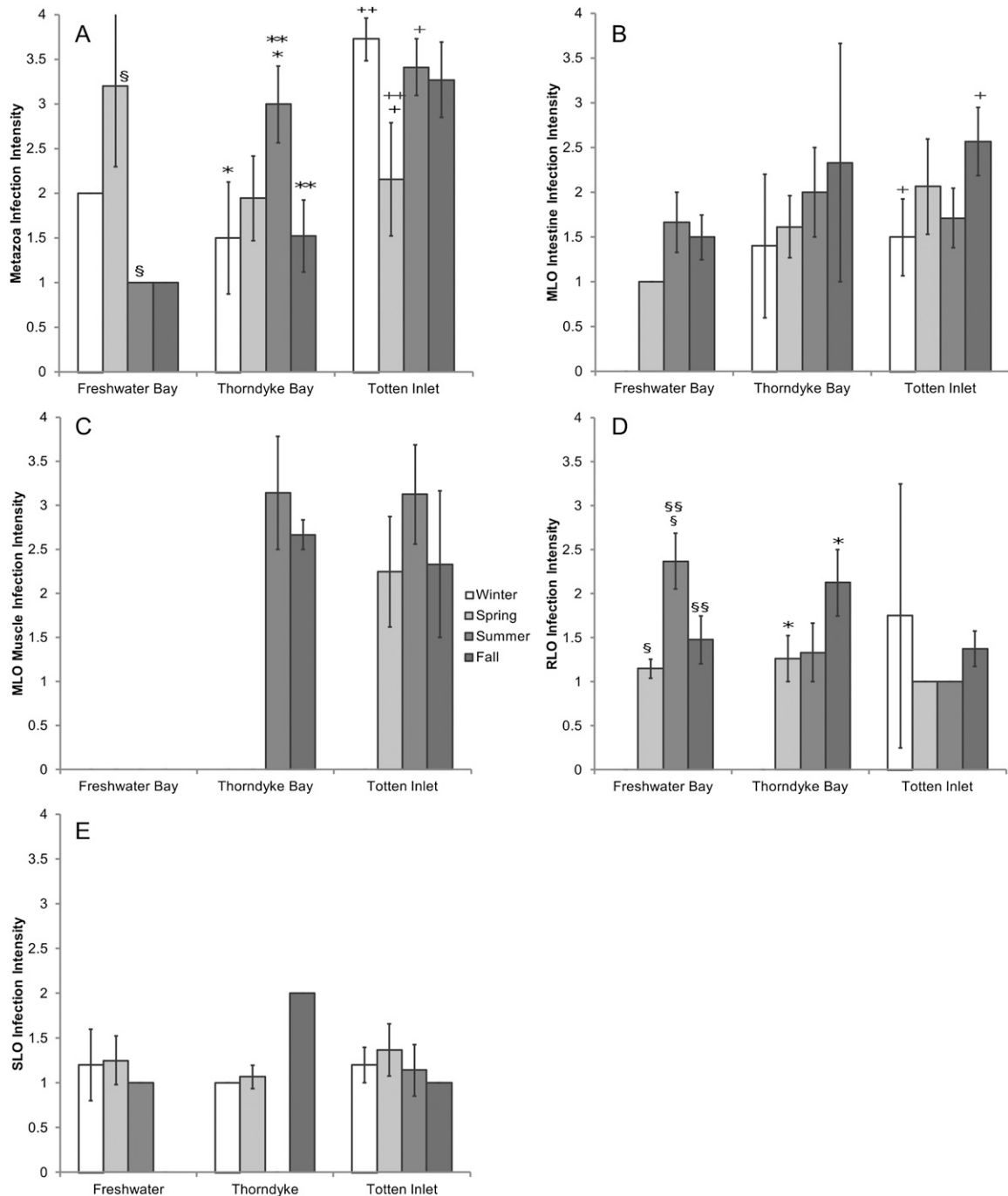


Figure 3. (A–E) Infection intensity in *Panopea generosa* by site and season. Parasite groups metazoa (A), intestinal microsporidia (microsporidia-like [MLO] intestine) (B), siphon muscle microsporidia (MLO muscle) (C), *Rickettsia*-like organism (RLO) (D), and *Steinhausia*-like organism (SLO) (E) observed from histology in geoducks collected from Freshwater Bay, Thorndyke Bay, and Totten Inlet. Error bars represent 95% confidence intervals. § or §§ Freshwater Bay pairwise comparisons indicating significant difference between seasons. * or ** Thorndyke Bay pairwise comparisons indicating significant difference between seasons. + or ++ Totten Inlet pairwise comparisons indicating significant difference between seasons.

reproduction may also increase with elevated temperature and may lead to the trends observed.

In the current study, metazoan infections in geoducks 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 at 168 y. Animals collected in this study were

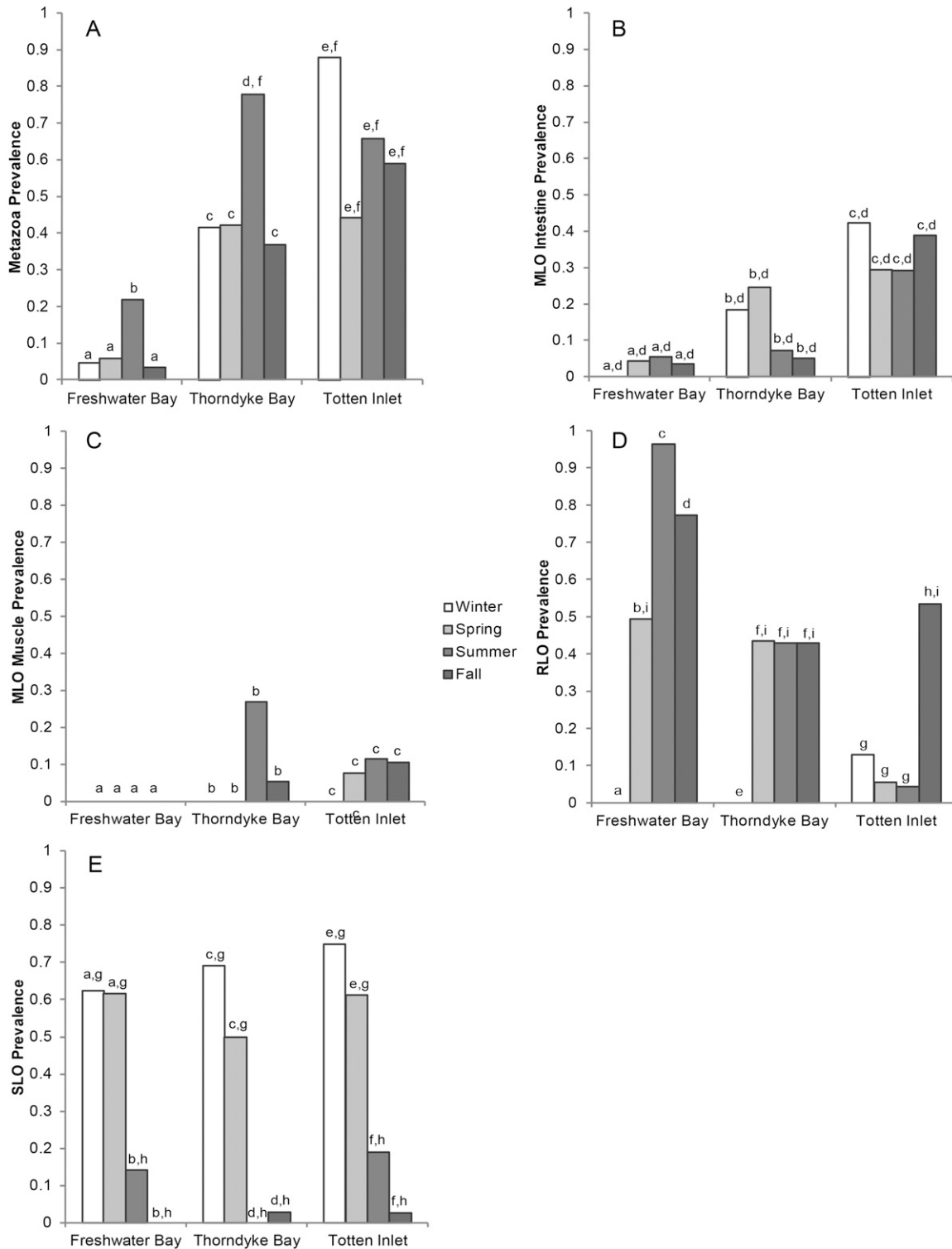


Figure 4. Proportions of parasite groups metazoa (A), intestinal microsporidia (microsporidia-like [MLO] intestine) (B), siphon muscle microsporidia (MLO muscle) (C), *Rickettsia*-like organism (RLO) (D), and *Steinhausia*-like organism (SLO) (E) observed from histology in geoducks collected from Freshwater Bay, Thorndyke Bay, and Totten Inlet. Bars with the same letters are statistically similar, while differing letters represent significant differences in the measured response.

recruits and assumed to be collected at random with respect to age. Although shell length was collected for all specimens, shell length correlates poorly with age after asymptotic length is attained at age 5–15 y (Goodwin & Pease 1991,

Hagen & Jaenicke 1997, Hoffmann et al. 2000, Campbell et al. 2004).

Microsporidian infections have not been identified previously in geoducks. Currently, microsporidia have been reported

TABLE 2.
Parasite prevalence and intensity among sites and seasons.

| Parasite | Freshwater Bay (n = 237) | | | Thornlyke Bay (n = 173) | | | Totten Inlet (n = 224) | | |
|--|--------------------------|--------------------------|----------------------------|-------------------------|--------------------------|----------------------------|------------------------|--------------------------|----------------------------|
| | Prevalence (%) | Intensity (mean ± SE) | Seasonal prevalence trends | Prevalence (%) | Intensity (mean ± SE) | Seasonal prevalence trends | Prevalence (%) | Intensity (mean ± SE) | Seasonal prevalence trends |
| <i>Rickettsia</i> -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 |
| Metazoan | 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 |
| <i>Steinhausia</i> -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 | NA | No trend | 6 ^b | 3.00 ± 0.30 ^A | No trend | 9 ^c | 2.67 ± 0.26 ^A | No trend |

F, fall; NA, not applicable; Sp, spring; Su, summer; W, winter. Different letters indicate significant differences in prevalence (lowercase) or intensity (uppercase) among sites ($P < 0.05$).

only 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 MLO observed in geoducks in the current study, only those observed within oocytes (SLOs) were consistent morphologically with a known microsporidian genus observed previously in oocytes of some bivalve species. This parasite was morphologically similar to members of the genus *Steinhausia*, such as *Steinhausia mytiloyum*, which 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 have all 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, with the infectious stage responsible for host-to-host transmission (Garcia 2002). Both of these stages were observed in geoducks. However, the two life stages were not always observed within the same individual. Of all geoducks examined with either intestinal or siphon muscle MLO parasites, nine were observed with both MLO life stages (7%). The intestinal MLO parasites in geoducks had a plasmodium-like morphology, which may represent meronts, whereas 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 is 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 greatest prevalence of SLO infections was observed in geoducks during colder months (February through May), whereas SLO parasites in warmer months were rarely observed. This observation is consistent with the annual oocyte maturation cycle in geoducks (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 with 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 geoducks in Puget Sound in June and July (Friedman & Vadopalas, unpubl. data). Of geoduck cases with SLO parasites, infection intensity was generally low, possibly because of elimination by the host when oocytes are released during spawning. Vertical transmission of *Steinhausia* is suspected to occur in *Mytilus galloprovincialis*, which may explain the perpetuation of infection in 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 (Emmett 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; although, in general, Thorndyke Bay exhibited intermediate parasite abundance and infection intensity. Intestinal MLO and metazoan parasites were observed in greatest prevalence at Totten Inlet (mean, 63%), and they showed the lowest abundance at Freshwater Bay (mean, 9%). In contrast, trends in RLO prevalence were the inverse of those observed for metazoan and intestinal microsporidia; Totten Inlet exhibited the lowest RLO prevalence (mean, 19%), whereas RLOs were commonly observed in Freshwater Bay (mean, 62%). Sample site did not influence the presence of the SLO, which was limited to reproductively active female geoducks 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. Furthermore, 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

The presence of several previously unreported parasites in Puget Sound geoducks was reported. Parasite presence in geoduck populations was influenced significantly 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 assess the potential risks of geoduck diseases more completely, continued exploration of individual parasite distributions, virulence, and physiological tolerances is needed. Gathering additional 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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DISTRIBUTION, ABUNDANCE, AND HABITAT ASSOCIATIONS OF A LARGE BIVALVE (*PANOPEA GENEROSA*) IN A EUTROPHIC FJORD ESTUARY

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ABSTRACT Marine bivalves are important ecosystem constituents and frequently support valuable fisheries. In many nearshore areas, human disturbance—including declining habitat and water quality—can affect the distribution and abundance of bivalve populations, and complicate ecosystem and fishery management assessments. Infaunal bivalves, in particular, are frequently cryptic and difficult to detect; thus, assessing potential impacts on their populations requires suitable, scalable methods for estimating abundance and distribution. In this study, population size of a common benthic bivalve (the geoduck *Panopea generosa*) is estimated with a Bayesian habitat-based model fit to scuba and tethered camera data in Hood Canal, a fjord basin in Washington state. Densities declined more than two orders of magnitude along a north–south gradient, concomitant with patterns of deepwater dissolved oxygen, and intensity and duration of seasonal hypoxia. Across the basin, geoducks were most abundant in loose, unconsolidated, sand substrate. The current study demonstrates the utility of using scuba, tethered video, and habitat models to estimate the abundance and distribution of a large infaunal bivalve at a regional (385-km²) scale.

KEY WORDS: assessment, Bayesian model, bivalve, cryptic, geoduck, habitat, Hood Canal, hypoxia, *Panopea generosa*, Puget Sound, scuba, videography

INTRODUCTION

Wild and cultured marine bivalves can act as important ecosystem engineers in marine and estuarine ecosystems (e.g., Jones et al. 1994), where they provide habitat, regulate primary productivity, and couple energy and nutrients in pelagic and benthic environments (Prins et al. 1998, Newell et al. 2002, Burkholder & Shumway 2011, Carmichael et al. 2012, Dame 2012). Wild bivalve populations also support important fisheries worldwide, with capture production exceeding 1.6 million t in 2012 (FAO 2014). These fisheries are economically important because unit price values for bivalves tend to exceed those for finfish and other invertebrates (Gosling 2003). In many nearshore areas, human disturbance can affect the distribution and abundance of wild bivalve populations, which may complicate ecosystem or fishery management approaches (Dame et al. 2002, Dame 2012). Moreover, chronic perturbations associated with continued human population growth and development in urban and suburban watersheds are expected to accelerate declines in shellfishery yields through the effects of nutrient loading and other disturbances (Kennish 2002).

The inland marine waters of Washington state (i.e., Puget Sound and the Strait of Juan de Fuca) support valuable wild subtidal bivalve harvests and a growing human population. Puget Sound in particular experiences significant stressors, including habitat alteration, contamination, as well as eutrophication resulting in low dissolved oxygen (LDO). Seasonal LDO and periods of hypoxia (defined as dissolved oxygen

concentrations less than 2 mg/L [Diaz 2001]) are most intense in Hood Canal, a narrow and deep fjord basin that comprises the westernmost portion of Puget Sound. Oxygen levels typically decline in deeper waters of the southern reaches of Hood Canal throughout the course of the boreal summer, and hypoxic conditions may expand to depths less than 20 m for short periods (Newton et al. 1995). Localized wind events can cause upwelling of this hypoxic layer to the surface, leading to precipitous decreases in dissolved oxygen throughout the water column on the scale of hours to days (Palsson et al. 2008, Kawase & Bang 2013). Although historical reconstructions suggest LDO and periodic hypoxia are regular features of the basin (Brandenberger et al. 2011), “fish kill” events and evidence of stress and mortality among invertebrates in 2002 to 2004 and 2006 have focused attention on the impact of seasonal conditions on ecosystem health (Fagergren et al. 2004, Newton et al. 2007, Palsson et al. 2008). Subsequent studies have characterized a north–south gradient of declining dissolved oxygen levels with a strong seasonal component (see Kawase and Bang [2013] and references therein) that is associated with changes in the distribution and behavior of fish and macroinvertebrates (Parker-Stetter & Horne 2008, Essington & Paulsen 2010, Froehlich et al. 2014). Notably, these previous studies have not specifically evaluated LDO effects on regional bivalve populations.

One of the most commercially and culturally valuable bivalve species in these waters is the geoduck *Panopea generosa* (Gould 1850), which has historically been harvested for subsistence, and in more recent decades by recreational clam diggers and commercial operations. Geoducks are large (>2 kg), long-lived (>100 y),

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deeply burrowing (~1 m) bivalves that inhabit soft, unconsolidated sediments in intertidal and subtidal areas. The species often dominates the benthic biomass of Puget Sound, the Strait of Juan de Fuca, and the Strait of Georgia, British Columbia, in suitable subtidal habitats (Goodwin & Pease 1989). Recent concern for geoduck populations in Hood Canal—particularly those in areas affected periodically by LDO—has prompted resource management agencies to call for concerted monitoring and assessment efforts within the basin (Sizemore & Blewett 2006).

Determining the abundance and distribution of infaunal clams is complicated by their cryptic habit. In subtidal areas, assessments for several species are done using dredges or grabs, but efficiency varies with sediment type and other environmental factors, as well as the apparatus used (Kennish & Lutz 1995, Ragnarsson & Thórarinsdóttir 2002, Gosling 2003). Moreover, these methods are typically very labor intensive and require a large number of samples, particularly for patchily distributed species that occur at low densities. Dredges also fail to capture small-scale patterns in sediment type, topography, and ecological interactions, which provide important context for understanding patterns (Ragnarsson & Thórarinsdóttir 2002). In Washington, British Columbia, and Alaska, geoduck assessments are conducted by divers in conjunction with harvest, which yields high-resolution data (Campbell et al. 1998, Muse 1998, Bradbury et al. 2000, Siddon 2007). However, these visual surveys are restricted to *a priori* designated tracts at relatively shallow depths (<21 m [Goodwin & Pease 1989]) compared with the maximum reported for the species (at least 110 m, as reported by Goodwin and Pease [1991]). Although remote photographic and videographic methods have been used elsewhere to assess populations of infaunal bivalves in deeper waters (Ragnarsson & Thórarinsdóttir 2002), this approach has not yet been used for geoducks.

The scale and extent of environmental stressors affecting Hood Canal underscores the importance of developing a suitable, scalable approach for evaluating broad-scale changes in geoduck distribution across depths and regions of the basin. Moreover, concerns about resilience of geoduck populations in general (Orensanz et al. 2004, Valero et al. 2004) and sustainability of geoduck fisheries (Khan 2006) provide the impetus for assessing abundance. Herein the distribution and substrate affinities of geoducks are documented in Hood Canal with scuba and tethered camera surveys. Abundance of present-day geoduck populations is estimated using a Bayesian habitat-based model, and this information is used to evaluate patterns among habitats across the entire basin.

MATERIALS AND METHODS

Study Area

Hood Canal is a long (~90 km), narrow (~2.4 km) fjord that constitutes the westernmost subbasin of Puget Sound (Burns 1985) (Fig. 1). The volume of Hood Canal (~ 2.1×10^{10} m³; based on MHW datum) is nearly 13% of all Puget Sound marine waters. To account for the heterogeneous hydrographic conditions in the overall study area, survey stations were selected in four distinct geographic regions; North, Middle, South, and Lynch. The North region is bordered by South Point (47°50'3.18" N, 122°41'15.25" W), and Lowfall (47°48'53.78" N, 122°39'18.36" W) in the north, and approximates the location of

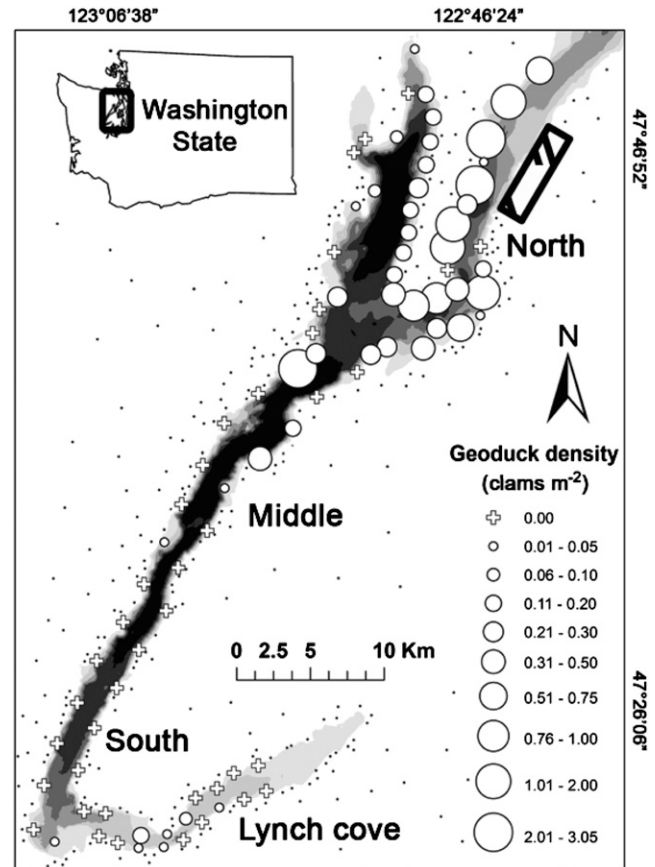


Figure 1. Geoduck density estimates in scuba surveys by transect position. Estimated densities are proportional to the area of each circle. Crosses indicate transect locations where no geoducks were observed. The four regions used in the analysis are shown: North, Middle, South, and Lynch Cove. Relative water depth is indicated by shading; land area is stippled. No surveys were conducted within the secure area of the Naval Base Kitsap installation at Bangor, Washington (box).

a prominent sill at the only opening to the subbasin. The Middle region is demarcated to the north by Quatsop Point (47°38'47.35" N, 122°54'15.83" W), where it abuts the North region, and Chinom Point (47°31'42.33" N, 123°1'2.56" W) to the south, where it abuts the South region. Data describing the physical oceanography of Hood Canal are available from the Hood Canal Dissolved Oxygen Program (<http://www.hoodcanal.washington.edu>). Since 2000, data have been collected as part of a citizen's monitoring program (Hood Canal Dissolved Oxygen Program 2007a) and the Oceanic Remote Chemical Analyzer system (Hood Canal Dissolved Oxygen Program 2007b). The North region is typically normoxic whereas the Middle region rarely experiences LDO or hypoxia in bottom waters. The South region encompasses the Great Bend and is divided from the Lynch region at Sister's Point (47°21'41.04" N, 123°02'08.82" W). The South region experiences seasonally protracted LDO in deeper waters, with hypoxic conditions extending periodically into shallow water (<20 m), whereas bottom waters in the Lynch region experience chronic hypoxia.

Scuba Surveys

A total of 87 sites were surveyed using scuba to assess the distribution and densities of geoducks in nearshore waters of

Hood Canal (Fig. 1). A stratified design was used, with sampling sites placed at 1.6-km increments around the entire shoreline, starting in Lynch Cove. A total of 63 and 23 sites were sampled from June through September in 2007 and 2008, respectively. In 2009, one site was surveyed near Tahuya in the South region, which was not surveyed during the first 2 y because of logistical constraints. Some areas falling within the Bangor Naval Base restricted access area were not sampled (Fig. 1). Diving protocol and survey methods were modified from Miller et al. (1994) and Bradbury et al. (2000). At each site, two divers descended to an initial starting depth of 21 m MLLW and swam a strip transect on a compass heading toward shore and perpendicular to depth contours; each diver surveyed a 1-m swath. The divers stopped every 5 m along the strip transect (10 m²; henceforth, “segment”) to record clam counts, habitat features, depth, and predominant (>90% coverage) substrate. Substrate type was classified using the following categories: bedrock/boulder (diameter, >256 mm), cobble (diameter, <256 to ≥4 mm), sand (diameter, <4 to ≥0.06 mm), and fines (diameter, <0.06 mm). Where cobble and sand substrates were well mixed and neither comprised more than 90% coverage, the substrate was called cobble/sand. The number of segments surveyed per site varied as a result of differences in bottom slope.

Because geoduck survey counts are based on observations of siphons, which are visible when clams are actively filtering water (i.e., the “show”), only a proportion of the total population of geoducks in a given area can be counted at any time. Therefore, a “show-factor” multiplier (i.e., the proportion of geoducks with visible siphons) must be estimated at the time and location of the survey, and applied to the uncorrected diver counts post hoc. The Washington Department of Fish and Wildlife/Washington Department of Natural Resources maintain three geoduck show plots within Hood Canal that are used to estimate the local show factor at the time of surveys. Within a show plot, the geoduck density is known, so by comparing survey counts with the known densities in these areas, the show factor can be estimated (Bradbury et al. 2000). The nearest available show plot was surveyed once per week during all survey weeks in all 3 y to generate weekly show-factor estimates for survey data. This sampling frequency was deemed appropriate after analysis of 2007 show-plot data (when show plots were surveyed more frequently) indicated strong temporal autocorrelation in show factor on a scale of 7–10 days.

Tethered Camera Surveys

To determine the distribution of geoducks beyond safe diving depths, tethered camera surveys were conducted offshore on a random stratified sample of 40 of the 87 sites sampled using scuba (20 sites each in 2008 and 2009). Sites were selected randomly within the four geographic regions to ensure broad spatial coverage throughout Hood Canal. The tethered camera (Deep Blue Pro Color Underwater Video System; Ocean Systems Inc., Everett, WA) was equipped with two lasers (beam width, 5.5 cm) and two underwater lights, and was tethered to a digital video recorder (Canon HV20 Camcorder; Canon USA, Lake Success, NY) on the support vessel. At each site, a survey consisted of four deployments targeting depth strata of 20 m, 30 m, 40 m, or 50 m for 5-min “drifts” in each stratum. The position of the boat and the water depth at 1-min intervals were recorded while allowing the boat to drift above the target

coordinates using an integrated GPS/depth sounder on the support vessel; these intervals were used for binning video analyses in the laboratory. All showing geoducks were counted, and habitat features, depth, and predominant substrate were recorded. Estimates of the area surveyed were calculated as the product of the distance drifted during all 1-min intervals of each deployment and the mean width (in meters) of the camera’s field of view, as determined from the ratio of mean distance between lasers on the viewing monitor and the entire viewing area (actual distance between lasers, 10 cm).

Statistical Analyses

To determine the set of predictor variables that best explained the field data, and to estimate the effects of those parameters on geoduck density, generalized linear mixed models (GLMM) (Breslow & Clayton 1993) with a Poisson error distribution and a log-link function were used. The response variable in these models was the count of geoducks per transect segment, and the predictor variables were region, depth, and substrate; sampling site was a categorical variable with random effects. From these effects, a set of candidate models was developed consisting of all combinations of terms, as well as an intercept-only “null” model. For all models, fixed terms were included on the linear set of predictors to account for area sampled (10 m² for all scuba transect segments, variable for tethered camera; *AREA*) and for the show factor (*showFactor*) for each survey date. Thus, the statistical model was

$$Y = \exp[\eta + \ln(\text{AREA}) + \ln(\text{showFactor})]$$

$$\eta = X\beta + Z\alpha,$$

where Y is the number of geoducks observed in a transect segment, X is the matrix of fixed-effects independent variables, β is a vector of fitted coefficients for fixed effects, Z is the design matrix indicating the site for each datum, and α is a random effect parameter assumed to be drawn from a normal distribution, with a mean of zero and a variance estimated from the data.

Multiple models with unique combinations of covariates were fit to the data by minimizing the negative log-likelihood using the lme4 package for the R statistical system (R Development Core Team 2008). Akaike’s information criterion corrected for overdispersion (QAIC) was used to select the subset of models that best fit the data (Burnham & Anderson 2002). Overdispersion implies a greater frequency of extreme events (i.e., greater geoduck densities) than expected from the probability density function, and introduces a bias toward selecting more complicated models. In addition to correcting for overparameterization, QAIC penalizes more complicated models based on the degree of overdispersion that is estimated from each model. The analysis was performed separately for the scuba data and the tethered camera data.

Bayesian Estimation and Prediction

The best-fitting model from the likelihood-based GLMM was used to estimate Bayesian posterior probability density functions for each model parameter, which were then combined with the GIS output to estimate probability density functions

for total geoduck abundance in each region of Hood Canal. Five Markov-Chain Monte Carlo (MCMC) simulations, each for 400,000 iterations, were run using openBUGS software, and every 10th iteration was saved. Starting values for each chain were randomized around the maximum likelihood estimate. Convergence of chains was evaluated using the Gelman–Rubin test on each parameter and by confirming low autocorrelation in the MCMC. A uniform prior was used for the fixed-effects coefficients and the hyperdistribution mean (−10 to 10), as well as the hyperdistribution standard deviation (0.01–10).

The Bayesian posterior probability distributions were used to predict geoduck densities in each region in Hood Canal based on the areas containing each depth and substrate classification. For each iteration of the MCMC simulation, a predicted geoduck density was generated for any area in Hood Canal based on its depth, substrate type, and region in which the area occurs. By summing these predictions over all areas within a region, total abundance of geoducks in each region was estimated. This was repeated for each simulated draw from the posterior distribution, thereby generating probability distributions of total geoduck abundance by region. Posterior inferences were drawn by fitting nonparametric kernel density smoothers to the posterior draws.

This analysis requires geographic information on the amount of area in each region within specified depth bins and with characteristic substrate types. Geographic information system data layers were generated for the three ecogeographic variables that were included in the GLMM: region, depth, and substrate. All calculations were performed on geospatial data sets using the built-in functionality of ArcGIS 9.0 and Spatial Analyst tools (ESRI, Redlands, CA). The starting point for the geospatial modeling was a digital elevation model of combined bathymetry and topography for the Puget Lowland available through the University of Washington, School of Oceanography (Finlayson 2005). All data, regardless of native projection, were reprojected as UTM Zone 10N, WGS 1984.

The depth layer was generated by reclassifying the digital elevation into 1-m- and 20-m-depth bins for depths of 0–60 m and more than 60 m, respectively, with the analysis extent set to the Region layer. Sediment data were obtained for the North region from the Naval Oceanographic Office (Stennis Space Center, Bay St. Louis, MS) and reduced from the original 28 sediment categories to five broad sediment types: bedrock/boulder, cobble, cobble/sand, sand, and fines. Mapped substrate data are not available from the other regions. Instead, data collected in scuba surveys were used to generate estimated proportions of the total area in each region consisting of each substrate category; this approach provides a reasonable approximation in the absence of detailed bottom mapping and substrate classification data. To use these data, each region and depth-specific polygon had to be subdivided into subareas based on the proportion of area that consisted of each substrate type.

RESULTS

Geoduck Distribution and Abundance

Geoduck densities varied markedly across regions and depths. The scuba data indicated strong regional contrasts in geoduck densities (Fig. 1). Geoduck densities were substantially

greater in the North region compared with the other three regions. For instance, geoduck densities at intermediate depths ranged from 0–9 geoducks/m² in the North, 0–2 geoducks/m² in the Middle region, and 0–0.6 geoducks/m² in the South and Lynch Cove regions. In all regions, geoduck densities were generally low at shallow depths (<3–4 m), peaked at intermediate depths, and showed some indication of declining thereafter (Fig. 2). Within the North region, the trend with depth was difficult to discern because of the high variability in geoduck counts among segments. A strong declining trend with depth was evident in the Middle region, whereas a possible increasing trend with depth was apparent in the Lynch Cove region. However, variation in depth trends across regions cannot be determined conclusively because of substantial patchiness in geoduck densities, which can give rise to spurious differences resulting from chance events.

The tethered camera surveys confirmed that geoduck densities declined with depth beyond 20 m (Fig. 2). The North region had the most informative data because densities were generally greatest there. Within the depth range surveyed by both scuba

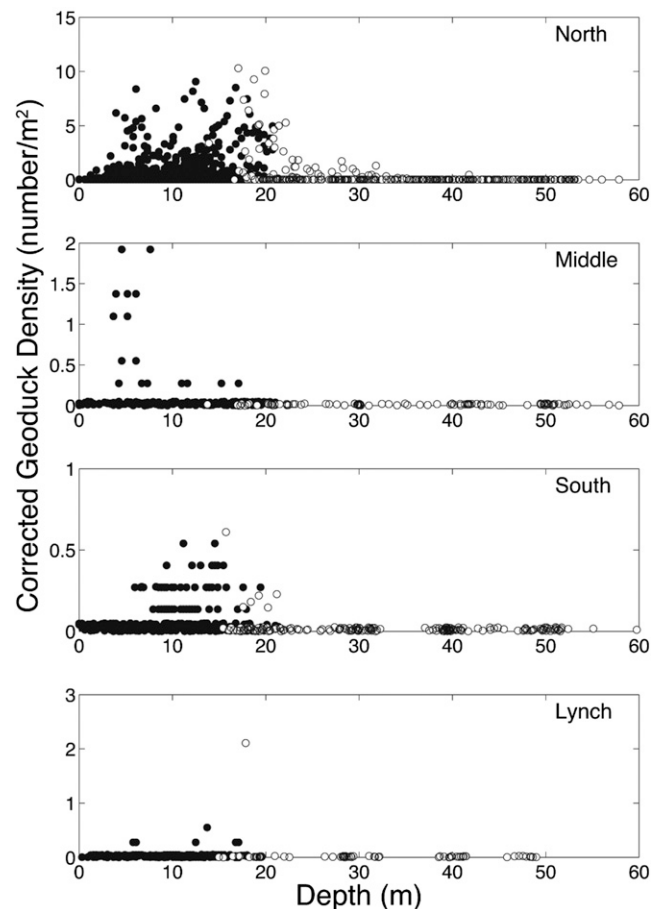


Figure 2. Mean geoduck density (corrected for show factor) versus depth based from scuba (black circles) and tethered camera (open circles) surveys. Show factor ranged from 22.7%–84.1% (mean, 58.0%) and 46.0%–74.0% (mean, 58.0%) of known geoducks in show plots for scuba and tethered camera surveys, respectively (see text for details). Each black circle represents a single 10-m² transect segment in scuba surveys; each open circle represents a density estimate from a single 1-minute drift in tethered camera surveys. A small amount of jitter was added to densities so that the number of observations can be seen.

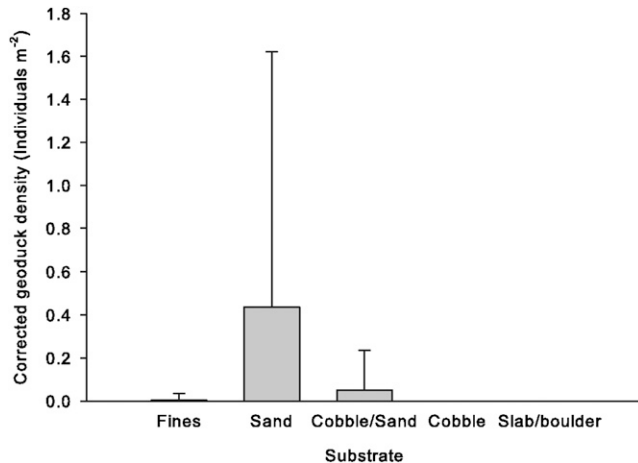


Figure 3. Mean geoduck density (\pm SE) by substrate type, estimated from scuba data.

and tethered camera, geoduck densities were similar between the two sampling methods (Figs. 2). The tethered camera data show a sharp decline in geoduck densities at depths greater than 20 m, generally declining to near zero around 30 m. Only a single geoduck was observed at depths greater than 40 m (Fig. 2).

Geoduck densities also varied substantially by substrate type (Fig. 3). Densities were greatest in scuba transect segments classified as sand, and cobble/sand mixture sites had notable but still reduced geoduck densities. Sites categorized as fines had virtually no geoducks present, and no geoducks were observed in sites categorized as cobble or bedrock/boulder.

The initial GLMM fits indicated an unexpected fitted relationship with depth; density was predicted by the models to increase with depth. These fitted values were inconsistent with the general observation that geoduck densities declined with depth, and resulted from the apparent nonlinearity in the effect of depth, particularly the decline in geoduck densities at shallow depths. To account for this, a new predictor variable was added that described how densities declined with depth at shallow but not deep depths. Specifically, depth was standardized so that the data had a mean of zero and a standard deviation of one. A predictor variable was then added equal to standardized depth squared (depth^2) if the standardized depth was less than zero (i.e., the actual depth was below the mean of all sampled depths), and was zero otherwise. This allowed for a “dome-shaped” effect of depth expressed as a collection of linear coefficients on predictor variables.

Model selection indicated that the additional nonlinear depth predictor variable greatly improved the model fits, so this term was added to all models that also had depth as a predictor (Table 1). The full model provided the best fit and contained all fixed effects, including the modified depth^2 predictor, indicated as $\text{depth}^2(-)$ to denote that it only applies to those sites with a negative standardized depth. The next best fitting model (depth, $\text{depth}^2[-]$, and substrate) had a ΔQAIC value of 11, providing further support that the collection of depth and substrate predictor variables was informative.

The model coefficients provide further information about the magnitude and direction of fixed effects (Fig. 4). When fitting the GLMM to categorical data, the algorithm uses the first level for each categorical predictor as the reference state to which all others are relative. In this case, all parameter estimates denote the predicted geoduck density relative to sand substrate in the North region. All other parameter estimates are negative, indicating that densities are greatest in the North and in sand substrates (Fig. 4). Because these effects are on a log scale, the model predicts stark differences between the North and all other regions, after accounting for differences in substrate types and depths. For instance, geoduck densities in the Middle, South, and Lynch regions are estimated to be 3.9%, 0.7%, and 3%, respectively, of those in comparable depths and substrates in the North region. Even stronger effects are evident for substrate. Mixed cobble/sand is predicted to have 10% of the densities of sites categorized as sand, whereas sites categorized as fines have densities that are 7% of those in sand (Fig. 4). The model estimates near-zero density in bedrock/boulder and cobble substrates (SE could not be calculated for these estimates using numerical approximations).

The same model selection procedures were used on the tethered camera data, for which each datum was a single 1-min segment of a tethered camera deployment, and site was a random effect. However, because there were multiple data segments per deployment, a second random effect (called deployment) was included and nested within each site random effect. Because the data did not span the shallow depths where geoduck densities appeared to decline (based on scuba data), the $\text{depth}^2(-)$ term was not used in model selection; only a log-linear depth effect was used. The best-fitting model included depth only as a predictor variable (Table 2). There was weak evidence for a region effect ($\Delta\text{QAIC} = 0.8$), although by convention more complicated models are not favored over simpler models that have lower QAIC scores. The best-fitting

TABLE 1.

Model selection results for 8 GLMM to predict geoduck densities in scuba data (selected model in bold type).

| Model | No. of fixed effects | ΔQAIC^* | QAIC* weight | Cumulative weight |
|--|----------------------|-----------------------|--------------|-------------------|
| Depth + $\text{depth}^2(-)$ + substrate + region | 10 | 0 | 1.00 | 1.00 |
| Depth + $\text{depth}^2(-)$ + substrate | 7 | 11.1 | 0.00 | 1.00 |
| Substrate + region | 7 | 108 | 0.00 | 1.00 |
| Substrate | 5 | 120 | 0.00 | 1.00 |
| Depth + $\text{depth}^2(-)$ + region | 6 | 269.8 | 0.00 | 1.00 |
| Depth + $\text{depth}^2(-)$ | 3 | 279.1 | 0.00 | 1.00 |
| Region | 4 | 362.2 | 0.00 | 1.00 |
| Depth | 2 | 377.8 | 0.00 | 1.00 |

* Akaike's information criterion corrected for overdispersion.

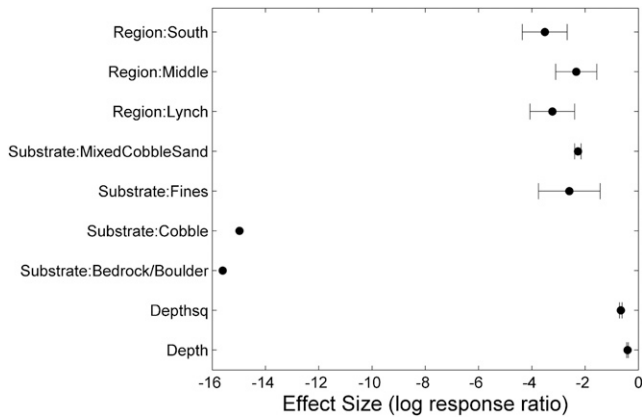


Figure 4. Estimated coefficients (\pm SE) from the generalized linear model depicting the effect of depth, depth squared (Depthsq), region, and substrate on geoduck densities. Coefficients are log-response ratios, and within categorical variables are expressed as a log-ratio relative to a reference level within that category. Reference levels were sand and North for substrate and region, respectively.

model had depth coefficient (\pm SE) equal to -0.18 ± 0.02 , which equates to a 16% decline in geoduck densities with each 1 m of depth over the full range of depths (13–61 m). The lack of significant substrate and region effects in this analysis is a result of the fact that this analysis spanned the maximum depth extent of geoducks, so that differences among substrates and regions at these depths were relatively few compared with the more important influence of depth. The data and model therefore predict that geoduck densities are reduced to 99.5% of their maximum value at depths greater than 30 m.

For each vector of model parameters produced by the MCMC output, the predicted geoduck abundance in each polygon was calculated based on its depth, substrate, and region. It was necessary to specify a maximum depth range at which geoduck densities were of an appreciable level to warrant consideration and to extrapolate the estimated depth effect via scuba data (depth range, 0–22 m) out to alternative maximum depth extents of geoduck ranges. Thus, four alternative models were considered about the maximum depth extent: 25 m, 30 m, 35 m, and 40 m. Of these, the 30-m and 35-m maximum depth range are most consistent with the tethered camera data.

The application of the GLMM to predict region-specific densities indicated large differences in total geoduck abundances between regions (Table 3). Because of the aforementioned region effects, the high proportion of area that had favorable substrate, and the larger total area, the estimated geoduck abundances in the North region were roughly two orders of magnitude greater than the South and Lynch regions, and roughly 50-fold greater than in the Middle region. In fact, the modal geoduck abundance in the North region was roughly 30-fold greater than the summed abundance over all other areas (Table 3). The 95% Bayesian credibility intervals were fairly large, spanning a roughly fourfold range of abundance in the North region and between 30-fold (South, Middle) and 40-fold (Lynch Cove) in the other regions. However, the credibility intervals were narrow compared with the large difference among regions. The lower bound for the North region (between 1.5 and 1.7 million; Table 3) was substantially greater than the upper bound for any other region (range, 0.07–0.4 million). The estimated abundances were not highly sensitive to the estimated maximum depth extent, especially compared with the precision of the estimates. For instance, in the North region, the 95% prediction interval for the scenario that assumes a 25-m maximum depth extent was 1.5–5.0 million geoducks, which shifted to 1.7–5.5 million geoducks for the 35-m maximum depth scenario.

DISCUSSION

The current study represents one of the first attempts to estimate the distribution and total abundance of a large infaunal bivalve (geoduck) at the scale of an entire fjord estuary (385 km²). Geoduck density exhibited regional variability and was greatest near the mouth of the fjord (i.e., North region) at depths between 10 m and 15 m (Figs. 1 and 2). Across the basin, geoduck distribution was spatially patchy and highest in loose, unconsolidated sediments, which is similar to patterns reported by Goodwin and Pease (1991). Unlike that study, geoduck densities in the current study associated strongly with sand substrate (Fig. 3) rather than other unconsolidated sediments (e.g., mud, gravel). Goodwin and Pease (1991) also suggest that geoducks may be abundant at depths up to 61 m in Puget Sound based on their own work and analysis of other studies; however, in the current study, densities declined dramatically beyond 20 m, and no geoducks were observed at depths of more than 31 m in Hood Canal (Fig. 2).

TABLE 2.

Model selection results to predict geoduck densities in tethered camera data (selected model in bold type).

| Model | No. of fixed effects | Δ QAIC* | QAIC* weight | Cumulative weight |
|--|----------------------|----------------|--------------|-------------------|
| Depth | 2 | 0 | 0.47 | 0.47 |
| Depth + region | 5 | 0.8 | 0.31 | 0.78 |
| Depth + substrate | 6 | 2.5 | 0.13 | 0.91 |
| Depth + substrate + region | 9 | 3.5 | 0.08 | 0.99 |
| Depth + substrate + region + depth \times region | 12 | 8.3 | 0.01 | 1.00 |
| Null | 1 | 87.6 | 0.00 | 1.00 |
| Substrate | 5 | 90.6 | 0.00 | 1.00 |
| Region | 4 | 91.5 | 0.00 | 1.00 |
| Substrate + region | 8 | 94.7 | 0.00 | 1.00 |

* Akaike's information criterion corrected for overdispersion.

TABLE 3.
Bayesian prediction intervals of geoduck abundance ($\times 1,000$) by region and model (maximum depth range).

| | Posterior mode | Posterior median | 50% Interval | 80% Interval | 95% Interval |
|------------|----------------|------------------|--------------|--------------|--------------|
| North | | | | | |
| 25 | 2,622 | 2,813 | 2,294–3,420 | 1,901–4,084 | 1,528–4,997 |
| 30 | 2,796 | 3,002 | 2,448–3,651 | 2,029–4,360 | 1,631–5,333 |
| 35 | 2,875 | 3,122 | 2,546–3,798 | 2,110–4,537 | 1,696–5,549 |
| 40 | 2,949 | 3,199 | 2,609–3,893 | 2,162–4,652 | 1,737–5,689 |
| Middle | | | | | |
| 25 | 47.49 | 80.31 | 45.8–136.0 | 25.82–213.4 | 12.1–353.3 |
| 30 | 50.57 | 85.58 | 49.8–144.9 | 27.51–227.4 | 12.8–376.1 |
| 35 | 52.88 | 89.47 | 51.0–151.5 | 28.75–237.7 | 13.4–392.9 |
| 40 | 54.15 | 91.61 | 52.2–155.1 | 29.42–243.3 | 13.7–402.1 |
| South | | | | | |
| 25 | 6.95 | 13.1 | 7.26–22.7 | 4.1–36.07 | 1.86–60.57 |
| 30 | 7.37 | 13.88 | 7.69–24.05 | 4.34–38.23 | 1.97–64.2 |
| 35 | 7.78 | 14.64 | 8.11–25.39 | 4.58–40.35 | 2.08–67.78 |
| 40 | 8.21 | 15.47 | 8.57–26.83 | 4.84–42.66 | 2.19–71.67 |
| Lynch Cove | | | | | |
| 25 | 25.44 | 39.44 | 21.73–68.5 | 11.61–111.42 | 5.17–188.3 |
| 30 | 26.86 | 41.89 | 23.08–72.74 | 12.35–118.32 | 5.51–200.09 |
| 35 | 27.85 | 43.66 | 24.07–75.8 | 12.89–123.31 | 5.75–208.7 |
| 40 | 28.13 | 44.16 | 24.35–76.67 | 13.04–124.72 | 5.82–211.15 |

Average densities estimated in the North region of Hood Canal were roughly one third those observed by Goodwin and Pease (1991) in central and south Puget Sound. Nevertheless, densities are far greater in the North region near the mouth of the fjord (north of Quatsop Point) than in any other portion of Hood Canal, and southern regions, which experience more frequent and intense periods of LDO and hypoxia, had the lowest geoduck densities. Differences in the availability of optimal substrate magnify disparities in total abundance between northern and southern regions, because sand is proportionally more available in the North region, where geoducks are more abundant, than all other regions combined. Peak mean densities were approximately 0.3 geoduck/m², although local densities are highly variable, reaching 2 geoducks/m² in some locales. That said, the analyses distinguished between the effects of substrate and region on local geoduck densities to account for the availability of preferred substrate. Notably, densities were greatly reduced in southern regions of Hood Canal, even in areas that otherwise had optimal (sand) substrate.

Although commercial harvest of geoducks has occurred in Hood Canal since 1970, fishing pressure does not explain the pattern of distribution and abundance described here. In fact, fishing intensity has historically been—and continues to be—greatest in the North region, and geoducks have never been harvested commercially in the South and Lynch regions (Washington Department of Fish and Wildlife, unpubl. data). Moreover, shell aging of extant populations suggests that geoducks in southern regions are, on average, smaller and younger than their counterparts in northern regions, and patches of relict shell indicative of a mass mortality event have been observed in some locations of southern Hood Canal (Valero 2011). Mass mortality of bivalves has been attributed to bottom-water hypoxia in several coastal embayments (e.g., Buzzelli et al. 2002, Seitz et al. 2009). Given the data and other

evidence presented here, the most parsimonious explanation for the pattern of distribution and abundance observed in this study is that current geoduck populations are responding to environmental gradients in Hood Canal, most notably dissolved oxygen. Geoducks, like other bivalves, have limited behavioral responses available to reduce exposure to potentially lethal hypoxia events (e.g., Long et al. 2008), and previous work has already demonstrated significant impacts of LDO on other sessile organisms in Hood Canal (Essington & Paulsen 2010).

Although patches of relict shell suggest that southern Hood Canal once harbored greater geoduck densities, this study and recent work (e.g., Valero 2011) suggest that current conditions may prohibit recovery of geoduck populations presumably affected by past mass mortality events. Local bathymetry and patterns of geoduck larval advection and diffusion do not favor large recruitment pulses of exogenous larvae to the region. Simulation models indicate substantial transport potential for larval propagules released in the North region, but dispersal distance within southern Hood Canal, including Lynch Cove, is very limited (Valero 2011). In general, the pattern reflects the slow exchange between regions and the particularly long residence time of water in southern Hood Canal (about 85.5 days [Babson et al. 2006]).

Bayesian habitat models are useful for estimating abundance of patchy species with strong habitat affinities. The analysis described here considered uncertainty and precision of region-specific density estimates explicitly. Because of patchiness in geoduck densities observed in surveys, modeled distributions were broad, which contributed to the range of population estimates. Estimating abundance from field studies required several assumptions that can influence absolute abundance estimates, yet relative patterns in geoduck distribution were robust to those assumptions. In general, geoduck densities were assumed to follow a nonmonotonic pattern with depth,

first increasing and then decreasing at depths greater than approximately 10 m. Error distributions on estimated quantities (e.g., mean densities) were also assumed to follow a Poisson probability density function typical of count or density data, reflecting the high probability of observing few individuals and the long tail of decreasing probabilities of high densities of geoducks.

Results of the current study suggest that future work should examine the likely impact of environmental stressors, including deteriorating water quality, on the abundance and distribution of geoducks and other sessile organisms in Hood Canal. Monitoring for and assessing impacts require suitable, scalable methods for estimating abundance of these cryptic and frequently patchy organisms. An additional ethical consideration for routine monitoring in ecosystems under stress is the degree to which sampling methods create further disturbance; common sampling gear can disrupt substrate and damage or kill associated fauna (Gosling 2003). Although visual methods provide an alternative to dredges and grabs, such monitoring is a particular challenge for organisms inhabiting depths beyond routine safe limits for the use of scuba. Concordance

between scuba and tethered camera results in the current study (Fig. 2) suggest these methods are complementary. Overall this work demonstrates the utility of using a combination of scuba, tethered video, and Bayesian habitat models to develop estimates of regional population abundance that consider uncertainty and precision of the survey methods.

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REDUCED GENETIC VARIATION AND DECREASED EFFECTIVE NUMBER OF BREEDERS IN FIVE YEAR-CLASSES OF CULTURED GEODUCKS (*PANOPEA GENEROSA*)

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ABSTRACT Aquaculture for the Pacific geoduck (*Panopea generosa*) is a small but expanding industry in Washington state, where geoducks are native and genetic interactions between wild and cultured geoducks are likely. To examine the potential genetic implications of geoduck aquaculture, genetic diversity, and effective number of breeders (N_b), five contiguous year-classes of cultured geoducks were compared with a wild population. The results from five microsatellite loci indicate the cultured year-classes exhibited reduced allelic richness and N_b as well as increased mean pairwise genetic relatedness. However, examination of relationships within year-classes using sibship assignment revealed that many parents contributed progeny to each year-class. The geoducks in each year-class were comprised of 9 to 25 full-sib groups as well as a large number of individuals unrelated to others at the full-sib level. No clear pattern emerged regarding changes in genetic diversity during the 5-y time span of this study. To decrease the genetic risk to wild geoducks, the results suggest that hatcheries should increase the genetic diversity of cultured geoducks by adopting a partial factorial mating scheme, or they should minimize gene flow from cultured to wild populations by culturing sterile triploid geoducks.

KEY WORDS: geoduck, *Panopea generosa*, aquaculture, effective population size, effective number of breeders

INTRODUCTION

The culture of native taxa is often advocated as a way to reduce negative environmental impacts of aquaculture (e.g., Naylor et al. 2001, De Silva et al. 2009); however, culture of native species also carries risks, including genetic risks (Utter & Epifanio 2002, Hedgecock & Coykendall 2007, Camara & Vadopalas 2009). If wild populations exhibit local adaptation, aquaculture may homogenize these groups and reduce overall fitness through outbreeding depression (e.g., Gilk et al. 2004, Tymchuk et al. 2007, Roberge et al. 2008). In addition, because cultured shellfish tend to exhibit lower genetic diversity than their wild counterparts (e.g., Evans et al. 2004, Li et al. 2007, Lemay & Boulding 2009, Lind et al. 2009), genetic introgression from cultured to wild conspecifics may reduce the genetic diversity of wild populations (Allendorf & Ryman 1987, Hedgecock & Coykendall 2007, Camara & Vadopalas 2009).

Aquaculture for geoducks [*Panopea generosa* Gould, 1850, formerly *Panopea abrupta* Conrad, 1849 (Vadopalas et al. 2010)] is an expanding industry in Puget Sound, Washington. Wild geoduck populations are common in this region, where they support an economically valuable fishery (Hoffmann et al. 2000, Washington Department of Natural Resources 2000) and influence the ecosystem through filter feeding and biodeposition, as documented in other bivalves (Newell 2004, Norling & Kautsky 2007, Clavier & Chauvaud 2010). Geoduck aquaculture may put wild conspecifics at risk if (1) cultured geoducks mature and spawn before they are harvested, (2) culture occurs in close proximity to wild conspecifics, and (3) cultured geoducks are genetically distinct from wild geoducks. The first two conditions appear to have been met. Although estimates of geoduck maturation range from 2 y (Campbell & Ming 2003) to 8 y (Sloan & Robinson 1984), evidence from Puget Sound

suggests that cultured geoducks mature and spawn during the typical 5-y culture cycle, with 50% maturity in both sexes occurring at age 2 y, with concomitant gamete release (Vadopalas et al. 2015). Many geoduck farms are close enough to wild populations that cultured and wild gametes may interact directly. In addition, because geoduck larvae are pelagic for approximately 6 wk (Goodwin 1976), larvae of cultured provenance may settle broadly within Puget Sound. If these propagules survive to maturity, their gametes may interact with those of wild geoducks. Thus, geoduck aquaculture may put wild geoduck populations at genetic risk if cultured geoducks are genetically distinct from wild conspecifics. Previous work using allozymes and microsatellites revealed little evidence of neutral population structure among populations of geoducks from Puget Sound (Vadopalas et al. 2004, Miller et al. 2006). Thus, outbreeding depression and homogenization of populations are not of primary concern. However, these studies found very high microsatellite variation among geoducks. Although microsatellites are considered neutral markers, high microsatellite diversity may suggest high diversity in other genomic regions that could be perturbed by geoduck aquaculture.

Because wild geoducks likely serve many important ecological roles within Puget Sound and because they are the basis of a very valuable fishery, it is important that aquaculture does not develop at the expense of wild geoduck populations. The relatively new and small-scale (began in the mid 1990s; ~80 ha under cultivation [Washington Department of Natural Resources 2013]) geoduck aquaculture industry affords the opportunity to evaluate the potential for genetic risk of this activity. In this study, genetic diversity of cultured geoducks was compared with wild conspecifics. Specifically, five microsatellite markers were used to compare allelic richness, heterozygosities, effective number of breeders (N_b), and relatedness among a wild population and five year-classes of cultured geoducks. These geoducks, planted by the emerging geoduck

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aquaculture industry beginning in 1999, were sampled 1–5 y later; these samples represent hatchery seed geoducks planted on a farm and surviving for several years. Genetic diversity found in the samples is thus representative of geoducks cultured during this time period. The results provide insight into whether culture practices effectively maintain genetic diversity observed in wild geoducks—information that is essential for sustainable management of this emerging industry.

MATERIALS AND METHODS

Tissue Samples, DNA Extraction, Polymerase Chain Reaction, and Genotyping

In 2004, 96 cultured geoducks, age 1–5 y, comprising the 1999 to 2003 year-classes were collected from a geoduck farm on Hartstine Island, Puget Sound, Washington. Wild geoducks ($n = 96$) from a proximate wild aggregation were obtained for a previous study. Both cultured and wild geoducks were collected by hand after using pressurized water to liquefy the sand substrate. Siphon tissue samples were taken from all samples and stored in 95% ethanol until DNA extraction.

DNA was extracted according to the protocol developed by Ivanova et al. (2006), with a few modifications. Vertebrate lysis buffer was used and the 5,000-g centrifuge steps were conducted at 1,928g (top speed of the centrifuge used) for 13 min (5 min, Ivanova protocol) and 5 min (2 min, Ivanova protocol). Eluted DNA was diluted 1:20 with LoTE buffer before use in polymerase chain reaction (PCR). Five microsatellite loci were amplified in all individuals using PCR (Table 1). PCRs were conducted in 10- μ L reactions containing 1 μ L diluted template DNA, 5 μ L 2X SensiMix (Bioline, London, UK). The final concentrations were 3 mM MgCl₂ and 0.5 μ M each primer (except for Pab 3, which had a final concentration of 0.25 μ M each primer). Thermal cycling was conducted in a DNA engine thermal cycler (Bio-Rad, Hercules, CA). Thermal cycling programs for all PCRs began with an initial denaturation step of 95°C for 10 min followed by five cycles of 95°C for 30 sec, a locus-specific annealing temperature for 30 sec (Table 1), and 72°C for 30 sec, followed by 35 cycles of 90°C for 15 sec, locus-specific annealing temperature for 15 sec (Table 1), and 72°C for 30 sec, with a final extension at 72°C for 40 min.

After amplification, 1 μ L PCR product was added to 3.9 μ L Hi-Di formamide (Applied Biosystems, Foster City, CA) and 1 μ L GeneScan 500 LIZ size standard (Applied Biosystems), and

was denatured by heating to 95°C for 2 min followed by rapid cooling. These products underwent capillary electrophoresis on an Applied Biosystems 3730 automated sequencer. Allele sizes were calculated using GeneMarker v. 1.8 (SoftGenetics, State College, PA). Each plate was run with three control samples of known genotype to enable quantification of genotyping error.

Statistical Analysis

All analyses were performed on the wild geoduck collection and each of the five year-classes of cultured geoducks. In addition, data were analyzed examining 3-y-old, 4-y-old, and 5-y-old geoducks together as a single group because these three year-classes potentially interbreed during a typical 5-y geoduck culture cycle. This group is referred to as the breeding group. Microchecker v. 2.2.3 (Van Oosterhout et al. 2004) was used to detect genotyping errors and calculate null allele frequencies. Expected and observed heterozygosities in each group were calculated using HW-Quickcheck (Kalinowski 2006), whereas both allele counts and allelic richness after rarefaction were estimated using HP-Rare (Kalinowski 2005). The nonparametric Kruskal-Wallis test (Zar 1999) was used to test for differences in mean allelic richness and average expected heterozygosity between hatchery and wild samples using R (R Core Team 2010). Significance was tested using the F test (Zar 1999). Differences were identified using the Nemenyi test, a nonparametric analog to the Tukey test for multiple comparisons (Zar 1999). Maximum likelihood pairwise estimates of relatedness were calculated using the program ML-Relate (Kalinowski et al. 2006). From these data, mean pairwise relatedness values were calculated using Microsoft Excel. Sibship was estimated in each geoduck group using a full-maximum likelihood model as implemented by Colony v. 2.0.0.1 (Wang 2004, Wang & Santure 2009). Colony assigns sibling relationships based on shared alleles given allele frequencies in the population and both null allele and genotyping error rates. The following parameters were specified for all Colony runs: polygamous males and females, long run length, full-likelihood analysis, high-likelihood precision, update allele frequencies during run, and no prior information.

Effective number of breeders (N_b) was estimated using three different methods. The linkage disequilibrium method (LD [Hill 1981]) was implemented in NeEstimator v. 1.3 (Peel et al. 2004). A sibship assignment-based method (sibship) was implemented in Colony v. 2.0.0.1 (Wang 2004, Wang & Santure 2009) and the parentage without parents method (PwoP [Waples & Waples 2011]) was implemented in Python v. 2.6.4 (Python Software Foundation, 2010) using relationship data generated in ML-Relate (Kalinowski et al. 2006).

RESULTS

More than 95% of samples were genotyped successfully at all five microsatellite loci. Individuals where amplification failed at any locus were removed from analysis. The average genotyping error was 1.1% but varied by locus, ranging from 0.00% in Pab 101e and Pab 112e–3.1% in Pab 6 (Table 1). Null allele rates varied per locus and population, and are shown in Table 2. Analysis using MicroChecker (Van Oosterhout et al. 2004) revealed no evidence of scoring error resulting from stutter or large allele dropout.

TABLE 1.

Microsatellite markers used for genetic analysis of *Panopea generosa*.

| Locus | Fluorescent label | T _A (°C) | Genotyping error | Reference |
|----------|-------------------|---------------------|------------------|------------------------------|
| Pab 3 | FAM | 60 | 0.015 | Vadopalas and Bentzen (2000) |
| Pab 6 | FAM | 56 | 0.031 | Vadopalas and Bentzen (2000) |
| Pab 101e | VIC | 58 | 0.000 | Miller et al. (2006) |
| Pab 106e | NED | 56 | 0.007 | Miller et al. (2006) |
| Pab 112e | PET | 56 | 0.000 | Miller et al. (2006) |

TABLE 2.
Genetic diversity statistics for wild and cultured *Panopea generosa*.

| | Wild | 1999 | 2000 | 2001 | 2002 | 2003 | Breeding |
|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| <i>n</i> | 96 | 93 | 92 | 92 | 91 | 94 | 281 |
| Pab 3 | | | | | | | |
| A | 31 | 21 | 23 | 22 | 22 | 23 | 28 |
| AR | 30.7 | 20.9 | 23.0 | 22.0 | 22.0 | 22.9 | 23.6 |
| H_e | 0.95 | 0.88 | 0.92 | 0.91 | 0.92 | 0.91 | 0.93 |
| H_o | 0.75 | 0.70 | 0.67 | 0.82 | 0.85 | 0.91 | 0.75 |
| Null | 0.104 | 0.099 | 0.128 | 0.047 | 0.040 | 0.000 | 0.115 |
| Pab 6 | | | | | | | |
| A | 33 | 18 | 18 | 20 | 22 | 18 | 24 |
| AR | 32.5 | 18.0 | 18.0 | 19.9 | 22.0 | 18.0 | 20.1 |
| H_e | 0.93 | 0.90 | 0.92 | 0.85 | 0.89 | 0.88 | 0.92 |
| H_o | 0.90 | 0.98 | 0.92 | 0.89 | 0.79 | 0.95 | 0.92 |
| Null | 0.000 | 0.000 | 0.000 | 0.000 | 0.052 | 0.000 | 0.000 |
| Pab 101e | | | | | | | |
| A | 21 | 17 | 17 | 13 | 19 | 16 | 18 |
| AR | 20.8 | 16.9 | 17.0 | 13.0 | 19.0 | 16.0 | 15.8 |
| H_e | 0.94 | 0.90 | 0.91 | 0.87 | 0.90 | 0.90 | 0.92 |
| H_o | 0.81 | 0.85 | 0.87 | 0.79 | 0.91 | 0.93 | 0.83 |
| Null | 0.065 | 0.000 | 0.000 | 0.045 | 0.000 | 0.000 | 0.059 |
| Pab 106e | | | | | | | |
| A | 44 | 29 | 28 | 27 | 31 | 34 | 40 |
| AR | 43.6 | 28.8 | 27.9 | 26.9 | 31.0 | 33.7 | 32.2 |
| H_e | 0.96 | 0.97 | 0.9 | 0.87 | 0.95 | 0.93 | 0.92 |
| H_o | 0.95 | 0.92 | 0.93 | 0.92 | 0.92 | 0.96 | 0.94 |
| Null | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Pab 112e | | | | | | | |
| A | 51 | 35 | 25 | 34 | 36 | 33 | 46 |
| AR | 50.2 | 34.7 | 25.0 | 33.9 | 36.0 | 32.7 | 35.9 |
| H_e | 0.97 | 0.91 | 0.92 | 0.9 | 0.93 | 0.94 | 0.95 |
| H_o | 0.97 | 0.9 | 0.89 | 0.97 | 0.97 | 0.99 | 0.93 |
| Null | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Mean | | | | | | | |
| A | 36 | 24 | 22.2 | 23.2 | 26 | 24.8 | 31.2 |
| AR | 35.6 | 23.9 | 22.2 | 23.1 | 26.0 | 24.7 | 25.5 |
| H_e | 0.95 | 0.91 | 0.91 | 0.88 | 0.92 | 0.91 | 0.93 |
| H_o | 0.88 | 0.87 | 0.86 | 0.88 | 0.89 | 0.95 | 0.87 |
| Null | 0.034 | 0.020 | 0.026 | 0.019 | 0.018 | 0.000 | 0.035 |

A, number of alleles; AR, allelic richness; H_e , expected heterozygosity; H_o , observed heterozygosity; Null, null alleles. Bold text for H_o indicates the population is significantly out of the Hardy–Weinberg expectation at this locus.

Genetic Diversity

Number of alleles (A), allelic richness (AR), observed heterozygosity (H_o), and expected (H_e) heterozygosity for Hardy–Weinberg equilibrium for each geoduck group at each locus are shown in Table 2. The wild geoduck collection and all year-classes of cultured geoduck were polymorphic at all loci but fewer alleles were observed in cultured than wild geoduck groups. Mean AR across the five loci (mean \pm 95% confidence interval [CI]) was 35.6 ± 10.1 in the wild collection, which was significantly greater than that observed in the cultured geoducks (mean AR across five seed cohorts, 24.0 ± 2.6 ; Kruskal–Wallis test, $P < 0.01$). On average, the cultured groups exhibited $32.6\% \pm 3.6\%$ fewer alleles than that observed in the wild aggregation. Mean AR in the breeding group (25.5 ± 6.6) was not significantly different than that found in the cultured year-classes.

Mean H_e across the five loci was greater in wild geoducks (0.95 ± 0.014) than in any seed cohort; however, this difference was only significant in the wild 2001 year-class comparison (mean H_e , 0.88 ± 0.021 ; Kruskal–Wallis test, $P < 0.001$). The breeding group (mean H_e , 0.93 ± 0.011) also showed significantly greater H_e than that observed in the 2001 year-class; H_e in the breeding group was not significantly different than the wild population or other individual year-classes. No differences in mean AR or H_e were observed among the year-classes of cultured geoducks (Kruskal–Wallis test, $P > 0.05$). Deviations from the Hardy–Weinberg expectations were observed in both wild and cultured geoducks and were characterized by both heterozygote deficiencies and heterozygote excess (Table 2).

Relatedness and Sibship

Mean pairwise relatedness values were significantly lower in the wild collection than in the cultured year-classes of geoducks (Fig. 1). The wild collection was characterized by a mean relatedness (mean \pm 95% CI) of 0.041 ± 0.002 , whereas the mean relatedness in the cultured geoduck groups ranged from 0.066 ± 0.003 – 0.083 ± 0.004 . The breeding group exhibited a significantly higher degree of relatedness than the wild group, but significantly lower relatedness than that observed in any individual year-class (0.061 ± 0.003).

Sibship reconstruction revealed that 93.7% of the wild geoducks were unrelated to any other individual in the sample at the full-sib level, with three full-sib pairs each comprising 2.1% of the population (Fig. 2). In contrast, in the cultured geoduck, between 37% (2001 year-class) and 55% (2002 year-class) of individuals were unrelated to other geoducks in the sample at the full-sib level. Both the number and size of full-sib families varied widely among the five year-classes of cultured geoduck. The 2001 year-class was characterized by 25 families, none of which included more than 5.4% of the year-class. In contrast, the 2003 year-class was comprised of nine full-sib families; one family constituted 13.8% and a second constituted 10.6% of the year-class. In the breeding group, 52% of individuals were unrelated to other geoducks in the sample at the full-sib level. Twenty-one families comprised the related proportion of the breeding group, with no single family including more than 4% of the total individuals (Fig. 2).

Effective Number of Breeders

The N_b estimates for each geoduck group are shown in Table 3. The N_b estimates varied widely according to the method used. The LD (Hill 1981) and PwoP (Waples & Waples 2011) methods gave similar N_b estimates for the cultured year-classes. Across the five cultured year-classes, mean N_b estimate (mean \pm 95% CI) was 57.0 ± 6.4 using the PwoP method and 46.4 ± 7.0 using the LD method. The sibship (Wang 2004, Wang & Santure 2009) method gave lower estimates; mean N_b across the five cultured year-classes was 22.4 ± 3.8 . In contrast, the wild collection was characterized by substantially higher N_b estimates using all three methods. The sibship and PwoP estimates were similar (N_b , 108 [95% CI, 77–152] and 120 [95% CI not provided by the program]), respectively, whereas the LD estimate was much larger [N_b , 3,241; 95% CI, 909– ∞].

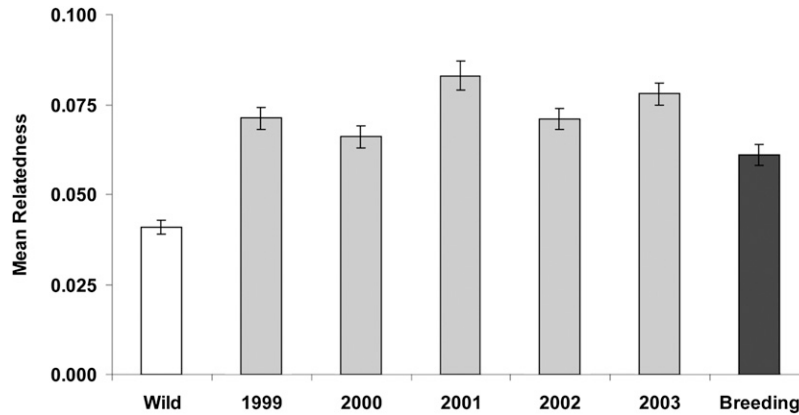


Figure 1. Relatedness values (mean \pm 95% confidence interval) for wild and cultured geoducks (hatchery year-classes 1999 to 2003 and the three year-class breeding group). The wild geoduck collection is shown with a white bar, individual year-classes are shown with gray bars, and the breeding group is shown with a black bar.

DISCUSSION

This study compared genetic diversity in five separate year-classes and an aggregate population (the “breeding group”) of cultured geoducks with an adjacent wild population. The three mature year-classes were combined into a breeding group because this better approximated the potentially interbreeding geoducks than looking at individual year-classes in isolation, and enabled a more realistic assessment of potential genetic impacts of geoduck aquaculture on wild conspecifics.

Results reveal that cultured geoducks exhibit decreased genetic diversity as evidenced by reduced AR, increased relatedness, and reduced N_b when compared with a wild population.

The lower genetic diversity is characterized by reduced AR; the five cultured year-classes exhibit an average of 32.6% fewer alleles than the wild aggregation (Table 2). A comparable reduction in allelic richness (28.2%) was also observed in the breeding group. Although each year-class had significantly lower AR than the wild aggregation, across all loci, only 22.4 private alleles (12.6%) found in the wild aggregation are absent from all five year-classes combined. The cultured groups as a whole thus retained more of the low-frequency alleles present in the wild population than any single year-class. In addition, 15.5 private alleles were found in the cultured year-classes that were not found in our sample of wild geoducks. That numerous private alleles were found in both the wild and the cultured groups emphasizes the magnitude of diversity found at these microsatellite alleles in geoducks. Comparable declines in AR

have been reported previously in cultured shellfish, including abalone (Evans et al. 2004, Lemay & Boulding 2009) and oysters (Lind et al. 2009). Such declines are worrisome because reduced diversity at microsatellite loci may indicate reduced diversity at other areas of the genome and may imply reduced adaptive potential. Observed decreases in allelic richness are often seen in conjunction with significant declines in expected heterozygosity (Li 2004, Hara & Sekino 2007, Lemay & Boulding 2009). In contrast, the current study demonstrated a significant reduction in H_e in only one of the five hatchery year-classes. This pattern has also been reported in aquaculture settings (Evans et al. 2004, Lind et al. 2009) and may indicate a short-term genetic bottleneck (Nei et al. 1975, Allendorf 1986). Bottlenecks are expected in even the first hatchery generation because cultured groups simply cannot contain all the alleles present in a large wild population.

The N_b is a parameter of central importance in conservation biology because it influences the degree of genetic drift and inbreeding that will occur in a population and is intimately related to a population’s persistence probability (Wright 1931, Wright 1938, Frankham et al. 2002). Although estimates of N_b varied widely depending on the method used (Table 3), N_b estimates for the wild collection were substantially greater than those for the cultured year-classes. Depending on the method used, N_b estimates for the wild population were about twofold (PwoP [Waples & Waples 2011]), fivefold (sibship [Wang 2004, Wang & Santure 2009]), or 70-fold greater (LD [Hill 1981]) than

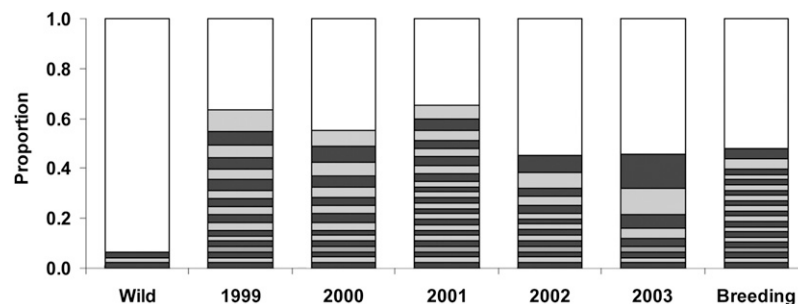


Figure 2. Full-sib assignment in wild and cultured geoduck groups. The proportion of individuals not related to any other individuals at the full-sib level is shown in white. The gray and black bars represent the proportion of individuals assigned to each different full-sib family.

TABLE 3.

Effective number of breeders (N_b) in wild and cultured *Panopea generosa* groups estimated using three methods: parentage without parents (Waples & Waples 2011), linkage disequilibrium (Hill 1981), and sibship assignment (Wang 2004, Wang & Santure 2009).

| Group | Parentage without parents | | Linkage disequilibrium | | Sibship assignment | |
|--------------------------|---------------------------|-----------|------------------------|-----------|--------------------|-----------|
| | N_b | 95% CI | N_b | 95% CI | N_b | 95% CI |
| Wild | 120 | | 3,241 | 909–∞ | 108 | 77–152 |
| 1999 | 55.7 | | 41.5 | 38.1–45.3 | 17 | 10–34 |
| 2000 | 61.6 | | 50.2 | 45.3–55.9 | 22 | 13–40 |
| 2001 | 54.5 | | 34.9 | 32.2–37.9 | 21 | 12–39 |
| 2002 | 46.8 | | 54.7 | 49.8–60.2 | 29 | 18–49 |
| 2003 | 66.2 | | 50.6 | 46.2–55.7 | 23 | 14–42 |
| Cultured year-class mean | 57.0 | 50.6–63.4 | 46.4 | 39.4–53.4 | 22.4 | 18.6–26.2 |
| Breeding | 56.1 | | 50.6 | 46.2–55.7 | 32 | 19–52 |

the N_b estimates for the cultured year-classes. The conservation implications of reduced N_b vary dramatically depending on the magnitude of the N_b reduction. Further work is needed to understand the variation in N_b estimates and to determine which is the most appropriate for predicting the genetic risks of geoduck aquaculture.

The five year-classes of cultured geoduck exhibited greater mean relatedness than the wild collection (Fig. 1). Although mean relatedness values in the cultured year-classes were 1.5–2 times greater than that observed in the wild aggregation (0.041), overall relatedness values remained relatively low in each year-class (0.066–0.083). These numbers are lower than have generally been reported for cultured shellfish. For example, although wild silver-lipped pearl oysters (*Pinctada maxima*) exhibit relatedness values of 0–0.01, cultured *P. maxima* show relatedness values ranging from 0.07–0.28, with all but one group more than 0.15 (Lind et al. 2009). Abalone (*Haliotis rubra* and *Haliotis midae*) relatedness in six cultured groups ranged from 0.16–0.44, although relatedness in a seventh cultured group was estimated to be zero (Evans et al. 2004). Relatedness in cultured Pacific lion-paw scallops (*Nodipecten subnodosis*) ranged from 0.15–0.55 whereas wild conspecifics exhibited relatedness values ranging from 0–0.06 (Petersen et al. 2010).

Analysis of the sib group assignments (Fig. 2) sheds some light on how these low levels of relatedness may have been achieved. In each of the five hatchery year-classes, 35%–55% of geoducks were not related to a single other geoduck from the study sample at the full-sib level. These numbers are surprising given the extremely high fecundities of geoducks (estimated at 40 million eggs per year [Beattie 1992]) that would theoretically enable hatchery personnel to produce ample geoduck seed using only a few broodstock. In fact, previous studies of other cultured molluscan shellfish revealed that very few parents produce an extremely large proportion of the progeny (e.g., Selvamani et al. 2001, Boudry et al. 2002, Lemay & Boulding 2009, Lind et al. 2009). In contrast, the Washington state geoduck hatchery that produced these seed must have spawned quite a large number of broodstock and successfully husbanded larvae and seed to ensure survival of many different families. The study sib group assignments bear this out. In addition to the large proportion of individuals unrelated to others at the full-sib level, 9–25 full-sib groups comprise each year-class, with

more than 50% of these groups made up of only two individuals. No clear pattern emerged regarding changes in relatedness over the 5-y time span of this study. The most recent year-class of this study (2003) exhibits a high proportion of unrelated individuals (54%), and also exhibits among the highest relatedness (0.078). This apparent contradiction is a result of family size; the two largest full-sib groups are observed in this year-class, comprising 13.8% and 10.6% of the total.

The decreased genetic diversity in cultured geoducks observed as reduced AR, increased relatedness, and reduced N_b suggests that intraspecific introgression from cultured to wild geoducks may reduce the genetic diversity of wild populations (Allendorf & Ryman 1987, Ryman & Laikre 1991, Lynch & O'Hely 2001, Ford 2002, Hedgecock & Coykendall 2007, Camara & Vadopalas 2009). When wild and cultured populations are more differentiated, the potential for negative genetic interactions between wild and cultured populations is increased. Lynch and O'Hely (2001) modeled these dynamics and demonstrated that even low levels of gene flow from cultured to wild populations would likely shift the average phenotype of the wild population toward the average culture phenotype. The likelihood that the observed genetic diversity in cultured geoducks will reduce the genetic diversity in wild geoducks will be estimated via a genetic risk model specific to geoducks, currently under development. In the meantime, the aquaculture industry can make two changes to decrease the genetic risk to wild geoducks: (1) increase the genetic diversity of cultured geoducks and (2) minimize gene flow from cultured to wild populations.

Changing the fertilization protocol in geoduck hatcheries can likely increase the genetic diversity of, and decrease divergence from, wild populations. Fertilizing with pooled sperm, a common practice in shellfish hatcheries, can increase the variance in reproductive success and decrease the effective number of breeders resulting from sperm competition (Withler 1988, Withler & Beacham 1994, Campton 2004). In oysters, Boudry et al. (2002) estimated that this practice was responsible for a 20% decrease in effective population size. Isolating both males and females to release gametes individually would enable factorial crosses and avoid sperm competition. A complete factorial breeding scheme without equalizing family size comes

closest to the goal of maintaining genetic diversity while maximizing progeny production (Fiumera et al. 2004, Busack & Knudsen 2007), but partial factorial designs as small as two by two provide many of the benefits of full-factorial mating schemes (Busack & Knudsen 2007) and may be more manageable for hatchery personnel to conduct.

An alternate strategy to reduce the genetic risk of geoduck aquaculture would be to culture only sterile geoducks and thus minimize the gene flow from cultured to wild geoducks. Sterility can be conferred on shellfish via triploid induction, and triploid shellfish have been used extensively in aquaculture because they exhibit reduced or absent gametogenesis and often show increased growth (Brake et al. 2004, Nell & Perkins 2005, Mallia et al. 2006). Triploidy techniques developed for geoducks (Vadopalas & Davis 2004) appear to confer sterility (Vadopalas & Davis, unpublished) and are currently undergoing further evaluation.

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ECOLOGICAL EFFECTS OF THE HARVEST PHASE OF GEODUCK (*PANOPEA GENEROSA* GOULD, 1850) AQUACULTURE ON INFAUNAL COMMUNITIES IN SOUTHERN PUGET SOUND, WASHINGTON

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ABSTRACT Intertidal aquaculture for geoducks (*Panopea generosa* Gould, 1850) is expanding in southern Puget Sound, Washington, 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, the authors used a “before–after–control–impact” experimental design, emphasizing spatial scales comparable with those used by geoduck culturists to evaluate the effects of harvesting market-ready geoducks on associated benthic infaunal communities. Infauna were sampled 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 the edges of cultured plots to assess the effects of harvest activities in adjacent uncultured habitat. Using multivariate statistical approaches, strong seasonal and spatial signals in patterns of abundance were found, but there was scant evidence of effects on the community structure associated with geoduck harvest disturbances within cultured plots. Likewise, no indications of significant “spillover” effects of harvest on uncultured habitat adjacent to cultured plots were noted. Complementary univariate approaches revealed little evidence of harvest effects on infaunal biodiversity and indications of modest effects on populations of individual infaunal taxa. Of 10 common taxa analyzed, only three showed evidence of reduced densities, although minor, after harvests whereas the remaining seven taxa indicated either neutral responses to harvest disturbances or increased abundance either during or in the months after harvest events. It is suggested 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.

KEY WORDS: aquaculture, benthic, disturbance, extralimital, geoduck, infauna, intertidal, *Panopea generosa*, Puget Sound, spillover

INTRODUCTION

Aquaculture operations are proliferating and diversifying in nearshore marine habitats across the globe (e.g., Naylor et al. 2000, Chopin et al. 2001, Goldberg & Naylor 2005, Buschmann 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 & Fresh 1995, Newell 2004, Sara 2007, Dumbauld et al. 2009, Forrest et al. 2009, Coen et al. 2011, Hedgcock 2011). Since the early 2000s localized but intensive political controversy has emerged in communities near southern Puget Sound, Washington, regarding development of geoduck (*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\$20 million. As a consequence of expanding geoduck aquaculture operations,

many questions and concerns have emerged regarding ecological effects of harvesting activities.

The focus of the current study was on the evaluation of possible ecological changes to marine ecosystems as a result of habitat disturbances associated with geoduck aquaculture activity in southern Puget Sound. Ecological disturbance is considered here 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, p. 8). Disturbances in general 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 placed PVC tubing extending above the sediment surface. Young

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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/m². The tubes and netting are removed 1–2 y 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 y 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 seawater 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 sec 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-size 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 unharvested substrata, causing extralimital changes in benthic communities. There is significant management interest in potential “spillover” effects of geoduck harvest, particularly relating to the regulation of the spatial scope of cultured geoduck plots and the 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 after harvest. The export of benthic organisms, sediment, detritus, and nutrient materials could affect resident infaunal populations at intensities varying with distances from the edges of harvested plots.

Reported here are the results of a field study to determine whether geoduck aquaculture harvest operations alter benthic infaunal invertebrate assemblages of intertidal sandflats in southern Puget Sound. Infaunal assemblages as response variables were chosen for three reasons. First, the opinion of the authors *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 the current 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.

Three related hypotheses (identified by number in the subsequent text) were tested 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 for 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 after completion of harvests as a consequence of harvest-related disturbances.

MATERIALS AND METHODS

Study Areas

The study was conducted at intertidal locations in the southern basin of Puget Sound, Washington. 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). The surface area of the basin is 449 km² at mean high water, including 67.4 km² 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 2.7–3.2 m in a mixed semidiurnal pattern (Mofjeld et al. 2002), with a maximum range of 6.5 m for single tidal exchanges at the extreme southern limit of the basin (National Ocean Service, National Oceanic and Atmospheric Administration 2014). Surface water temperatures range annually from ~8 to ~16°C, and salinities range from 27–30, with the exception of periods of dilution from riverine flooding (Collias et al. 1974, Dethier & Schoch 2005).

Three study sites were chosen (Fig. 1) based on three criteria. First, selected sites were involved in production-scale commercial aquaculture at the time of the anticipated field sampling. The study site selections had the purpose of fostering relevance of the current 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, which allowed sampling before, during, and after harvest at

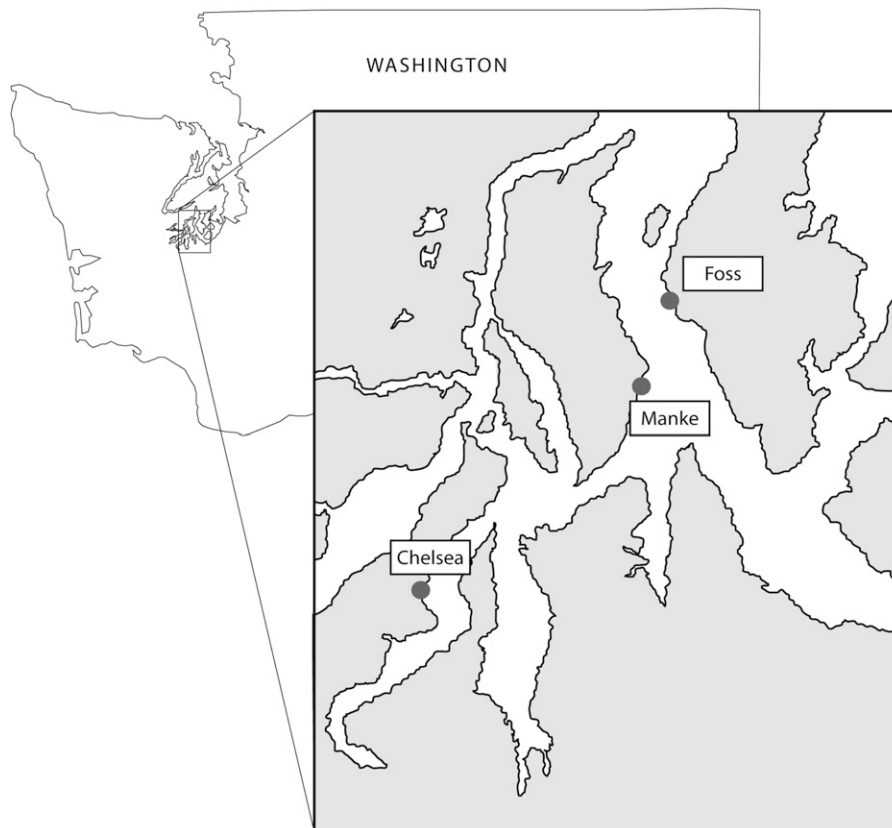


Figure 1. Locations of study sites in southern Puget Sound, Washington. Coordinates (latitude and longitude) for each site are provided in the text. Shaded areas are land; white areas are water.

treatment and adjacent reference plots in time periods ≤ 30 mo. 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 used 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, WA) specifically for geoduck aquaculture at the time of the current study. “Chelsea” (47.13° N, 122.96° W) was on the northwestern shore of Eld Inlet. At the time of this study, the cultured plot at Chelsea was owned by Chelsea Farms, LLC (Olympia, WA), 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 geoducks. Neither Taylor Shellfish, Inc., nor Chelsea Farms, LLC, made any effort whatsoever to influence study design, sampling procedures, generation and analysis of resulting data, or interpretations of results as provided herein or elsewhere.

Sampling Design and Methods

We used a “before–after–control–impact” design (Green 1979), establishing a cultured (i.e., “impact”) plot containing

mature geoducks and an unplanted reference (i.e., “control”) plot, each measuring at least $2,500 \text{ m}^2$, at each of the three sites. Cultured plots at each site were subject to geoduck harvest throughout the course of the study whereas reference plots experienced no harvest activity. None of the study plots had been used for geoduck aquaculture prior to this 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 resulting from location, and simultaneously provided adequate separation distance to reduce potential extralimital effects of the harvest process on the reference plot (Fig. 2A). Plots were marked with PVC stakes at the two shoreward corners. Cultured and reference plots were divided into 100×100 -unit Cartesian grids, and 10 sampling points were selected randomly 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 the shoreline for a distance 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 geoducks or other types of aquaculture except at Chelsea, where the first 10 m of the transect crossed over a young cohort of

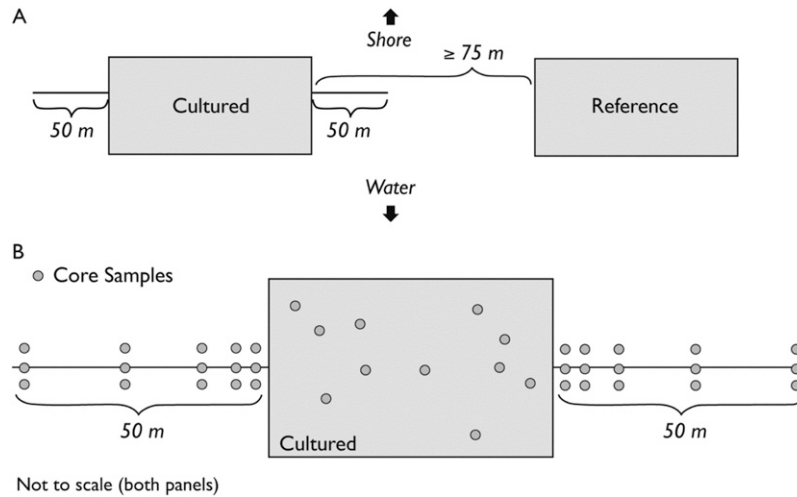


Figure 2. Diagram of physical layout (plan view) used for each of the three study areas. (A) Relative positions of cultured and reference plots at each site and placement of extralimital transects at Foss (only one transect was established at Manke and Chelsea, respectively). (B) Example random placement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

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, and 60 m at Chelsea). At each distance, one 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 resampling the same point during subsequent sampling events.

Benthic core samples were 5 cm in diameter with a surface area of 19.6 cm², a depth of 10 cm, and a volume of 196 cm³. All contents of each core sample were placed unscreened in 500-mL jars and preserved in 10% buffered formalin solution immediately after collection. According to 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- μ m 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 to be 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. Infaunal biomass densities were not estimated in the current 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. The 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 after completion of the harvest. The study design did not include sampling targeted specifically to times immediately after 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 as a result of 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 square meter). 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 later. 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 plots vs. 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 (preharvest), during harvest (midharvest or harvest period), and after harvest (postharvest).

For assessment of extralimital effects of harvesting based on transect sampling, categories were treatment (samples collected in cultured plots and reference plots vs. 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, with subcategories as indicated earlier).

Patterns of abundance in a species of particular interest in a management context—the benthic gammaridean amphipod

TABLE 1.

Dominant infaunal taxa in core sample data selected on the basis of frequencies of occurrence or (for *Americorophium salmonis*) ecological significance.

| Taxon | Category | Frequency | Ecological notes |
|---|---------------------|-----------|------------------|
| <i>Americorophium salmonis</i> (Stimpson, 1857) | Amphipod crustacean | 0.71 | TD, SDSS |
| <i>Cumella vulgaris</i> (Hart, 1930) | Cumacean crustacean | 0.92 | EFDF |
| <i>Rochefortia</i> spp. Vélain, 1877 | Bivalve mollusc | 0.98 | CTD, SF |
| <i>Micrura</i> 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 |
| Polynoidea Malmgren, 1867 | Polychaete annelid | 0.81 | MCOS |

Frequency calculations are based on all core samples taken during all sampling events within cultured and reference plots at all three study sites during the study. In the Spionidae, mode of habit (tube dweller or mobile) varies by species. 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.

Americorophium salmonis (Stimpson, 1857)—were evaluated along with organisms occurring frequently in samples. The amphipod *A. salmonis* is known to be an important prey species for juvenile out-migrating salmonid fish populations in Puget Sound, particularly Chinook salmon (*Oncorhynchus tshawytscha* [Walbaum, 1792]).

Multivariate Analyses

Permutation-based analyses of variance (perMANOVAs [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 \times treatment interaction term indicated an effect of the harvest state on one of the treatments—specifically, the effect of the midharvest 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 plot data and locations on extralimital transects. Homogeneity of multivariate dispersion 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 and Cohen (1991). In addition, a number of environmental impact studies have reported that the variability of species

TABLE 2.

Mean densities (measured in individuals per square meter SE) rounded to nearest integer by site and plot type for all sampling dates during the study as determined from core samples.

| Taxon | Foss | | Manke | | Chelsea | | Culture mean | Reference mean | Overall mean |
|--------------------------------|-------------|---------------|---------------|-------------|---------------|-------------|--------------|----------------|--------------|
| | Culture | Reference | Culture | Reference | Culture | Reference | | | |
| <i>Americorophium salmonis</i> | 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) |
| <i>Cumella vulgaris</i> | 567 (194) | 490 (127) | 435 (80) | 1,531 (307) | 1,611 (540) | 1,630 (637) | 862 (203) | 1,291 (254) | 1,077 (163) |
| <i>Rochefortia</i> 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) |
| <i>Micrura</i> 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 (2,501) | 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) |
| Polynoidea | 97 (33) | 146 (58) | 123 (26) | 332 (56) | 187 (51) | 207 (88) | 137 (22) | 242 (41) | 190 (24) |

Listed taxa are those identified and described in Table 1.

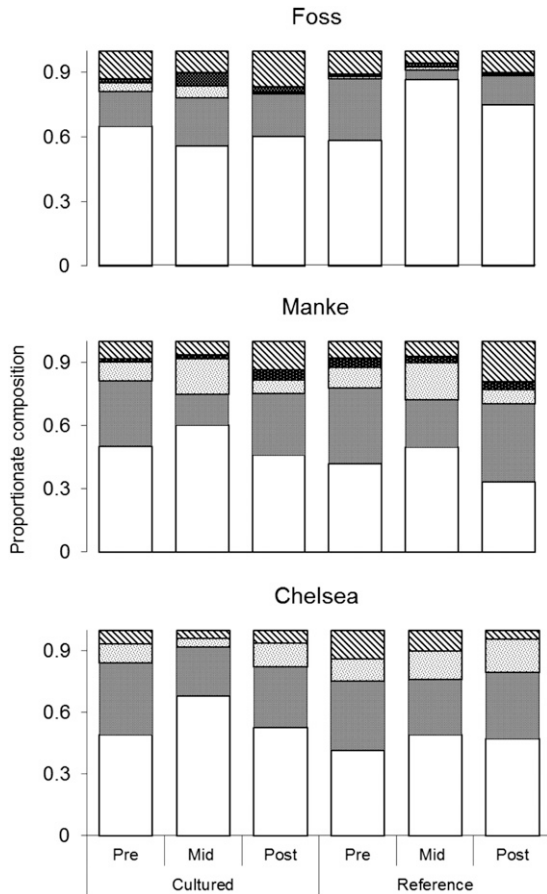


Figure 3. Taxonomic composition of all infauna summed as proportions of numbers of individuals in samples in cultured and reference plots during preharvest, midharvest, and postharvest states at each study site. In each plot, taxonomic categories are from bottom to top, polychaetes, crustaceans, bivalves,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

abundance in samples collected from disturbed areas was greater than the variability of samples collected from undisturbed 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 because averaging samples could mask important intersample 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), the Shannon-Wiener index, and the 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 (ANOVAs) 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). Analysis of variance methods have been applied in a number of other studies with data characteristics similar to ours (e.g., Smith & Brumsickle 1989, Warwick et al. 1990, Thrush et al. 1996, Kaiser et al. 1996, Anderson & Underwood 1997, Kaiser et al. 2006).

Generalized linear mixed models (McCullagh & Nelder 1989) were used assuming Poisson-distributed data to examine the factors contributing to abundance of selected individual infaunal taxa from our core samples. These analyses were applied to *Americorophium salmonis* and the nine other individual taxa (species, genera, or families) identified from high frequencies of occurrence in core samples (Table 2). For 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 used to compare models formally, including the harvest state \times 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, 50 discernible animal taxa were identified in the samples. The numerically dominant taxa were generally small (maximum length of individuals, <1 cm) 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 (Fig. 3). Taxonomic compositions of the samples generally resembled those reported previously for southern Puget Sound (Dethier et al. 2003, Dethier 2005, Dethier & Schoch 2005, Dethier 2010, Dethier et al. 2010, Dethier et al. 2012).

Multivariate Contrasts by Site and Plot Type

Infaunal abundance was 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 and Fig. 4, top and middle panels). At Chelsea the core sample data from the two plots did not differ significantly (perMANOVA; Table 3 and Fig. 4, bottom panel).

The 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 \times treatment were found to be significant (perMANOVA; Table 3). For

TABLE 3.
Summary of permutation-based analyses of variance results
for contrasts at scales of study sites and plots.

| Scale | Contrast | R^2 | df | P value |
|---|-------------------|-------|------|-----------------------|
| Among sites | All sites | 0.37 | 2 | <0.001 |
| | Foss vs. Manke | 0.19 | 1 | <0.001 |
| | Foss vs. Chelsea | 0.44 | 1 | <0.001 |
| | Manke vs. Chelsea | 0.27 | 1 | <0.001 |
| Among sites within plot type, cultured plots | Foss vs. Manke | 0.19 | 1 | <0.001 |
| | Foss vs. Chelsea | 0.41 | 1 | <0.001 |
| | Manke vs. Chelsea | 0.24 | 1 | <0.001 |
| Among sites within plot type, reference plots | Foss vs. Manke | 0.39 | 1 | <0.001 |
| | Foss vs. Chelsea | 0.56 | 1 | <0.001 |
| Within site between plot type, by treatment | Foss | 0.41 | 1 | <0.001 |
| | Manke | 0.45 | 1 | <0.001 |
| | Chelsea | 0.09 | 1 | NS |
| Within site between plot type, by date | Foss | 0.60 | 10 | $0.01 \leq P < 0.05$ |
| | Manke | 0.62 | 16 | <0.001 |
| | Chelsea | 0.75 | 13 | <0.001 |
| Within site between plot type, by harvest state | Foss | 0.18 | 2 | $0.01 \leq P < 0.05$ |
| | Manke | 0.17 | 2 | <0.001 |
| | Chelsea | 0.08 | 2 | NS |
| Within site between plot type, harvest state \times treatment interaction | Foss | 0.02 | 2 | NS |
| | Manke | 0.03 | 2 | NS |
| Within site within plot type, by date, cultured plots | Foss | 1.00 | 10 | <0.001 |
| | Manke | 1.00 | 16 | <0.001 |
| | Chelsea | 1.00 | 13 | <0.001 |
| Within site within plot type, by harvest state, cultured plots | Foss | 0.25 | 2 | NS |
| | Manke | 0.25 | 2 | <0.001 |
| | Chelsea | 0.13 | 2 | NS |
| Within site within plot type, by date, reference plots | Foss | 1.00 | 10 | <0.001 |
| | Manke | 1.00 | 16 | <0.001 |
| | Chelsea | 1.00 | 13 | <0.001 |
| Within site within plot type, by harvest state, reference plots | Foss | 0.32 | 2 | $0.01 \leq P < 0.05$ |
| | Manke | 0.25 | 2 | $0.001 \leq P < 0.01$ |
| | Chelsea | 0.11 | 2 | NS |

NS: $P \geq 0.05$.

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 \times treatment interaction term is the uniquely informative metric for assessment of harvest effects within the current study design. Analytical results were inconsistent with hypotheses 1 and 3 as defined earlier. 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 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 were 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 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

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

Significant effects of harvest state \times 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, and 7). In comparison, 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 hypothesis 2.

Within each site, the HMD values for community data from the preharvest 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 midharvest state whereas values in reference plots either remained relatively constant or decreased. For both sites, HMD calculations for cultured plots during the midharvest state were significantly different from values at most transect distances and the reference plot (Table 9). During the postharvest state at Foss, HMD values in the cultured plot remained high whereas values for most transect locations and the reference plot returned to near preharvest levels. At Manke, postharvest HMD values were similar to preharvest values at most transect distances and in cultured and reference plots. Homogeneity of multivariate dispersion values increased for most distances on the Chelsea transect during the midharvest state. However, permutation tests revealed that infaunal data from Chelsea were most similar among locations during midharvest (Table 9). In summary, HMD analyses for transect data were generally 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 (Fig. 6, top and bottom panels). At Manke, index values fluctuated more among dates on both plots, but the cultured plot had consistently lower diversity indices (Fig. 6, middle panel). When diversity values were averaged by harvest state, there was a mixture of significant and nonsignificant values in contrasts between plots for each harvest state and within plots among harvest states (Table 10).

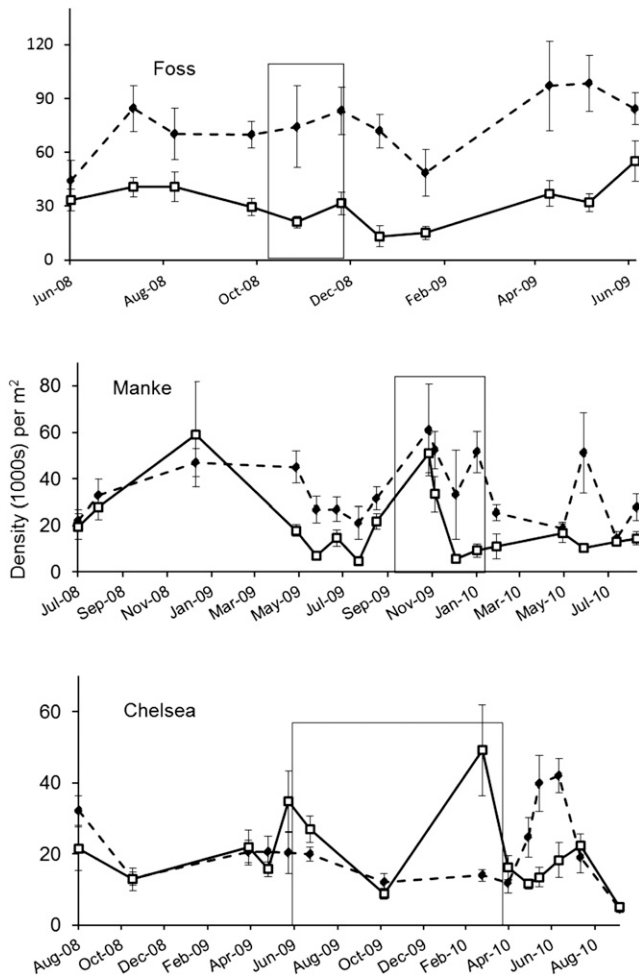


Figure 4. Mean densities of all infauna summed as thousands of individuals per square meter (± 1 SE) from samples in each plot for each sampling date at each study site. Data from cultured plots are shown with white boxes and solid lines, and from reference plots with black diamonds and dashed lines. Vertically oriented rectangles represent midharvest periods on cultured plots. Note that scales on both the horizontal and vertical axes differ among study sites.

Species-specific contrasts, using generalized linear mixed models, provided results in six categories for the 10 taxa analyzed (Table 11). As noted the analyses were based on the protocol that a significant interaction result for harvest state \times 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 abundance in harvest plots compared with reference plots both during and after harvest activities. Conversely, two other taxa, the bivalve genus *Rochefortia* and the polychaete family Phyllodocidae, experienced reductions in harvest plots compared with 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 after 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 compared with reference plots, but the effect did not persist after completion of harvest. The polychaete family Polynoidae was not influenced numerically during harvest, but declined in harvest plots compared with reference plots after harvest was completed. Last, the polychaete family Hesionidae was affected negatively by harvest activities during harvests compared with reference plots, but the negative effect did not persist after harvest was completed.

DISCUSSION

The current 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, little evidence of a significant “spillover” effect of cultured geoduck harvest operations was found on resident infaunal communities. Univariate ANOVAs provided no evidence of significant impacts of cultured clam harvest on the biodiversity of resident infauna. Of the 10 most frequently sampled infaunal taxa, only three indicated evidence of reduction in abundance persisting as long as 4 mo after conclusion of harvest activities. None of the proportionate changes in the three affected taxa approached local extinction.

Our results led to the rejection of the three hypotheses listed earlier. Some of the data suggested consistency with hypothesis 1, with significant differences between treatment category at the Foss and Manke sites. However, analyses of the harvest state \times treatment interaction term revealed that the subject differences were the result of plot properties independent of harvest-related disturbance effects. Despite scattered temporary exceptions, it is apparent that none of the hypotheses is generally applicable to the study sites.

The results are similar to a recent experimental study of ecosystem-level effects of geoduck aquaculture done in British Columbia, Canada (Department of Fisheries and Oceans 2012). Abundance 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 British Columbia study compared with commercially operated geoduck farms.

The benthic community data collected in the current study revealed variation in community composition among sites. Sediment grain-size distribution at the 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 affect 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

TABLE 4.
Summary of homogeneity of multivariate dispersion analytical results for contrasts at scales of study sites and plots.

| Scale | Contrast | df | P value |
|--|------------------------------------|----|-----------------------|
| Among harvest states within plot type, Foss cultured plots | Preharvest vs. midharvest | 1 | $0.001 \leq P < 0.01$ |
| | Preharvest vs. postharvest | 1 | NS |
| | Midharvest vs. postharvest | 1 | $0.001 \leq P < 0.01$ |
| Among harvest states within plot type, Manke cultured plots | Preharvest vs. midharvest | 1 | NS |
| | Preharvest vs. postharvest | 1 | NS |
| | Midharvest vs. postharvest | 1 | NS |
| Among harvest states within plot type, Chelsea cultured plots | Preharvest vs. midharvest | 1 | $0.01 \leq P < 0.05$ |
| | Preharvest vs. postharvest | 1 | $0.01 \leq P < 0.05$ |
| | Midharvest vs. postharvest | 1 | NS |
| Among harvest states within plot type, Foss reference plots | Preharvest vs. midharvest | 1 | NS |
| | Preharvest vs. postharvest | 1 | $0.001 \leq P < 0.01$ |
| | Midharvest vs. postharvest | 1 | NS |
| Among harvest states within plot type, Manke reference plots | Preharvest vs. midharvest | 1 | $0.001 \leq P < 0.01$ |
| | Preharvest vs. postharvest | 1 | NS |
| | Midharvest vs. postharvest | 1 | $0.01 \leq P < 0.05$ |
| Among harvest states within plot type, Chelsea reference plots | Preharvest vs. midharvest | 1 | NS |
| | Preharvest vs. postharvest | 1 | $0.01 \leq P < 0.05$ |
| | Midharvest vs. postharvest | 1 | NS |
| | Midharvest vs. postharvest | 1 | NS |
| Within sites within plot type, among harvest states | All states, Foss culture plot | 2 | $0.001 \leq P < 0.01$ |
| | All states, Foss reference plot | 2 | $0.001 \leq P < 0.01$ |
| | All states, Manke culture plot | 2 | NS |
| | All states, Manke reference plot | 2 | $0.01 \leq P < 0.05$ |
| | All states, Chelsea culture plot | 2 | NS |
| | All states, Chelsea reference plot | 2 | $0.01 \leq P < 0.05$ |
| Within sites between plot type, within harvest states | Foss, preharvest | 1 | NS |
| | Foss, midharvest | 1 | $0.001 \leq P < 0.01$ |
| | Foss, postharvest | 1 | $0.01 \leq P < 0.05$ |
| | Manke, preharvest | 1 | $0.001 \leq P < 0.01$ |
| | Manke midharvest | 1 | <0.001 |
| | Manke postharvest | 1 | NS |
| | Chelsea preharvest | 1 | NS |
| | Chelsea midharvest | 1 | NS |
| Chelsea postharvest | 1 | NS | |

NS, $P \geq 0.05$.

& Schoch 2005). Intertidal sand flats in Case Inlet, the location of the 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 the habitat variations described earlier, it was determined that the three study sites could not be considered replicates. As a result the data were analyzed separately for each site. Such an approach had the unavoidable effect of reducing statistical power for detection of significant differences. Nevertheless, a number of significant differences were found in the data relating to date, a proxy for both season and harvest state, and between study plots within the current study sites. The resulting contention is that the current 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 differences were found in abundance patterns between plots within study sites associated with harvest state, it was invariably also found that harvest state was effectively a proxy for seasonal variation in harvested plots. Thus, harvest state unavoidably covaried with date and associated seasonal effects, and was not an informative stand-alone treatment factor for understanding harvest effects. Consistently,

the most informative metric in this study for an unambiguous harvest impact, the harvest state \times treatment interaction term, was not significant in the analyses. Interaction term R^2 values were consistently low, typically explaining less than 5% 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 data set.

With regard to multivariate assemblage contrasts and univariate biodiversity analyses used in the current study, the decision to analyze data from different study sites independently raises questions regarding the propriety of applying ANOVAs to the data (e.g., Hurlbert 1984). The dilemma in design of the current study was the large size and relative scarcity of potential study plots that fit the selection criteria. Hurlbert's (1984) design rubrics to the contrary notwithstanding, Oksanen (2001) has argued that large-scale field studies with attributes such as those used in the current study are fully appropriate for the application of ANOVAs. It is noted that Hurlbert's (1984) dogmatic perspective on design and analysis in field ecology has become increasingly questioned (e.g., Oksanen 2001, Schank & Koehnle 2009). Oksanen (2001) asserts that reflexive application of Hurlbert's dogma to cases

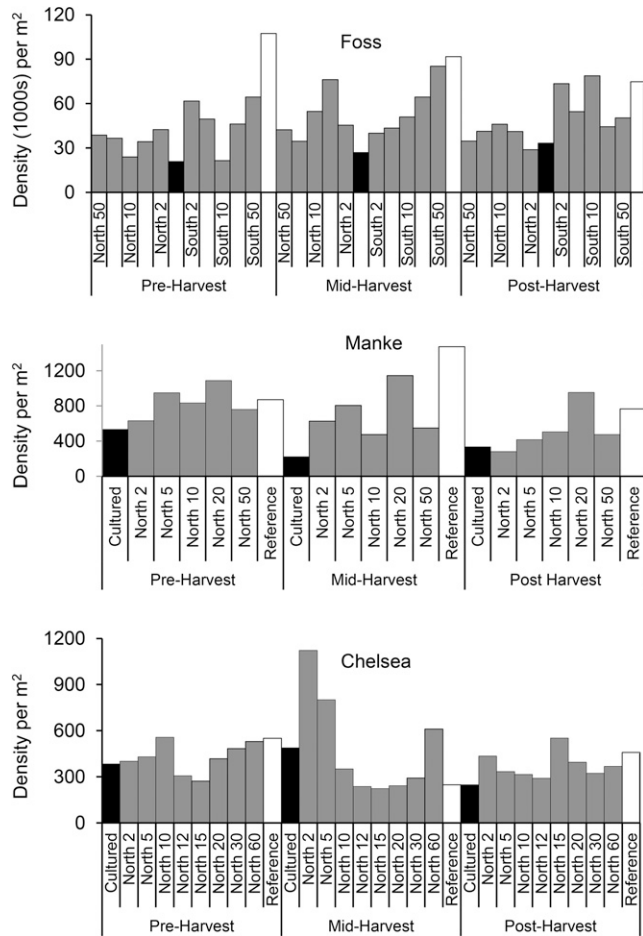


Figure 5. Mean densities of all infaunal organisms summed as individuals per square meter from samples in cultured and reference plots, and on extralimital transects at each distance, within harvest states. Black bars represent densities within cultured plots and white bars represent reference plots. Gray bars indicate densities at specific distances (in meters) from cultured plot edges on transects. Note that scales on both the horizontal and vertical axes differ among study sites.

of design dilemmas such as that in the current study amounts to “entirely unwarranted stigmatization of a reasonable way to test predictions referring to large-scale systems (p. 27).”

In contrast to the results of the current study, 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 the current 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 mo (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 the current 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

TABLE 5.

Summary of permutation-based analyses of variance results for contrasts within plots and transect locations within study sites by date and by harvest state.

| Transect and contrast | Location on transect (m) | R ² | df | P value |
|------------------------------|--------------------------|----------------|--------|------------------|
| Foss North, date | 2 | 1.00 | 10 | <0.001 |
| | 5 | 1.00 | 10 | <0.001 |
| | 10 | 1.00 | 10 | <0.001 |
| | 20 | 1.00 | 10 | <0.001 |
| | 50 | 1.00 | 10 | <0.001 |
| Foss North, harvest state | 2 | 0.38 | 2 | 0.01 ≤ P < 0.05 |
| | 5 | 0.33 | 2 | NS |
| | 10 | 0.26 | 2 | NS |
| | 20 | 0.27 | 2 | NS |
| | 50 | 0.25 | 2 | NS |
| Foss South, date | 2 | 1.00 | 10 | <0.001 |
| | 5 | 1.00 | 10 | <0.001 |
| | 10 | 1.00 | 10 | <0.001 |
| | 20 | 1.00 | 10 | <0.001 |
| | 50 | 1.00 | 10 | <0.001 |
| 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 | 0.01 ≤ P < 0.05 |
| Manke North, date | 2 | 1.00 | 16 | <0.001 |
| | 5 | 1.00 | 16 | <0.001 |
| | 10 | 1.00 | 16 | <0.001 |
| | 20 | 1.00 | 16 | <0.001 |
| | 50 | 1.00 | 16 | <0.001 |
| Manke North, harvest state | 2 | 0.23 | 2 | 0.001 ≤ P < 0.01 |
| | 5 | 0.16 | 2 | 0.001 ≤ P < 0.01 |
| | 10 | 0.27 | 2 | <0.001 |
| | 20 | 0.24 | 2 | <0.001 |
| | 50 | 0.12 | 2 | 0.001 ≤ P < 0.01 |
| Chelsea North, date | 2 | 1.00 | 13 | <0.001 |
| | 5 | 1.00 | 13 | <0.001 |
| | 10 | 1.00 | 13 | <0.001 |
| | 12 | 1.00 | 13 | <0.001 |
| | 15 | 1.00 | 13 | <0.001 |
| | 20 | 1.00 | 13 | <0.001 |
| | 30 | 1.00 | 13 | <0.001 |
| 60 | 1.00 | 13 | <0.001 | |
| 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 |
| | 60 | 0.26 | 2 | NS |

Locations include cultured plot, reference plot, and each sampled distance on transect lines. NS, P ≥ 0.05.

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 0 abundance by definition. The method by which geoducks are harvested has the potential to displace benthic organisms

TABLE 6.

Summary of permutation-based analyses of variance results for contrasts within plots within study sites and within transect locations by treatment, date, and harvest state (part 1).

| Transect and contrast | Location on transect (m) | R ² | df | P value |
|---|--------------------------|----------------|----|------------------|
| Foss North, cultured plot, treatment | 2 | 0.10 | 1 | 0.01 ≤ P < 0.05 |
| | 5 | 0.17 | 1 | <0.001 |
| Foss North, cultured plot, date | 2 | 0.62 | 10 | 0.001 ≤ P < 0.01 |
| | 5 | 0.59 | 10 | 0.01 ≤ P < 0.05 |
| | 10 | 0.67 | 10 | <0.001 |
| | 20 | 0.68 | 10 | <0.001 |
| Foss North, cultured plot, harvest state | 50 | 0.68 | 10 | <0.001 |
| | 2 | 0.21 | 2 | <0.001 |
| | 5 | 0.18 | 2 | 0.001 ≤ P < 0.01 |
| | 10 | 0.19 | 2 | 0.001 ≤ P < 0.01 |
| Foss North, cultured plot, harvest state | 20 | 0.18 | 2 | 0.01 ≤ P < 0.05 |
| | 50 | 0.17 | 2 | 0.01 ≤ P < 0.05 |
| | 2 | 0.23 | 1 | <0.001 |
| | 5 | 0.28 | 1 | <0.001 |
| Foss North, reference plot, treatment | 10 | 0.17 | 1 | 0.001 ≤ P < 0.01 |
| | 20 | 0.17 | 1 | <0.001 |
| | 50 | 0.11 | 1 | 0.01 ≤ P < 0.05 |
| | 10 | 0.64 | 10 | 0.001 ≤ P < 0.01 |
| Foss North, reference plot, date | 20 | 0.59 | 10 | 0.01 ≤ P < 0.05 |
| | 50 | 0.66 | 10 | <0.001 |
| | 2 | 0.18 | 2 | 0.01 ≤ P < 0.05 |
| Foss North, reference plot, harvest state | 10 | 0.16 | 2 | 0.01 ≤ P < 0.05 |
| | 20 | 0.16 | 2 | 0.01 ≤ P < 0.05 |
| | 50 | 0.18 | 2 | 0.01 ≤ P < 0.05 |
| | 2 | 0.15 | 1 | <0.001 |
| Foss South, cultured plot, treatment | 5 | 0.14 | 1 | <0.001 |
| | 10 | 0.11 | 1 | 0.01 ≤ P < 0.05 |
| | 20 | 0.13 | 1 | <0.001 |
| | 50 | 0.19 | 1 | <0.001 |
| Foss South, cultured plot, date | 2 | 0.58 | 10 | 0.01 ≤ P < 0.05 |
| | 5 | 0.62 | 10 | 0.001 ≤ P < 0.01 |
| | 10 | 0.64 | 10 | <0.001 |
| | 20 | 0.60 | 10 | 0.001 ≤ P < 0.01 |
| | 2 | 0.16 | 2 | 0.01 ≤ P < 0.05 |
| Foss South, cultured plot, harvest state | 5 | 0.17 | 2 | 0.01 ≤ P < 0.05 |
| | 10 | 0.18 | 2 | 0.01 ≤ P < 0.05 |
| | 20 | 0.16 | 2 | 0.01 ≤ P < 0.05 |
| | 2 | 0.19 | 1 | <0.001 |
| Foss South, reference plot, treatment | 5 | 0.21 | 1 | <0.001 |
| | 10 | 0.16 | 1 | <0.001 |
| | 50 | 0.18 | 1 | 0.001 ≤ P < 0.01 |
| | 10 | 0.58 | 10 | 0.01 ≤ P < 0.05 |
| Foss South, cultured plot, date | 20 | 0.70 | 10 | <0.001 |
| | 50 | 0.64 | 10 | 0.01 ≤ P < 0.05 |
| | 2 | 0.16 | 2 | 0.01 ≤ P < 0.05 |
| Foss South, cultured plot, harvest state | 5 | 0.17 | 2 | 0.01 ≤ P < 0.05 |
| | 10 | 0.17 | 2 | 0.01 ≤ P < 0.05 |
| | 20 | 0.18 | 2 | 0.001 ≤ P < 0.01 |
| | 50 | 0.19 | 2 | 0.01 ≤ P < 0.05 |

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.

TABLE 7.

Summary of permutation-based analyses of variance results for contrasts within plots within study sites and within transect locations by treatment, date, and harvest state (part 2).

| Transect and contrast | Location on transect (m) | R ² | df | P value |
|--|--------------------------|----------------|----|------------------|
| Manke North, cultured plot, treatment | 5 | 0.05 | 1 | 0.01 ≤ P < 0.05 |
| | 20 | 0.10 | 1 | <0.001 |
| Manke North, cultured plot, date | 2 | 0.66 | 16 | <0.001 |
| | 5 | 0.62 | 16 | <0.001 |
| | 10 | 0.65 | 16 | <0.001 |
| | 20 | 0.57 | 16 | 0.001 ≤ P < 0.01 |
| | 50 | 0.63 | 16 | <0.001 |
| Manke North, cultured plot, harvest state | 2 | 0.16 | 2 | <0.001 |
| | 5 | 0.16 | 2 | <0.001 |
| | 10 | 0.18 | 2 | <0.001 |
| | 20 | 0.14 | 2 | <0.001 |
| | 50 | 0.17 | 2 | <0.001 |
| Manke North, reference plot, treatment | 2 | 0.09 | 1 | <0.001 |
| | 5 | 0.05 | 1 | 0.01 ≤ P < 0.05 |
| | 10 | 0.06 | 1 | 0.001 ≤ P < 0.01 |
| | 20 | 0.06 | 1 | 0.01 ≤ P < 0.05 |
| Manke North, reference plot, date | 2 | 0.57 | 16 | 0.001 ≤ P < 0.01 |
| | 5 | 0.67 | 16 | <0.001 |
| | 10 | 0.64 | 16 | <0.001 |
| | 20 | 0.66 | 16 | <0.001 |
| | 50 | 0.64 | 16 | <0.001 |
| Manke North, reference plot, harvest state | 2 | 0.16 | 2 | <0.001 |
| | 5 | 0.19 | 2 | <0.001 |
| | 10 | 0.17 | 2 | <0.001 |
| | 20 | 0.16 | 2 | <0.001 |
| | 50 | 0.14 | 2 | <0.001 |
| Chelsea North, cultured plot, treatment | 60 | 0.07 | 1 | 0.01 ≤ P < 0.05 |
| Chelsea North, cultured plot, date | 2 | 0.72 | 13 | <0.001 |
| | 5 | 0.69 | 13 | <0.001 |
| | 10 | 0.75 | 13 | <0.001 |
| | 12 | 0.68 | 13 | <0.001 |
| | 15 | 0.66 | 13 | <0.001 |
| | 20 | 0.67 | 13 | <0.001 |
| | 30 | 0.69 | 13 | <0.001 |
| | 60 | 0.66 | 13 | <0.001 |
| Chelsea North, cultured plot, harvest state | 5 | 0.11 | 2 | 0.01 ≤ P < 0.05 |
| | 20 | 0.11 | 2 | 0.01 ≤ P < 0.05 |
| | 60 | 0.12 | 2 | 0.01 ≤ P < 0.05 |
| Chelsea North, reference plot, treatment | 30 | 0.07 | 1 | 0.01 ≤ P < 0.05 |
| | 60 | 0.12 | 1 | <0.001 |
| Chelsea North, reference plot, date | 2 | 0.69 | 13 | <0.001 |
| | 5 | 0.68 | 13 | <0.001 |
| | 10 | 0.70 | 13 | <0.001 |
| | 12 | 0.66 | 13 | <0.001 |
| | 15 | 0.64 | 13 | <0.001 |
| | 20 | 0.67 | 13 | <0.001 |
| | 30 | 0.67 | 13 | <0.001 |
| | 60 | 0.58 | 13 | 0.001 ≤ P < 0.01 |
| Chelsea North, reference plot, harvest state | 60 | 0.11 | 2 | 0.01 ≤ P < 0.05 |

Analyses were done and are presented as described in Table 6.

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 used small patches (surface area, <5 m²) to quantify disturbance effects and implemented a spatially uniform disturbance regime. Geoduck harvest occurs on large

TABLE 8.
Summary of homogeneity of multivariate dispersion analytical results within study sites and plots among transect locations.

| Site | Harvest state | P value |
|---|---------------|-----------------------|
| Within site within harvest state, among transect locations, Foss North | Preharvest | NS |
| | Midharvest | $0.001 \leq P < 0.01$ |
| | Postharvest | $0.001 \leq P < 0.01$ |
| Within site within harvest state, among transect locations, Foss South | Preharvest | $0.01 \leq P < 0.05$ |
| | Midharvest | $0.001 \leq P < 0.01$ |
| | Postharvest | $0.001 \leq P < 0.01$ |
| Within site within harvest state, among transect locations, Manke North | Preharvest | $0.01 \leq P < 0.05$ |
| | Midharvest | <0.001 |
| | Postharvest | $0.01 \leq P < 0.05$ |
| Within site within harvest state, among transect locations, Chelsea North | Preharvest | $0.001 \leq P < 0.01$ |
| | Midharvest | $0.01 \leq P < 0.05$ |
| | Postharvest | NS |

Transect locations include cultured plot and reference plot as well as each sampled location on transects. All indicated contrasts had 6 degrees of freedom. NS, $P \geq 0.05$.

spatial scales (plots that are typically 2,500 m² or larger in surface area) and creates a nonuniform 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 multiyear production cycle, often resulting in nonuniform 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 compared with the uniform disturbance and small scale of other experimental disturbance studies could diffuse any impacts over such a large area so that the effect of harvest is undetectable and possibly trivial from the ecosystem perspective.

The univariate analyses in the current study 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). Three taxa were identified with abundance that increased during the harvest phase in cultured plots and remained elevated in the months after completion of harvest. Such patterns suggest the possibility that the presence of adult geoducks at high densities near the termination of the culture cycle had a negative effect on the subject populations, and that

TABLE 9.
Summary of homogeneity of multivariate dispersion analytical results within study sites between cultured plots and transect locations (the latter include the reference plot as well as each sampled location on transects) for each study site.

| Contrast and location (m) | Harvest state | P value, Foss North | P value, Foss South | P value, Manke North | P value, Chelsea North |
|----------------------------------|---------------|-----------------------|-----------------------|-----------------------|------------------------|
| Cultured plot vs. reference plot | Preharvest | NS | NS | <0.001 | NS |
| | Midharvest | <0.001 | <0.001 | <0.001 | NS |
| | Postharvest | $0.01 \leq P < 0.05$ | $0.01 \leq P < 0.05$ | NS | NS |
| Cultured plot vs. 2 m | Preharvest | NS | NS | NS | NS |
| | Midharvest | NS | <0.001 | <0.001 | NS |
| | Postharvest | <0.001 | $0.01 \leq P < 0.05$ | NS | <0.001 |
| Cultured plot vs. 5 m | Preharvest | NS | NS | NS | NS |
| | Midharvest | <0.001 | $0.001 \leq P < 0.01$ | <0.001 | NS |
| | Postharvest | <0.001 | $0.01 \leq P < 0.05$ | $0.001 \leq P < 0.01$ | NS |
| Cultured plot vs. 10 m | Preharvest | NS | $0.01 \leq P < 0.05$ | $0.001 \leq P < 0.01$ | $0.001 \leq P < 0.01$ |
| | Midharvest | $0.01 \leq P < 0.05$ | NS | <0.001 | NS |
| | Postharvest | NS | $0.01 \leq P < 0.05$ | NS | NS |
| Cultured plot vs. 12 m | Preharvest | — | — | — | NS |
| | Midharvest | — | — | — | NS |
| | Postharvest | — | — | — | NS |
| Cultured plot vs. 15 m | Preharvest | — | — | — | NS |
| | Midharvest | — | — | — | NS |
| | Postharvest | — | — | — | NS |
| Cultured plot vs. 20 m | Preharvest | NS | NS | $0.001 \leq P < 0.01$ | NS |
| | Midharvest | $0.001 \leq P < 0.01$ | $0.01 \leq P < 0.05$ | <0.001 | NS |
| | Postharvest | NS | $0.001 \leq P < 0.01$ | $0.01 \leq P < 0.05$ | NS |
| Cultured plot vs. 30 m | Preharvest | — | — | — | <0.001 |
| | Midharvest | — | — | — | NS |
| | Postharvest | — | — | — | $0.01 \leq P < 0.05$ |
| Cultured plot vs. 50 m | Preharvest | NS | NS | NS | — |
| | Midharvest | <0.001 | <0.001 | $0.001 \leq P < 0.01$ | — |
| | Postharvest | $0.01 \leq P < 0.05$ | <0.001 | NS | — |
| Cultured plot vs. 60 m | Preharvest | — | — | — | $0.001 \leq P < 0.01$ |
| | Midharvest | — | — | — | $0.001 \leq P < 0.01$ |
| | Postharvest | — | — | — | NS |

NS, $P \geq 0.05$.

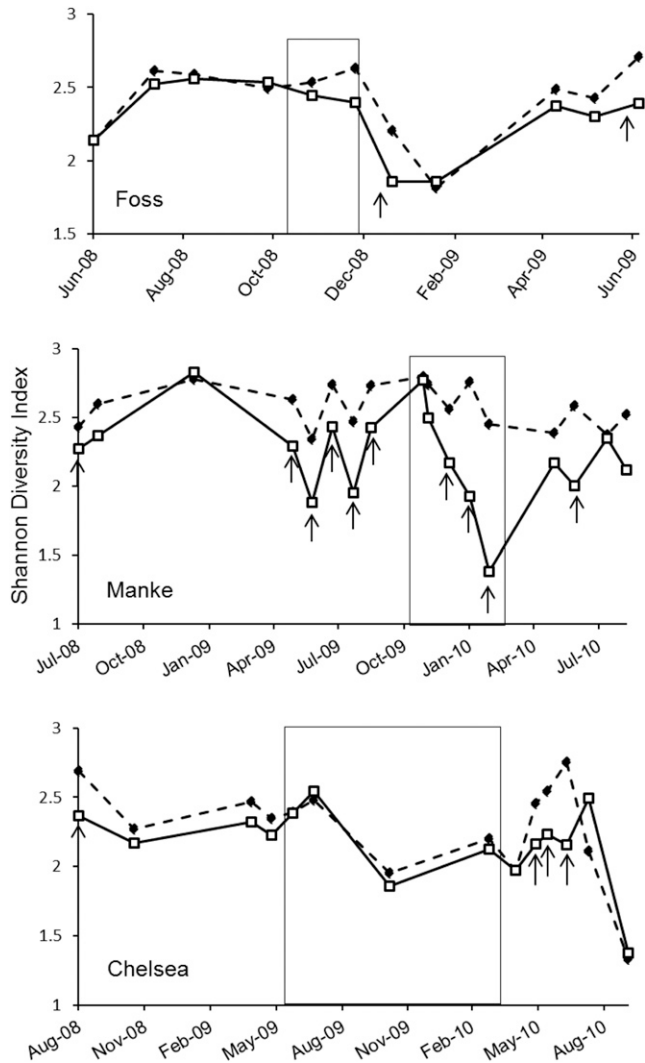


Figure 6. Shannon diversity index values from samples in each plot for each sampling date at each study site. Data from cultured plots are shown with white boxes and solid lines, and from reference plots with black diamonds and dashed lines. Arrows indicate sample dates with significant differences between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

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 microscale patterns of water movement as a consequence of the high living biomass density of geoducks in cultured plots. Cummings et al. (2001) identified variations in abundance 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 geoducks at harvest and subsequent infaunal abundance patterns was beyond the scope of the current study. The matter would be an informative topic for future study.

It is suggested 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 & Keogh 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 (1) small waves resulting from normal wind shear (e.g., Maunder 1968, Anderson 1972, Clarke et al. 1982, Gabrielson & Lukatelich 1985), (2) wakes from vessel passage (e.g., Crawford 1984, Garrad & Hey 1987, Osborne & Boak 1999, Bishop 2007), (3) thermal stress associated with daytime low tides in summer months (e.g., Dethier 2010, Dethier et al. 2010, Dethier et al. 2012), (4) large waves caused by wind storms (e.g., Lynott & Cramer 1966, Reed 1980, Steenburgh & Mass 1996, Mass & Dotson 2010), (5) flooding events caused by maxima in rainfall or snowmelt in watersheds draining to Puget Sound (e.g., Ferber et al. 1993, Zhu & Newell 1998, Colle & Mass 2000, Frascari et al. 2006, Lohrer et al. 2006, Forrest et al. 2007, Hermand et al. 2008, Warner et al. 2012), and (6) 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, Williams & Hutchinson 2000, Sherrod 2001, González 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 greater than rates of significant disturbances caused by geoduck aquaculture operations in a given plot. In addition, it is noted that Puget Sound is quite young in geological and oceanographic contexts, being only 5,000 y of age in current configuration after 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 disturbances of various types. Thus, it is argued that the prevailing natural disturbance climate in the region has effectively selected the infaunal assemblage toward tolerance of and resilience to the types of disturbances associated with geoduck aquaculture operations. Naturally evolved characteristics preadaptive to effects of anthropogenic disturbances are known for a number of marine and freshwater benthic species across many habitat types (e.g., Pearson & Rosenberg 1978, Tomassetti & Porrello 2005, Melzner et al. 2009, Gabel et al. 2011).

As also noted in McDonald et al. (2015), it is cautioned that projection of the current study results to larger temporal or spatial scales may be inappropriate in the absence of additional

TABLE 10.
One-way analysis of variance results for Shannon indices of diversity for samples at all sites.

| Study site and scale | Contrast | F value | P value |
|--|----------------------------|---------|-----------------|
| Foss, between treatments | Preharvest | 0.68 | NS |
| | Midharvest | 0.24 | NS |
| | Postharvest | 3.49 | NS |
| Manke, between treatments | Preharvest | 19.24 | <0.001 |
| | Midharvest | 30.12 | <0.001 |
| | Postharvest | 12.92 | <0.001 |
| Chelsea, between treatments | Preharvest | 5.35 | 0.01 ≤ P < 0.05 |
| | Midharvest | 0.001 | NS |
| | Postharvest | 1.60 | NS |
| Foss, within cultured plot, between harvest states | Preharvest vs. midharvest | 0.17 | NS |
| | Preharvest vs. postharvest | 17.74 | <0.001 |
| | Midharvest vs. postharvest | 13.59 | <0.001 |
| Manke, within cultured plot, between harvest states | Preharvest vs. midharvest | 15.36 | <0.001 |
| | Preharvest vs. postharvest | 4.97 | 0.01 ≤ P < 0.05 |
| | Midharvest vs. postharvest | 2.41 | NS |
| Chelsea, within cultured plot, between harvest states | Preharvest vs. midharvest | 0.04 | NS |
| | Preharvest vs. postharvest | 4.79 | 0.01 ≤ P < 0.05 |
| | Midharvest vs. postharvest | 3.04 | NS |
| Foss, within reference plot, between harvest states | Preharvest vs. midharvest | 0.56 | NS |
| | Preharvest vs. postharvest | 3.70 | NS |
| | Midharvest vs. postharvest | 0.67 | NS |
| Manke, within reference plot, between harvest states | Preharvest vs. midharvest | 0.37 | NS |
| | Preharvest vs. postharvest | 4.08 | 0.01 ≤ P < 0.05 |
| | Midharvest vs. postharvest | 4.84 | 0.01 ≤ P < 0.05 |
| Chelsea, within reference plot, between harvest states | Preharvest vs. midharvest | 10.38 | <0.001 |
| | Preharvest vs. postharvest | 3.58 | NS |
| | Midharvest vs. postharvest | 0.14 | NS |

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 1 degree of freedom. NS, $P \geq 0.05$.

studies. The sites for the current study were relatively isolated from other geoduck aquaculture plots, and were being used for aquaculture of geoducks for the first time. The data may not provide a sufficient basis for unequivocal extrapolation to cases when a given plot is exposed to a long series of successive

geoduck aquaculture cycles. Likewise, it may not be appropriate to extend the findings of the current study to cases when 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

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.

| Taxon | Results of likelihood ratio tests | | Apparent effect of harvest on populations | |
|--------------------------------|-----------------------------------|-----------------|---|---------------|
| | Chi square | P value | During harvest | After harvest |
| <i>Americorophium salmonis</i> | 108.54 | <0.001 | Positive | Positive |
| <i>Cumella vulgaris</i> | 82.13 | <0.001 | Positive | Positive |
| <i>Rochefortia</i> spp. | 38.19 | <0.001 | Negative | Negative |
| <i>Micrura</i> spp. | 0.82 | NS | Neutral | Neutral |
| Capitellidae | 271.51 | <0.001 | Positive | Positive |
| Goniadidae | 15.89 | <0.001 | Positive | Neutral |
| Spionidae | 1.41 | NS | Neutral | Neutral |
| Hesionidae | 362.82 | <0.001 | Negative | Neutral |
| Phyllodoceidae | 24.32 | <0.001 | Negative | Negative |
| Polynoidae | 8.07 | 0.01 ≤ P < 0.05 | Neutral | Negative |

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 for which harvest phase is before harvest, mid harvest, or postharvest, and treatment is either cultured plot or reference plot. All indicated contrasts had 2 degrees of freedom. Taxa are those described in Tables 1 and 2. NS, $P \geq 0.05$.

temporal scales will be a major challenge for geoduck farmers as they continue production on existing plots and expand into new areas, and will be an important research goal in the interests of informed management policies by natural resource agencies.

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EFFECTS OF GEODUCK (*PANOPEA GENEROSA* GOULD, 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, –structure), (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., 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, altering species diversity dramatically. Moreover, macroalgae growing on aquaculture structures can further enhance emergent struc-

ture 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., Dealeris 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 near-shore 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.

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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, 8 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

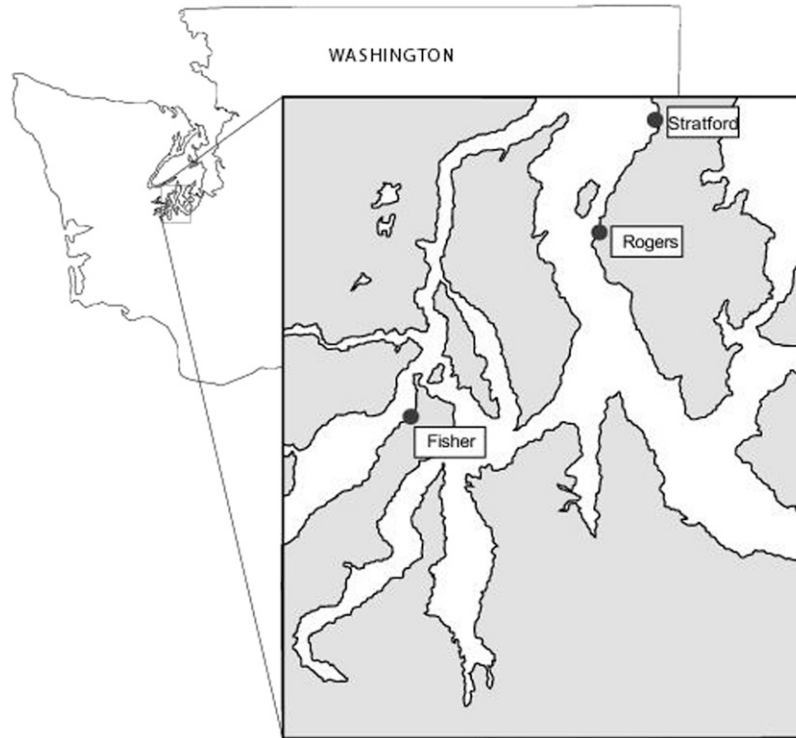


Figure 1. Locations of study sites in south Puget Sound, Washington. Inset shows the region of interest; most geoduck aquaculture in Washington state occurs within the area demarcated by the box.

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).

| Site/status | Description | Biota |
|---|---|--|
| Stratford site: gear placed 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). 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. | Horse clams and cockles are present; sand dollars, patchy. |
| Rogers site: gear placed 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). 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. | Horse clams and cockles are present; graceful crab is abundant; sand dollars, patchy |
| Fisher site: gear placed June 2009 to July 2009; 90% of 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). The substrate is muddy sand (grain size, ~250 μm). The reference area is 75 m to the east on private property. | Horse clams are present; crabs, sea stars, and moon snails are abundant. |

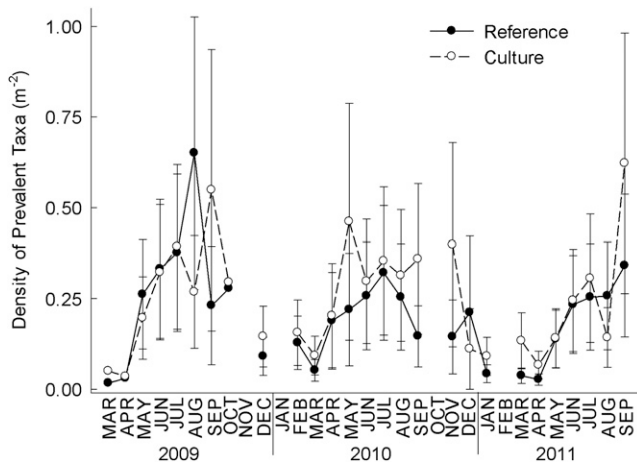


Figure 2. Summed density of prevalent taxa in scuba surveys of transient macrofauna (fish and invertebrates) defined as species present in at least 10% of surveys. Data were collected on culture plots (Culture) and adjacent reference areas (Reference) at three sites in southern Puget Sound during scuba surveys in 2009 to 2011. Note: The northern kelp crab (*Pugettia producta*) is excluded. Error bars are \pm SE.

sediments settled to the bottom and the elutriated organisms floated to the surface. Water was decanted through a 500- μ m sieve and organisms were retained on the collection screen. This process was repeated several times for each sample to ensure 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, the processing method just described was used to examine beach spawning by Pacific sand lance (*Ammodytes hexapterus*) opportunistically at study sites during the peak spawning period (November to April). Although our methods did not target spawning specifically (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, unpubl.).

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 (pregear, gear present, and postgear) separately for each site (Fisher, Rogers, and Stratford). In addition to the main effects, the interaction of plot type and culture phase was tested, and 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 the principal metric for determining the effect of culture practices. Analyses were conducted in R software (R Development Core Team 2011); significance was set at $\alpha = 0.05$.

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. Homogeneity of multivariate dispersion 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 and Cohen (1991) hypothesized a positive relationship between multivariate dispersion of samples and disturbance, and previous assessments of disturbance effects have pointed to greater variability of species abundance in samples collected from disturbed areas relative to undisturbed 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 intersample 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 $\alpha = 0.05$.

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.

Surveys 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

TABLE 2.

Functional groups for commonly observed taxa in scuba surveys of three geoduck aquaculture sites in Puget Sound, Washington, 2009 to 2011.

| Functional group | Common name | Scientific name | Frequency in surveys (%) |
|----------------------------|-------------------------|---------------------------------------|--------------------------|
| Cockle | Heart cockle | <i>Clinocardium nuttallii</i> | 29.6 |
| Crab (true crab) | Graceful crab | <i>Cancer (Metacarcinus) gracilis</i> | 89.4 |
| | Kelp crab | <i>Pugettia product</i> | 47.0 |
| | Red rock crab | <i>Cancer productus</i> | 29.6 |
| | Graceful decorator crab | <i>Oregonia gracilis</i> | 7.6 |
| Hermit crab | Black-eyed hermit crab | <i>Pagurus armatus</i> | 65.2 |
| | Bering hermit crab | <i>Pagurus beringanus</i> | 15.9 |
| Moon snail | Pacific moon snail | <i>Lunatia lewisii</i> | 55.3 |
| Other benthic invertebrate | Dendronotid nudibranch | <i>Dendronotus</i> spp. | 10.6 |
| | Black-tailed crangon | <i>Crangon nigricauda</i> | 4.6 |
| | Giant sea cucumber | <i>Parastichopus californicus</i> | 0.8 |
| Sea star | Sunflower star | <i>Pycnopodia helianthoides</i> | 53.0 |
| | Pink sea star | <i>Pisaster brevispinus</i> | 38.6 |
| | Mottled sea star | <i>Evasterias troschelli</i> | 22.7 |
| | Ochre sea star | <i>Pisaster ochraceus</i> | 15.9 |
| Flatfish | Speckled sanddab | <i>Citharichthys stigmaeus</i> | 42.4 |
| | Starry flounder | <i>Platichthys stellatus</i> | 18.9 |
| | Sand sole | <i>Psettichthys melanostictus</i> | 6.8 |
| Gunnel | Saddleback gunnel | <i>Pholis ornata</i> | 6.1 |
| | Pinpoint gunnel | <i>Apodichthys flavidus</i> | 1.5 |
| | Crescent gunnel | <i>Pholis laeta</i> | 0.8 |
| Other demersal fish | Plainfin midshipman | <i>Porichthys notatus</i> | 4.6 |
| | Sturgeon poacher | <i>Podothecus accipenserinus</i> | 5.3 |
| Other nearshore fish | Bay pipefish | <i>Syngnathus leptorhynchus</i> | 18.9 |
| | Snake prickleback | <i>Lumpenus sagitta</i> | 8.3 |
| | Tubesnout | <i>Aulorhynchus flavidus</i> | 0.8 |
| Sculpin | Staghorn sculpin | <i>Leptocottus armatus</i> | 37.1 |
| | Roughback sculpin | <i>Chitonotus pugetensis</i> | 3.0 |
| Sea perch | Shiner surf perch | <i>Cymatogaster aggregate</i> | 6.1 |
| | Striped surf perch | <i>Embiotoca lateralis</i> | 0.8 |

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 gear in place at culture plots [+geoducks, +structure]), and postgear (in 2011, protective tubes and nets were removed but geoducks remained during grow-out [+geoducks, -gear]). Although the 2010 to 2011 data represent periods in which aquaculture was active, farming occurred at culture plots only; thus, there was no change in epibenthic structure at reference areas.

Data from the three survey sites were not analyzed individually because all sites were considered to have functionally similar habitat for mobile macrofauna. In addition, 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 $\alpha = 0.05$ for statistical tests of significance.

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

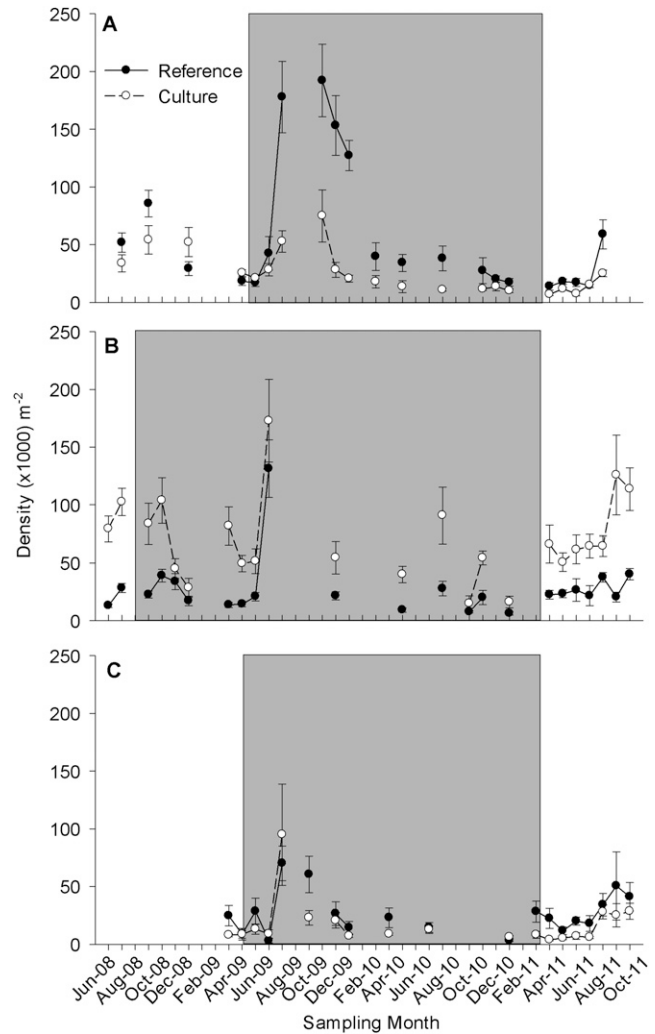


Figure 3. (A–C) Density of total taxa in surveys of resident macrofauna (infauna and epifauna). Data were collected on culture plots (Culture) and adjacent reference areas (Reference) at three sites in southern Puget Sound: Fisher (A), Rogers (B), and Stratford (C). Shaded areas illustrate the aquaculture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

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 $24.2 \pm 11.9/\text{m}^2$ and $278.6 \pm 115.7/\text{m}^2$, 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.

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 \times phase interaction terms (Fisher site: pseudo- $F = 0.049$, $P = 0.116$; Rogers site: pseudo- $F = 0.023$, $P = 0.643$; Stratford site: pseudo- $F = 0.029$, $P = 0.529$).

TABLE 3.

Permutational analysis of variance results for multivariate abundance data for all resident macroinfaunal taxa in core samples.

| Site | Factor | df | SS | MS | R ² | F value | P value |
|-----------|------------|----|--------|--------|----------------|---------|---------|
| Fisher | Month* | 9* | 1.269* | 0.141* | 0.266* | 2.2528 | 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* | 0.1488 | 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 | | | | |

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 $\alpha = 0.05$.

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 \times 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 Spiro-nidae (chi square = 22.89, $df = 2$, $P < 0.001$) and Orbiniidae (chi square = 109.17, $df = 2$, $P < 0.001$). Abundance of the amphipod *Americorhium 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 but

TABLE 4.

Results of the test of multivariate homogeneity comparing multivariate dispersion (HMD test) of resident macroinvertebrate communities of culture plots and reference areas.

| Site | Phase | Multivariate dispersion | | F value | P value |
|-----------|--------------|-------------------------|-----------|---------|---------|
| | | Culture | Reference | | |
| Stratford | Pregear | 0.34 | 0.33 | 0.007 | 0.93 |
| | Gear present | 0.32 | 0.35 | 0.178 | 0.68 |
| | Postgear | 0.35 | 0.25 | 14.608* | <0.01* |
| Rogers | Pregear | 0.18 | 0.19 | 0.162 | 0.70 |
| | Gear present | 0.28 | 0.31 | 0.480 | 0.69 |
| | Postgear | 0.21 | 0.23 | 1.026 | 0.34 |
| Fisher | Pregear | 0.20 | 0.22 | 0.355 | 0.57 |
| | Gear present | 0.27 | 0.28 | 0.261 | 0.64 |
| | Postgear | 0.25 | 0.22 | 0.790 | 0.44 |

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 $\alpha = 0.05$.

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 during the postgear phase in culture plots relative to reference areas. In addition, the abundance of the polychaete Family Goniadidae (chi square = 10.94, $df = 2$, $P = 0.004$) and anemone Family Edwardsiidae (chi square = 20.505, $df = 2$, $P < 0.001$) increased when gear was present, and recovered to pregear levels after gear was removed. The bivalve genus *Rochefortia* (chi square = 6.99, $df = 2$, $P = 0.030$), nemertean genus *Micrura* (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.

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

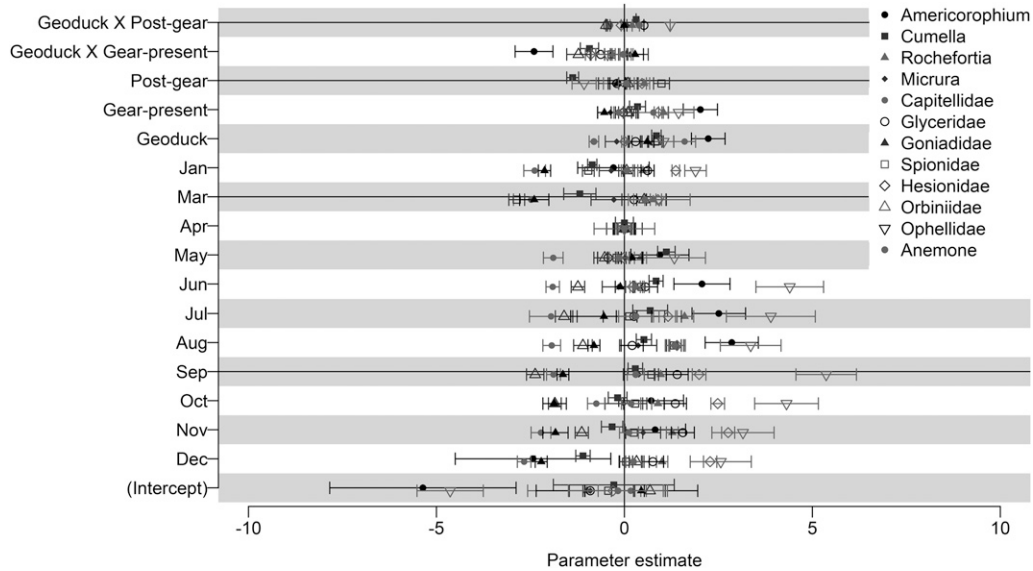


Figure 4. Parameter estimates and 95% confidence intervals for generalized linear mixed models of selected macrofauna. The models included main effects of month of sampling, plot type (geoduck culture or reference area), phase (pregear, gear present, postgear), and their interaction, as well as random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

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

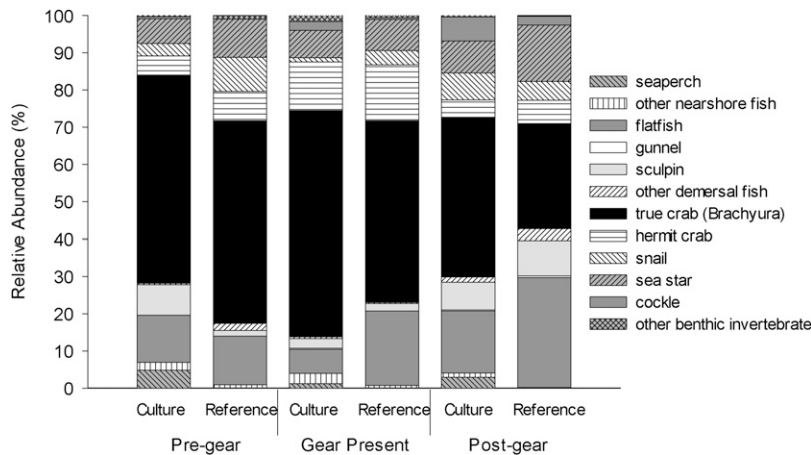


Figure 5. Relative abundance of 10 functional groups of transient fish and macroinvertebrates on geoduck culture plots (Culture) and adjacent reference beaches (Reference) during scuba surveys at three sites in southern Puget Sound (2009 to 2011). Data are presented in three April to October periods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

TABLE 5.

Results of two-way, crossed analysis of similarity (ANOSIM) 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.

| Test groups | ANOSIM R | P value |
|--|----------|---------|
| Pregear reference area vs. culture plot | -0.0501 | 0.761 |
| Gear-present reference area vs. culture plot | 0.0808* | 0.035* |
| Postgear reference area vs. culture plot | -0.0254 | 0.789 |
| Pregear vs. gear-present reference area | 0.1176 | 0.093 |
| Pregear vs. gear-present culture plot | 0.1557* | 0.040* |
| Pregear vs. postgear reference area | -0.0268 | 0.600 |
| Pregear vs. postgear culture plot | -0.0851 | 0.842 |
| Gear present vs. postgear reference area | 0.0900* | 0.029* |
| Gear present vs. postgear culture plot | 0.1604* | 0.003* |

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

(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 postgear 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 postgear 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 the 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 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 \times 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.

| Phase | Plot type | Shannon diversity index (H') | t-Test results for diversity values | Total organisms observed (n) |
|--------------|-----------|----------------------------------|-------------------------------------|------------------------------|
| Pregear | 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 | | 1,692 |
| Postgear | Reference | 1.163 | $t = 0.309, df = 25, P = 0.760$ | 621 |
| | Culture | 1.207 | | 694 |

Differences among culture plots and reference areas were examined with Welch's t-test with $\alpha = 0.05$. Total abundance of all observed organisms is included.

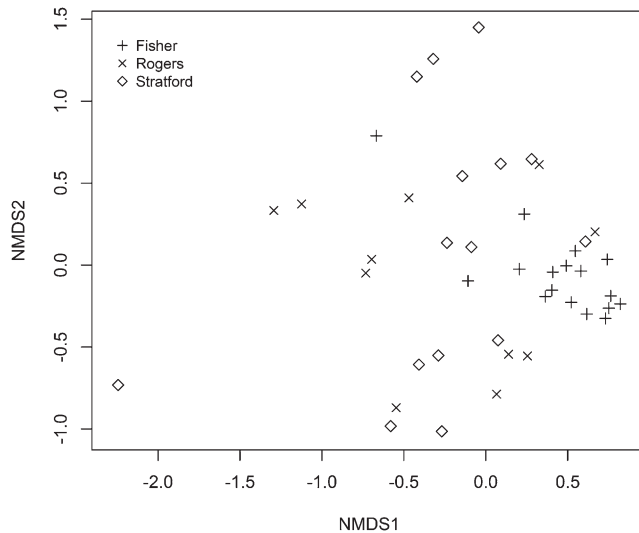


Figure 6. Two-dimensional nonmetric multidimensional scaling (NMDS) ordination of scuba surveys at reference areas during 2010, which corresponds to when aquaculture gear was in place (gear present) at the culture sites. Stress = 17.24. Stress tested statistically significant with the Monte Carlo randomization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

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 Orbinidae—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 pre-gear 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

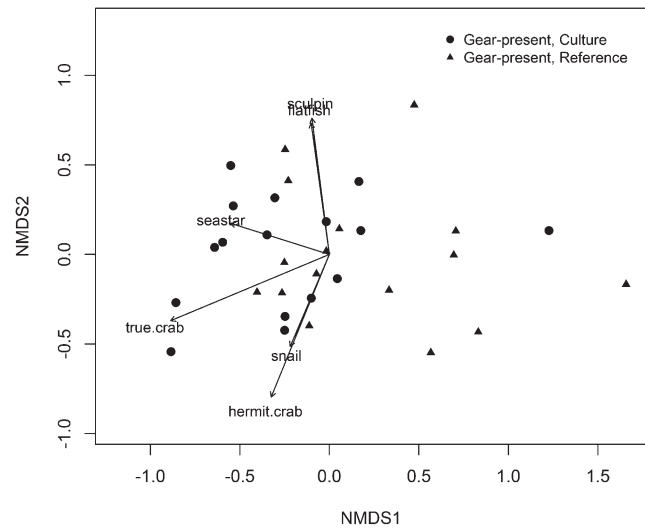


Figure 7. Two-dimensional nonmetric multidimensional scaling (NMDS) plot of scuba surveys at culture plots (solid circles) and reference areas (solid triangles) when aquaculture gear was in place (gear present). The functional group vectors shown are those with $P < 0.05$. Stress = 13.87. Stress tested statistically significant with 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 ($P < 0.05$).

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; $R = 0.081$, $P = 0.035$). 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

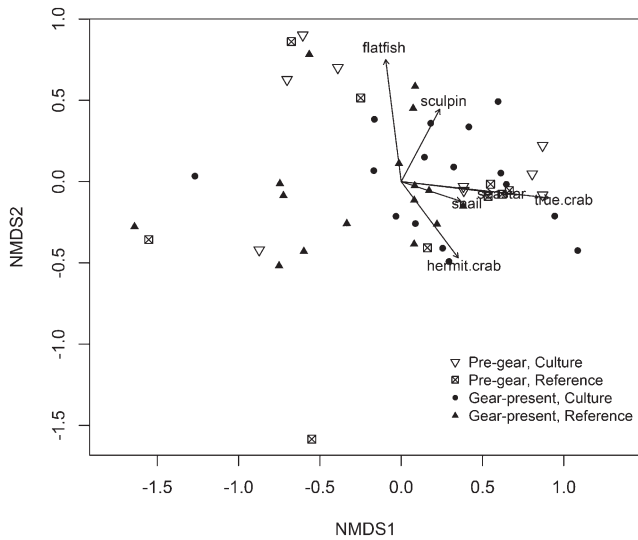


Figure 8. Two-dimensional nonmetric multidimensional scaling (NMDS) plot of scuba surveys at culture plots and reference areas prior to deployment of aquaculture gear (pregear) and when aquaculture gear was in place (gear present). The functional group vectors shown are those with $P < 0.05$. Stress = 14.498. Stress tested statistically significant with 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 ($P < 0.05$).

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 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 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

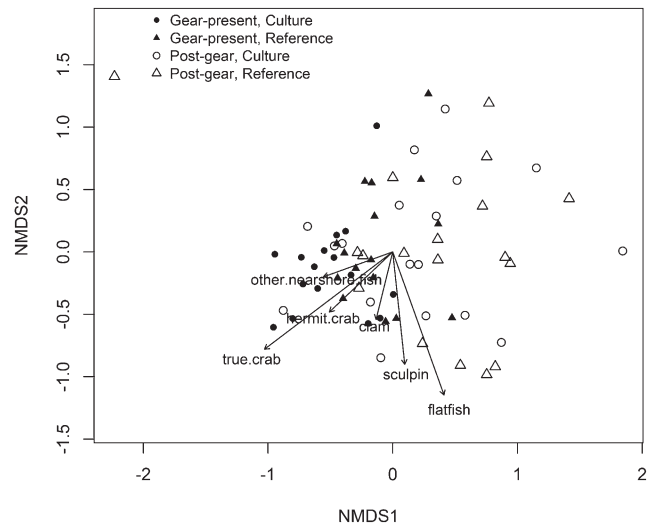


Figure 9. Two-dimensional nonmetric multidimensional scaling plot (NMDS) of scuba surveys at culture plots and reference areas when aquaculture gear was in place (gear present) and after gear was removed (postgear). The functional group vectors shown are those with $P < 0.05$. Stress = 18.08. Stress tested statistically significant with 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 ($P < 0.05$).

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 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 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 (*Ammodytes 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 pallasii*), 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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