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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

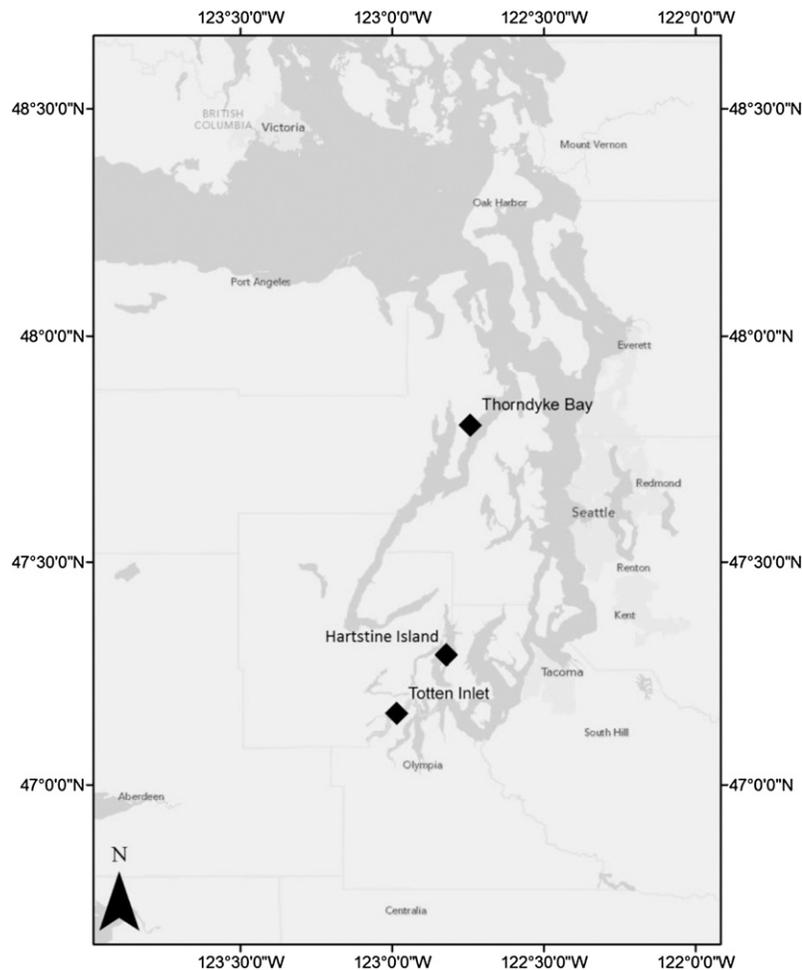


Figure 1. Map showing collection locations for farmed and wild Pacific geoduck *Panopea generosa* in Puget Sound, Washington.

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

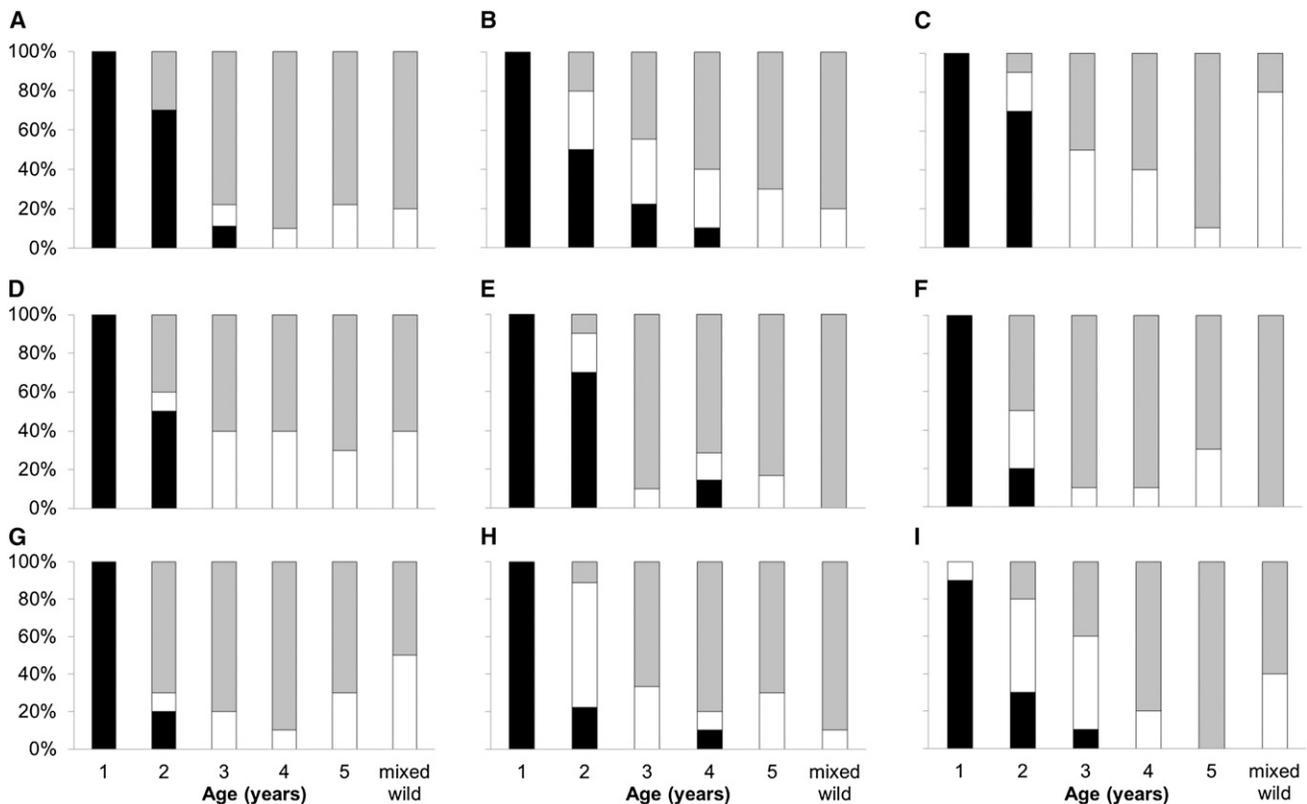


Figure 2. (A–I) Maturation proportions for the farmed and wild geoduck *Panopea generosa*. Immature, black bars; mature, white bars; gamete release, gray bars. Differences among proportions are nonsignificant among sites (by row: Thorndyke Bay, A, B, C; Hartstine Island, D, E, F; and Totten Inlet, G, 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).

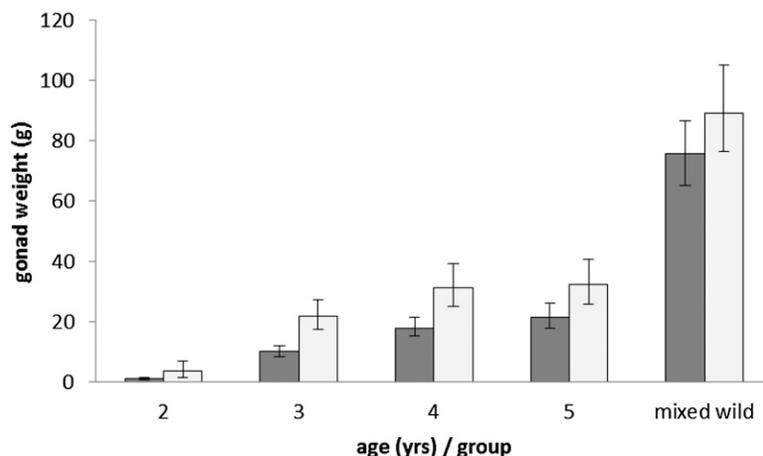


Figure 3. Male (dark bars) and female (light bars) mean ($\pm 95\%$ bias-corrected and accelerated confidence intervals) gonad weights for 2-, 3-, 4-, and 5-year-old farmed and a random sample of wild Pacific geoducks (*Panopea generosa*).

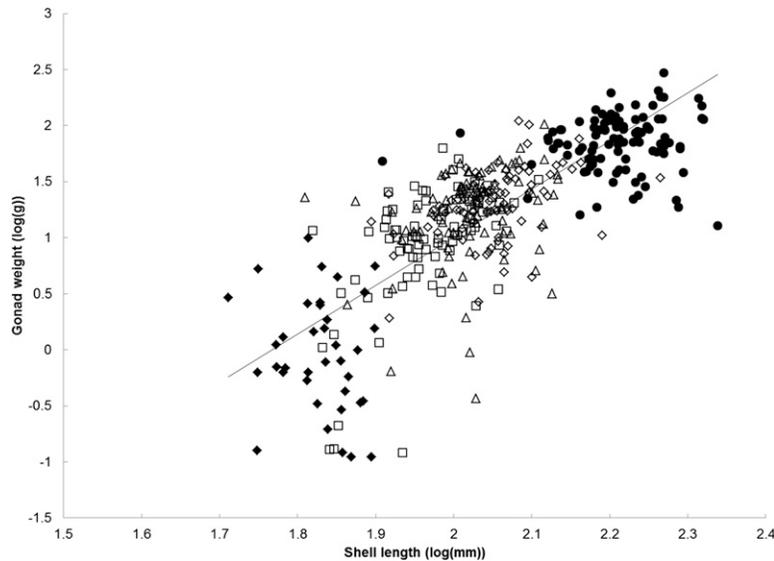


Figure 4. Relationship of gonad weight (log) to shell length (log) for 2- (solid diamonds), 3- (boxes), 4- (triangles), and 5- (open diamonds) year-old farmed and a random sample of wild (solid circles) Pacific geoducks (*Panopea generosa*). Regression equation: $y = 4.2973x - 7.5906$; $R^2 = 0.6313$.

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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