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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 1,928*g* (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-μL reactions containing 1 μL diluted template DNA, 5 μL 2X SensiMix (Bioline, London, UK). The final concentrations were 3 mM $MgCl_2$ and 0.5 μM each primer (except for Pab 3, which had a final concentration of 0.25 μM 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 μL PCR product was added to 3.9 μL Hi-Di formamide (Applied Biosystems, Foster City, CA) and 1 μL 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.

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)

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 2.

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

	Wild	1999	2000	2001	2002	2003	Breeding
n	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 ± 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% ± 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 ± 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 ± 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–∞]).

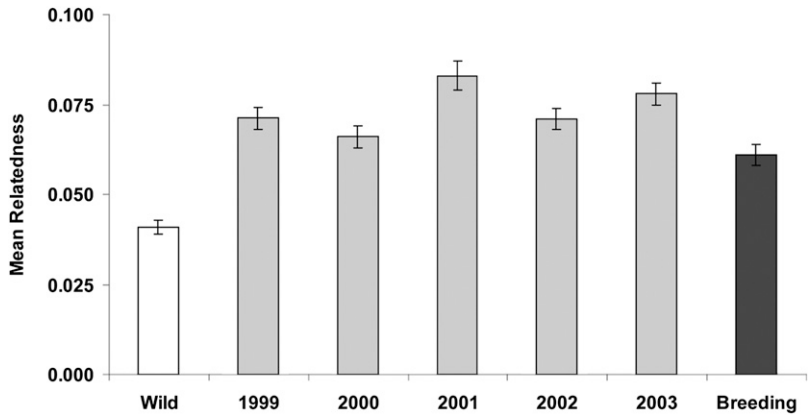

Figure 1. Relatedness values (mean ± 95% confidence interval) for wild and cultured geoducks (hatchery year-classes 1999 to 2003 and the three year-class breeding group). The wild geoduck collection is shown with a white bar, individual year-classes are shown with gray bars, and the breeding group is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

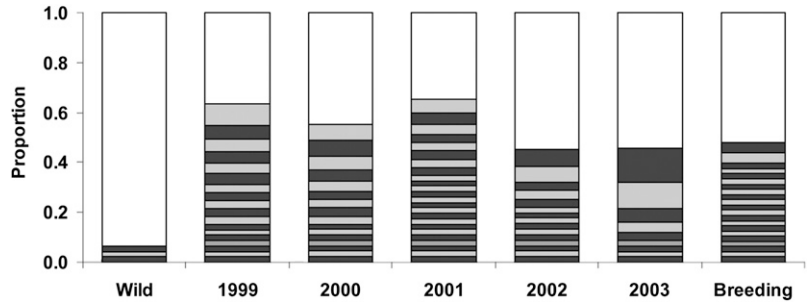

Figure 2. Full-sib assignment in wild and cultured geoduck groups. The proportion of individuals not related to any other individuals at the full-sib level is shown in white. The gray and black bars represent the proportion of individuals assigned to each different full-sib family.

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).

	Parentage without parents		Linkage disequilibrium		Sibship assignment	
Group	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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