Assessment of the current knowledge of geoduck and other shellfish population structure, with general recommendations for the genetic management of cultured and wild geoduck clams.

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1. INTRODUCTION

In the early 1990s, commercial shellfish growers began using and refining the protocols for commercial production of geoduck clams on private intertidal beds within the state of Washington. Commercial sized geoducks, grown from hatchery 'seed' on intertidal shellfish beds, have been produced and are currently marketed. These aquaculture activities may impose genetic risk (Currens and Busack, 1995) to wild geoduck populations within Puget Sound, if there are cultured-wild interactions (CWI).

In the sections that follow, we 1) summarize the theoretical effects of shellfish aquaculture on wild conspecifics; 2) discuss the evidence for geoduck clam population structure and local adaptation; 3) cite examples of documented genetic effects of CWI; 4) recommend aquaculture practices that a) reduce the likelihood of CWI, and b) mitigate deleterious effects of CWI; 5) outline fundamental research needed to understand ecological and genetic interactions of cultured and wild geoducks; and 6) conclude with a summary of the main points.

1. OVERVIEW OF GENETIC EFFECTS OF CWI IN SHELLFISH

Genetic change in naturally occurring populations takes place via migration, genetic drift, mutation, mating system, and natural selection (Box 1). Changes wrought by human action that may imperil naturally occurring marine mollusks are of significant concern. More specifically, the actions we consider here are the outcomes of localized culture activities of endemic species. Negative genetic impacts result in population reduction and, hence, in decreased ability of populations to adapt to a changing environment. This decrease in adaptive potential is directly related to the genetic variability within the population. An assessment of naturally occurring levels of genetic variability is a necessary prerequisite to any discussion of genetic risks and impacts.

A hatchery population is essentially analogous to a small wild population in many respects. Mutation, selection, migration, and drift also affect the genetic makeup of the hatchery population, and by virtue of often small population sizes in bivalve hatcheries, cultured population changes the dynamics and contribution of these factors relative to a larger population. Some of these factors can be manipulated through breeding programs and hatchery management, and can reduce the potentially deleterious effects of the relatively small hatchery population interacting with the much larger wild population. Small population size, for example, intensifies the effects of genetic drift and increases the likelihood of inbreeding, yet the effects of selection are reduced. Therefore, hatchery management actions taken to decrease drift and inbreeding can mitigate negative genetic effects of CWI.

Box 1. Mechanisms of Genetic Change

Mutation

The effects of mutation are difficult to quantify. Mutation rates vary across the genome, being very low for highly conserved genes to very high in some repetitive non-coding regions of the genome. The time scales for regeneration of variation in quantitative characters (10^2-10^3 generations) is much less than for neutral variation (10^5-10^7 generations) (Lande and Barrowclough, 1987).

Mutations are an important source of genetic variation. Mutations can be favorable, neutral, or deleterious. Favorable mutations are rare, many are neutral, and most strongly deleterious mutations are rapidly removed by selection. However, slightly deleterious mutations accumulate in a population resulting in what is termed genetic load.

Genetic Drift

Genetic drift arises from the random sampling of gametes resulting in subsequent generations comprising a different subset of genotypes than the original population. The effect of genetic drift is inversely related to population size, therefore smaller populations tend to be more influenced by genetic drift than by selection, although the rate of diversity loss due to drift for neutral loci is often less than for adaptive traits such as reproductive fitness (Frankham, et al., 1999). Overlapping generations can buffer against drift via the availability of more genotypic variability from multiple year classes. The important genetic parameter, effective population size or N_e, is a theoretical metric based on a population with equal sex ratio that experiences genetic drift equal to a population of size N. With overlapping generations, N_e is equal to the annual effective number of spawners times the generation length. Genetic drift in hatcheries can be countered by boosting the hatchery N_e, and will be covered in more detail below.

Migration

Migration is perhaps the easiest factor to understand yet among the most difficult to study. Genetic migration can be defined as movement that results in genetic interaction among members of different subpopulations, or demes. If there is movement at virtually any life history stage that results in viable between group progeny, migration of genetic material has occurred. In marine mollusks, migration can take place via movement of adults, juveniles, larvae, or gametes, as long as successful reproduction ultimately takes place. Genetic migration is only an issue if there are adaptive genetic differences between wild and cultured animals. By implementing hatchery management strategies designed to minimize genetic differences between cultured and wild as outlined below, deleterious genetic effects of CWI will be reduced, (but see Waples 1999).

Selection

Differences in reproductive fitness and differential survival of genotypes gives rise to selection. Because fitness characters may differ between the hatchery and natural environments, selection pressures will be correspondingly different as well. The relative influence of selection on quantitative genetic variation is dependant upon the type of selection (directional, balancing, or disruptive), the degree of selection pressure, and the heritability level. Small hatchery populations typically have lower genetic diversity than large wild populations, the influence of selection should be lower. However, the degree of selection pressure in the hatchery environment can be inordinately large due to culture methods, and to environmental homogeneity relative to the wild. In addition, artificial selection pressure can have large consequences in breeding programs. Thus domestication selection, whether intentional or not, creates genetic differences between hatchery and wild populations (Waples 1999). Culture and hatchery management methods can be implemented to reduce the selection pressures in the hatchery. Once outplanted, however, purifying selection will not necessarily purge effects of domestication in the same or subsequent generations, because the genes normally under selection during the hatchery period will not necessarily be the same genes subjected to selection during adulthood or subsequent generations.

Reproductive isolation and local adaptation

Among broadcast spawning bivalves, gene flow and hence population structure is governed by larval dispersal, juvenile dispersal, and to a lesser degree, adult migration. Reproductive isolation can occur in a number of ways in the marine environment. Isolation by watercourse distance can separate populations, either by virtue of the maximum migratory distance of pelagic larvae or by the temporal viability of drifting gametes. Spawning behaviors can isolate individuals within the same region. The extent of gene flow and effective population size (N_e, Box 1) determines the potential for genetic differentiation among natural populations. Gene flow is correlated with dispersal ability in many organisms (Bohonak, 1999), including many marine fish and shellfish (reviewed in Shaklee and Bentzen, 1998), and may be correlated with spatial distribution in marine mollusks (Johnson, et al., 2001). In sedentary marine bivalves such as geoduck clams, dispersal and gene flow occur primarily during the pelagic larval phase, with some limited dispersal of small juveniles via pedal locomotion across the substrate, and, in some species, byssal drifting (Sigurdsson, et al., 1976).

Large broadly distributed populations, high fertilities, and planktotrophic larvae with high dispersal potential characterize many species of marine molluscs, and these attributes are often correlated with genetic homogeneity (panmixia) over broad geographic areas (Bohonak, 1999). In many of these species it is only over relatively large distances that the common pattern of isolation by distance (IBD) emerges (Shaklee and Bentzen, 1998). A number of investigators have failed to detect genetic heterogeneity (falsify the null hypothesis of panmixia) among collections at broad geographic scales in a variety of broadcast spawning marine species with pelagic larvae (e.g. *Littorina striata*, De Wolf, et al., 2000; *Mytilus galloprovincialis* Skalamera, et al., 1999). In some marine invertebrates, however, genetic differentiation that deviates from the common pattern has been observed among populations, e.g. urchins (*Strongylocentrotus franciscanus*, Moberg and Burton, 2000, abalone *Haliotis cracherodii*, Hamm and Burton, 2000, oysters *Crassostrea angulata*, Michinina and Rebordinos, 1997, giant clams *Tridacna gigas* Benzie and Williams, 1995, blue crab *Calinectes sapidus*, McMillen-Jackson, et al., 1994, and queen conch *Stombus gigas*, Mitton, et al., 1989).

Most population genetic studies of marine invertebrates have focused on populations that are distributed along open coasts or island populations with discontinuous distributions separated by deep oceanic water. Even on smaller geographic scales, however, genetic differences were detected in the limpet *Siphonaria jeanae* (Johnson and Black, 1984), the oyster *Crassostrea virginica* (King, 1986) and cockles *Cerastoderma glaucum* (Mariani, et al., 2002). Parsons (1996) demonstrated significant genetic subdivision (9-locus F_{ST} = 0.16) over an area of only 75 km^2 in the intertidal gastropod, *Austrocochlea constricta*. Thus, in some cases, genetic and geographic distances are not correlated on either broad or narrow spatial scales, leading investigators to consider hypotheses other than IBD to explain the biological significance of the observed genetic patchiness.

It is becoming increasingly clear that spatial genetic differentiation can be influenced by temporal differentiation (Jorde and Ryman, 1995), since differences among year classes or cohorts within a locality can be equal to or larger than those among spatially distinct stocks (e.g. Laikre, et al., 1998; Planes and Lenfant, 2002). Temporally-based genetic differences would be expected to produce heterozygote deficiencies in samples composed of multiple year classes or cohorts (i.e., a temporal Wahlund effect). Conversely, the magnitude of heterozygote deficiencies (and F_{IS} estimates) would be expected to be lower within year classes or cohorts compared to the population as a whole. As an explanation of stochastic spatial variation, a model of sweepstakes reproduction (Hedgecock, 1994b) was proposed, where a strong bias in reproductive success can reduce the genetically effective population size (N_e) per year class, causing local allele frequency fluctuations that may yield temporally unstable genetic differences among geographic locations. Investigators have suggested that studies of spatial genetic stock structure should also consider temporal stability (Heath, et al., 2002; Hedgecock, 1994a; Laikre, et al., 1998), but there has been a paucity of studies specifically testing the sweepstakes hypothesis.

Examination of neutral genetic variation is valuable, yet apparent panmixia discerned via neutral molecular markers can mask adaptive variation present among population groups (Utter, 2004), since neutral genetic variation essentially measures the effects of genetic drift. Genetic differences produced by natural selection, however, are best measured via quantitative genetic variation rather than neutral markers (Reed and Frankham, 2001), because such measures reflect local adaptation. The correlation between quantitative and molecular markers is weakest for life history traits (Reed and Frankham, 2001), and has generally tended toward greater divergence in quantitative traits (Merila and Crnokrak, 2001). For example, Luttikhuizen, et al. (2003) found Q_{ST} of 0.416 but F_{ST} of .0114 in *Macoma balthica*. The relationship is illustrated by McKay and Latta (2002) (Fig. __).

In a number of cases, however, divergence in quantitative traits appears less frequently than predicted by neutral markers (e.g. Edmands and Harrison, 2003; Lee and Frost, 2002) Adaptive variation is related to reproductive fitness, and is measured using quantitative characters. The variation in quantitative characters is partitioned between environmental and genetic causes; quantitative characters are typically influenced by multiple loci in the genome, as well as by the environment.

An important first step in the evaluation of possible genetic effects of CWI is the identification of genetic resources in wild populations; genetic changes cannot be monitored without an established basis.

Culture operations must be managed to avoid disruption of extant stock structure and local adaptation. Since mutations cannot be relied upon to replenish genetic variation, conservation efforts should be directed towards the maintenance of the critical level of effective population size.

1.1 Loss of genetic variation within populations

Loss of genetic diversity within the wild population is a primary genetic risk associated

with CWI, and may be the result of inbreeding, drift, or breakdown of adaptive substructure (e.g. shallow or deep water adaptations). Loss of within-population genetic diversity as a result of drift can be predicted by comparing estimates of effective population sizes in cultured and wild populations.

If effective sizes are much smaller in the hatchery than in wild populations, and large numbers of cultured individuals are released, the wild population gene pool may be "swamped" with less diverse hatchery genotypes.

Both heterozygosity and allelic diversity can be reduced in populations of small effective population size. There are three types of effective population size. Inbreeding effective population size refers to the rate of inbreeding, and variance effective size refers to rate of genetic drift (Ryman, 1994), while eigenvalue effective population size refers to the rate of loss of genetic diversity (Templeton and Read, 1994). In most situations, however, the values are similar (Nei, 1975). Franklin (1980) suggests an inbreeding effective size of at least 50 individuals to guard against inbreeding, although variance effective population sizes of up to 5000 may be necessary to avoid long-term losses of variability (Lande and Barrowclough, 1987). If cultured individuals are released in the wild and genetic interaction occurs, the influence of the cultured population on the wild effective population size depends on the effective population size of the wild population, the effective population size of the cultured population, and the proportion of cultured animal reproductive output relative to the whole population (Ryman and Laikre, 1991). Clearly, if the relative reproductive contribution of cultured animals is very low, there is very little risk to the wild population regardless of the effective population size of the cultured population. If the proportion is high, however, the effective population size of the cultured population will affect the total effective population in either direction according to the Ryman-Laikre model

$$\frac{1}{N_e} = \frac{x^2}{N_c} + \frac{(1-x)^2}{N_w}$$

Where N_e is the total effective population size, N_c is the effective number of cultured animals, N_w is the effective number of wild animals, and x is the census proportion of offspring produced by parents from the cultured population. If x is very small, then N_e approaches N_w. Conversely, as x approaches unity, N_e is approximated by N_c. These three parameters are rarely known for species where CWI may occur (Hedgecock and Coykendall, in press). The impacts of hatchery releases may be both positive or negative. For example, Hedrick, et al. (2000) measured these three parameters in a Chinook salmon supplementation program, and found increased effective sizes in the total population as a result of hatchery supplementation through uniform sizes of individual cultured families.

Replenishing broodstock from the wild, maintaining high hatchery N_e, and conducting pair matings between individuals with rare alleles will conserve within-population diversity

and will reduce the impacts of cultured on wild strains.

Among cultured shellfish, however, there are no examples in the literature where the three Ryman-Laikre (1991) parameters (N_w, N_c, and x) have been estimated. Gaffney, et al. (1996) reported very low effective population size estimates in a reseeded population of red abalone, *Haliotis rufescens* compared to a natural population (Table 3, SC92 and NC92, respectively) and a genetic signature of the reseeding effort. However, a subsequent reassessment by Burton and Tegner (2000) found no evidence of enhancement, and attribute the results of Gaffney et al. (2000) to either rapid rebound from a genetic bottleneck, or genotyping errors.

Inbreeding and Inbreeding Depression

Drift due to low effective population size is one mechanism for a reduction of within-population genetic diversity. Inbreeding also affects within-population genetic diversity. Inbreeding, or the mating of related individuals, can cause genetic change by decreasing heterozygosity in the population because related individuals tend to carry the same alleles.

Inbreeding depression may follow inbreeding, and is defined as a decrease in fitness due to matings of related individuals. In wild populations of geoducks, pairwise relatedness levels are typically low (B. Vadopalas, unpublished data; but see Vadopalas and Rothaus, 2003). Thus, as long as each new set of broodstock are not derived from cultured animals, there is little likelihood of inbreeding in the hatchery setting. However, inbreeding can be realized inadvertently among hatchery outplants under the following conditions:

1) Small Ne in the hatchery leading to high degree of relatedness among progeny/outplants
2) Related outplants are proximate, or close enough to breed with one another
3) Outplants are sexually mature
4) Progeny of outplants can survive to reproduce.

The first two conditions are likely under current geoduck culture systems, since relatively few broodstock are needed to produce millions of seed, and the outplants are planted at high densities. The age at which outplants reach sexually maturation, the third condition, is the subject of a current investigation at the University of Washington. Fourth, the offspring of cultured geoducks may reproduce with their wild counterparts, but the hybrids may be less fit then their wild counterparts. Thus, there may be a loss of population viability and "wasted" reproduction by wild individuals.

Maintaining a high effective population size in the hatchery by replenishing broodstocks from wild populations, conducting single pair matings, and sustaining high genetic diversity among outplants will help avoid inbreeding depression.

Domestication selection

Selection in the hatchery, whether deliberate or inadvertent, will result in genetic

differences between hatchery and wild populations. Genetic drift is random genetic change, whereas domestication selection is non-random but sometimes inadvertent within hatcheries. The outcomes of domestication selection are dependent on the size of the captive population, the difference between the wild and captive environments (the *selection differential*) and the genetic variation underlying the fitness trait experiencing selection. In the presence of CWI, there are particular hatchery management techniques to reduce domestication beyond the obvious removal of deliberate selection. First, maintaining gene flows from the wild population via regular broodstock procurement, as currently practiced in geoduck culture, will aid in the randomization of genotypes and reduce several generations' exposure to the hatchery environment. Second, equalizing family size has been shown to maintain higher levels of reproductive fitness (Borlase, et al., 1993) and reduce domestication selection (Allendorf, 1993).

Domestication selection is not necessarily deleterious; adaptation to the hatchery/high intertidal environment may be beneficial as long as reproduction of cultured and wild populations remains distinct.

1.2 Loss of genetic variation among populations

The loss of genetic variation among/between populations occurs by mixing of genetically distinct population segments. If genetic differences among populations are temporally and spatially stable, care must be taken not to disrupt the differentiation via excess gene flow. For cultured animals, care must be taken to procure broodstocks from the population proximate to the outplant sites.

With geoducks, the neutral genetic differences detected within Puget Sound do not appear related to reproductive isolation or lack of gene flow (Vadopalas, 2003). Adaptive differences, however, may exist and warrant further investigation.

Outbreeding depression

It is difficult to predict whether geoduck transplants from one region to another will have negative consequences on the performance of wild populations via outbreeding. There are two types of outbreeding depression. Type 1 is a decrease in fitness of the hybrid due to traits expressed by the hybrid that are maladaptive in the environment. If hatchery and wild geoducks are genetically different and reproduce, their hybrid offspring may exhibit lower survivorship and be less fit than 100% wild individuals. Type 2 outbreeding depression is a decrease in fitness due to the breakup of adaptive gene complexes in the wild strain through recombination with exogenous genomes. Type 1 outbreeding depression is expressed in the first generation of hybrids, while Type 2 outbreeding depression will not appear until the second or later generation of hybrids. In general, if low genetic distances can be maintained between hatchery and wild stocks by using new wild broodstock and via maintaining high survivorship in the hatchery, outbreeding depression is much less likely to occur. However factors such as large population size, low mutation rate, and high rate of recombination may exacerbate outbreeding depression (Edmands and Timmerman, 2003). With low genetic distances, the magnitude of and recovery period from outbreeding depression are low (Fig. 1).

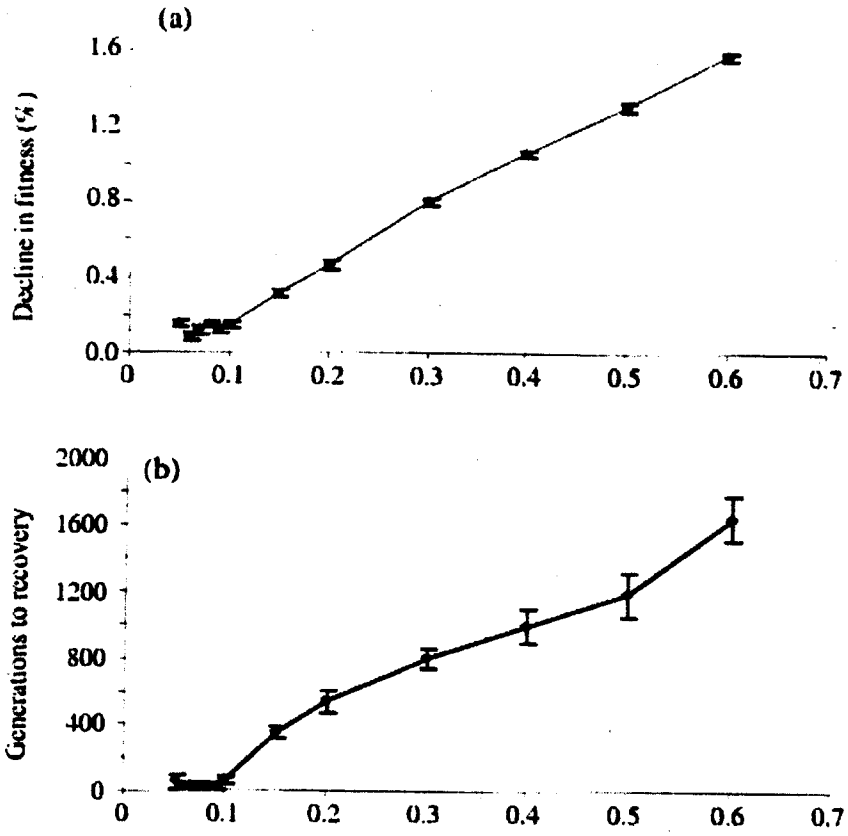

Figure 1. Modelled effect of genetic distance, D, (Nei, 1975) on the (a) magnitude and (b) duration of outbreeding depression (20 diallelic loci, 1 chromosome, population size = 100, mutation rate 0.001/locus/generation). Magnitude and duration were calculated from generation averages for 100 replications. This was repeated 10 times to determine mean and standard error. Figure from (Edmands and Timmerman, 2003).

2. DOCUMENTED GENETIC DIFFERENCES AMONG GEODUCK POPULATIONS

Neutral markers

Identification and safeguarding the genetic variation in natural populations of Puget Sound geoduck clams is essential for effective and sustainable management of this important resource. A pilot project to provide preliminary information regarding levels of variation and potential stock differentiation in geoduck clams was conducted in 1989 by WDFW using allozyme loci. This study revealed that geoducks are highly polymorphic: based on a sample of thirteen hatchery-reared adults and 110 of their progeny, allozyme polymorphisms appeared to exhibit Mendelian inheritance (J. Shaklee, WDFW, pers. comm). A preliminary analysis of 190 geoducks from three Puget Sound locations (Pitt Is./Steamboat Is. in the Southern Basin, Port Townsend in Admiralty Inlet, and Lofall in the Hood Canal Basin) revealed an average heterozygosity summed over all three locations of 35% and statistically significant differences in allozyme allele frequencies between the Hood Canal and South Puget Sound and the Port Townsend and South Puget Sound collections (based on a simultaneous chi-square analysis at all variable loci, S.R. Phelps 1993, WDFW unpublished report). The analyses were hampered by loss of enzyme activity at more than 10 loci in over 50% of one of the samples, so the data are questionable. The report concludes that gene flow is restricted among the three collections from Hood Canal, South Puget Sound, and Port Townsend.

Using the cytochrome oxidase III subunit (COIII) of the mitochondrial genome, Van Koeveringe (1998) found no significant population differences among collections of geoducks within British Columbia, Canada. However, statistical power to detect subdivisions was low, owing first to the use of a single locus, and second to small sample sizes averaging 6.5 individuals with a low of three individuals. More recently, an additional population genetic study was conducted on British Columbia geoducks funded by the Underwater Harvesters Association. This study used microsatellite DNA and larger sample sizes, so the results may be more robust, but the results of this study have not yet been published (K.M. Miller, DFO Pacific Biological Station, Nanaimo, personal communication).

Investigators from the University of Washington and Washington Department of Fish and Wildlife (Vadopalas, 2003) examined population differentiation among collections from sites in the Strait of Juan de Fuca – Georgia Strait - Puget Sound complex using 11 variable allozyme and seven microsatellite loci. This survey (Vadopalas, 2003) of 1645 specimens from 17 locations analyzed at 18 loci (allozymes and microsatellites) revealed a general pattern of apparent panmixia with statistically significant differences interspersed among a minority of collections. A pattern of isolation by distance was not evident, nor was there support for high variance in reproductive success in this species. Both marker classes were concordant in the detection of genetic differentiation of the Freshwater Bay collection in the Strait of Juan de Fuca from others. There was also concordance in the apparent genetic homogeneity among most other collections.

Local Adaptation

Local adaptation may be driving the differences noted in the survey of Puget Sound geoducks. Many of the differences among geoduck populations seen at allozyme loci were primarily driven by a minority of loci that could be linked to loci under selection. While selection operating directly at certain allozyme loci has been implicated in other species (Riginos, et al., 2002), the survey by Allendorf and Seeb (2000) substantiates assumptions of general selective neutrality for this marker class. Nevertheless, both temperature and salinity vary less at the Freshwater Bay site compared to other more estuarine localities. Among the allozyme loci, the significant differentiation of Freshwater Bay from other collections was driven primarily by the allozyme locus GPI. Without this locus, jackknifing over collections indicated that GPI has the strongest effect among allozymes loci on global F_{ST}. Without Freshwater Bay, jackknifing over loci resulted in a reduced F_{ST} for GPI, further indication that the differentiation may be due to this locus. The allozyme locus GPI appears to be under temperature selection in *Mytilus edulis* (Hall, 1985) with a latitudinal gradient among alleles (Koehn, et al., 1976; Koehn, et al., 1984). A similar mild selective effect might explain the observed differences in GPI allele frequencies in geoducks, although no evidence for selection at this locus was detected in geoducks using the method of Beaumont and Nichols (1996). The microsatellite locus Pab4 gave similar results albeit a weaker signal: after jackknifing, this locus likewise showed the strongest effect on F_{ST} and the jackknifed value for the locus without the Freshwater Bay collection was likewise low. Although we detected no linkage disequilibrium among any of the loci in this study, the microsatellite locus Pab4 may be linked to a locus under selection. Selection cannot be ruled out as a possible cause of the genetic differentiation observed in the Freshwater Bay collection, although differences of similar magnitude were detected at neutral microsatellite loci between Carr Inlet and collections within basins with temperature and salinity profiles similar to Freshwater Bay.

3. DOCUMENTED GENETIC EFFECTS OF CWI IN MARINE MOLLUSCS

There are a number of documented cases of genetic effects of aquaculture on conspecific wild populations of finfish, with many from the salmon literature. A striking example is from Thailand, where the endangered Mekong giant catfish (*Pangasianodon gigas*) is reared for release in a restoration aquaculture project. Parentage analyses indicated that 95% of the 10,000 fingerlings released in 2001 were from the same two parents (Hogan, et al., 2004). This loss of genetic variability may ultimately jeopardize survival of this species. Stock enhancement efforts in the Japanese flounder *Paralichthys olivaceus* include breeding efforts via mass spawning in mesocosms, where N_e was reduced by 80% from the relatively small hatchery census size (Sekino, et al., 2003). Busack and Currens (1995) discuss the risks associated with CWI in Pacific salmon, and Utter (1998) provides examples from the extensive salmonid literature on the hybridization effects of CWI. The most important point to be taken from the extensive literature on the effects of interactions between cultured and wild fish populations is that most negative outcomes are the result of poor management decisions (Campton, 1995). Theoretically, many of these consequences can be avoided with careful planning at the onset of a culture program followed by

subsequent monitoring and adaptive management.

There are few published investigations of genetic effects of CWI in marine invertebrates. A study of genetic variability differences between cultured and wild Pearl oysters found no significant differences in the number of alleles or observed heterozygosity (Arnaud-Haond, et al., 2003). In this system, wild spat were collected for use in culture operation. Therefore, unless the process of spat collection somehow modifies the genetic composition of the cultured oysters, genetic differences would not be expected.

Apte, et al. (2003) attempted detection of cultured Greenshell mussel (*Perna canaliculus*) introgression into wild populations using three classes of genetic marker: allozymes, mtDNA, and RAPDs. There were no significant differences in observed heterozygosities between cultured and wild individuals at allozymes. Haplotype diversity for the mtDNA loci in the cultured mussels was significantly lower than in wild populations, but the number of RAPD bands was not different between cultured and wild collections. There were no private alleles in the cultured populations that would allow direct detection of introgression.

In another study designed to detect introgression from cultured to wild populations of the hard clam *Mercenaria mercenaria*, Metzner-Roop (1994) used two allozyme locus (GPI*) alleles present at high frequencies in cultured and rare in wild populations as a genetic marker. Despite repeated outplants of cultured stocks over the course of eight years, elevated frequencies of marker alleles were not detected in collections of 300 individuals from four proximate wild locations. The lack of introgression suggests that introgression was low from cultured to wild stocks.

Reporting genetic effects of aquaculture of marine invertebrates on wild conspecifics, Natsukari et al. (1993) detected significant genetic differences among wild, cultured, and mixed populations of the sea urchin *Pseudocentrotus depressus* in Japan using nine allozyme loci. In addition to allele frequency differences, the authors found reduces heterozygosities and polymorphic loci in cultured populations. These seeding efforts may be compromising the genetic variability in the wild stocks if interbreeding occurs due to CWI.

Another example of genetic effects involved two species of abalone, *Haliotis rubra* and *H. midae*, where approximately 40% of relatively infrequent microsatellite alleles present in wild collections were lost in cultured samples (Evans, et al., 2004). In addition, alleles relatively rare in the wild collections were often the most frequent in the cultured groups, and relatedness levels were high in two cultured groups. These results suggest that the practices associated with the two programs has resulted in changes in the genetic composition of populations, and that the potential for inbreeding is probably high.

Despite the paucity of empirical evidence for genetic effects of CWI, low effective population sizes in cultured populations of mollusks engender concern (Hedgecock &

Coykendall, *in press*). High variance in reproductive success, coupled with high fertilities in many species, may reduce effective population sizes. In marine invertebrates, a very large variance in reproductive success has been hypothesized to constrain effective population size (Hedgecock, 1994b) and effective sizes in marine populations are typically very low (Gaffney, et al., 1992; Hedgecock and Sly, 1990; Hedgecock, et al., 1992; Saavedra, 1997). Empirical evidence for reductions following release of cultured shellfish has been scarce. However, there is ample evidence in the literature on cultured oysters that N_e can be much lower in hatchery than in wild populations (Gaffney, et al., 1992; Hedgecock and Sly, 1990; Hedgecock, et al., 1992; Saavedra, 1997). In the Pacific oyster, *Crassostrea gigas*, Boudry, et al. (2002) demonstrated that some of the high variance in reproductive success likely arises from gamete quality, sperm-egg interactions, and genotype dependent viability.

Ryman, et al. (1995) suggested that the variance effective population size is the most important parameter in a breeding program where genetic interaction between cultured and wild is an expectation, since this dynamic is associated with the loss of genetic diversity. Thus, maximizing the Ne/N ratio in the hatchery can maximize the genetic variability in the cultured stock. In the extreme case, as we shall see below, minimization of family size variance can lead to a higher genetic variability in cultured than in wild stocks. As Hedgecock suggests, a high variance in reproductive success creates a low Ne/N ratio in wild populations, and a much higher Ne/N ratio is maintained in cultured populations; in this case CWI may have neutral or even positive effects on genetic diversity in wild populations (Hedgecock and Coykendall, in press). With no particular family or set of families dominating, the negative effects of any genetic interaction with wild stocks will be minimized. In nature, the ratio of effective to census population size is, on average, 0.11 in land vertebrates (Frankham, 1995). Among long-lived marine species with high fertilities, however, this ratio can be much lower. Hauser, et al. (2002) demonstrated a Ne/N ratio of 10^{-5} in red snapper, and in red drum Turner (Turner, et al., 1999) estimated the Ne/N ratio to be 0.004. Herbinger, et al. (1997) also found correspondingly low ratios in Atlantic cod.

Among marine invertebrates, there have been relatively few empirical estimates of effective population sizes in wild populations. Li and Hedgecock's (1998) study showing high variance in reproductive success in the Pacific oyster provides indirect evidence of low effective population size relative to the census population. This study occurred, however, in Dabob Bay Washington where the Pacific oyster was introduced approximately 100 years ago. Natural spawning is known to occur in the inland waters of Washington, but mass spawns, historically, take place only sporadically and in isolated embayments. Thus, relatively narrow environmental windows of opportunity necessary for successful spawning and fertilization may obviate the reproductive success of many adults oysters in the population. Geoduck clams, on the other hand, are in the center of their distribution in Puget Sound, and so may be maximally adapted to successful spawning in this environment. Mass spawnings have been observed by commercial divers, but the degree to which this occurs and the mechanism for spawning synchrony in geoducks has not been established.

Again, refreshing broodstock from the wild, maintaining high hatchery N_e, and conducting pair matings between many individuals will conserve extant wild within-population diversity.

Because of the potential for reductions in N_e via CWI, the *hatchery N_e may be the single most important factor to monitor in a cultured population.*

4. BEST PRACTICES

While the following section contains general recommendations for the genetic management of cultured geoducks; the development of a more comprehensive strategy necessitates a number of studies as outlined in section 5. Thus, the recommendations below provide a foundation for identifying and understanding the available options, risks, and benefits, but do not represent specific operational recommendations for geoduck culture.

The most risk-averse strategy for aquaculture of a native species is isolation. Segregating cultured animals from their wild conspecifics essentially negates genetic concerns of effective population size, domestication selection, inbreeding depression, outbreeding depression, and neutral and adaptive differences between hatchery and wild. Isolation of cultured animals places these issues firmly within the purview of growers, along with concomitant disease transmission issues. If isolation management is not practicable and some CWI is inevitable, a secondary course of action is correct hatchery management. Culture practices necessary for reduction of negative genetic effects of CWI are addressed in the second part of this section.

Culture Practices to Avoid CWI

Isolation management is focused on the separation of cultured and wild populations. Isolation management is perhaps easiest to envision in a land-based culture operation, where no genetic interactions are possible and effluent water is treated. In addition to spatial segregation, temporal isolation has been used in a number of cases to achieve separation. In Pacific salmonids, selection for run-timing differences can isolate populations, but newly introduced populations can rapidly adapt to new conditions and in some cases, start hybridizing with wild populations (McLean *et al.* 2004). Temporal isolation is only possible in geoduck clams via harvest prior to maturation.

When cultured and wild conspecifics will exist in the same region, cultured stocks are essentially released into a natural system and may negatively impact wild populations. Thus, if possible, maintaining the temporal and/or spatial separation of cultured and wild geoducks is the best solution to many of these issues. There are a variety of ways this isolation can be maintained, in general, but practical considerations severely limit the available options (Box 2).

Box 2. Isolation Management Options for Geoduck Clam Culture

Land based culture

Land based geoduck culture, for example, would require many hectares of simulated subtidal substrate at least one meter deep, with downwelling outflows. The expense associated with such an operation, were it permissible under the shoreline management provisions, would be prohibitive.

Off-shore

An offshore operation involving a benthic infaunal species such as the geoduck clam would be difficult to plant, maintain, and harvest, unless significant progress were made toward artificial substrates for infauna. Also, the distance from wild geoducks would have to be greater than larval dispersal distance.

Harvest prior to maturity

If harvested at the rate of 100% before maturation occurs, intertidal aquaculture of geoduck clams would have no effect on wild populations. This approach is probably the most practical.

Monosex culture

The production of all-male populations may mitigate risk if sperm viability/potential dispersal distance is relatively low. This may be the most feasible solution if geoducks, as reported in the literature, mature first as males. Some geoducks may be protandrous hermaphrodites, maturing first as males and then changing to female later in life (Campbell and Ming, 2003). In either case, a highly skewed sex ratio has been reported among young geoducks. Andersen (1971) reported 94.4% males among individuals with shell length <100 mm, and in another study the proportion of males for individuals <11 years old was 90% (Sloan and Robinson, 1984). Cambell and Ming (2003) also reported a sex ratio of 92.5% males among mature geoducks < 90 mm. In this study, however, 41% of geoducks <90 mm shell length were immature. If such strongly skewed sex ratios remain among commercially grown geoducks until harvest, the likelihood of reproductive success within a population of cultured geoducks would be significantly reduced. However, it must be stressed that a reliance on monosex culture is only feasible if gamete transport to neighboring populations is low. Both gamete age and density affect fertilization success. The age at which gametes become nonviable affects the distance gametes may travel before fertilization and successful zygote formation. Little information is available on geoduck gamete viability, but eggs are viable for at least six hours in culture (Vadopalas and Davis, unpublished data). The further gametes travel from the adult, however, the lower the gamete density and likelihood of successful fertilization. If the watercourse distance between cultured and wild geoduck aggregations is great enough to ensure adequate gamete cloud dilution and/or low

gamete survival, downstream fertilizations with sperm from cultured individuals would be precluded.

Triploid induction or other sterility measures

Isolation via sterility/incompatibility is another strategy to separate reproduction in cultured and wild animals. If cultured animals are incapable of reproduction, risk is low. Development of techniques to confer sterility on hatchery outplants could mitigate potential genetic risk. Induced triploidy has been utilized by the shellfish aquaculture industry since the mid-1980's, in some cases to confer sterility (Beaumont and Fairbrother, 1991). Triploid Manila clams (*Venerupis phillipinarum*) were produced in an attempt to avoid genetic introgression of an introduced species (e.g. Beaumont and Contaris, 1988, Laing and Utting, 1994). For the same reason, triploid *Crassostrea gigas* oysters (Thorgaard and Allen, 1988) and *C. ariakensis* have been contemplated for widespread outplanting into Chesapeake Bay, where *C. virginica* is the native oyster. If total or partial sterility can be conferred on hatchery- produced geoduck clams, it would decrease the potential genetic risk to naturally occurring populations.

The induction of triploidy in geoduck clams, and the first potential use of triploidy to retard gametogenesis to limit intraspecific introgression (as defined by (Futuyma, 1998) from hatchery to naturally occurring bivalve populations, was conducted by Vadopalas and Davis (2004). They found the optimal concentration and contact time for a chemical triploidy induction agent, as defined by both ploidy and survivorship of treated post-embryonic larval geoduck clams, was 600 μM 6-DMAP using a 20 minute contact time. The larval triploid yield of 92.5% with survivorship of 30% was the optimal response achieved; subsequent studies are underway to determine the efficacy of chemical triploid induction of geoduck clams in according sterility.

Triploid induction carries a number of imperfections. Low triploid survivorship both at the larval and juvenile stages do not compare favorably with their diploid counterparts. Also, in some species, triploids may revert over time to diploids (Allen, et al., 1996; Allen, et al., 1999). Whether recovery of reproductive potential occurs, and if so, whether it occurs over growout time scales, is the subject of an ongoing investigation (J.P. Davis, Taylor Resources, personal communication). In addition, preliminary studies on triploid geoducks suggest a lower growth rate for triploid versus their diploid counterparts.

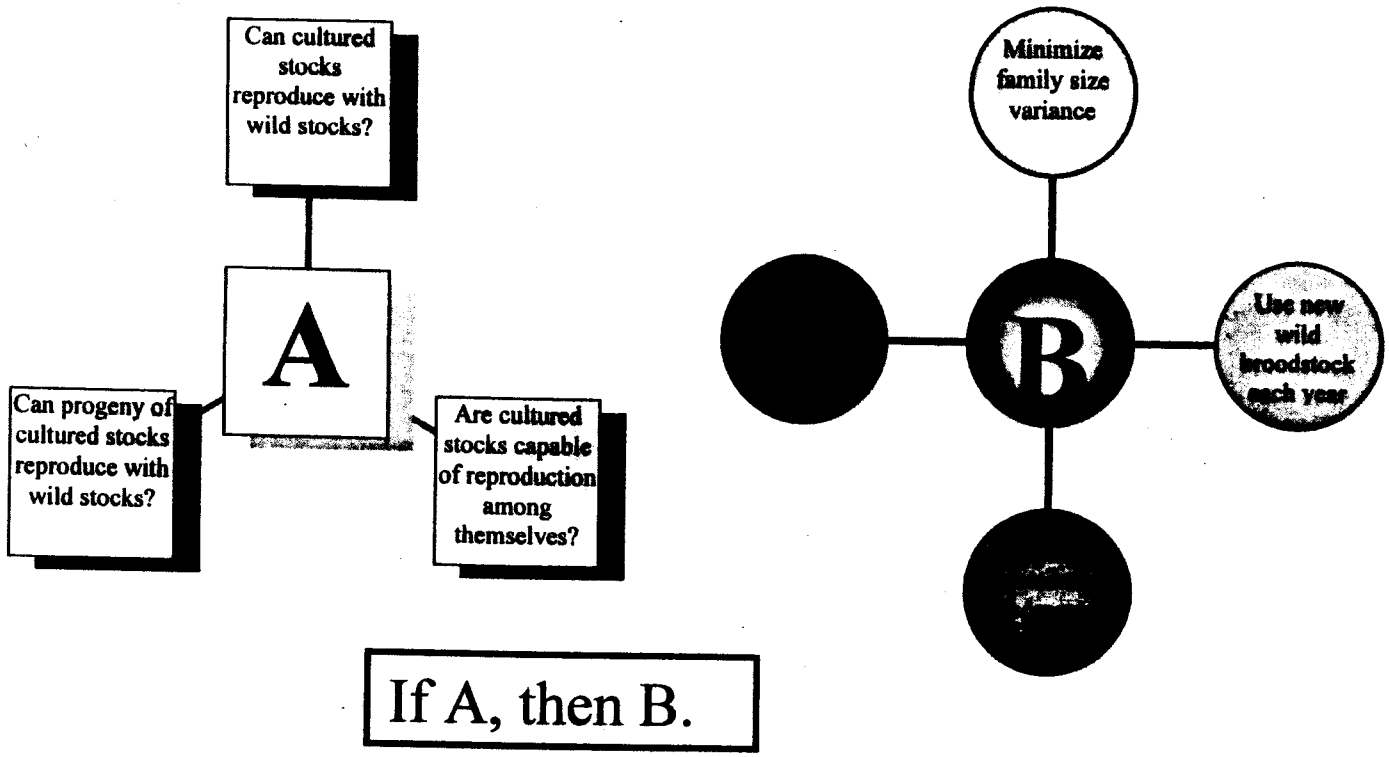

Figure 2. Schematic diagram of modes of genetic interaction between cultured and wild conspecifics, in the presence of which management to maximize hatchery Ne/N and hatchery genetic diversity is necessary to reduce deleterious genetic impacts on wild populations. Sex control methods such as production of single sex or sterile triploids would obviate the outlined hatchery management steps.

Culture Practices to Reduce Negative Effects of CWI

If practical or biological parameters obviate isolation management, then hatchery management must be implemented to avoid negative genetic consequences of CWI. There are three primary management strategy categories to increase genetic diversity in cultured outplants and circumvent negative genetic effects of hatchery culture. These strategies involve 1) maximizing the effective population size, and 2) maximizing genetic diversity (Fig. 2). A third strategy would be to avoid domestication selection as far as possible. One such strategy would be to create a culture environment as similar as possible to the natural environment. This specific strategy will not be addressed here, since eliminating differences between the hatchery and natural environments is difficult. Geoducks, however, are exposed to the hatchery environment for a very short time, and domestication selection during the relatively short hatchery period is likely to be of much less concern than overall loss of genetic variability (Utter, 2004). Also, to the extent family size variance can be kept at a minimum, selection can be reduced (Allendorf 1993, insofar that one family will not be favored over another. However, as Waples (1999) argues, selection occurs over the entire life history of the animal, and mortality is typically higher immediately following release. Efforts to equalize family effects may thus be negated after release. The outcome will be that genetic change will almost inevitably occur in hatchery stocks relative to the wild.

Maximize Ne/N

- *Found broodstock with a large number of individuals*
  Several criteria have been offered for determining the correct number of founding broodstock (Frankham *et al.* 2002) if loss of genetic diversity is to be avoided. The most common criterion is based on the probability of sampling an allele of frequency 0.05 with 95% certainty (Fig 3). Thus, an effective size of about 30-40 founders are required, and most recommend 50 (HSRG, 2000).

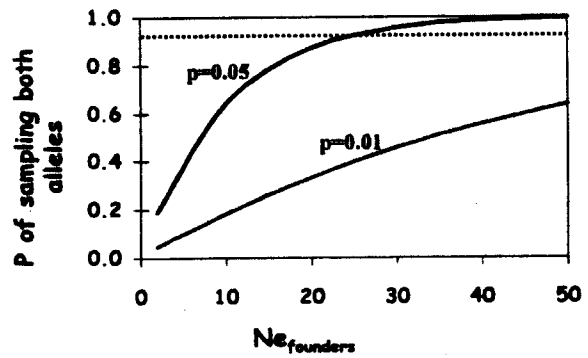

Fig 3. Relationship between probabilities of sampling an allele with frequency of 0.05 (blue line) or 0.01 (green line) and effective size of founding population.

- *Maintain 1:1 sex ratio in breeding plan*
  The effect of sex ratio on the effective population size is well known, and can be quantified as

$$N_e = \frac{4N_{ef}N_{em}}{(N_{ef} + N_{em})}$$

Where N_{ef} is the effective number of breeding females and N_{em} is the analogous number of males. Significant deviations from a 1:1 ratio of reproductively successful parents have a profoundly negative effect on effective population size. These deviations may occur due to unequal sex ratios in the population or to the mating system (Fig. 4).

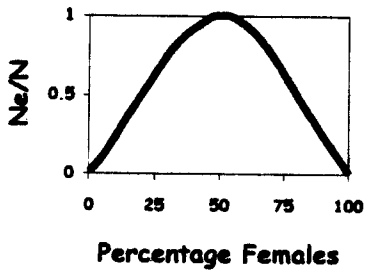

Fig 4. Relationship between sex ratio and the ratio of effective population size to census size

Wild adult geoducks are typically found in roughly equal sex ratios, but the mass spawning mating system undoubtedly distorts the sex ratio of effective parents and thus the N_e/N ratio (Fig. 4). Hatchery mating systems can be designed to minimize deviations from a 1:1 effective sex ratio, which will maximize hatchery N_e, and in fact can increase the N_e in wild populations (Hedrick *et al.* 2001). There are a number of mechanisms that can be utilized to maintain a 1:1 sex ratio. Discrete pair matings can be conducted in pairwise, nested, or factorial crosses.

- *Reduce family size variance*
  In order to maintain a high N_e/N ration in the hatchery, it is insufficient to merely conduct single pair matings. Variance in family size reduces effective population size (Fig 5). Thus to maintain maximum effective population size in the hatchery, the variance in family size must be kept to a minimum. To ensure equal contribution from each cross (a family), family cultures need to be maintained separately until outplanted. Outplanting equal numbers of progeny from each cross maximizes effective population size. Differential family survival reduces the effective population size of the broodstock. A number of studies have demonstrated high variance in family reproductive success in the hatchery environment, both when group spawnings are performed and in more controlled breeding

programs. Boudry et al. (2002??) focused on family size variance in the Pacific oyster, *Crassostrea gigas*, and carefully controlled gamete contribution in single pair matings. Nevertheless, these investigators found that a number of factors contribute to the large variance in reproductive success, including gamete quality, gamete interactions, and differential viability of certain genotypes. Longwell and Styles (1973) found evidence for gamete cross incompatibility in *Crassostrea virginica*. This phenomenon may exist as a function of high genetic load. Launey and Hedgecock (2001) calculated the genetic load in *C. gigas* to be as high as 14 deleterious mutations. High genetic load certainly exacerbates the high variance in reproductive success found in *C. gigas* (Li and Hedgecock, 1998), and if ubiquitous in other cultured marine mollusks presents a challenge for general conservation of genetic diversity.

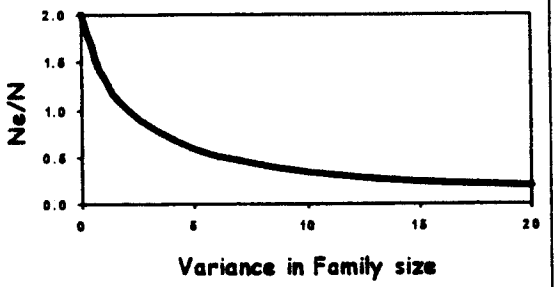

Figure 5. Relationship between variance in family size and the ratio between effective and census size.

Family size variance affects the effective population size according to

$$N_e = \frac{4N - 2}{2 + V_k}$$

Where N_e is the variance effective population size, N is the population size, and Vk is the family size variance. Equal sex ratios are assumed. Thus, when family sizes are equal, V_k is zero, and the equation reduces to

$N_e = 2N - 1$

and N_e can actually exceed N.

Maximize Genetic Diversity

- *Minimize kinship in matings*
  By having genotypes of broodstock on hand, matings can occur between pairs of maximally unrelated individuals. This strategy is relatively easy to implement, by using

either pedigrees or by genotyping broodstock. For efficient genotyping a number of variable loci are necessary, with precision increased either by increasing the number of loci, or number of alleles per locus. With loci such as microsatellites, expected herozygosities can be on the order of 0.95. Depending on the frequency of the most common allele, individuals can by genotyped at as few as two loci. Using these shared microsatellite alleles as proxy for relatedness, individuals can be sorted into mating pairs in accord with low relatedness scores. This method is conservative, since shared alleles may not be identical by descent.

- *Procure new wild broodstock annually*
  Instead of developing broodstock lines, which by definition would result in genetic change (domestication) over time, using a different set of wild broodstock for each spawning can help maximize representation of the wild gene pool. This management strategy runs contrary to common molluscan culture operations, and precludes the common aquaculture practice of improving traits such as growth and siphon color through selective breeding. In the Pacific oyster, *Crassostrea gigas*, not native to the eastern Pacific, concerted efforts are underway to establish improved broodstock lines through line breeding (Molluscan Broodstock Program) and cross breeding of inbred lines (WRAC inbred oyster project). Both of these breeding strategies have potential value for an essentially domesticated species and merit pursuit in such species; if applied to geoduck culture, however, these approaches could have dire consequences for wild stocks if interbreeding were to occur between cultured and wild. It is essential, therefore, to determine whether cultured geoduck can be isolated from wild geoduck before these practices be considered.

Minimize Domestication Selection

Many conservation hatcheries for fish species are attempting to mimic the wild environment as far as possible, in order to minimize environmental differences between capture and wild environments. While preferable, we do not believe this approach feasible, since geoduck are maintained in the hatchery during a very short phase of their life cycle. If anything, we may well be relaxing selection during the crucial settlement phase (at which Pacific oysters are known to be under intense selection; Launey & Hedgecock 2001), and hence may be outplanting less fit individuals into the wild. This concern emphasizes the need to reduce interactions between cultured and wild stocks; reproduction between less fit cultured individuals with wild geoduck would result in suboptimal reproduction by the latter.

However, there are other ways to reduce domestication selection in geoduck hatcheries:

- Randomize matings between broodstock. Every attempt to avoid mating "like with like" (for example, large animals with large) should be avoided.
- It is known that selection is more efficient on larger, rather than on smaller, populations. We strongly recommend that broodstock collection, spawning and larval rearing be performed over a number of hatcheries, rather than one hatchery provide

seed for all operations.

- The number of generations should be reduced in the hatchery. This point is made above, but the more frequent the collection from the wild, the less opportunity for selection. Broodstock should be collected from wild, rather than from outplanted individuals.

Minimize negative interactions with wild populations

- *Derive broodstock from local sources only*

- *Avoidance of "broodstock mining"*

One danger of geoduck culture is the use of wild geoduck as sources for cultured individuals – without the replacement of these individuals in the wild. In other words, we may reduce the genetic diversity of wild populations by "mining" these individuals. Every attempt should be made to replace wild broodstock after spawning, preferable to the location from which they were taken.

- *Use an outplanting strategy that reduces the proximity of related individuals*

Ongoing assessment of the affects of geoduck culture on wild populations

We emphasize the need for "adaptive management" of geoduck culture. However, such approaches should be implemented from the start of this endeavor, rather than several generations after implementation. Thus, we strongly recommend an effective monitoring program that incorporates the following;

- Collection and archiving of tissue samples from all broodstock
- Collection of data on traits that may be related to fitness in geoduck – weight, length, sex, (what other traits – can we do age at maturity without killing the animal?)
- Maintenance of pedigrees in each hatchery, and maintenance of family records (survivorship of offspring per family, number of individuals outplanted from each family, survivorship in the wild)
- Genotyping of wild populations to examine the incidence of reproduction between wild and cultured geoduck. Such work should be budgeted into the operation of any hatchery or outplant site.

5. RESEARCH NEEDS

Ideally, geoduck aquaculture efforts would pose no risk to wild populations. Irrespective of whether or not isolation management is feasible, risks associated with different management strategies should be assessed. Geoduck aquaculture may be viewed as a stocking or enhancement effort, where, under some management and/or biological scenarios, the proportion of reproductive contributions of the stocked population varies from zero to one. A

risk assessment using a quantitative model should be conducted once basic demographic parameters for geoduck clams are in place. Although a model will be flawed by myriad assumptions, reliable results may be attained if we have accurate estimates of:

Age at harvest-
Harvest rate-how many left after harvest?
Reproductive potential at age
Intrapopulation proximity
Interpopulation proximity
Sex ratio at ages
Number planted
Gamete age and viability
Spawning synchrony—mechanisms and prevalence

Sex ratio and maturity at age are the subjects of a current investigation being conducted at the UW. Some of the data needs may be readily available from the industry. The following subjects remain to empirically investigate:

Possibility for sex control (single sex/sterile)
Range/scale of Allee effects in geoduck
Magnitude and duration of larval and juvenile migration
Reproductive success of cultured animals
Levels of outbreeding depression
Neutral genetic differences between cultured and wild
Quantitative genetic differences between cultured and wild
Gamete age and viability
The relationship between reproductive contribution and depth.
If different reproductive contributions to the maintenance of native stocks are made by geoducks at different depths, commercial outplant sites and harvest strategies may affect the level of genetic risk of CWI.

6. CONCLUSIONS

The existence of cultured bivalves alongside their wild counterparts is unusual in Puget Sound. The identification of genetic variability and stock structure in wild populations of geoduck clams is a necessary prerequisite for monitoring genetic change to wild populations. The neutral genetic differences detected within Puget Sound do not appear related to reproductive isolation or lack of gene flow, but more work is needed to address whether adaptive differences exist.

In the absence of more data, it is only possible to make very general operational recommendations for the genetic management of geoduck clam culture. Maintaining a high effective population size in the hatchery by replenishing broodstocks from wild populations, conducting single pair matings, equalizing family sizes, and sustaining high genetic diversity among outplants will reduce many of the potential impacts of cultured on wild strains. Both the establishment of a genetic monitoring program for hatchery outplants, and the further exploration of isolation management strategies, would be prudent and proactive at this stage.

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