# Shellfish aquaculture and conservation of two Puget Sound molluscs: the Pinto abalone (*Haliotis kamtschatkana kamtschatkana*) and the Pacific geoduck (*Panopea generosa*)

Kristina M. Straus

A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington

2010

Program Authorized to Offer Degree:
Aquatic and Fishery Sciences

University of Washington
Graduate School

This is to certify that I have examined this copy of a doctoral dissertation by

Kristina M. Straus

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made

Chair of the Supervisory Committee:

Carolyn S. Friedman

Reading Committee:

Brent Vadopalas

Carolyn S. Friedman

Willie J. Swanson

Date: 12/15/10

In presenting this dissertation in partial fulfillment of the requirements for the doctoral degree at the University of Washington, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of the dissertation is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Requests for copying or reproduction of this dissertation may be referred to ProQuest Information and Learning, 300 North Zeeb Road, Ann Arbor, MI 48106-1346, 1-800-521-0600, to whom the author has granted "the right to reproduce and sell (a) copies of the manuscript in microform and/or (b) printed copies of the manuscript made from microform."

Signature Kristina M. Straus

Date December 15, 2010

University of Washington

## Abstract

Shellfish aquaculture and conservation of two Puget Sound molluscs: the Pinto abalone (*Haliotis kamtschatkana kamtschatkana*) and the Pacific geoduck (*Panopea generosa*)

Kristina M. Straus

Chair of the Supervisory Committee:
Associate Professor Carolyn S. Friedman
School of Aquatic and Fishery Sciences

I examined aquaculture and conservation of two taxa native to Washington, USA (WA): Pinto abalone (*Haliotis kamtschatkana kamtschatkana*) and Pacific geoduck (*Panopea generosa*). Because pinto abalone populations continue to decline in WA despite fisheries closures, conservation aquaculture may be necessary. To determine appropriate culture methods, juveniles were reared in habitat-enriched or conventional tanks. No differences in survivorship or growth were observed but abalone behavior differed between rearing treatments. Abalone from habitat-enriched tanks changed habitats more often and spent different proportions of time in available habitats. Results demonstrate that rearing conditions affect abalone behavior and should be considered for abalone restoration.

Abalone are commonly misidentified, increasing the challenge of abalone management and conservation. I developed sequence-based genetic markers for species identification of Eastern Pacific abalone. I applied these tools and determined that flat abalone (*H. walallensis*) are a cryptic species in WA. Several individuals collected for the pinto abalone conservation aquaculture program were identified as flat abalone. Use of these individuals as broodstock may have led to interspecific hybridization and been detrimental to pinto abalone restoration. Results highlight the importance of molecular tools in abalone management, especially if conservation aquaculture is used.

To examine the potential genetic implications of geoduck aquaculture, I used five microsatellite loci to conduct two studies comparing genetic diversity in wild and cultured geoducks. In both studies, cultured geoduck showed reduced genetic diversity and effective number of breeders ($N_b$). In one study, I examined geoduck seed produced in two hatcheries. Parentage assignment revealed that in one hatchery, many parents contributed to each seed cohort, with the largest full-sib family comprising 11-31% of the offspring. In contrast, 94% of the seed from the second hatchery were from a single full-sib family. In a complementary study, I examined five year classes of cultured geoducks. Sibship assignment revealed that year classes were comprised of nine to 25 full-sib families and many individuals unrelated to others at the full-sib level. Results from both studies demonstrate that hatchery practices affect genetic diversity; these results may aid in developing geoduck culture practices that minimize genetic risk to wild populations.

## TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

# Chapter I
# Literature Review

## Background

Human beings have likely always made changes to their immediate surroundings, but due to human population growth and increasing resource use, the rate at which humans perturb natural ecosystems has increased dramatically since the industrial revolution. Although it took many thousands of years for the human population to reach one billion, the population increased from one billion in 1804 to six billion in 1999 (United Nations Population Division 2000), and has since grown to 6.8 billion (U.S. Census Bureau, 2010). Concurrent with the increasing population, per capita consumption has increased 3% per year starting in the 1970s (Hawken et al. 1999). The escalation of human population and consumption has led to increasing pressures on organisms and ecosystems from factors including habitat loss, over exploitation, exotic species, pollution, and climate change (Groom et al. 2006). There are few, if any, environments left on earth that are undisturbed by humankind (Sanderson et al. 2002) and many species are in decline (International Union for the Conservation of Nature 2010). Given this situation, the need to understand the "principles and tools" necessary to conserve biodiversity in a landscape of human perturbation grows greater with each passing year (Soule 1985).

The need to conserve a species becomes obvious, and urgent, when that species has declined to the point that extinction is likely without conservation efforts. The United States (U.S.) Endangered Species Act and the Convention on International Trade in Endangered Species are powerful examples of national and international legislation,

respectively, that aim to conserve species that have declined to this degree. However, it is also important to consider conservation while species remain relatively healthy. Scientists must aim to understand the likely effects of human activity and work to provide solutions that could mitigate adverse environmental effects, before damage is realized (Redford and Sanjayan 2003). The conservation spectrum can thus include "rescuing" endangered species from extinction (e.g. by captive rearing and release of offspring; Meretsky et al. 2000) as well as preserving the long-term viability of a species in the context of changing human use of an environment. My dissertation research examined conservation of two species residing in Puget Sound that are at opposite ends of this conservation continuum.

Puget Sound is an estuarine fjord comprising the inland waters of Washington State. Over four million people reside in the Puget Sound region, and the human population of this area has grown by an average of 40,000 people per year since the 1940s (Culliton 1998, Fraser et al. 2006). This increase in human population has been accompanied by changes to the Puget Sound ecosystem including habitat alteration, increased contaminant levels, and increased harvest. The Puget Sound shoreline has been extensively altered by activities including diking, filling, and removing vegetation (Rice 2006). Harvest within Puget Sound has also altered the ecosystem; many taxa have experienced substantial declines due in part to over exploitation. The reduced abundance and population density of these taxa may have changed the ecosystem in ways that scientists and managers do not fully understand.

Inhabitants of Puget Sound face multiple threats and many taxa are at risk. Currently, three molluscs, 25 fish, and five marine mammal species that spend at least a portion of their lives in the Puget Sound are protected as endangered, threatened, or of concern (WDFW 2009b). Many taxa including some rockfishes (*Sebastes* spp; Parker et al. 2000), salmonids (*Oncorhynchus* spp.; WDFW 2009a), and the pinto abalone (*Haliotis kamtschatkana kamtschatkana*; Rothaus et al. 2008) continue to decline despite fisheries management efforts. Conservation aquaculture is a tool that may be used to conserve or restore aquatic species in such cases (McCormick and Brogan 2003, Preston et al. 2007, Steffens 2008). In fact, the U.S. Fish and Wildlife Service have used conservation aquaculture for 30% of the freshwater fish species listed under the Endangered Species Act (Johnson and Jensen 1991). However, aquaculture also has potentially deleterious effects on marine environments; aquaculture has been implicated in disease introductions (Burreson et al. 2000), habitat changes (Krost et al. 1994, Nizzoli et al. 2006), and decline of marine populations (Ford and Myers 2008). Aquaculture may also expose wild populations to genetic risk such as homogenization of populations or loss of genetic diversity (Gilk et al. 2004, Utter 1998, Utter and Epifanio 2002). I examined aquaculture and conservation of two taxa native to the Puget Sound: restoration aquaculture for pinto abalone (*Haliotis kamtschatkana kamtschatkana* Jonas, 1845) and the potential genetic effects of commercial geoduck (*Panopea generosa* Gould 1850) aquaculture on wild conspecifics.

## PINTO ABALONE RESTORATION AQUACULTURE

Abalone are herbivorous gastropod molluscs in the genus *Haliotis*, which includes 56 recognized species (Geiger 2000) that inhabit tropical and temperate oceans worldwide. Abalone are patchily distributed, tending to be found in nearshore subtidal rocky habitats with abundant kelp. Eight abalone taxa are found in the Eastern Pacific (*H. rufescens, H. kamtschatkana kamtschatkana, H. k. assimilis, H. walallensis, H. fulgens, H. corrugata, H. cracherodii, H. sorenseni*). These abalone were once abundant along the west coast of North America but have suffered catastrophic declines due to over-harvest (Hobday et al. 2001, Rothaus et al. 2008), climate changes (Rogers-Bennett 2007, Tegner et al. 2001, Vilchis et al. 2005), and disease (Altstatt et al. 1996, Haaker et al. 1992b, Miner et al. 2006, VanBlaricom et al. 1993). Two abalone species (*H. cracherodii* and *H. sorenseni*) are federally protected by the U.S. Endangered Species Act as endangered and three additional species are listed as species of concern (*H. fulgens*, *H. corrugata*, and *H. k. kamtschatkana*; (National Oceanic and Atmospheric Administration 2009).

The pinto abalone (*H. k. kamtschatkana*) ranges from Point Conception, California to Yakutat, Alaska and is the northernmost abalone species worldwide (Geiger 2000). The pinto abalone is the predominant abalone in Washington State and the only known abalone in British Columbia, Canada (B.C.) and Alaska. Pinto abalone populations throughout the range experienced substantial declines during the second half of the twentieth century, and most fisheries were closed in the 1990s. Commercial harvest of pinto abalone in B.C. peaked at over 400 metric tons in 1979 and declined until the fishery was closed in 1990 (Jamieson 2001). Population densities continued to decline

despite a complete harvest moratorium, falling by 43% between 1993 and 1997 (Campbell 2000). The pinto abalone was designated as threatened under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2000 and was uplisted to endangered in 2009 (COSEWIC 2009). In the United States, commercial fisheries for Pinto abalone existed in California and Alaska but were closed in the 1990s due to declines in abalone abundance. Pinto abalone were never commercially fished in Washington State, but a recreational fishery existed for many years. This fishery was closed due to sustainability concerns in 1994. In California and in Washington State, pinto abalone abundance has continued to decline despite complete harvest closures (Rogers-Bennett 2007, Rothaus et al. 2008). The Pinto abalone was federally listed as a species of concern in 2004 (National Oceanic and Atmospheric Administration 2009) and is currently recognized as both a State Candidate species and a Species of Concern within Washington State (WDFW 2009b).

At ten index stations monitored by the Washington Department of Fish and Wildlife (WDFW) in the San Juan Island Archipelago, abalone abundance declined by 77% between 1992 and 2006 (Rothaus et al. 2008). Such reduced numbers make pinto abalone vulnerable to the Allee effect: a situation in which decreased population size leads to decreased reproduction and survival (Allee et al. 1949). In abalone, fertilization success may be limited when spawning animals are separated by more than one to two meters (Babcock and Keesing 1999, Riffell et al. 2004). Recruitment declines suggestive of the Allee effect have been documented when abalone population densities decrease below a threshold level (0.15-0.3 abalone per $m^2$), which varies by taxa and location (Richards

and Davis 1993, Shepherd and Brown 1993). In 1994, half of the WDFW index stations contained abalone at densities suitable for successful fertilization; by 2006, densities at all ten index stations were below this threshold (Rothaus et al. 2008). Additional data from both length frequency analyses and juvenile abundance surveys indicate very low recruitment over several years (Bouma 2007, Rothaus et al. 2008). Taken together, these data suggest low population density induced recruitment failure. The Allee effect has been implicated in the failure of multiple sedentary marine species to recover from over-fishing (Bell 2008, Stoner and Ray-Culp 2000). Population densities of pinto abalone may be too depressed for natural recovery; restoration efforts such as aggregating reproductive adults so that they are in close proximity to one another when they spawn (Tegner 1992) and supplementing wild populations with hatchery-reared juveniles may be necessary to successfully restore pinto abalone populations in Washington State.

Restoration efforts involving the release of hatchery-reared larvae or juveniles into the wild to conserve or restore wild populations (hereafter conservation aquaculture) have been conducted with several species of fishes (Araki et al. 2007, Cummings et al. 1997a, DeHaan et al. 2008) and invertebrates (McCormick and Brogan 2003, Preston et al. 2007). Release of aquacultured larvae or juveniles has also been used to enhance fished populations limited by recruitment (hereafter enhancement or stock enhancement) in a wide variety of taxa (Arnold 2008, Bell et al. 2008, Bell et al. 2005, Dixon et al. 2006, McEachron et al. 1998). Despite years of efforts in many taxa, success rates in stock enhancement projects are often low (reviewed by Bell et al. 2006 and Leber et al. 2005). For example, hatchery production of pink salmon (*Oncorhynchus gorbuscha*) is thought

to have replaced rather than augmented wild fish production in Prince William Sound, Alaska (Hilborn and Eggers 2001) and yellow croaker (*Larimichthys crocea*) stock enhancement efforts in China failed to reverse population declines and restore the fishery (Liu and de Mitcheson 2008).

Abalone have been outplanted for conservation or enhancement purposes in many countries including Japan (Ino 1966, Kojima 1995), New Zealand, the U.S. (Tegner and Butler 1985b) and others (reviewed by McCormick et al. 1994 and Tegner and Butler 1989). Survival of outplanted juveniles after one year has varied from less than 1% (Goodsell et al. 2006, Rogers-Bennett and Pearse 1998, Tegner and Butler 1985a) to over 30% (Davis 1995, Dixon et al. 2006, Schiel 1992). Variable success may be due to many factors, including the health, size, and shell color of juvenile abalone, habitat where abalone are planted, and predation (McCormick et al. 1994).

Behavioral differences between cultured and wild abalone may also contribute to juvenile mortality. Behavioral differences between hatchery-reared and wild fishes are thought to contribute to predation in outplanted fishes - much of which occurs shortly after release (reviewed by Olla et al. 1998 and Salvanes and Braithwaite 2005a). Although this phenomenon is less well known in invertebrates, hatchery-reared blue crabs (*Callinectes sapidus*) bury in sediment less often than do wild conspecifics and hatchery crabs experience increased predation in the wild (Davis et al. 2004). In laboratory experiments, hatchery-reared abalone differed in movement patterns and habitat-selection and were more easily preyed upon than were wild conspecifics (Schiel and Welden 1987, Tegner

and Butler 1989). Rearing conditions within the hatchery have been shown to affect behavior in a variety of aquatic species (Berejikian et al. 2000, Brown et al. 2003, Lee and Berejikian 2009, Salvanes and Braithwaite 2005b). For example, Berejikian et al. (2000) illustrated that steelhead (*Oncorhynchus mykiss*) reared in habitat-enriched tanks (supplemented with structures, overhead cover and underwater feeders) socially dominated conspecifics reared in conventional tanks.

Given the increasing numbers of threatened and endangered aquatic species worldwide and the desire to supplement declining populations with captive-reared animals, it is imperative to optimize culture methods for successful restoration. Rearing conditions for conservation aquaculture must be designed to minimize behavioral differences between cultured and wild abalone, while simultaneously maximizing growth and survivorship in the hatchery. The balance between growth and survivorship in the hatchery and subsequent survival in the wild must be considered in order to develop a cost-effective and successful enhancement program. As a first step towards determining whether rearing conditions can affect this balance, I examined the growth, survivorship and behavior of pinto abalone reared in habitat-enriched (supplemented with coralline algae encrusted rocks, sea urchins, and native macroalage) and conventional tanks. A better understanding of the culture conditions necessary to produce juveniles that demonstrate high survival in the wild is critical to effectively restore abalone populations in Washington State.

If conservation aquaculture is to be used to help restore pinto abalone, genetic concerns must also be incorporated into the development of captive rearing strategies. Aquaculture poses genetic threats to wild populations including loss of genetic variability and homogenization of populations (Utter 1998, Waples 1991); first and foremost, a restoration program must do no harm. As pinto abalone conservation aquaculture commences, we must understand how genetic diversity is structured in this species and incorporate this knowledge into hatchery management. Otherwise, outplanting hatchery-reared juveniles may result in declines in the genetic diversity of wild populations and homogenization of populations. Population structure varies widely among abalone species, and the detection of this structure varies depending on the markers used. In the red abalone (*H. rufescens*), one allozyme locus (Kirby et al. 1998) and 41 of 163 amplified fragment length polymorphism (AFLP) markers (Gruenthal et al. 2007) showed significant genetic divergence among populations. However, this genetic divergence was not detected using additional allozyme loci, microsatellites, and mitochondrial DNA (mtDNA) sequences (Burton and Tegner 2000, Gruenthal et al. 2007, Kirby et al. 1998). In contrast, black abalone (*H. cracherodii*), a sympatric species, shows significant population structure at all markers examined (allozyme, AFLP, microsatellite, and mtDNA loci (Chambers et al. 2006, Gruenthal and Burton 2008, Hamm and Burton 2000), with the pattern in AFLP and microsatellite markers suggesting isolation by distance (Gruenthal and Burton 2008). In Australia, weak genetic differentiation at allozyme loci was found among populations of both *H. laevigata* and *H. rubra* (Brown 1991b, Brown and Murray 1992). A later study using three molecular markers (randomly amplified polymorphic DNA, minisatellites, and microsatellites)

found significant population structure in Australian *H. rubra* with a strong signature of isolation by distance (Huang et al. 2000). The Taiwanese abalone (*H. diversicolor* Reeve, 1846) shows extremely high population structure, with fixed mtDNA differences between populations separated by only 35 km (Jiang et al. 1995).

Previous research detected little population structure in the pinto abalone (Withler et al. 2003). Withler et al. (2003) used eight microsatellite loci to analyze genetic diversity in pinto abalone from 31 sites in B.C. and one site in Southeast Alaska. Little evidence of differentiation was found among coastal B.C. locations but these sites differed from sites in the Queen Charlotte Islands and Alaska. Results indicated that 99.6% of variation was found within abalone samples with only 0.4% partitioned among samples. In initial studies examining mtDNA genetic diversity within Washington State abalone, we uncovered evidence of several genetically distinct abalone. At the mtDNA genes encoding cytochrome oxidase *c* subunit I (COI) and cytochrome oxidase B (CytB), these animals diverged strongly from other pinto abalone. At COI, these divergent individuals grouped not with pinto abalone but with flat abalone (*H. walallensis*). Although the historical distribution of flat abalone may have extended to southern Washington State, these animals are not known to be here presently and have not been in Washington's inland marine waters (Geiger 2000).

It should be noted that these divergent abalone cannot be distinguished from pinto abalone using gross morphology; they are morphologically similar to but genetically distinct from pinto abalone. There are several ways to explain this observation: 1) the

genetically divergent individuals may form a reproductively isolated sympatric population of pinto abalone, 2) they may be flat abalone, 3) they may belong to another species of abalone or 4) they may be hybrids between pinto abalone and a second species.

Abalone color and morphology vary substantially depending upon habitat and diet (Leighton 2000) and abalone are commonly misidentified due to phenotypic plasticity. In one case, twenty white abalone (*H. sorenseni*) were collected for use as broodstock in the white abalone recovery program (McCormick and Brogan 2003). These individuals were morphologically identified as white abalone, but genetic analyses revealed that one abalone was *H. kamtschatkana* (either pinto or threaded abalone (*H. k. assimilis*), the southern sub-species; Gruenthal and Burton 2005). In another case, researchers examining phylogenetic relationships among Indo-Pacific abalone using the mtDNA gene cytochrome oxidase II (Degnan et al. 2006) found that two abalone identified as *H. varia* were actually members of a previously unknown taxon, a morphologically cryptic species basal to the entire Indo-Pacific clade.

Before we ascertain which abalone taxa are present in Washington State, managers cannot accurately assess abalone populations nor plan appropriate conservation actions. My work to examine genetic diversity and species identification in Washington State abalone is particularly timely because pilot scale pinto abalone restoration programs which require identification (e.g. conservation aquaculture and adult aggregation) are currently being conducted by WDFW in collaboration with the University of Washington and the Puget Sound Restoration Fund. Several genetically divergent individuals are now held in the conservation aquaculture facility, but their use as broodstock has been

discontinued until their taxonomic status is determined. If these individuals are not pinto abalone, their inclusion as broodstock could have serious consequences for pinto abalone recovery. At the least, using these animals in culture could lead to wasted effort, as interspecific abalone hybrids often show reduced survivorship and fertility (Coleman and Vacquier 2002). No long term increase in abalone numbers would be expected if non-viable offspring were outplanted. Outplanting viable hybrid offspring could also negatively affect pinto abalone; hybridization has led to the extirpation or extinction of many species (Rhymer and Simberloff 1996).

## POTENTIAL GENETIC EFFECTS OF GEODUCK AQUACULTURE

The Pacific geoduck (*Panopea generosa* (until recently = *P. abrupta*, (Vadopalas et al. 2010); hereafter geoduck) is an extremely large hiatellid clam; the largest individuals have shells over 200 mm long and weigh more than 3.25 kg (Goodwin 1976, Goodwin and Pease 1991a). Geoducks are found in the Eastern Pacific from Baja California, Mexico to Alaska, USA (Anderson 1971, Coan et al. 2000, Morris et al. 1980). Geoducks are filter-feeders that generally burrow 50-60 cm into soft sediments (Goodwin 1976) from the low intertidal to subtidal habitats more than sixty meters deep (Goodwin 1976), with pilot video-surveys suggesting that geoducks may be found to 110 meters (Jamison et al. 1984). Geoducks are very long-lived; many individuals have been aged at over 100 years (Campbell and Ming 2003, Goodwin 1976, Shaul and Goodwin 1982, Sloan and Robinson 1984). Geoducks likely influence the ecosystem through filter feeding and biodeposition, as has been documented in other bivalves (Newell 2004).

Geoducks are thought to be dioecious (Goodwin 1976) but some evidence suggests that they may be protandrous hermaphrodites. A highly skewed sex ratio is observed in young clams; two studies identified more than 90% of small (SL< 100 mm, (Anderson 1971) or young (<11 years, (Sloan and Robinson 1984) clams as male. At three locales within Puget Sound, 77% of two year old geoduck and 67% of three to five year old geoduck were identified as male (B. Vadopalas, unpublished data). In histological examinations of 253 geoducks, one individual was observed with both oocytes and spermatozoa (Campbell and Ming 2003); researchers at the University of Washington have observed a similar rate of occurrence (B. Vadopalas, unpublished data). Geoduck clams broadcast spawn. Their fertilized eggs develop into plankotrophic larvae (Sloan and Robinson 1984) that remain planktonic for 47 days at 14°C (Goodwin et al. 1979). Geoducks are fished commercially in Washington State, Alaska, and B.C. (Hoffmann et al. 2000). Commercial geoduck fisheries began in Washington State in 1970 (Washington Department of Natural Resources 2000) and in B.C. in 1976 (Muse 1998). Market demand for geoduck was limited when the fishery commenced but both Asian and domestic markets have grown over time (Washington Department of Natural Resources 2000). Between 1992 and 1999, an average of 1.6 million pounds of geoducks were harvested per year, which generated between five and seven million dollars annually in Washington State (Washington Department of Natural Resources 2000). Between 1999 and 2008, an average of 4.3 million pounds of geoduck clams were harvested annually (Mel Stanley, WDFW, personal communication to Brent Vadopalas on Oct. 15, 2009). The geoduck fishery is now the most lucrative clam fishery along the Pacific coast of North America (Washington Department of Natural Resources 2000).

Methods to culture geoduck clams were developed by Washington State to speed recovery of fished beds in 1991 (Beattie 1992). These methods were adapted by the shellfish industry; geoduck aquaculture is lucrative and is now expanding in Washington State (J.P. Davis, Taylor Resources, Inc., Quilcene, Washington, personal communication). Although some advocate culturing native taxa to reduce negative environmental impacts of aquaculture (e.g. De Silva et al. 2009, Naylor et al. 2001), aquaculture with native species also carries risks, including genetic risks. Genetic risk may be defined as exposing a natural population to genetic change by human action (Currens and Busack 1995). In native species, such as geoduck in Puget Sound, two risks are the primary concerns. First, if wild populations are locally adapted to their specific micro environments, aquaculture may homogenize these groups and reduce overall fitness through outbreeding depression. Second, regardless of genetic structure of wild populations, aquaculture may reduce the natural genetic diversity, which enables the population to adapt to a changing environment. Drastic reductions in genetic diversity can reduce the fitness of a population and can even lead to extinctions (Frankham and Ralls 1998, Saccheri et al. 1998). Previous work using allozymes and microsatellites found little evidence of neutral population structure but high levels of genetic variation among collections of Puget Sound-Georgia Basin geoduck clams (Kaukinen et al. 2004, Miller et al. 2006, Vadopalas et al. 2004). Although microsatellites are thought to be neutral, high microsatellite diversity may suggest high adaptive potential in local geoducks, which may be perturbed by aquaculture of conspecifics. Because wild geoducks likely serve many important ecological roles within Puget Sound and because

they are the basis of an economically valuable fishery, it is important that aquaculture expansion does not come at the expense of wild geoduck populations. It is thus imperative to assess the potential genetic effects of geoduck aquaculture on wild conspecifics. Wild geoduck populations may be at risk if: 1) geoduck aquaculture occurs in close proximity to wild populations, 2) cultured geoducks mature and spawn before they are harvested, and 3) cultured geoducks are genetically distinct from wild geoducks.

Geoduck aquaculture occurs in close proximity to wild populations and preliminary evidence suggests that cultured and wild gametes may directly interact (B. Vadopalas, unpublished data). Additionally, because geoduck larvae are planktonic for over six weeks (Goodwin 1976), larvae produced within cultured beds may settle nearly anywhere within Puget Sound. If such larvae survive to maturity, their gametes may interact with those of wild geoduck. Thus, distance likely does not preclude indirect genetic interactions between wild and cultured geoducks. If cultured geoduck do not mature and spawn before they are harvested, the genetic risk of geoduck aquaculture is nearly eliminated. The age at which geoducks mature and release gametes is unclear; two B.C. studies found widely disparate results (Campbell and Ming 2003, Sloan and Robinson 1984). However, evidence suggests that geoducks cultured at multiple locations within Puget Sound mature and spawn before they are harvested, with 50% maturity at age two years (B. Vadopalas, unpublished data). Because cultured geoducks within Puget Sound spawn before they are harvested, and because distance does not preclude genetic interactions between wild and cultured geoduck clams, geoduck aquaculture may put

wild geoduck populations at genetic risk. If cultured geoducks are genetically distinct from wild conspecifics, the genetic risk to wild geoducks may increase.

Shellfish reared in an aquaculture facility may be genetically distinct from wild conspecifics for many reasons. Genetic drift, random changes in allele frequency from one generation to the next, is one such reason. Genetic drift plays substantial roles in the evolutionary processes that occur in hatcheries because random effects play large roles in small populations. Reduced genetic diversity, random allele frequency changes, and diversification among populations are expected in hatchery shellfish due to random genetic drift. The "founder effect" (Holgate 1966, Mayr 1942) is one type of genetic drift that affects hatcheries. Whenever a new population is established by a small subset of individuals from a larger population, as is the case when a hatchery population is established, genetic diversity is lost because the smaller population simply cannot contain all of the genetic variation found in the original population. Due to random chance, the founders may carry genetic variants that are uncommon in the larger population; these variants become common in the hatchery population.

Selection and adaptation also contribute to genetic differentiation between hatchery and wild shellfish. Broodstock collected distantly from the culture location may have genomes reflecting adaptation to a different environment. Mounting evidence suggests that local adaptation may occur even in marine invertebrates with pelagic larvae (Bertness and Gaines 1993, Gartner-Kepkay et al. 1983, Koehn et al. 1976, Marshall et al. 2010, Riginos and Cunningham 2005, Yanick et al. 2003). Yanick et al. (2003) reared

newly settled blue mussels from two locations (150 km apart) in a common garden experiment at one collection location. The mussels collected from the rearing location showed both better survival and growth, suggesting local adaptation (Yanick et al. 2003). Selection plays a role in the differentiation between hatchery and wild shellfish because the selective pressures acting upon cultured shellfish differ from those acting upon wild shellfish in the natural environment. Shellfish hatcheries alter selection pressures by culling slow growing larvae, changing environmental parameters such as temperature, salinity, and food availability, and actively selecting for commercially valuable traits.

Many shellfish are characterized by extremely high fecundities (e.g. the Pacific Oyster (*Crassostrea gigas*) can release 55 million eggs during one spawning and may spawn multiple times per season; Galtsoff 1930). Due to this extreme fecundity, entire hatchery cohorts can be produced with very few broodstock pairs which will reduce the hatchery genetic effective population size ($N_e$) (Hedgecock and Sly 1990). Even when many broodstock are used, differential fertilization success or survival at the family level can reduce $N_e$ and shift the genetic makeup of the hatchery population towards that of a few successful families. For example, in the silver-lipped pearl oyster (*Pinctada maxima)*, despite the use of more than 25 broodstock, up to 40% of individuals in hatchery cohorts derived from a single full-sib family (Lind et al. 2009). Multiple studies have documented significantly lower $N_e$ in hatchery oysters than in their wild counterparts (Gaffney 1992, Hedgecock et al. 1992, Hedgecock and Sly 1990, Saavedra 1997). Reduced $N_e$ can reduce genetic variation in the hatchery population and may contribute to genetic differences between hatchery and wild shellfish.

Examples of cultured shellfish that are genetically distinct from wild conspecifics have been documented in a range of taxa (Apte et al. 2003, Evans et al. 2004, Hedgecock et al. 1992, Kong and Li 2007, Li et al. 2007, Lind et al. 2009, Yu and Chu 2006). For example, three hatchery populations of the Japanese scallop (*Patinopecten yessoensis*) were characterized by fewer alleles per locus and lower heterozygosities than wild populations at six microsatellite loci (Li et al. 2007). Similarly, Evans et al. (2004) demonstrated that progeny of wild brood stock were genetically distinct from wild abalone in two species (*H. rubra* and *H. midae*). The hatchery populations had reduced allelic richness and different allele frequencies than the wild population; some relatively rare alleles in the wild population were absent in the hatchery population while other relatively rare wild alleles were common in the hatchery populations (Evans et al. 2004). In pinto abalone (*H. k. kamtschatkana*), first generation progeny of wild brood stock had reduced heterozygosity and allelic richness when compared with the wild population (Lemay and Boulding 2009). In addition, relatedness was higher in the hatchery progeny; although many broodstock were used, offspring from each spawning event were comprised of only four to six kin groups (full and half sib groups), and each spawning cohort was dominated by a single kin group. In the most extreme example, a single male sired all of the progeny during one spawning event (Lemay and Boulding 2009). Similar results were observed in silver-lipped pearl oysters (*Pinctada maxima*); hatchery spawning cohorts were characterized by higher relatedness than populations of wild conspecifics and some cohorts were dominated by one or two full-sib groups (Lind et al. 2009).

If hatchery geoducks are genetically distinct from their wild counterparts, these differences may compromise the fitness of cultured geoducks and cultured-wild hybrid geoducks in the natural environment (Ford 2002, Lynch and O'Hely 2001, Ryman and Laikre 1991). Ryman and Laikre (1991) modeled the effects of captive breeding on wild effective population size ($N_e$) using $N_e$ of the captive and wild populations and the relative proportions of progeny from captive and wild adults. Models indicate that if captive $N_e$ is low, and relative contribution is high, a captive rearing program can substantially reduce $N_e$ in the wild population. The genetic risk to wild geoducks will also vary depending on the rates of gene flow between the cultured and wild populations, the genetic distinction among captive and wild populations, and the degree to which selection varies from the captive to the wild environment (Ford 2002, Lynch and O'Hely 2001). For example, alleles that are deleterious in wild geoducks may increase in frequency in the hatchery due to altered selection. Fitness consequences to the wild population depend upon the rate at which these alleles are introgressed, which in turn depends on effective population sizes and rates of gene flow between the two groups. It should be noted that when census size is very high, as is the case with geoduck in Puget Sound, or if the cultured population has a higher $N_e/N$ ratio than the wild population (possible with culture practices such as factorial mating or single pair mating combined with minimizing family size variance (Busack and Knudsen 2007, Fiumera et al. 2004)), gene flow from cultured to wild populations may actually increase $N_e$ and genetic diversity in wild populations (Gaffney 2006, Hedgecock and Coykendall 2007).

# Chapter II
# Restoration aquaculture of the pinto abalone (*Haliotis kamtschatkana kamtschatkana* Jonas): impacts of rearing method on behavior, growth, and survivorship in the hatchery


## ABSTRACT

Pinto abalone (*Haliotis kamtschatkana kamtschatkana*) populations in Washington State (USA) and British Columbia (Canada) continue to decline despite fisheries closures. For successful recovery, supplementation may be necessary. To determine appropriate culture methods, juveniles were reared in habitat-enriched tanks (supplemented with rocks, macroalgae, and sea urchins) or conventional aquaculture tanks and assessed for growth and survivorship in the laboratory over fifteen months. No differences in survivorship or growth were observed. Subsequent experiments examined whether abalone behavior (habitat selection and movement patterns) differed between rearing treatments. Abalone were exposed to one of three predator treatments (sea star arm, small crab, or no predator (control)) and filmed for eight hours. Abalone from habitat-enriched tanks changed habitats significantly more often than abalone from conventional tanks regardless of predator treatment. Significant differences in the percent time that abalone occupied the various habitats were also observed. Abalone in the sea star and control treatments primarily occupied the rocks, while abalone in the crab treatment behaved differently depending on rearing method; conventionally reared abalone spent more time in corners, while abalone from habitat-enriched tanks spent more time exposed. These results demonstrate that rearing conditions can affect abalone behavior and should be considered for abalone restoration efforts worldwide.

## INTRODUCTION

Abalone populations declined worldwide in the late twentieth century (Shepherd et al. 2001, Tarr 2000, Tegner 1993). In the northeastern Pacific, precipitous declines have been observed in all eight abalone species and have been attributed to a variety of causes including fishing pressure (Bouma 2007, Sloan and Breen 1988, Tegner 1993) and disease (Altstatt et al. 1996, Friedman et al. 2000, Haaker et al. 1992). A key impediment to abalone recovery may be the Allee effect: low population densities prevent successful reproduction (Allee et al. 1949). Fertilization success may be limited when distances separating spawning abalone exceed 1- 2 meters (Babcock and Keesing 1999, Riffell et al. 2004), and recruitment declines have been documented when abalone population densities decrease by 50% or more (Richards and Davis 1993). Responses to abalone population declines have included fishery closures and federal listings as well as captive rearing and supplementation programs (Campbell 2000, Gruenthal and Burton 2005, Moore et al. 2002).

The pinto or northern abalone (*Haliotis kamtschatkana kamtschatkana* Jonas) is the only common haliotid species in Washington State and Alaska, USA and British Columbia, Canada (Geiger 2000). Commercial harvest of pinto abalone in British Columbia peaked at over 400 tons in 1977-78 and declined until the fishery was closed in 1990 (Jamieson 2001). Despite complete harvest closure, population densities declined by 43% between 1993 and 1997 (Campbell 2000). The Washington State fishery was closed in 1994 but abundance and population densities have continued to decline. Length frequency analyses (Rothaus et al. 2008) and surveys of juvenile abundance (Bouma 2007) indicate that little

recruitment has occurred for several years. The pinto abalone is now listed as a species of concern in the USA and as endangered in Canada. Due to the Allee effect, population densities of pinto abalone in Washington State may be too depressed for natural recovery; supplementation may be necessary for their successful restoration.

Enhancing populations with hatchery-reared animals has been conducted with a wide variety of fishes (Salvanes and Braithwaite 2005a, Smith and Fuiman 2004, Weber and Fausch 2005) and invertebrates (Castro and Cobb 2005, Davis et al. 2004, Dixon et al. 2006). Despite years of supplementation efforts in many taxa, success rates in enhancement projects are often low (Bell et al. 2006, Olla et al. 1998, Salvanes and Braithwaite 2005a). Abalone have been outplanted in many countries including Japan (Kojima 1995), New Zealand (Schiel 1992), the United States (Tegner and Butler 1989), South Africa (Sweijd et al. 1998) and others (reviewed by (McCormick et al. 1994, Tegner and Butler 1989). Survival of seeded abalone after one year has varied from less than 1% (Goodsell et al. 2006, Rogers-Bennett and Pearse 1998, Tegner and Butler 1985b) to over 30% (Davis 1995, Dixon et al. 2006, Schiel 1992). Variable success may be due to many factors, including the health, size, and shell color of juvenile abalone, habitat where abalone are planted, and predation (McCormick et al. 1994). Morphological differences that may increase predation may also play a role. Such differences have been observed between hatchery and wild invertebrates including shorter lateral spines in blue crab (Davis et al. 2004), weaker shells in scallops (Grefsrud and Strand 2006) and different claw morphology in lobsters (Govind and Kent 1982, Van der Meeren and Uksnoy 2000).

Behavioral differences between cultured and wild abalone may also contribute to juvenile mortality. In laboratory experiments, hatchery-reared abalone differed in movement patterns and habitat-selection and were more easily preyed upon than were wild conspecifics (Schiel and Welden 1987, Tegner and Butler 1989). Rearing conditions have been shown to affect behavior in aquatic species (Berejikian et al. 2000, Brown et al. 2003, Lee and Berejikian 2009). Berejikian *et al.* (2000) illustrated that steelhead (*Oncorhynchus mykiss*) reared in habitat-enriched tanks (supplemented with structures, overhead cover and underwater feeders) socially dominated same-sized competitors reared in conventional tanks. To date, this approach has not been applied to an invertebrate species.

Given the increasing numbers of threatened and endangered aquatic species worldwide and the desire to supplement declining populations with captive bred animals, it is imperative to optimize culture methods for successful restoration. Optimal rearing conditions for restoration aquaculture must be designed to minimize behavioral differences between cultured and wild abalone, while simultaneously maximizing growth and survivorship in the hatchery. The balance between growth and survivorship in the hatchery and subsequent survival in the wild must be considered in order to develop a cost-effective and successful enhancement program. As a first step towards determining whether rearing conditions can affect this balance, we examined the growth, survivorship, and behavior of pinto abalone reared in habitat-enriched (supplemented with coralline algae encrusted rocks, sea urchins, and native macroalgae) versus conventional tanks. We

hypothesized that pinto abalone reared in habitat-enriched tanks would not significantly differ from those reared in conventional aquaculture tanks in terms of growth or survivorship, but would show significant behavioral differences in habitat-selection and movement patterns. These laboratory experiments were undertaken in order to determine whether a larger scale field trial was warranted.

## MATERIALS AND METHODS

*Study population and rearing treatments*

Pinto abalone collected in 2002 from the San Juan Island Archipelago, Washington, USA were induced to spawn in July 2003 at the Taylor Resources Hatchery in Quilcene, Washington, USA. Full-sibling offspring (n = 192, shell length (mean ± standard error (SE)) = 11.7 mm ± 0.17 mm) were reared using conventional hatchery methods for 10 mo and transferred to the NOAA field station in Mukilteo, Washington, USA in June 2004. Abalone were placed in 220-L white fiberglass tanks, supplied with flow-through filtered (25 μm) seawater at ambient temperatures (8 to 13 °C) and were kept on a 12:12 diel cycle.

Abalone received one of the following two treatments (n = 32 per tank, 3 tanks per treatment): 1) Conventional tanks contained corrugated fiberglass plates ("wave plates") as a surface for benthic diatom growth, a method commonly employed in abalone farms or 2) Habitat-enriched tanks contained coralline algae encrusted rocks and sea urchins (*Stronglylocentrotus droebachiensis* (n = 12) and *S. franciscanus* (n = 2)). Sea urchins were included in the habitat-enriched tanks because many species of juvenile abalone

closely associate with urchin spine canopies (Rogers-Bennett and Pearse 1998, Tarr et al. 1996, Tegner and Dayton 1977). Urchin spine canopies may provide protection from predation; Experimental removal of urchins resulted in a decrease in juvenile abalone abundance both in California (USA) and South Africa (Rogers-Bennett and Pearse 2001, Tarr et al. 1996). Rocks and plates were added to the enriched and conventional tanks, respectively, so that the surface area available for diatom growth in each tank was approximately 2.5 $m^2$. Native macroalgae including *Palmeria mollis*, *Chondracanthus exasperatus*, and *Nereocystis luetkeana* were added to the enriched tanks weekly but were occasionally unavailable in the late winter.

*Growth and survivorship*

Individuals were counted and measured monthly for 15-mo from June 2004 through August 2005. Length was measured to the nearest 0.1 mm using vernier calipers. After removal of excess sea water using paper towels, wet weight was quantified to the nearest 0.001g.

*Abalone habitat selection and movement*

Behavior experiments were initiated using the pinto abalone reared in the habitat-enriched and conventional tanks. To determine whether habitat selection or movement patterns differed between abalone reared in the conventional and habitat-enriched tanks, each abalone was subjected to one of 24 treatments in a 2 x 3 x 2 x 2 factorial design. Factors included rearing method (habitat-enriched, conventional), predator (sea star, crab, and no-predator control), lighting (light, dark) and illumination order (light during first 4

h vs, second 4 h of the 8 h experiment). As abalone are normally more active at night, this experimental design allowed us to test habitat selection under both light and dark conditions as well as to control for an order effect.

Tanks for the behavior experiment contained one of three predator treatments: Sunflower sea star (*Pycnopodia helianthoides*), Dungeness crab (*Cancer magister*) or control (no predator). Both *P. helianthoides* and *C. magister* prey on juvenile pinto abalone (Tomascik and Holmes 2003). To reduce abalone predation, one arm of a *P. helianthoides* (185 mm diameter length) was used as the "predator" in the sea star treatment and a juvenile *C. magister* (23-36 mm carapace length) was used in the crab treatment. Crabs of this size can consume juvenile pinto abalone (Griffiths and Gosselin 2008)(Griffiths and Gosselin, 2008) and pinto abalone of all sizes demonstrate an escape response when contacted with *Pycnopodia helianthoides* arms (Don Rothaus, Washington Department of Fish and Wildlife, personal communication, 2008). The predator was introduced into the tank before the abalone and data acquisition via video camera began when a single abalone (mean shell length ± SE = 29.1 mm ± 0.7 mm) was introduced to each tank. Experiments were run for 8 h, during which time the abalone were free to move within the tank. The lighting changed midway through each experiment: Half of the experimental runs were illuminated for 4 h followed by 4 h of darkness and half of the runs experienced the reverse lighting regime (4 h dark followed by 4 h light). Eight simultaneous trials were conducted in each experimental run, half (n = 4) of the abalone from habitat-enriched tanks and half (n = 4) from conventional tanks. Six temporal replicates were conducted for each predator treatment such that on any

given run, four animals were exposed to the same predator (either sea star or crab) and four animals were controls (not exposed to any predator). The experiment was run on 12 different dates each with eight simultaneous trials so that the habitat selecting behaviors of 96 different abalone (24 abalone exposed to each predator and 48 controls) were recorded under these conditions.

White plastic dishpans were used as replicate tanks for these experiments because abalone were more easily visualized on video recordings when filmed against a white background than against clear glass aquaria. The tanks were rectangular (25 cm x 21.5 cm x 14 cm), contained 1839.5 $cm^2$ of available surface area and held 7.5 L of seawater. A 10 cm x 10 cm area in one corner of each tank was delineated, and three coralline algae encrusted rocks were placed into this area at the start of each experiment. Sea urchins were not included in the behavior experiment because no association between juvenile abalone and sea urchins was observed during the growth and survivorship experiment. The tanks were filled with 0.25 µm-filtered unheated seawater and placed in a water bath to maintain ambient temperature (10 to 11 °C). Tanks were left without aeration or flow for the duration of the experiment to avoid changing olfactory variables that could impact animal behavior and to avoid visual distortion caused by aeration.

All habitat selection experiments were filmed using two SONY video cameras; each camera filmed four tanks. Cameras were ceiling-mounted 3 m above the tanks and connected to standard VHS recorders. Tanks were illuminated with ceiling lights during the light period and filmed using the cameras' NightShot infrared mode during the dark

period. Videos were viewed by a single experimenter (K.M.S.) who recorded time and abalone location. Abalone locations were recorded as exposed (floor and wall), corner, or rocks. The number of times each abalone changed habitat (e.g. from floor to rocks to corner) was also recorded.

*Data analysis*

Differences in abalone growth between treatments were assessed using a mixed effects model, an ideal statistical technique for analyzing data that include repeated measures on the same individuals (Crawley 2002). Differences in survivorship among treatments were determined using an analysis of deviance on proportion data with binomial errors. Due to overdispersion, significance was tested using the F test (Crawley 2002).

The number of times that abalone changed habitats (e.g. from floor to rocks to corner) was compared between abalone reared in habitat-enriched and conventional tanks and among abalone exposed to the different predator treatments using an analysis of deviance GLM. Error structure in this model was defined using log link to constrain the predicted counts to non-negative and a variance that increased with the square of the mean (Crawley 2002).

The proportion of time that abalone spent in different habitats was analyzed using compositional data analysis (Aitchison 1986) using an additive log-ratio transformation which allows compositional data to be analyzed using conventional parametric statistics. This technique is ideal for statistical analyses of data sets such as time budgets where

variables are not independent of one another because they sum to 1. Although any component can be used as the denominator in the ratio (Aitchison 1986), we used each component (percent time in exposed, corner, rocks) separately as the denominator and ran the analyses three times to ensure that this selection did not change our results. The Pillai-Bartlett trace multivariate analysis of variance (MANOVA; Morrison 1967) was used to test the effects of rearing conditions, predator, lighting, and order on abalone behavior. Differences were identified using Tukey's honestly significant difference (Zar 1999). Normality of the transformed data was assessed using the Kolmogorov-Smirnov test (Zar 1999). All analyses were conducted using S-Plus® 7.1 for Windows (Insightful).

## RESULTS

*Growth and survivorship*

Although growth and survivorship varied among tanks within each rearing treatment, no significant differences in abalone survivorship (analysis of deviance, $P > 0.05$) or growth (mixed effects model, weight, $P > 0.05$; length, $P > 0.05$) were observed. Juvenile pinto abalone reared in conventional tanks grew (mean ± confidence interval (CI)) 16.02 mm ± 5.38 mm and increased wet weight 3.07 g ± 1.58 g during the 15-mo experiment. Pinto abalone reared in habitat-enriched tanks grew 13.88 mm ± 2.44 mm and increased wet weight 2.43 g ± 0.78 g during this period (Fig 1). Survival (mean ± CI) was 50.60% ± 9.38% in the conventional tanks and 63.04% ± 8.56% in the habitat-enriched tanks (Fig 2).

*Abalone behavior—habitat selection and movement*

Under light and dark conditions and during the first and second four hours of the experiment, no differences were detected in either the number of habitat changes (analysis of deviance; order: $P > 0.05$; lighting: $P > 0.05$) or the proportion of time spent occupying different habitats (MANOVA; $P > 0.05$). Because no order or light effects were detected, these factors were omitted and all further analyses examined abalone behavior over the entire 8 h experiment.

Significant differences were observed in the number of habitat changes between abalone reared in habitat-enriched tanks and those reared in conventional tanks (analysis of deviance, $F = 19.54$, $P < 0.001$; Fig 3). Abalone reared in habitat-enriched tanks changed habitats more frequently ((mean ± CI), 21.21 ± 6.76 times) than did those reared in conventional tanks (7.82 ± 1.35 times) during each 8 hr experiment. Presence or absence of a predator did not impact the number of habitat changes observed (analysis of deviance, $P > 0.05$).

Significant differences in the percent time that abalone occupied the various habitats (Fig 4) were also observed. Highly significant differences were observed among abalone exposed to the different predator treatments (MANOVA: $F = 7.81$, $P < 0.001$); no behavioral differences were detected between abalone in the sea star and no predator treatments, while abalone in the crab treatment behaved differently. In the sea star and the no predator treatments abalone spent (mean ± CI) 67.25% ± 8.02% of the time in the rocks, 25.98% ± 6.68% of the time exposed, and 6.77% ± 3.76% of the time in the

corners. In contrast, abalone in the crab treatment, regardless of rearing, spent less time in the rocks (26.70% ± 12.02%) than those in the sea star or no predator treatments. A significant interaction was also observed (MANOVA: $F = 4.126$, $p = 0.019$); abalone in the crab treatment reared in conventional tanks spent more time in the corners (39.70% ± 19.44%), while abalone in the crab treatment reared in habitat-enriched tanks spent more time exposed (61.13% ± 16.84%, Fig 4).

## DISCUSSION

### *Abalone behavior—habitat selection and movement*

This study, the first to examine habitat-enrichment and behavior in an invertebrate species, clearly demonstrates that rearing environment affects behavior in pinto abalone. In both metrics tested (percent time spent in different habitat types and number of changes in habitat type), juvenile abalone reared in habitat-enriched tanks behaved differently than those reared in conventional tanks. Research on a variety of aquatic species support our finding that culture methods can influence behavior. Abnormal behaviors have been documented in hatchery fishes in predator recognition, predator evasion, aggression, habitat selection, and daily or seasonal activity rhythms (reviewed by Olla et al. 1998; Salvanes and Braithwaite 2005). Although fewer studies have been conducted using invertebrates, results indicate that rearing environment can also affect shellfish behaviors. For instance, hatchery-reared blue crabs (*Callinectes sapidus*) buried in sediment less frequently than did wild crabs (Davis et al. 2004) and hatchery-reared juvenile American lobsters (*Homarus americanus*) spent less time using shelter refugia than did wild conspecifics (Castro and Cobb 2005).

Several laboratory studies have examined behavioral differences between cultured and wild abalone. Schiel and Welden (1987) demonstrated that cultured red abalone (*H. rufescens*) took substantially longer to attach to the substrate than did wild conspecifics, and also found that wild abalone moved rapidly to concealed rock habitats whereas cultured abalone remained immobile where placed, often for several hours. Tegner and Butler (1989) found that cultured green abalone (*H. fulgens*) changed habitats more frequently and selected different habitats than wild conspecifics. For example, in an experimental tank with four habitats, 100% of wild abalone from 10-20 mm were found in cobble, while only 44% of hatchery abalone in this size class were found in the cobble. Given these results, we hypothesized that pinto abalone habitat-selection and movement patterns would vary between animals reared in the two treatments.

We found clear behavioral differences between abalone reared in conventional and habitat-enriched tanks. Abalone reared in habitat-enriched tanks changed habitats more frequently than abalone reared in conventional tanks regardless of the presence or type of predator. Additionally, in the presence of a crab, abalone reared in conventional tanks spent significantly more time in the corners, while abalone reared in habitat-enriched tanks spent significantly more time exposed. Abalone reared in the habitat-enriched tanks may have been more accustomed to a complex environment with an excess of suitable habitat. Their increased movement in the behavioral experiment may be related to searching for preferred habitat within the experimental environment or may be a reflection of moving more frequently between suitable habitats in their rearing tanks.

When abalone from either rearing treatment were introduced into experimental tanks with a sea star or no predator, abalone spent the highest proportion of time in the rocks, suggesting that juvenile pinto abalone reared in either treatment will select the most concealed position in a novel environment in the presence or absence of a predator. Such behavior is consistent with normal juvenile abalone behavior: Wild juveniles tend to be cryptic under rocks or in crevices until they reach a certain emergent size which varies by species (Kiyomoto and Yamasaki 1999, Tissot 1995). Wild pinto abalone are cryptic until they reach 30-40 mm (D. Rothaus, pers. comm. 2006). Abalone in the crab treatment spent significantly less time concealed. However, this behavior also appeared to be an appropriate response as the crabs tended to select the rock habitat and the abalone avoided the predator by avoiding the rocks. Thus, abalone in both rearing treatments demonstrated predator avoidance, but they avoided predators differently. Whether one method is more advantageous in the field remains to be tested.

*Growth and survivorship*

Abalone reared in the two treatments showed no significant differences in growth or survival over the course of the fifteen month experiment. Additionally, although cultured abalone often develop discolored shells (McCormick 2000), the animals in our two treatments were visually indistinguishable. Although not quantified, animals in both treatments developed shells with red and green mottled coloration, as is commonly observed in wild juvenile pinto abalone (D. Rothaus, pers. comm. 2008). As abalone with discolored shells lack the cryptic coloration of wild abalone and may experience

increased predation when outplanted, shell coloration may affect the success of enhancement efforts (McCormick 2000). Thus, with respect to growth, survival, and shell coloration, both rearing treatments appeared to be equally effective in producing juvenile abalone for supplementation.

*Conclusions and Future Directions*

High survival of captive bred animals under field conditions is the goal of every supplementation program, but many studies have shown higher predation on hatchery-reared than on wild individuals in both fishes (Kellison et al. 2000, Stunz and Minello 2001) and invertebrates (Castro and Cobb 2005, Davis et al. 2004, Stoner and Davis 1994). Previous work has demonstrated that hatchery-reared juvenile abalone suffer increased predation in both laboratory (Schiel and Welden 1987, Tegner and Butler 1989) and field (Saito 1984, Schiel 1992) experiments. As juvenile abalone have many predators, the ability to avoid predation is vital for seed survival (Shepherd 1973, Tegner and Butler 1985b). Schiel (1992) examined differences in survival between outplanted hatchery-reared and wild black-foot abalone (*H. iris*). After 1-2 months in the field, 40-72% fewer hatchery than wild abalone were recovered. Despite these trends, Guzmán del Próo *et al.* (2004) observed similar annual survival in outplanted wild and hatchery-reared green abalone (*H. fulgens*; Guzman del Proo et al. 2004). Although the present study demonstrates that rearing environment can affect behavior in pinto abalone, it is not clear whether or how the behavioral differences we observed will impact survival of hatchery-reared abalone in the wild. Given these data, we have begun rearing large

numbers of juvenile pinto abalone in both conventional and habitat-enriched tanks in order to assess differential survival in the field.

In response to worldwide abalone population declines (Shepherd et al. 2001, Sloan and Breen 1988, Tarr 2000, Tegner 1993) there is global interest in using cultured abalone to supplement wild stocks. In North America, three abalone species (*H. sorenseni*, *H. cracherodii*, and *H. k. kamtschatkana*) are currently being cultured with the goal of using juveniles to augment wild populations in restoration programs (Campbell 2000, Gruenthal and Burton 2005, Moore et al. 2002). Internationally, many more abalone species are being cultured to supplement wild stocks. Some current programs include green abalone in Mexico (Guzmán del Próo *et al.* 2004), greenlip abalone (*H. laevigata*) in Australia (Dixon et al. 2006), perlemoen (*H. midae*) in South Africa (De Waal and Cook 2001), and ezo abalone (*H. discus hannai*) in Japan (Sekino et al. 2005). Our finding that rearing environment affects behavior in pinto abalone has implications for abalone restoration worldwide. If outplanting is to assist in the long-term recovery of abalone populations, we must improve our knowledge of abalone behavior and optimize culture methods for supplementation before large scale programs can be successfully implemented.

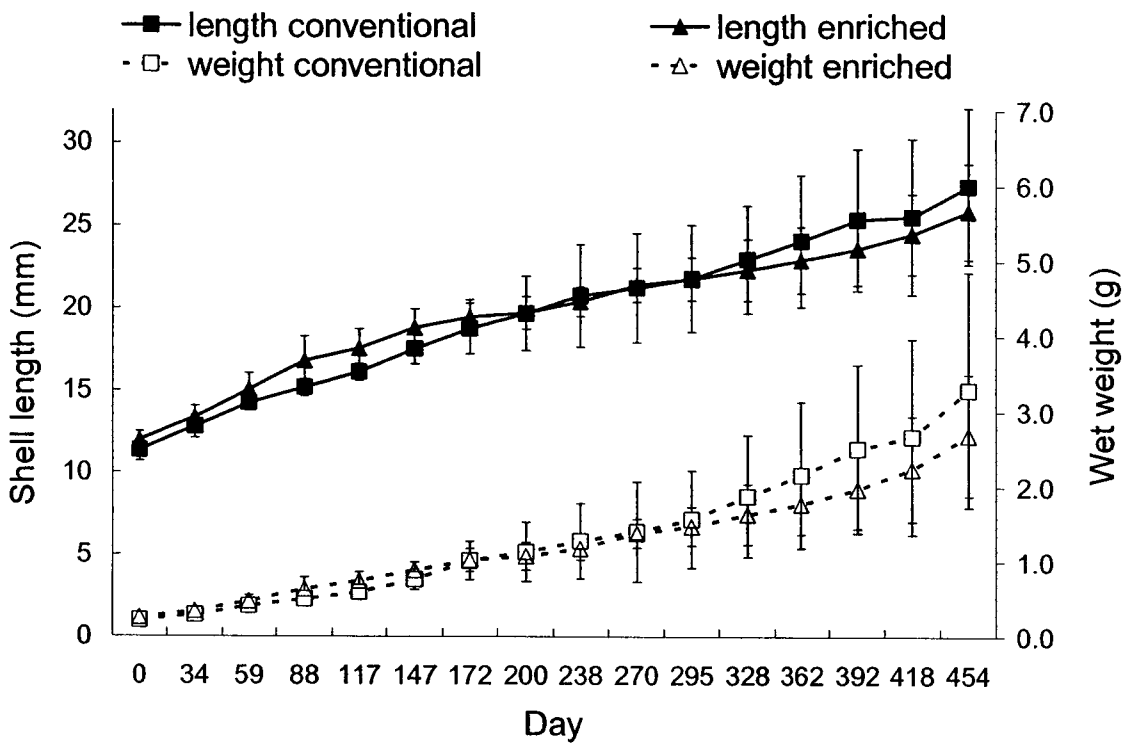



**Fig 1.** Juvenile pinto abalone (*Haliotis kamtschatkana kamtschatkana*) growth (mean ± confidence interval) over time in habitat-enriched and conventional tanks. No differences inshell length or wet weight were observed over the 15-month study (mixed effects model, $P > 0.05$).

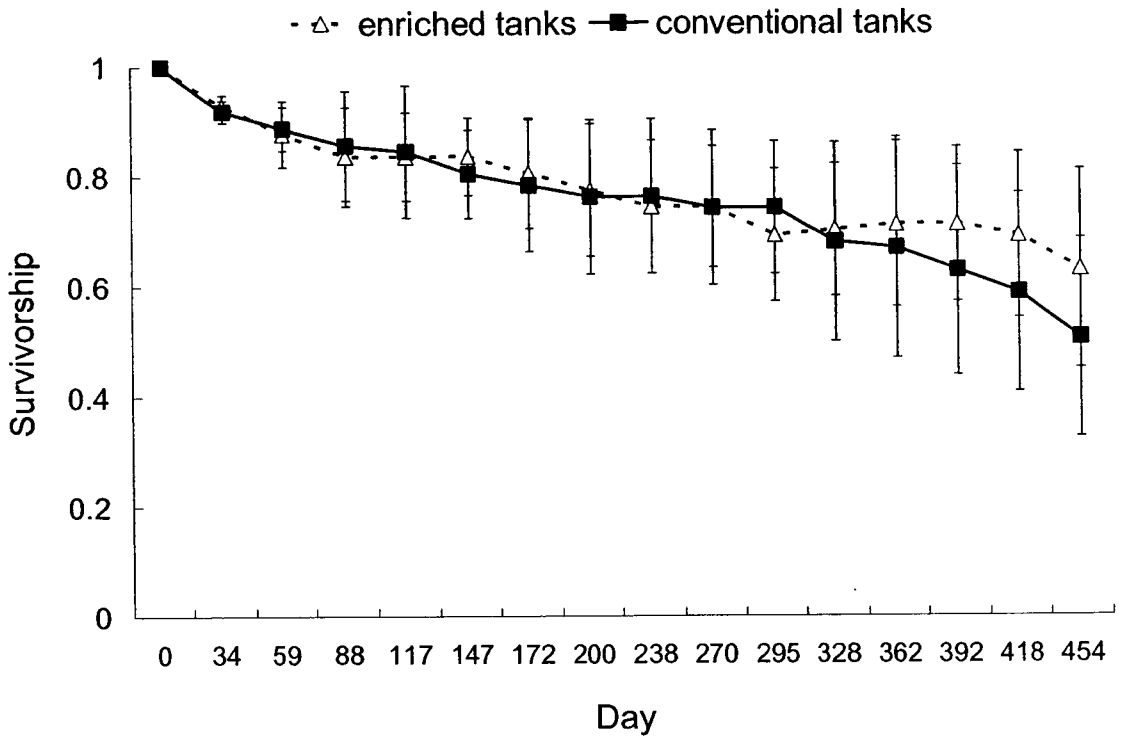



**Fig 2.** Juvenile pinto abalone (*Haliotis kamtschatkana kamtschatkana*) survivorship (mean ± confidence interval) over time in habitat-enriched and conventional tanks. No significant differences were observed between treatments (analysis of deviance, $P > 0.05$).

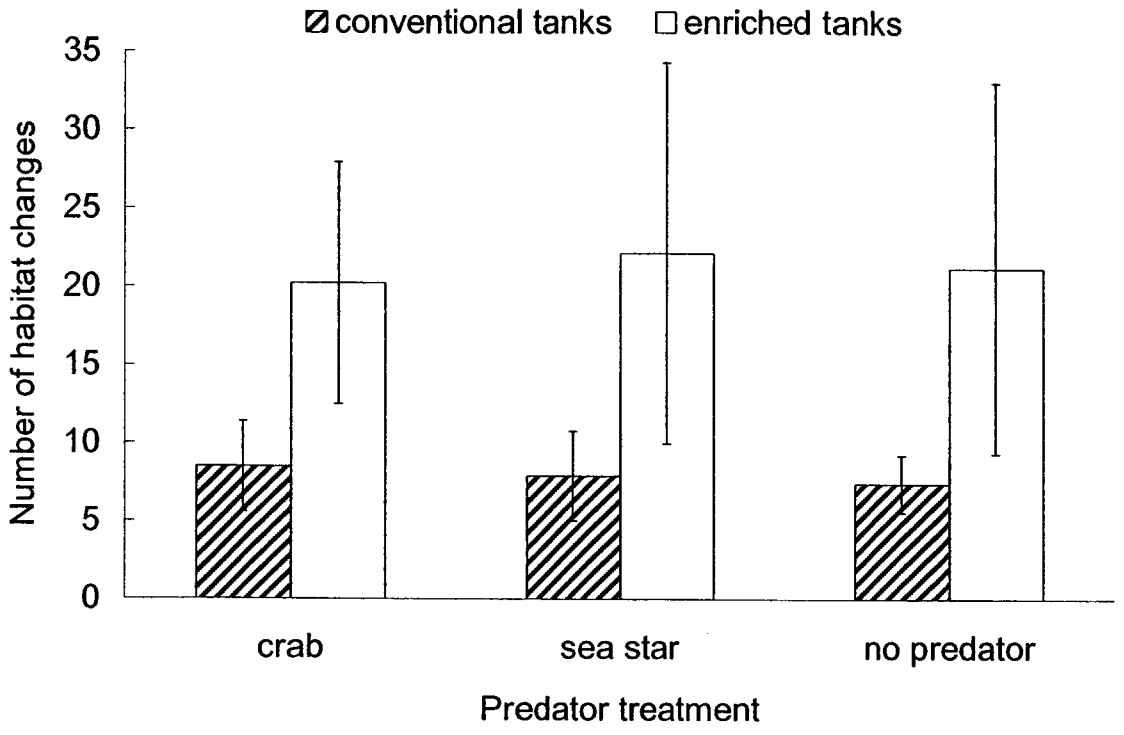



**Fig 3.** Number of habitat changes (mean ± confidence interval) per individual pinto abalone (*Haliotis kamtschatkana kamtschatkana*) observed during the 8-h experiment (n = 24 abalone in each predator treatment; n = 48 abalone in no predator controls). Significant differences in the number of habitat changes were observed between rearing treatments; however, presence or type of predator did not have an impact on the number of site changes observed (analysis of deviance: $F = 19.54$, $P < 0.001$).

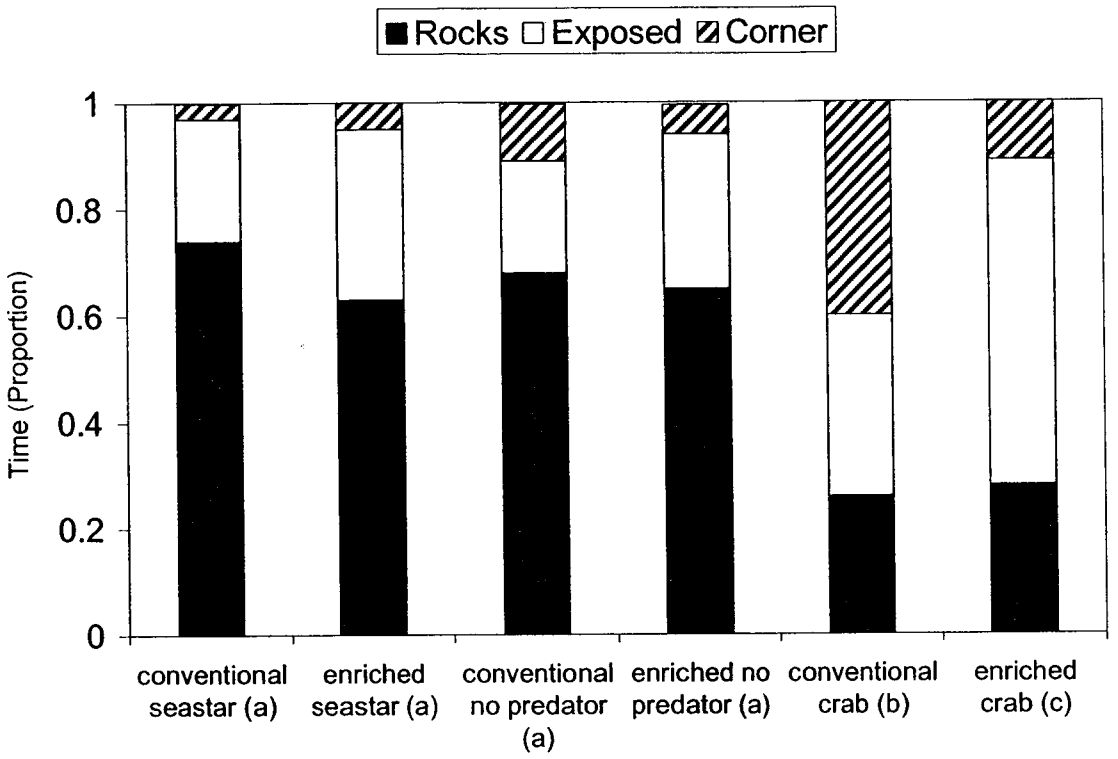



**Fig 4.** Proportion of time pinto abalone (*Haliotis kamtschatkana kamtschatkana*) spent in different habitats during the 8-h experiment (n = 24 abalone in each predator treatment; n = 48 abalone in no predator controls). (*a*) No differences in behavior were observed between the no predator (control) and sea star treatments, regardless of the rearing treatment. In the crab treatment, abalone from both rearing treatments spent less time in the rocks and behaved differently depending on rearing treatment (MANOVA: $F = 4.126$, $P = 0.019$). (*b*) Abalone reared in conventional tanks spent more time in the corners whereas (*c*) abalone reared in habitat-enriched tanks spent more time exposed.

# Chapter III
# Genetic detection of flat abalone (*Haliotis walallensis* Stearns, 1898) as a cryptic species in the inland marine waters of Washington State, USA


## ABSTRACT

Many abalone (*Haliotis* spp.) species are in decline world wide; in the Eastern Pacific, five of the eight abalone taxa are protected as endangered, threatened, or of concern by one or more jurisdictions. Because abalone are visually identified by plastic traits such as shell color and morphology, abalone misidentification may be common. The inability to accurately identify abalone to species increases the challenge of abalone management and conservation. We developed improved sequence based genetic markers at both a nuclear (lysin) and a mitochondrial (COI) locus for use in species identification of abalone in the Eastern Pacific. We applied these tools in conjunction with morphological data and determined that flat abalone (*H. walallensis*) are present as a cryptic species with pinto abalone (*H. kamtschatkana kamtschatkana*) in Washington State, USA. The range of the flat abalone was not thought to extend to Washington State, and these animals have never previously been recorded in Washington's inland marine waters. Several flat abalone were initially identified during genetic screening of animals collected for a pinto abalone restoration program. The use of these individuals as broodstock may have led to inadvertent interspecific hybridization which would have been detrimental to pinto abalone restoration. A second important finding is that, with our molecular tools, we cannot differentiate between pinto abalone and their subspecies, the threaded abalone (*H. k. assimilis*). Our results strongly advocate the use of species specific molecular tools for

abalone management, especially if restoration efforts such as adult aggregation or captive rearing are used.

## INTRODUCTION

The ability to accurately identify individuals to species is a necessary first step in biological research, management of living resources, and conservation of biodiversity. Misidentification of species clouds our understanding of biological phenomena and increases the challenges of conservation and management. Taxa may be misidentified due to either cryptic species or phenotypic plasticity. Cryptic species are morphologically similar taxa that are erroneously classified as a single species (Bickford et al. 2007). Identifying cryptic species complexes may have crucial implications for conservation and management as evidenced by several molecular studies that identified cryptic frog species in Southeast Asia (Bain et al. 2003, Matsui et al. 2005, Stuart et al. 2006). While the nominal frog species were thought to have a broad geographic range, each of the cryptic species within these complexes has a reduced range, making each more vulnerable to extinction. Phenotypic plasticity occurs when organisms with the same genotype express different phenotypes in different environments (Bradshaw et al. 1965, Houston and McNamara 1992). Phenotypic plasticity may result in individuals of a single species being identified as members of multiple species. In one such case, three sympatric species of freshwater snail with differing shell morphologies were synonymized after molecular data were examined (Wilke and Falniowski 2001). Misidentification of species due to phenotypic plasticity may influence management priorities and result in unnecessary conservation actions. For example, after substantial

effort was expended to conserve the endangered pocket gopher *Geomys colonus*, it became apparent that separation of this species from the common *G. pinetis* was not warranted (Laerm et al. 1982).

Cryptic species have been documented within all major marine taxa (reviewed by (Knowlton 1993, 2000) and both phenotypic plasticity and cryptic species are known in marine gastropods (e.g. Manriquez et al. 2009, Nakano and Spencer 2007). Abalone are marine gastropods in the genus *Haliotis* that inhabit tropical and temperate oceans worldwide. Abalone shell color and morphology vary substantially depending upon environmental conditions (Leighton 2000); because scientists use shell characteristics to identify abalone species, misidentification may be common. In one such case, an abalone collected as broodstock for the USA federally endangered white abalone (*H. sorenseni*) conservation aquaculture program was later identified as a second species (*H. kamtschatkana*) using a molecular approach (Gruenthal and Burton 2005).

Eight abalone taxa (Geiger 1999) (Table 1) were once abundant in the Eastern Pacific but have suffered catastrophic declines due to over-harvest (Hobday et al. 2001, Rothaus et al. 2008), climate changes (Rogers-Bennett 2007, Tegner et al. 2001, Vilchis et al. 2005), and disease (Altstatt et al. 1996, Haaker et al. 1992, Miner et al. 2006, VanBlaricom et al. 1993). The pinto abalone (*H. kamtschatkana kamtschatkana* Jonas, 1845) is the only common abalone in Washington State and Alaska, USA, and British Columbia, Canada. Pinto abalone are in decline throughout their range and are currently listed as endangered in Canada (COSEWIC 2009) and as a Species of Concern in the USA (National Oceanic

and Atmospheric Administration 2009). Despite complete fisheries closures, pinto abalone abundance and population density continue to fall in both British Columbia, Canada (Campbell 2000) and Washington State, USA (Rothaus et al. 2008). Restoration efforts for the pinto abalone in Washington State are in initial phases and include both aggregating reproductive adults so that they are in close proximity to one another when they spawn (e.g.Tegner 1992) and supplementing wild populations with hatchery-reared juveniles (e.g. Dixon et al. 2006, Goodsell et al. 2006).

In initial studies examining mitochondrial DNA (mtDNA) genetic diversity within hatchery animals, we uncovered evidence of several genetically distinct abalone. These abalone could not be distinguished from pinto abalone morphologically but diverged substantially at two mitochondrial loci. Many sequence based phylogenies of Eastern Pacific abalone exist, but these include only a few individuals per species (Gruenthal and Burton 2005, Lee and Vacquier 1992, Metz et al. 1998, Supernault et al. 2009). Our preliminary work showed greater than expected polymorphism within pinto abalone. Sequence diversity is great enough that, employing the commonly used cytochrome oxidase subunit I (COI) primers (Metz et al. 1998) we cannot always discriminate among abalone taxa. In particular, white abalone (*H. sorenseni*) and pinto abalone (*H. k. kamtschatkana*) are not always discriminated, a problem also noted by Gruenthal and Burton (2005). In this paper, we describe a sequence based method to differentiate among abalone of the Eastern Pacific Ocean using both a nuclear gene (abalone sperm lysin) and a mitochondrial gene (COI). The mtDNA locus COI is commonly used in species identification (Costa et al. 2007, Hebert et al. 2003a, Hebert et al. 2004); we developed a

longer marker within this gene which, together with the Metz (1998) COI primers, can discriminate all Eastern Pacific abalone species with high bootstrap support. However, because mtDNA is typically maternally inherited, this locus cannot discriminate interspecific hybrids. Abalone sperm lysin (lysin), a rapidly evolving gamete recognition protein, was selected as a biparentally inherited nuclear gene. Lysin, which contributes to species specificity in fertilization, has been called a "speciation gene" because it limits gene exchange between diverging lineages (Andres et al. 2008, Vacquier et al. 1990). Lysin is an ideal candidate for species discrimination because it shows great sequence divergence among species while remaining highly conserved within species (Lee and Vacquier 1992, Metz et al. 1998).

The goals of the present study were to 1) use our sequence based method in conjunction with a morphological approach to determine whether the divergent Washington State abalone simply represent the genetic diversity found within pinto abalone, whether these individuals belong to a cryptic abalone species, or whether they are hybrids between two abalone taxa and 2) use the genetic tools we have developed to determine the distribution and abundance of the genetically divergent abalone in Washington State.

## MATERIALS AND METHODS

### *Sample Collection*

Samples of ten individuals from each of the eight abalone taxa inhabiting the Eastern Pacific Ocean were obtained from collaborators. In conjunction with the Washington Department of Fish and Wildlife (WDFW), 190 abalone samples were collected from the inland waters of Washington State. Samples from each taxon were taken from as wide a range of locations as possible; in three species (*H. cracherodii*, *H. fulgens*, and *H. corrugata*), additional sequences from Mexican abalone were obtained from GenBank and used for comparisons (Table 2). Tissue samples were taken from live animals non-lethally by clipping two epipodial tentacles. Several individuals were sampled promptly after death by cutting a small sample of foot tissue. All tissue samples were immediately stored in 95% molecular grade ethanol until DNA extraction.

### *Collection and Analysis of Morphological Data*

Morphometric analyses were conducted on 125 pinto abalone, 35 flat abalone, and five genetically divergent Washington abalone. Photographs of representative shell morphology are shown in Figure 5. Shell length (SL), shell width (SW), and shell height (SH) were measured in all abalone to the nearest 0.1 mm with vernier calipers. The number of open tremata were counted in all abalone.

Because abalone SW and shell height SH vary with SL, patterns of morphological variation were visualized by plotting SL against all other variables and SW against SH. Morphological differences among pinto abalone, flat abalone, and the genetically divergent abalone were assessed statistically using one-way analysis of variance on size standardized data (all variables divided by SL), followed by a Tukey Test (Zar 1999). The number of open tremata was compared between pinto abalone, flat abalone, and the genetically divergent abalone using an analysis of deviance Generalized Linear Model with quasi-Poisson errors (Crawley 2007). Normality of all data was assessed using the Shapiro-Wilk test (Shapiro and Wilk 1965). The program R was used for all statistical analyses of morphometric data (R Development Team 2010)

*DNA extraction and Polymerase Chain Reaction*

Genomic DNA was extracted from a single epipodial tentacle or from a small piece of epipodial tissue using a QIAamp DNA Mini Kit (Qiagen, Alameda, CA, USA) following the manufacturer's tissue protocol. Polymerase chain reaction (PCR) was used to amplify a portion of the mitochondrial Cytochrome Oxidase I gene (COI) in all individuals (Table 2) using abalone specific primers (Metz et al. 1998; Table 3). To clarify taxonomy, a second reverse primer was designed to work in conjunction with the Metz (1998) forward primer to amplify a longer portion of the COI gene (Table 3). This primer set was used in all white (*H. sorenseni*), flat (*H. walallensis*), pinto (*H. k. kamtschatkana*) and threaded (*H. k. assimiis*) individuals. Lysin primers were designed based on the Genbank entry for pinto abalone lysin (M59970) to amplify lysin intron 2 and include a portion of the 3' end

of exon 2 and the 5' end of exon 3 (Table 3). To detect hybridization, lysin was amplified in all flat, pinto, and threaded individuals.

All PCR reactions were carried out in 20μl reactions containing 1μl template DNA, 7.4μl Bio-X-Act short mix (Bioline, London, UK) with the final solution of 2mM $MgCl_2$ and 0.4 μM each primer. Thermal cycling was conducted in either a DNA engine PTC-200 thermal cycler (Bio-Rad, Hercules, CA, USA) or a Mastercycler (Eppendorf North America, Hauppauge, NY, USA) thermal cycler. The shorter COI PCR included an initial denaturation at 94°C for one min followed by 38 cycles of 94 °C for 15s, 55 °C for 30s, and 72 °C for 1 min while PCR for the longer COI region included an initial denaturation at 94°C for 1 min followed by 38 cycles of 94 °C for 15s, 48 °C for 30s, and 72 °C for 75s with a final extension step of 72°C for 3 min. Thermal cycling conditions for lysin included an initial denaturation at 94°C for 1 min followed by 38 cycles of 94 °C for 10s, 48.2 °C for 30s, and 72 °C for 75s with a final extension step of 72°C for 3 min.

*DNA sequencing*

Before sequencing, the PCR products were purified using either a QIAquick PCR purification kit (Qiagen, Alameda, CA, USA) or Exo-SAPIT (USB, Cleveland, Ohio, USA). The purified PCR products were then directly sequenced in both directions using the PCR primers. Cycle sequencing was performed using the BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Sequencing reactions were cleaned using a standard ethanol precipitation or BigDye XTerminator purification kit (Applied Biosystems, Foster City,

CA, USA). Sequences were run on a 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA).

*Phylogenetic Analyses*

Sequences were edited using the program Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned using ClustalW (Thompson et al. 1997) as implemented in the program BioEdit 7.0.9 (Hall 1999) with minor adjustments by eye. After alignment, sequences were truncated to the length of the shortest sequence in BioEdit 7.0.9. The final sequence lengths for analysis were COI short (470bp, 10 individuals from each taxon), COI long (816 bp, 10 individuals from *H. k. kamtschatkana*, *H. k. assimilis*, *H. walallensis*, and *H. sorenseni*) and lysin (406 bp including indels, 10 individuals from *H. k. kamtschatkana*, *H. k. assimilis* and *H. walallensis*). Aligned sequences were imported into MEGA version 4 (Kumar et al. 2008) for phylogenetic analysis. All sequences used in this study have been submitted to the NCBI GenBank database with accession numbers included in Table 2. The appropriate evolutionary model to fit our data was determined using the Akaike information criterion (AIC) as implemented in the program jModelTest (Posada 2008). The Tamura-Nei model with rate variation among sites (TrN + G; Tamura and Nei 1993) was selected as the best fit for the COI data while the three parameter model with unequal base frequencies and rate variation among sites (TPM2uf + G; Tamura 1992) was selected as the best fit for the lysin data. These models were implemented in MEGA version 4.0 (Kumar et al. 2008) to calculate sequence divergence and construct neighbor joining trees (Saitou and Nei 1987) with 1000 bootstrap replicates

(Felsenstein 1985). Data were also visualized using a haplotype networking approach as implemented in the program TCS (Templeton et al. 1992).

## RESULTS

### *Morphometric Analyses*

Highly significant morphological differences were observed among pinto, flat, and genetically divergent Washington abalone. No significant morphological differences were observed between pinto and genetically divergent Washington abalone. However, in all cases, these abalone were morphometrically distinct from flat abalone. Flat abalone had significantly more open tremata (mean ± confidence interval (CI)) (7.56 ± 0.28) than pinto/ genetically divergent Washington abalone (4.69 ± 0.15 and 4.40 ± 0.48) respectively (analysis of deviance, $P<0.001$; fig. 6). Flat abalone were significantly flatter per unit length than pinto/ genetically divergent Washington abalone; standardized shell height (mean ± CI); was 0.20 mm ± 0.01 mm in flat abalone while in pinto and genetically divergent abalone, the standardized shell height was 0.30 mm ± 0.01 mm and 0.32 mm ± 0.01 mm respectively (ANOVA, $P<0.001$; fig. 7. Flat abalone were also significantly narrower than pinto/ genetically divergent Washington abalone; standardized shell width (mean ± CI) was 0.69 mm ± 0.01 mm in flat abalone while in pinto and genetically divergent abalone, the standardized shell width was 0.72 mm ± 0.01 mm and 0.74 mm ± 0.02 mm respectively (ANOVA, $P<0.001$; fig. 8.)

*Phylogenetic Analyses*

COI sequence data revealed differences among the eight Eastern Pacific abalone taxa examined. Analysis of a 470 bp region of COI from 80 individuals (n = 10 per taxa) revealed 25 haplotypes comprised of 378 conserved sites and 92 variable sites, of which 89 were parsimony-informative. Phylogenetic analysis generated a neighbor joining tree that distinguishes five of the nine taxa (*H. fulgens*, *H. corrugata*, *H. cracherodii*, *H. rufescens*, and *H. walallensis*) with high bootstrap support; the remaining three taxa (*H. sorenseni*, *H. k. kamtschatkana* and *H. k. assimilis*) are unresolved using this locus (Fig 5). Average pairwise distance corrected using the Tamura Nei model was (mean ± 95% confidence interval (CI)) 0.19% ± 0.10% within taxa and 7.91% ± 1.90% among taxa (Fig 9).

To further resolve abalone identification and taxonomy, a longer region of COI was sequenced in the taxa unresolved by the 470 bp region (10 individuals from *H. k. assimilis, H. k. kamtschatkana* and *H. sorenseni*). In addition, 10 *H. walallensis* individuals were included because preliminary molecular evidence suggested that the divergent Washington State abalone were genetically similar to flat abalone. Alignments of 816 bp of the COI gene revealed 15 unique haplotypes comprised of 782 conserved sites and 34 variable sites, of which 18 were parsimony informative. Phylogeny was inferred using a neighbor joining tree which distinguishes the three species with ≥ 70% bootstrap support (Fig 10). The subspecies of *H. kamtschatkana* remain unresolved using this longer COI region; pinto and threaded abalone share identical haplotypes. Average pairwise distances corrected using the Tamura-Nei model was (mean ± CI) 0.18% ±

0.18% within taxa and 1.02% ± 0.65% among taxa (Table 5). All Washington State abalone (n = 190) were sequenced at this locus and added to the phylogenetic trees (data not shown). Of these, 173 abalone were identified as *H. kamtschatkana* (pinto or threaded subspecies) and 17 individuals were identified as flat abalone, *H. walallensis*. Several Washington State abalone share identical haplotypes with flat abalone.

To confirm species identification and detect hybridization, a 406 bp region of lysin was sequenced in *H. k. kamtschatkana*, *H. k. assimilis*, and *H. walallensis* (n = 10 individuals per taxa). Alignments at this region revealed 12 haplotypes comprised of 345 conserved sites and 29 variable sites, of which 17 were parsimony informative. A neighbor joining tree clearly distinguishes the two species at this locus (Fig 11). Again, subspecies of *H. kamtschatkana* are not resolved; these subspecies share several identical haplotypes. Average pairwise distances corrected using the three parameter model was 0.1% within *H. k. assimilis*, 0.0% within *H. k. kamtschatkana* and 1.0% within *H. walallensis*. Average pairwise distances among taxa were 0.0% between *H. k. kamtschatkana* and *H. k. assimilis*, and 5.4 – 5.5% between *H. kamtschatkana* subspecies and *H. walallensis*. All Washington State abalone (n =190) were sequenced at this locus and added to the phylogenetic tree (data not shown). Of these, 173 individuals were identified as *H. kamtschatkana* (pinto or threaded subspecies) and sixteen abalone were identified as *H. walallensis* (flat abalone). Several Washington State abalone share identical haplotypes with flat abalone. In contrast to the above molecular data, no statistically significant differences in morphology were detected between the five of these abalone that remain in our possession and pinto abalone from Washington State. A single individual was

identified as *H. walallensis* at COI but had heterozygous bases at diagnostic lysin sites; this individual was identified as a hybrid between a male *H. kamtschatkana* and a female *H. walallensis*.

Statistical parsimony analyses of COI and lysin sequence data for *H. k. assimilis, H. k. kamtschatkana, H. k. wallensis* and divergent Washington State individuals produced similar haplotype networks. With a 0.95 confidence limit, 12 mutational steps could be drawn between COI (820 bp) haplotypes and eight mutational steps could be drawn between lysin haplotypes. Analyses of lysin produced two networks, with one including all *H. kamtschatkana* haplotypes and one containing all *H. walallensis* haplotypes; the networks are not connected because more than eight mutational steps separate them (data not shown). Analysis of COI produced a single network divided into two clades. A clade containing all of the *H. kamtschatkana* haplotypes was separated by 12 mutational steps from a clade that contained all of the *H. walallensis* haplotypes, including the divergent abalone from Washington State (data not shown).

## DISCUSSION

We developed improved molecular tools for Eastern Pacific abalone species identification and applied those tools in conjunction with a morphological approach to ascertain which abalone taxa are present in Washington State, USA. Genetic evidence from both a nuclear locus and a mitochondrial locus indicate that flat abalone, (*H. walallensis*) are present along with pinto abalone (*H. k. kamtschatkana*) in the inland waters of Washington State. However, morphologic and mersitic examinations focusing

on shell measurement ratios and counts of open tremata identify these individuals as pinto abalone. Together, these data indicate that flat abalone are present as a cryptic species with pinto abalone in Washington State, USA. This paper also documents the first flat abalone from Washington's inland marine waters. Although the historical distribution of flat abalone may have extended to southern Washington State, these animals are not known to be here presently and have not been recorded in Washington's inland marine waters (Geiger 2000). Flat and pinto abalone are sympatric in Northern California, where they may be visually distinguished due to differences in shell shell height, rugosity, and number of tremata (Haaker et al. 1986). It appears that unknown environmental parameters differing between California and the inland waters of Washington State exert significant control on shell morphology in flat abalone. This may be a unique case of phenotypic plasticity where two species are visually distinguishable in one location but cryptic in another.

Phenotypic plasticity is well documented in marine gastropods; shell color and morphology are frequently under environmental control in these animals (e.g.Creese and Underwood 1976, Hoffman et al. 2010, Lindberg and Pearse 1990, Manriquez et al. 2009), including abalone (Leighton 2000). For Eastern Pacific abalone, the conservation implications of this plasticity are stunning; five of the eight taxa are protected as endangered, threatened, or of concern by one or more jurisdictions (Table 1), and all of these abalone are sympatric with other abalone through at least a portion of their range. If shell color and morphology show enough plasticity that abalone are regularly misidentified, scientists may be unable to determine which abalone are present in what

proportions and managers will have difficulty assessing status and planning appropriate conservation actions. Correctly identifying abalone to species takes on paramount importance when managers use active restoration strategies such as aggregating reproductive adults (Campbell et al. 2003, Henderson 1988, Tegner 1992) and supplementing wild populations with hatchery reared juveniles (De Waal and Cook 2001, Dixon et al. 2006, Goodsell et al. 2006). Two abalone conservation hatcheries have been established in the United States (for pinto abalone (this ms) and for white abalone (McCormick and Brogan 2003)). It is noteworthy that in both cases, the use of genetic tools allowed scientists to determine that multiple individuals initially included as broodstock were not, in fact, the target species (this ms, Gruenthal and Burton 2005). The inclusion of these individuals as broodstock could have serious consequences for abalone recovery.

Many of the 56 recognized abalone species worldwide (Geiger 2000) are in decline and there is global interest in using hatchery-reared abalone for conservation or fisheries enhancement. Misidentifications of Southern Hemisphere abalone have also been reported; molecular analysis of 51 abalone indicated that three had been misidentified based on morphology; two abalone identified as *H. varia* were actually members of a previously unknown cryptic species and another identified as *H. varia* appeared to be *H. ovina* at a mitochondrial locus. (Degnan et al. 2006). Clearly, morphological data alone are not adequate for identifying abalone taxa and proper species identification of hatchery broodstock is crucial.

A second important finding is that we cannot differentiate between pinto abalone and their subspecies, the threaded abalone (*H. k. assimilis* Dall, 1878) using our molecular techniques. Pinto abalone range from Point Conception, California north to Alaska USA (Geiger 2000) while threaded abalone range from Point Conception south to Baja California, Mexico (Geiger 2000). Our data show that individuals from the two subspecies share identical sequences at both a mitochondrial and nuclear locus. That no differences were observed in either COI (816 bp), a marker advocated by the "barcode of life project" as a standard way to discriminate among species (Hebert et al. 2003a, Hebert et al. 2003b), or lysin, a rapidly evolving gamete recognition protein that shows great sequence divergence among species (Lee and Vacquier 1992, Metz et al. 1998) is noteworthy. The subspecies of *H. kamtschatkana* are recognized based solely on shell morphology; the pinto abalone is more flat, rugose, and elongate than the threaded abalone, with intermediate morphological forms documented (McLean 1966). The unique shell characteristics of the *H. kamtschatkana* subspecies may simply be another case of phenotypic plasticity. In fact, *Haliotis* subspecies have been synonymized in the past when shell morphology differences were found to be under environmental control. Three subspecies of the black abalone, *H. cracherodii* Leach, 1814, were once recognized due to differences in the number and size of the tremata. These subspecies were synonymized after the morphology of translocated individuals changed to that characteristic of the area, providing evidence that these diagnostic shell shape characters were environmentally controlled (Geiger 1998)

Previous researchers have also documented the inability to distinguish between pinto and threaded abalone using molecular tools (Gruenthal and Burton 2005, Supernault et al. 2009). Before subspecific status is called into question, three important caveats must be stated. 1) We examined the DNA from only ten individuals, which is not an adequate sample size to synonymize two taxa. 2) The DNA from threaded abalone was sent to us by fellow researchers; we did not have the opportunity to examine the shells and 3) The three papers noting molecular similarity between threaded and pinto abalone (this ms, Gruenthal & Burton 2005, Supernault et al. 2009) all used DNA supplied by K. Gruenthal/ R. Burton. It is possible that the same few individuals were used in all three papers. That said, current evidence suggests no molecular distinction among pinto and threaded abalone and we strongly recommend that further research is conducted to elucidate their taxonomic status and evolutionary relationship.

Our findings strongly advocate the use of species specific molecular tools for abalone management, especially if restoration efforts such as aggregating reproductive adults and supplementing wild populations with hatchery-reared juveniles are used. Given our results from Washington State, molecular identification is recommended even in areas where only one abalone species is thought to be present. Due to the imperiled status of many abalone worldwide, more research identifying abalone species and describing their distribution is needed.

Table 1. Eastern Pacific abalone taxa names and United States Federal protected status.

| Scientific name | Common name | USA Federal Protected Status |
|---|---|---|
| *H. kamtschatkana* | | Species of Concern in US, endangered in Canada |
| subspecies: *kamtschatkana* | Pinto/ Northern | NA |
| subspecies: *assimilis* | Threaded | NA |
| *H. walallensis* | Flat | Not Listed |
| *H. sorenseni* | White | Endangered |
| *H. rufescens* | Red | Not Listed |
| *H. cracherodii* | Black | Endangered |
| *H. corrugata* | Pink | Species of Concern |
| *H. fulgens* | Green | Species of Concern |

Table 2. Details of specimens used in this study.

| Species | Location | n | Code |
|---|---|---|---|
| *H. k. kamtschatkana* | Ketchikan, AK, USA | 3 | kamtschatkana 1-3 |
| *H. k. kamtschatkana* | Sitka, AK, USA | 1 | kamtschatkana 4 |
| *H. k. kamtschatkana* | Monterey, CA, USA | 3 | kamtschatkana 5-7 |
| *H. k. kamtschatkana* | San Juan Islands, WA, USA | 3 | kamtschatkana 8-10 |
| *H. k. assimilis* | Santa Barbara, CA, USA | 10 | assimilis 1-10 |
| *H. walallensis* | Monterey, CA, USA | 4 | walallensis 1-4 |
| *H. walallensis* | Mendocino, CA, USA | 1 | walallensis 5 |
| *H. walallensis* | Port Orford, OR, USA | 5 | walallensis 6-10 |
| *H. sorenseni* | Channel Islands, CA, USA | 10 | sorenseni 1-10 |
| *H. rufescens* | Crescent City, CA, USA | 2 | rufescens 1-2 |
| *H. rufescens* | Monterey, CA, USA | 4 | rufescens 3-6 |
| *H. rufescens* | Channel Islands, CA, USA | 3 | rufescens 7-9 |
| *H. rufescens* | Mendocino, CA, USA | 1 | rufescens 10 |
| *H. cracherodii* | Mexico | 5 | cracherodii 1-5 |
| *H. cracherodii* | Channel Islands, CA, USA | 3 | cracherodii 6-8 |
| *H. cracherodii* | Monterey, CA, USA | 2 | cracherodii 9-10 |
| *H. corrugata* | Mexico | 5 | corrugata 1, 5-8 |
| *H. corrugata* | San Diego, CA, USA | 3 | corrugata 2-4 |
| *H. corrugata* | Channel Islands, CA, USA | 2 | corrugata 9-10 |
| *H. fulgens* | San Diego, CA, USA | 4 | fulgens 1-4 |
| *H. fulgens* | Channel Islands, CA, USA | 6 | fulgens 6-10 |
| *H. rubra* | Australia | 1 | rubra |

n is the total number of COI sequences examined to determine intra- and inter-specific distance. Geographic locations for all samples are shown; sequences obtained from Genbank have accession number in parentheses. A subset of these taxa were also examined at a longer COI region and at lysin (details in text).

Table 3. Primer sequences.

| Marker | Sequence Length | Primer name and sequence | Citation |
|---|---|---|---|
| COI | 470 bp | Forward: ABCOIF: 5' TGATCCGGCTTAGTCGGACTGC | Metz (1998) |
| | | Reverse: ABCOIR: 5' GATGTCTTGAAATTACGGTCGGT | This ms |
| COI | 820 bp | Forward: ABCOIF: 5' TGATCCGGCTTAGTCGGACTGC | Metz (1998) |
| | | Reverse: ABCOIR2: 5' GAAATGCTGGGGAAAGAATG | This ms |
| Lysin | 403 bp | Forward: HKALYS2F: 5' TGGCCAAATGGCTTAGAGTT | This ms |
| | | Reverse: HKALYS2R: 5' GAGCATGTAATTCGCCCAGT | This ms |

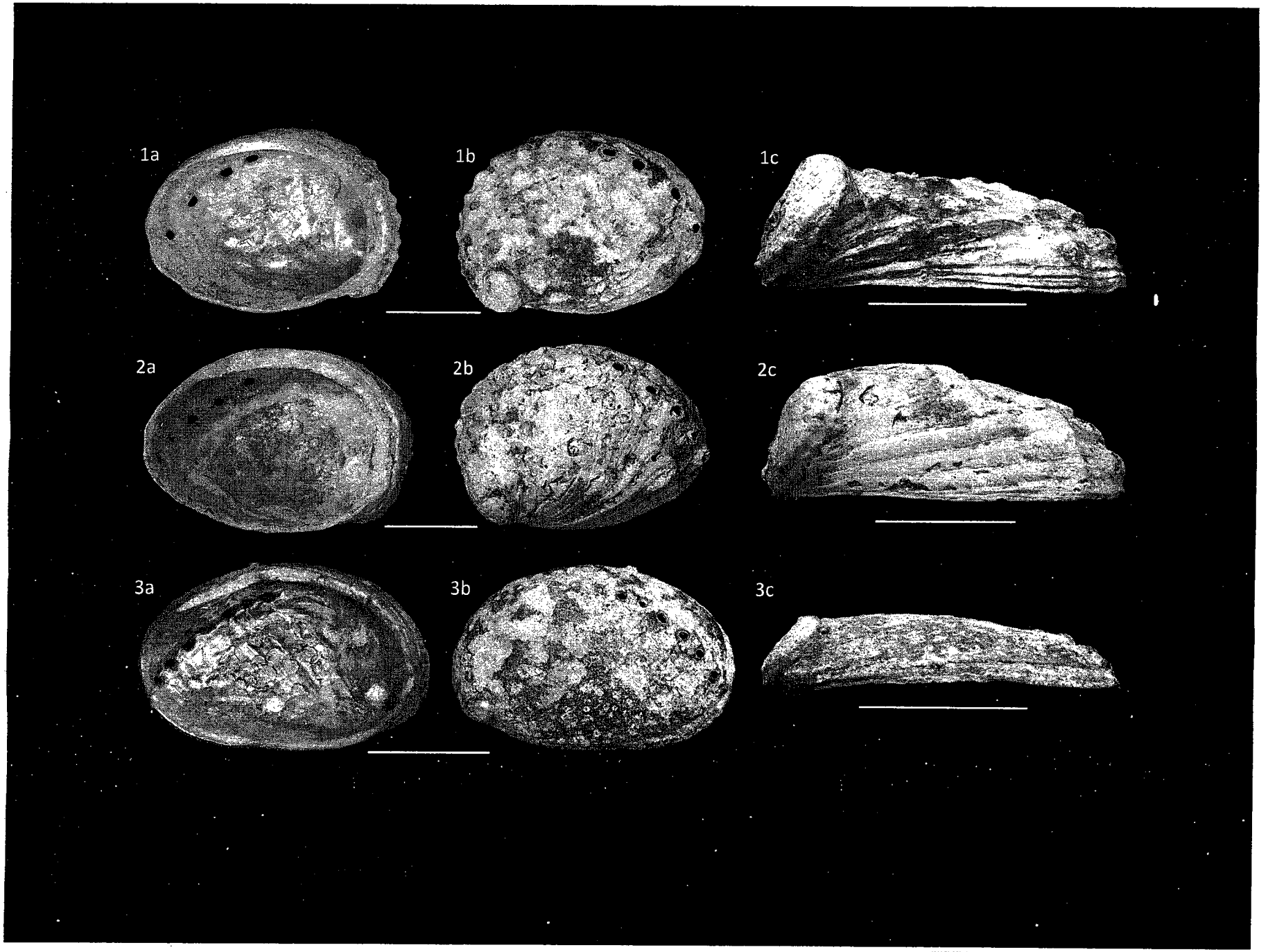



Figure 5. Photographs of representative shell morphology in flat abalone, pinto abalone, and genetically divergent Washington abalone

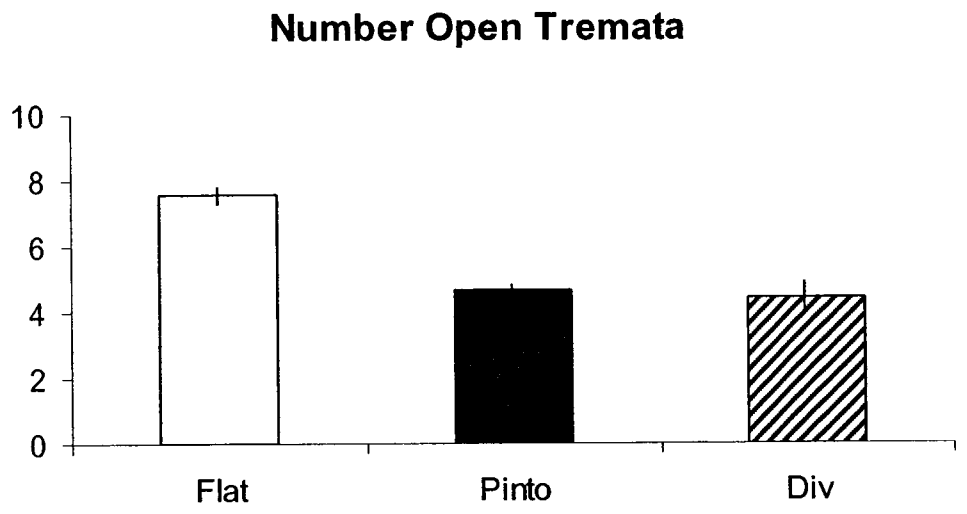



Fig 6. The number of open tremata (mean ± confidence interval) counted in flat abalone, pinto abalone, and genetically divergent Washington abalone (n = 125 pinto abalone, 32 flat abalone, and 5 genetically divergent Washington abalone). Flat abalone have significantly more open tremata than pinto or genetically divergent Washington abalone. No significant differences in the number of open tremata were observed between pinto and genetically divergent Washington abalone.

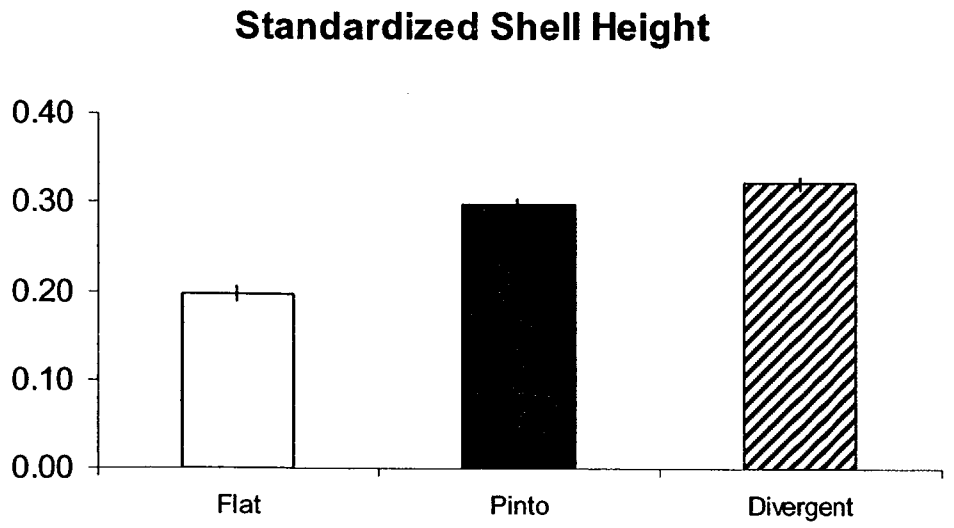



Fig 7. Standardized shell height (mean ± confidence interval) in flat abalone, pinto abalone, and genetically divergent Washington abalone (n = 125 pinto abalone, 32 flat abalone, and 5 genetically divergent Washington abalone). Flat abalone show significantly shorter spire height per unit length than pinto or genetically divergent Washington abalone. No significant differences in standardized shell height were observed between pinto and genetically divergent Washington abalone.

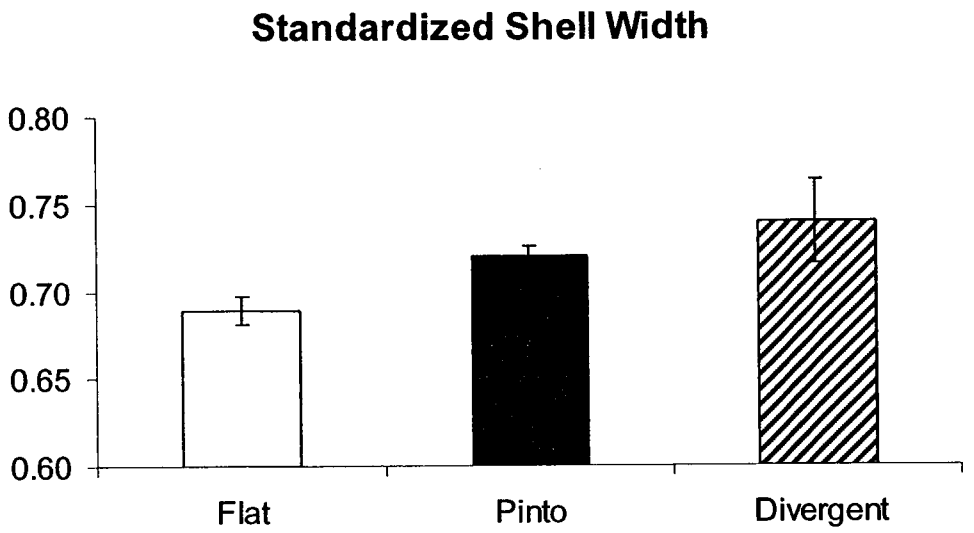
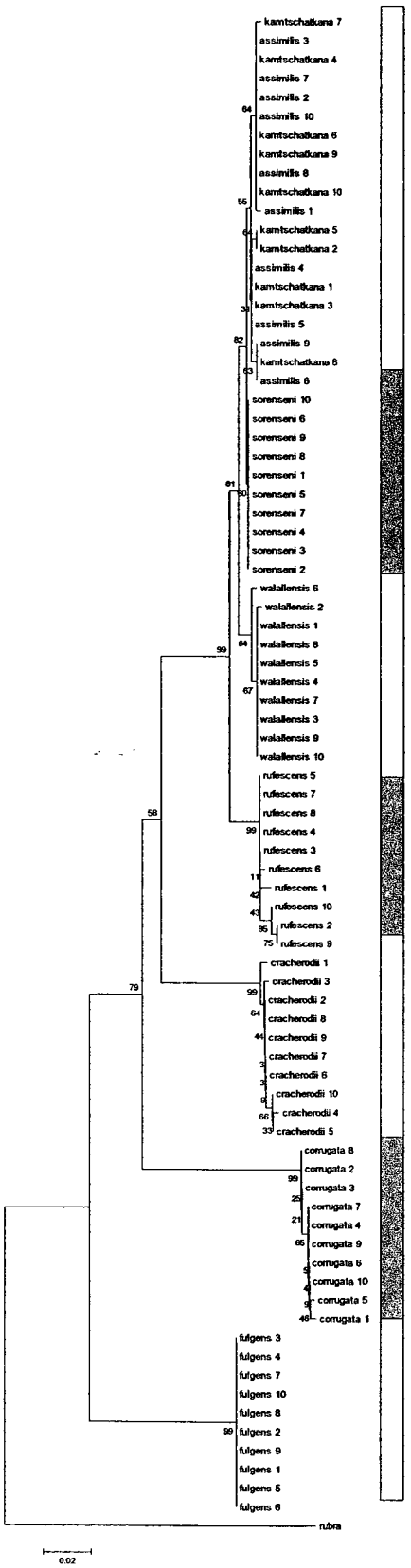



Fig 8. Standardized shell width (mean ± confidence interval) in flat abalone, pinto abalone, and genetically divergent Washington abalone (n = 125 pinto abalone, 32 flat abalone, and 5 genetically divergent Washington abalone). Flat abalone are significantly narrower per unit length than pinto or genetically divergent Washington abalone. No significant differences in standardized shell width were observed between pinto and genetically divergent Washington abalone.




Pinto (*H. k. kamtschatkana*) and Threaded (*H.k.assimilis*) Bootstrap support = 55%

White (*H. sorenseni*) Bootstrap support = 60%

Flat (*H. walallensis*) Bootstrap support = 84%

Red (*H. rufescens*) Bootstrap support = 99%

Black (*H. cracherodii*) Bootstrap support = 99%

Pink (*H. corrugata*) Bootstrap support = 99%

Green (*H. fulgens*) Bootstrap support = 99%

Fig 9. Neighbor-joining phylogenetic tree of short COI (470 bp) from the eight Eastern Pacific abalone taxa. *H. rubra*, an Australian abalone, serves as the outgroup. Numbers indicate percentage of replicate trees in which taxa clustered together (bootstrap test; 1000 replicates). Evolutionary distances were inferred using the Tamura Nei model with rate variation among sites and are in the units of number of base substitutions per site.

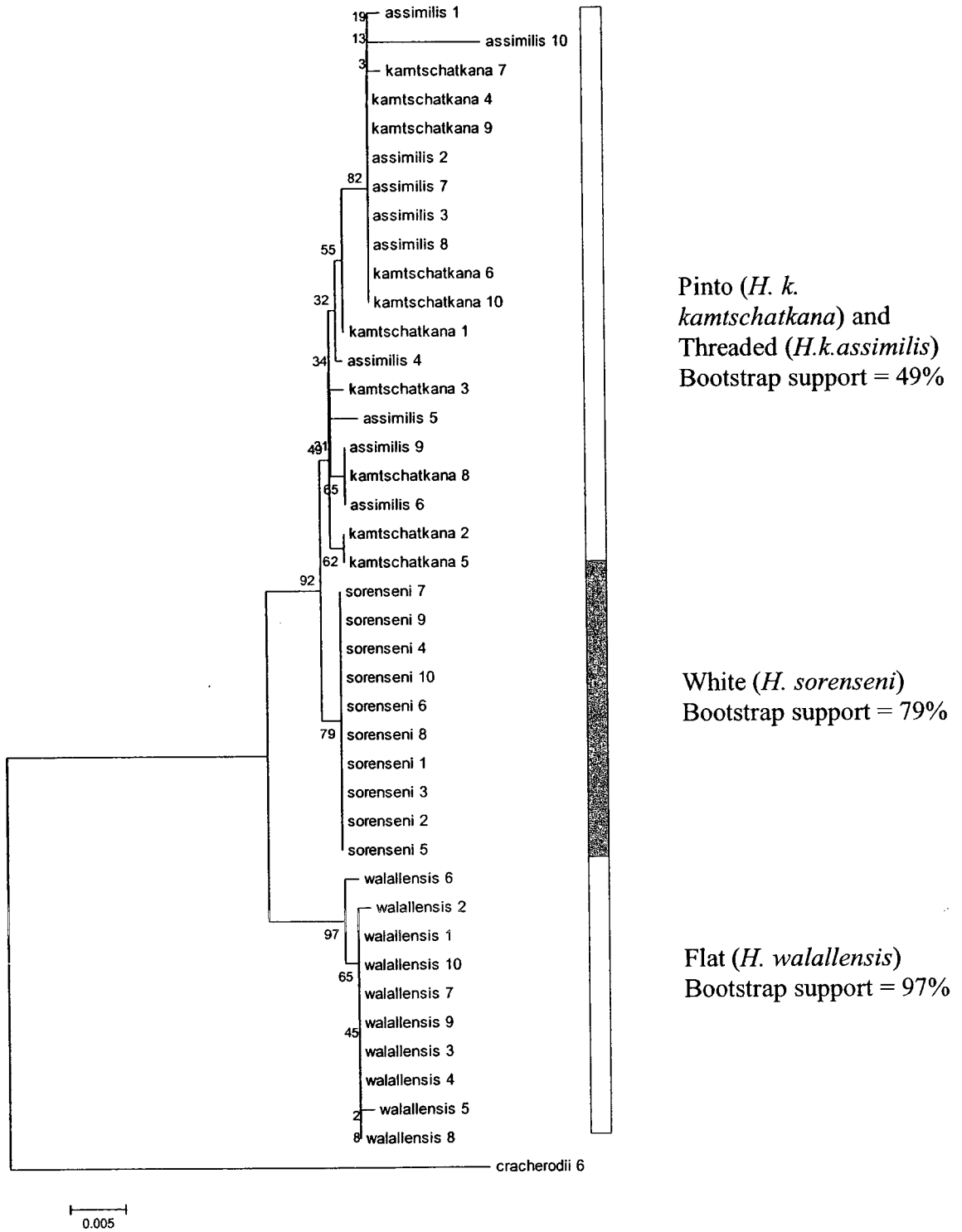



Fig 10. Neighbor-joining phylogenetic tree of long COI (820 bp) from pinto abalone (*H. k. kamtschatkana*), threaded abalone (*H. k. assimilis*), white abalone (*H. sorenseni*), and flat abalone (*H. walallensis*). Black abalone (*H. cracherodii*) serves as the outgroup. Numbers indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The shaded bar to the right of the tree delineates species groups. Evolutionary distances were inferred using the Tamura Nei model with rate variation among sites and are in the units of number of base substitutions per site.

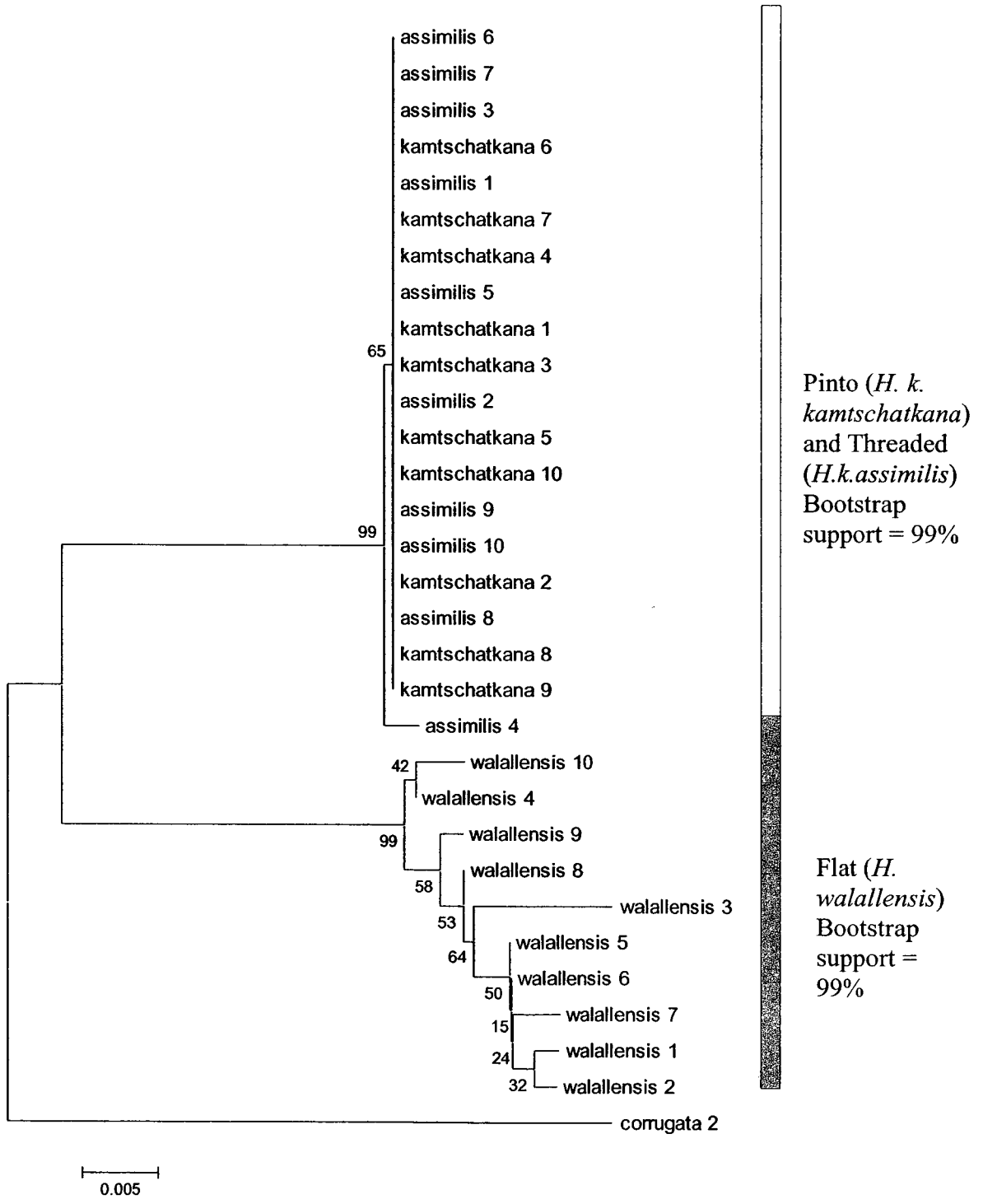



Fig 11. Neighbor-joining phylogenetic tree of lysin (405 bp) from pinto abalone (*H. k. kamtschatkana*), threaded abalone (*H. k. assimilis*), and flat abalone (*H. walallensis*). Black abalone (*H. cracherodii*) serves as the outgroup. Numbers indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The shaded bar to the right of the tree indicates species groups. Evolutionary distances were inferred using the Tamura 3-parameter model with rate variation among sites and are in the units of number of base substitutions per site.

# Chapter IV
# Effects of Geoduck Aquaculture on the Environment: A Synthesis of Current Knowledge

## Background

The Pacific geoduck, *Panopea abrupta* (Conrad, 1849, syn. *Panope generosa*, Gould 1850) is a large hiatellid clam, found in soft intertidal and subtidal substrates in the Northeast Pacific from California to Alaska, and may be found west to Japan. (Anderson 1971, Coan et al. 2000). Geoducks are found in soft substrate from the low intertidal to more than 60 m (Goodwin 1976). Geoducks are extremely long lived, with many examples of animals aged at over 100 years (Campbell and Ming 2003, Goodwin 1976, Shaul and Goodwin 1982, Sloan and Robinson 1984). Geoducks are broadcast spawners that commonly spawn in the spring and summer (Campbell and Ming 2003, Sloan and Robinson 1984) and produce larvae that remain planktonic for 47 days at 14 C (Goodwin et al. 1979). Postlarvae settle onto the substrate and develop into juveniles that burrow into the sediment. Lucrative commercial geoduck fisheries exist in Washington State, British Columbia Canada, and Alaska (Hoffmann et al. 2000).

There is a paucity of peer-reviewed information on *Panopea abrupta* or its congeners. This is particularly true for intertidal *Panopea abrupta,* as no tribe or regulatory agency currently surveys intertidal geoduck clams. Thus, although published reports on geoduck population parameters are available, these publications focus on subtidal geoduck clams. Part of our common understanding about geoduck clams is derived from the substantial amount of information and data on *P. abrupta* originally published in Washington State

and Canadian technical reports, which were not subjected to peer-review. There are two particularly noteworthy cases in point: whether geoducks are found in high abundance below 25 m is unclear from the peer-review literature; however, nearly every paper cites the same pilot video survey work that indicates geoducks are found to 110 m (Jamison et al. 1984b). Additionally, whether *P. abrupta* is found only from California to Alaska, or is also found south to Baja California (Morris et al. 1980) and/or West to Japan (Coan et al. 2000a), is unclear.

## TAXONOMY

| | |
|---|---|
| Phylum: | Mollusca |
| Class: | Bivalvia |
| Subclass: | Heterodonta |
| Order: | Myoida |
| Superfamily: | Hiatelloidea |
| Family: | Hiatellidae |
| Genus: | *Panopea* |
| Species: | *abrupta* |

## SHELL STRUCTURE AND AGE ESTIMATION

No detailed diagrammatic views of geoduck shell morphology have been published. For definitions of technical terms and detailed anatomical diagrams on bivalve shell morphology, we refer the reader to Coan et al. (2000). *Panopea abrupta* is a massive clam, with the largest individuals documented at more than 200 mm shell length (SL) and 3.25 kg (Goodwin 1976, Goodwin and Pease 1991). The valves of this species have a

broad, continuous pallial line with a short pallial sinus, smooth inner margins, a single cardinal tooth, an external ligament, and a porcelaneous interior. The two adductor scars are roughly equal in shape, and each valve has a hinge plate, or chondrophore. The valve comprises three layers, the outer two of which reveal seasonal growth patterns in the microstructure upon microscopic examination. Shaul and Goodwin (1982) developed an acetate peel technique that uses these growth patterns, or annuli, to estimate geoduck age. This technique has been used to determine size and age at maturity of geoduck clams in British Columbia (Campbell and Ming 2003) as well as to produce age–frequency distributions for Washington State and British Columbia geoduck collections (e.g. Breen and Shields 1983, Goodwin and Shaul 1984, Sloan and Robinson 1984). Important to any age-estimation technique is verification that the growth patterns tallied are in fact annual. Shaul and Goodwin (1982) conducted two verification experiments. The first examined growth-band counts from two groups of geoducks, sampled within and adjacent to a channel that had been dredged 26 yr previously. The authors projected that since clams could not have survived the dredging, only those that were sampled from the adjacent areas could exceed 26 yr of age. Annuli counts supported this hypothesis. However, patchiness in settlement of year classes coupled with spatially and temporally variable recruitment have been observed (e.g. Vadopalas 2003, Valero et al. 2004). Thus, highly variable numbers of successful progeny per year class could yield the observed results.

The second verification experiment (Shaul and Goodwin 1982) used a mark-and-recapture design. The authors marked shells of 91 hatchery-reared geoducks and then outplanted the clams. After seven years in the substrate, eight growth lines were

discerned in each of the three recovered geoducks—a confirmation of an annual growth pattern. More recently, concordance between mean sea-surface temperatures and growth-band width provides strong evidence for annual growth-band deposition in *P. abrupta* (Noakes and Campbell 1992, Strom et al. 2004).

Using these age estimation techniques, Sloan and Robinson (1984) determined the oldest geoduck recorded was age 146 and the oldest reproductive geoduck recorded was age 107. A technical report documents a geoduck from the Queen Charlotte Islands that was estimated to be 168 yr old (Bureau et al. 2002) but this report may not have been subject to peer-review.

## ANATOMY

The interior anatomy of *Panopea abrupta* is similar to other bivalves. However, geoducks have an extremely large, fused siphon and mantle that cannot be fully retracted into their shell, which distinguishes them from other clams in the region (Fig. 12). The mantle region has posterior siphon apertures and a pedal aperture (a small slit located dorsally on the anterior end). The geoduck orients itself with the posterior siphon towards the surface, where seawater containing dissolved oxygen and suspended microalgae is circulated via ciliated ctenidia down through the inhalant siphon. The ctenidia perform both gas exchange and feeding functions. The ctenidia trap, sort, and transport food particles to the labial palps, which then sort food particles into the esophagus (Yonge and Thompson 1976). Rejected food particles are bound with mucus and periodically ejected as psuedofeces via the exhalant siphon. After entering the esophagus, the mucus-bound

particles are transported via cilia to the stomach and crystalline style. The crystalline style is a freely rotating gelatinous rod that contains enzymes involved in digestion. The food moves from the stomach to the digestive gland, where most of the intracellular digestion takes place. After digestion, material enters the intestine and is discharged from the anus. Feces are expelled via the exhalant siphon. The gonad follicles are interspersed in the visceral mass, and depending on season and condition can vary from a few millimeters to > 1 cm thick.

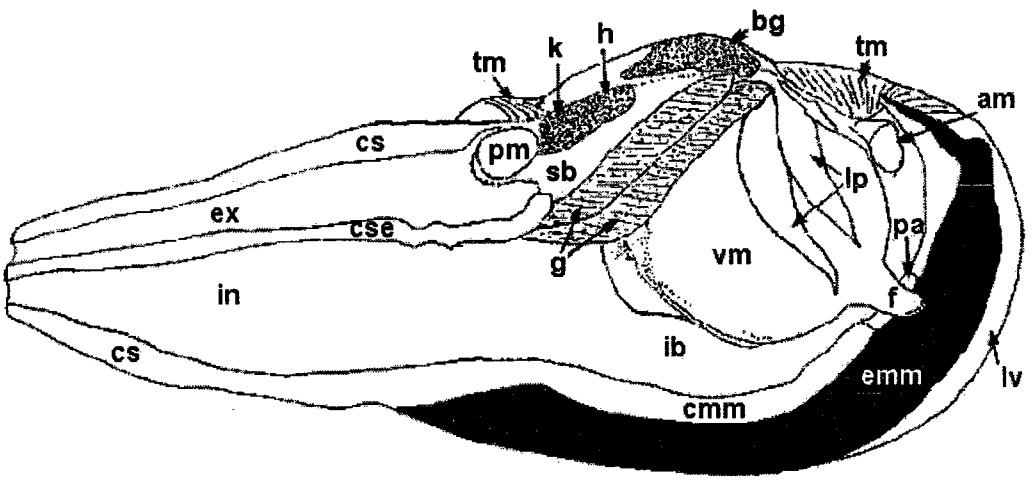



Figure 12. Sketch of the internal organization of the major organs of the geoduck clam, *Panopea abrupta*. The right valve and right side of the muscular mantle and siphon have been dissected away to reveal the fused siphons and the arrangement of the internal organs. The thin mantle (**tm**) that lines the inner surface of the right valve to the pallial line has been turned over the dorsal edge of the left valve (**lv**). Other labels on the sketch are: **am** - anterior adductor muscle, **bg** - brown gland, **cmm** - cut surface of muscular mantle, **cs** - cut surface of siphon, **cse** - cut surface of septum, **emm** - external surface of muscular mantle, **ex** - excurrent channel, **f** - foot, **g** - gills, **h** - heart, **ib** - infrabranchial chamber, **in** - incurrent channel, **k** - kidney, **lp** - labial palps, **lv** - left valve, **pa** - pedal aperture, **pm** - posterior adductor muscle, **sb** - suprabranchial chamber, **tm** - thin mantle, **vm** - visceral mass. Figure and legend used with permission from Bower et al. (2003).

## REPRODUCTION

Adult geoducks are highly fecund broadcast spawners but age of reproductive maturity is unclear. Sloan and Robinson (1984) examined 404 geoduck clams from British Columbia and aged the youngest mature male at seven years and the youngest mature female at eight years. However, Campbell and Ming (2003) examined 182 geoducks from two sites in British Columbia and found that age at 50% maturity was three years for geoducks from Gabriola Island and two years for geoducks from Yellow Bank. Reproductive senescence has not been observed in geoducks (Sloan and Robinson 1984). All "old" (> 50 yr) geoducks examined appeared reproductively active, with morphologically active sperm or ova. Ripe males as old as 107 yr and ripe females as old as 89 yr have been documented, with no apparent reduction in fecundity with age (Sloan and Robinson 1984).

Gametogenesis in geoducks follows an annual cycle. In Puget Sound (the Sound) and British Columbia, spawning commences in the spring and peaks in June and July (Anderson 1971, Campbell and Ming 2003, Goodwin 1976, Sloan and Robinson 1984). Goodwin (1976) examined histological sections of gonads from 124 geoducks from six locations in Puget Sound and characterized them into five phases of gametogenesis. He found that 50% were in the early active phase in September and 92% were ripe in November. Clams were 100% ripe in May and by August 50% were spent. Ripe males were found every month collected, from 14% in August to 100% in April. Females had a more contracted spawning season with no ripe females collected from August to October.

Sloan and Robinson (1984) reported similar seasonal changes in gametogenic condition for 365 geoducks from British Columbia.

Geoducks show an uneven sex ratio with higher proportions of males than females observed in the smaller size classes (Sloan and Robinson 1984, Campbell and Ming 2003). Sloan and Robinson (1984) observed a steady decrease in the proportion of males from 90% of all individuals $\leq$ 10 yr to 47% of all geoducks $\geq$ 51 yr. Campbell and Ming (2003) observed that 41% of geoducks < 90 mm SL were immature. Of the mature geoducks < 90 mm SL, 92.5% were male with only 7.5% female. In geoducks $\geq$ 90 mm SL, the sex ratio was essentially equal (52% males: 48% females). There are at least two possible explanations for these disparate sex ratios. Goodwin (1976) suggested that geoducks are dioecious, with sex determined by development and males maturing younger and smaller than females. Also, a portion of these young male geoducks may be protandrous hermaphrodites, which will reverse sexes at some point as they age. Of 253 geoducks sampled histologically by Campbell and Ming (2003), one individual was a hermaphrodite, with a gonad containing both oocytes and spermatozoa. Although most bivalves are dioecious (Coe 1943), hermaphroditism has been documented. The northern quahog, *Mercenaria mercenaria*, is generally considered protandrous, and bisexual gonads have been observed (Eversole et al. 1980). Protandrous hermaphrodism has also been observed in the Pacific oyster, *Crassostrea gigas* (Guo et al. 1998), and the pearl oyster, *Pinctada margarifera* (Dolgov 1991). Additionally, evidence suggests that a congener, the New Zealand geoduck, *Panopea zelandica*, experiences protandry (Gribben and Creese 2003).

## LIFE CYCLE

Reproductive development has been well documented in some bivalve species (reviewed in Sastry 1979). Longo (1987) describes the general meiotic process in clams, using *Spisula solidissima* as an example. Geoducks are thought to be dioecious (but see section 1.5), facultative repeat broadcast spawners. Synchronization of spawning in many marine invertebrates is not well understood, but the detection of sperm in seawater from one male may cue mass spawning in the aggregation (Sastry 1979). Fertilization occurs externally and meiosis progresses through expulsion of both polar bodies The duration of the meiotic cycle is affected by temperature: at 30 PSU salinity, meiosis took 106 min at 11 °C, 78 min at 15 °C, and 56 min at 19°C (Vadopalas 1999). Salinity also affects meiotic duration. At 15 °C, completion of meiosis took 106 min at 24 PSU, 81 min at 27 PSU, and 78 min at 30 PSU (Vadopalas 1999).

Subsequent to the completion of meiosis in the ova, the male and female pronuclei break down prior to the first mitotic division (Longo 1987). Goodwin (1973) described the combined effects of salinity and temperature on the timing of geoduck clam embryonic development. The optimal temperature and salinity ranges reported for embryonic development were 6-16°C and 27.5-32.5 PSU, respectively (Goodwin, 1973). Outside these ranges, a significant reduction in normal development from the embryonic to the larval stage was observed. However, temperature and salinity tolerance can vary significantly between developmental stages in clams (Sastry 1979).

After approximately 48 h of embryonic development, the geoduck trochophore develops into an actively swimming and feeding veliger larva (straight-hinge or D-stage; Goodwin et al. 1979). The veliger stage lasts 47 d at 14°C (Goodwin et al. 1979), during which the larva feeds on microalgae and grows from 111 to 381 μm in shell height (Goodwin et al. 1979). Studies on the behavior of geoduck veliger larvae are depauperate.

During early metamorphosis, geoduck veliger larvae settle to the bottom, lose their velum, develop primary ctenidia and spines on the shell and begin active crawling (Goodwin et al. 1979). Over the next several weeks, ctenidia formation is completed, the siphon grows and the mantle is fused. During this stage geoducks use their foot to crawl and to transfer detrital food to the mouth (pedal-palp feeding; King 1986). After two to four weeks as postlarvae, geoducks will have reached 1.5 to 2 mm SL and will have burrowed into the substrate and begun filter feeding (King 1986). Juveniles resemble adults, and valve length increases approximately 30 mm per yr for the first three years (Goodwin 1976).

Goodwin (1976) examined the growth of subtidal geoduck in Puget Sound using a mark and recapture method. Growth was fastest in the first 3 years of life, with valve length increases ranging from 20 to 30 mm per year. After ten years, growth rate slowed considerably (Goodwin 1976). Valves continue to increase in thickness throughout life, enabling age estimation based on shell layer visible in thin sections of the chondrophore. A recent study confirmed that geoduck growth is rapid for the first 10-15 years, but then growth rates decline, with shell length expansion essentially halted after 25 years (Strom

et al. 2004). Growth rate also varies significantly along environmental gradients such as temperature, substrate, and depth, and among geographic sites (Campbell et al. 2004, Goodwin and Pease 1991, Hoffmann et al. 2000).

Goodwin (1976) collected 2,037 geoduck from unexploited stocks in multiple Puget Sound locales and found an average shell length of 158 mm with an average range from 124 mm to 171 mm, depending on location. Only four individuals over 200 mm were collected (Goodwin 1976). A later study of 11,154 geoduck found the average shell length and weight to be 135 mm and 872 g, respectively, with a range of 49-212 mm and 28-3,250g (Goodwin and Pease 1991). Although many sources, (e.g. Campbell et al. 2004, Goodwin and Pease 1991, Zhang and Hand 2006) indicate that adult geoducks of this average shell length reach a burial depth of about 1 m, average adult burial depths observed by Anderson (1971) and Goodwin (1976) were 52 and 50-60 cm, respectively.

## DISTRIBUTION

Populations of various species of *Panopea* clams occur naturally world-wide, including Japan, Argentina, and New Zealand. *P. abrupta*, one of the more massive species in the genus, is reported to occur in coastal waters of the northeast Pacific from Baja California to Alaska (Morris et al. 1980) and in estuarine environments along the west coast of North America and in Japan (Coan et al. 2000). However, the congeners *P. japonica* and *P. globosa*—known to occur in Japan and the Gulf of California, respectively—may have been mistakenly identified as *P. abrupta*. Although introduction to the northwest Atlantic

was suggested as early as 1881 (Hemphill 1881), to our knowledge, there have been no intentional introductions of *P. abrupta* to the northwest Atlantic or other regions.

## HABITAT

Adults are found in sand, mud, mud–sand, mud–gravel, sand–gravel, and mixed loose substrates (Goodwin and Pease 1991). Adult geoducks can tolerate temperatures down to 8 °C (Goodwin et al. 1979): long-term temperature and salinity tolerances have not been established. Known geoduck aggregations in the Strait of Juan de Fuca occur where salinities are typically > 32 PSU (Herlinveaux and Tully 1961), and in South Puget Sound where temperatures can exceed 22°C.

In Puget Sound, geoducks are contagiously distributed in small patches and beds of high abundance with an average bed density of 1.7 geoducks per $m^2$ (Goodwin and Pease 1991). Goodwin and Pease (1991) observed that geoduck density ranged from 0 to 22.5 per $m^2$ and individuals tended to aggregate within the beds, in groups containing an average of 109 animals. Conspecific aggregation is common for many bivalve species and is important for spawning synchronization and fertilization success (Sastry 1979). It appears that geoduck density increases with depth to 25 m but mean length and weight decrease with depth between 3 and 20 m (Goodwin and Pease 1991).

Geoducks are found in the low intertidal to subtidal waters. Existing evidence of deep-water stocks in Puget Sound is limited to two pilot studies of a single area in Case Inlet

(South Puget Sound). Although not subjected to peer-review, the video surveys conducted in these studies revealed what appear to be substantial aggregations of geoduck clams starting below the 18-m mean lower low water (MLLW) fishing limit to a depth of 110 m (Jamison et al. 1984, Goodwin unpubl. data). There are additional anecdotal accounts of geoducks observed at even greater depths, but no thorough examinations resulting in peer-reviewed publications have looked for geoducks at depths > 25 m. From these few data, subtidal geoduck abundance in Puget Sound was estimated to be 25,800,000 individuals based on very limited video reconnaissance (Jamison et al. 1984). A postulation in the Washington State geoduck resource management plan is that these deep-water stocks contribute to recruitment and recovery of fished areas, yet data are lacking to support this important assumption.

## Spatial and Genetic Structure of Wild Geoduck Introduction

Many marine bivalves including geoducks tend to aggregate (Fegley 2001) and exhibit temporal changes in abundance. On broad spatial scales in the inland marine waters of Washington, subtidal geoducks are found in all subbasins and straits; on smaller spatial scales, geoduck distribution varies considerably (Goodwin and Pease 1991a). Although some information is available on subtidal geoduck clams, neither the Washington Department of Natural Resources (WDNR), the Washington Department of Fish and Wildlife (WDFW), nor the Treaty tribes regularly survey intertidal geoducks, so information about intertidal population size, density, and aggregation is lacking. Geoducks are occasionally reported in creel surveys of recreational harvesters, which

provide some anecdotal information on where geoducks are found but no information on population parameters.

## POPULATION SIZE

Subtidal geoducks are regularly surveyed to determine geoduck biomass and population size by the WDFW and several of the Washington Treaty tribes (Hoffmann et al. 2000). For these purposes, Puget Sound is divided into six geoduck management regions based on legal tribal fishing boundaries (Hoffmann et al. 2000); the regions have little to do with local oceanography or geoduck biology. Transects are conducted perpendicular to shore between 5.5 m and 21.5 m below MLLW. Counts are based on visual identification of either a geoduck siphon or a siphon depression sighted along a 0.91-m-wide band delineated by the transect line. Visual counts are corrected by a seasonal "show" factor, specific to each tract, to account for the portion of geoduck undetected by virtue of their retracted siphons (Goodwin 1977). WDFW uses these dive-survey data to make management decisions for the commercial geoduck fishery, such that 2.7% of the estimated available, harvestable biomass in each region is legal to harvest each year.

## POPULATION DENSITY

Goodwin and Pease (1991) analyzed subtidal geoduck density relative to geographic area, latitude, water depth, and sediment type in Puget Sound. On the basis of 8,589 transects, average geoduck density was 1.7 geoducks per $m^2$, with a range of 0–22.5 geoducks per $m^2$. Densities varied significantly among geographic area, latitude, water depth, and sediment type. The highest regional densities were observed in South Puget Sound (2.0

geoducks $m^{-2}$) and the lowest were recorded in North Puget Sound (0.2 geoducks $m^{-2}$). Within Washington State, an inverse relationship between geoduck density and latitude is observed, but this relationship does not extend to British Columbia where geoduck density is higher (Goodwin and Pease 1991). On the west side of Vancouver Island, geoduck densities ranged from 0 to 13 geoducks per $m^2$, with an average of 4.9 geoducks per $m^2$ (Fyfe 1984). Geoduck density was found to increase with depth to the extent studied in both Washington State (to 18 m; Goodwin and Pease 1991) and British Columbia (to 25 m; Campbell et al. 1998, but mean length and weight decreased with depth (Goodwin and Pease 1991). Geoduck densities also varied in different sediment types, across all regions; the lowest densities were observed in mud (1.2 geoducks $m^{-2}$) and the highest densities were observed in mud–sand and sand habitats (2–2.1 geoducks $m^{-2}$).

## AGGREGATION

In Puget Sound, subtidal geoducks are contagiously distributed in aggregated patches (Goodwin and Pease 1991). An average of 0.64 aggregations per 41.8 $m^2$ quadarat were found, with each aggregation containing an average of 109 animals (Goodwin and Pease 1991). Goodwin and Pease (1991) hypothesized that geoduck aggregations may result from larval attraction to adult conspecifics, patchy distribution of substrate type, or biotic attractants or deterrents, but these correlations have not been investigated. The aggregation pattern within a geoduck bed has been characterized as a Type III concentration (Hilborn and Walters 1992), with most locations within the bed exhibiting intermediate density, and fewer locations with either low or high abundance (Campbell et al. 1998).

Contagious distribution has also been observed in the northern quahog (*Mercenaria mercenaria*; Saila et al. 1967). In this study, 22% of quadrats sampled contained high *Mercenaria* densities while 29% contained very few or no northern quahog. Conspecific aggregation is common for many bivalve species and is important for spawning synchronization and fertilization success in broadcast spawners (Sastry 1979). For example, synchronous spawning was observed in an estimated 54,000 mussels (*Mytilus californianus*) and fertilization success was estimated to be 80% (Gosselin 2004); fertilization success in the red sea urchin (*Strongylocentrotus franciscanus*) was also shown to increase dramatically with proximity to conspecifics (Levitan et al. 1992).

## Recruitment and Temporal Changes

Groups of geoduck aggregations are structured as individual beds connected through the dispersal of planktonic larvae (Orensanz et al. 2004, Zhang and Hand 2006). Thus, the recruitment to a particular bed is not related to the reproductive capacity of geoducks within that bed (Orensanz et al. 2004). Instead, recruitment may depend on the reproductive and environmental conditions local to other beds as well as larger-scale environmental variables that may effect spawning, survival, and larval flow. There is currently no accurate way to model geoduck recruitment, as recruitment in one area is linked with reproductive capacity in unknown areas, and may be related to unknown geographic and oceanographic parameters that vary temporally and spatially.

Two recent studies examined geoduck density and recruitment in British Columbia over nine years in the same plots with controlled fishing pressure (Campbell et al. 2004, Zhang and Campbell 2004). Campbell et al. (2004) defined recruitment as the density of six to seven year-old geoducks per $m^2$ while Zhang and Campbell (2004) defined recruitment as the number of one year-old geoducks per $m^2$. Campbell et al. 2004 found that heavy fishing pressure reduced geoduck population densities and average geoduck age, but that densities slowly increased due to recruitment once fishing was halted. Zhang and Campbell (2004) found that severe harvesting (> 90% removal) negatively impacted recruitment at one site in the short term (≤ three years) but did not affect long-term recruitment patterns. At the second site, the most heavily harvested plot had the highest short- and long-term recruitment levels. However, the highest recruitment was also observed at this site before the experiment began, suggesting that recruitment is highly variable on a small spatial scale regardless of fishing pressure. Neither study documented long-term negative effects of geoduck fishing on subsequent recruitment and both studies observed that recruitment varied at large and small spatial scales.

Ripley (1998) suggested that, in addition to dispersal potential, an important advantage of broadcast spawning and pelagic larvae may be the periodic high success in recruitment. Long-lived species like geoduck can weather lengthy periods when environmental characteristics are not conducive to high recruitment because they may experience very large recruitment events when environmental conditions are ideal (Ripley 1998). Recent studies that have back-calculated historical recruitment patterns from age–frequency data (Orensanz et al. 2004, Valero et al. 2004, Zhang and Hand 2006) suggest that while

geoduck recruitment is characterized by substantial interannual variation, a multi-decadal recruitment trend can be observed across a huge geographic scale. Recruitment in British Columbia and Washington State declined during 1920–1975, reached a minimum around 1975, and then rebounded, reaching pre-decline levels in the early 1990s (Orensanz et al. 2004, Valero et al. 2004, Zhang and Hand 2006). The decline is not thought to be anthropogenic as it began long before the commencement of geoduck fisheries and is evident in pristine and disturbed locales. Instead, recruitment patterns appear to be correlated with environmental parameters including sea surface temperature (low temperatures = low recruitment) and discharge from large rivers (high discharge = low recruitment; Valero et al. 2004). Multiple environmental parameters shifted in the mid-1970s in the Pacific Ocean (Ebbesmeyer et al. 1991); collectively, these changes are referred to as a regime shift (Francis et al. 1998) and studies have documented their impact at both the ecosystem and the organismal level (Clark and Hare 2002, Hare and Mantua 2000, Tolimieri and Levin 2004). Previous studies have shown that environmental variables are correlated with bivalve recruitment; for example, sea surface temperature is positively correlated with year-class strength in native littleneck clams (*Protothaca staminea*; Orensanz 1989).

## POPULATION GENETICS, ADAPTATION, AND LARVAL DISPERSAL

In marine species, larval dispersal affects genetic stock structure and population dynamics; an understanding of larval dispersal is therefore vital for effective management. The extent to which populations are connected spatially and temporally is dependent on larval dispersal, as larvae are the primary migrating propagules in

broadcast-spawning marine invertebrates. Much of the research on dispersal and recruitment of broadcast-spawning marine invertebrates has relied on untested assumptions that larvae behave as passively drifting particles distributed randomly throughout the water column. There is mounting evidence, however, that larval dispersal of marine fish and invertebrates may be tied to complex interactions between the environment and larval behavior. In fact, Shanks and Brink (2005) recently falsified the hypothesis that bivalve larvae disperse passively via ocean currents. Studies by Taylor and Hellberg (2003) and Zacherl (2005), entailing genetic and microchemical analyses, respectively, have further challenged the idea that marine invertebrate larvae disperse passively.

Many clams can use their foot for some degree of active movement in response to wave action, tidal movement, substrate displacement by storms, strong currents, or disturbance (Prezant et al. 1990, Yonge and Thompson 1976). In contrast, geoduck adults have only a small vestigial foot and are not capable of much movement. Adult movement is restricted to siphon extension and retraction and, once exposed, adults cannot right themselves or dig back into the substrate. Thus, primary connectivity among geoduck aggregations is established via planktonic larval dispersal, with potential small-scale dispersal of juveniles.

In geoducks, larval dispersal plays the primary role in facilitating gene flow and determining population structure. Gene flow is correlated with dispersal in many organisms (Bohonak 1999), including many marine fish and shellfish species (reviewed

in Shaklee and Bentzen 1998). For example, marine species with planktonic larvae tend to have higher gene flow and less population differentiation than direct-developing species (Ayre and Hughes 2000, Collin 2001, De Wolf et al. 2000, Waples 1987, Ward 1990). Panmixia (random mating within a population) has been observed at broad geographic scales in broadcast-spawning invertebrates, especially those with a long larval stage (e.g., *Littorina striata,* De Wolf et al. 2000; *Strongylocentrotus franciscanus*, Miller et al. 2006; *Mytilus galloprovincialis*, Skalamera et al. 1999). However, genetic structure at a variety of spatial scales has also been observed in broadcast-spawning marine invertebrates including the Eastern oyster, *Crassostrea virginica* (Karl and Avise 1992), the sea urchins *Strongylocentrotus purpuratus* (Edmands et al. 1996) and *S. franciscanus* (Moberg and Burton 2000), the lagoon cockle, *Cerastoderma glaucum* (Mariani et al. 2002), the limpet *Siphonaria jeanae* (Johnson and Black 1984) and the black abalone *Haliotis cracherodii* (Chambers et al. 2006, Hamm and Burton 2000).

The complex hydrology and bathymetry of Puget Sound suggests the potential for restricted dispersal and population subdivision of marine invertebrates in the region. However, Puget Sound's freshwater inputs and surface outflow may increase the propensity of passive surface particles to disperse in a seaward direction. Molluscan populations colonized by pelagic larvae drifting seaward from populations in inner inlets could thus exhibit either genetic homogeneity or directional gene flow. A study examining population structure in the native littleneck clam (*Protothaca staminea*) and the macoma clam (*Macoma balthica*) within Puget Sound found that although the two species have similar reproductive and dispersal strategies, their population structure was

quite different (Parker et al. 2003). *P. staminea* showed substantial population structure at all loci examined while *M. balthica* populations were not highly differentiated. The level of population structure within Puget Sound is clearly species-dependent and should not be generalized, even for species that share reproductive characteristics.

In the last decade, several studies have examined population structure in geoduck clams (Miller et al. 2006, Vadopalas et al. 2004, Van Koeveringe 1998). Using the cytochrome oxidase III subunit (COIII) of the mitochondrial genome, Van Koeveringe (1998) investigated the population structure of geoduck clams in British Columbia and was unable to falsify the null hypothesis of panmixia. However, statistical power to detect population subdivision was low in this study because only a single locus was used and sample sizes were small.

Vadopalas et al. (2004) examined population differentiation in geoducks from sites in the Strait of Juan de Fuca–Georgia Strait–Puget Sound complex and one site from Southeast Alaska using 11 allozyme and 7 microsatellite loci. Similar patterns of genetic differentiation were detected with both marker classes. In general, little differentiation was detected among geoduck aggregations throughout the region although the Freshwater Bay collection, in the Strait of Juan de Fuca, was differentiated from other collections. The authors speculate about causes of this seemingly random genetic differentiation and suggest three possibilities. The observed pattern may represent genetic isolation, as Freshwater Bay is characterized by oceanographic conditions that may make emigration challenging. The observed pattern may also represent selection; the differentiation of

Freshwater Bay was driven by a locus (GPI) that is thought to be under temperature selection in *Mytilus edulis* (Hall 1985). Finally, the observed pattern may simply represent stochastic variation. Genetic homogeneity on a broad spatial scale and heterogeneity on a fine scale has been observed in other marine invertebrates including a barnacle (*Balanus glandula*; Sotka et al. 2004), a limpet (*Siphonaria jeanae*; Johnson and Black 1984) and a sea urchin (*Strongylocentrotus purpuratus*; Edmands et al. 1996). This geographical variation suggests that focusing on the average gene flow for a species can mask important within-species variation that may reflect selection or local oceanographic conditions.

Miller et al. (2006) used eight microsatellite loci to analyze population differentiation in geoducks from Washington to northern British Columbia and observed more genetic structure at broad spatial scales than was detected by Vadopalas et al. (2004). Overall, they report an isolation-by-distance structure. While Miller et al. (2006) and Vadopalas et al. (2004) observed panmixia at small (50–300 km) scales, Miller detected stepping-stone gene flow at larger (500–1,000 km) scales. The east and west coasts of Vancouver Island and the Queen Charlotte Islands were found to be considerably differentiated, possibly because oceanographic conditions limit gene flow between the regions but also because environmental parameters (e.g., high waves and disturbance on one side of Vancouver Island, the other sheltered) may impose adaptive constraints.

While we have a fairly good understanding of neutral genetic differentiation (i.e., gene flow) via microsatellite and allozyme analyses of wild geoduck aggregations (Vadopalas

et al. 2004, Miller et al. 2006), differences arising from selection (i.e., local adaptation) are more important for determining the consequences of gene flow from cultured stocks (Crandall et al. 2000, Pearman 2001). Panmixia indicated by neutral molecular markers can mask adaptive variation among populations (Utter 1998); Reed and Frankham (2001) found only weak correlation between quantitative variation in life history traits and neutral molecular markers. Adaptive differentiation, i.e. genetic differences produced by natural selection, are best measured with quantitative genetic approaches (Reed and Frankham 2001, Storfer 1996), but such information is currently lacking for geoduck clams.

## PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF THE PUGET SOUND SANDY INTERTIDAL ZONE INRODUCTION

A comprehensive review of the community characteristics and structure of the sandy intertidal of Puget Sound is beyond the scope of this review. Here, we briefly discuss topics which may contribute to our understanding of geoducks and geoduck aquaculture in Puget Sound, including natural biota, water quality, sediment quality, and recovery after natural disturbances. A common theme running through this discussion is that oceanographic and ecological conditions in Puget Sound vary dramatically on a variety of spatial and temporal scales.

## NATURAL BIOTA

A broad range of physical (e.g., current, substrate type, temperature, salinity) and biological (e.g., predation, competition) factors are known to affect the distribution and abundance of benthic marine organisms. In estuarine systems, the primary physical processes driving the distribution of benthic flora and fauna include wave energy, salinity, and sediment structure (Dethier and Schoch 2005). Salinity in particular plays a key role: low and variable salinity are correlated with reduced species diversity (Carriker 1967, Constable 1999, Smith and Witman 1999). One challenge to understanding patterns in estuarine systems is that oceanographic variables are often linked. For example, wave action may dictate sediment type and salinity may vary with temperature (Clarke and Green 1988). A second challenge is that estuaries tend to be extremely variable in environmental factors and the distribution and abundance of organisms, and that this variation or patchiness occurs on many spatial and temporal scales. Within-site variation is often considerable, which makes detecting patterns at larger spatial scales difficult (Morrisey et al. 1992).

A recent study overcame this problem of scale by using a nested sampling design to assess the distribution and abundance of benthic organisms in Puget Sound (Dethier and Schoch 2005). Because sediment type is known to influence benthic community (Coleman et al. 2007, Gray 1974, Kennish et al. 2004), only the most common beach type in Puget Sound (primarily sand with cobble and pebbles) was sampled. More than 165 taxa were identified in this study, with 85% of the taxa belonging to four phyla: annelida, mollusca, arthropoda, or rhodophyta. Of these, 134 were identified to species, 23 to

genus, and 10 were grouped into complexes. Twenty-six primary producers, 139 invertebrates, and 1 fish (a gunnel) were found. Unfortunately, geoducks were not identified to species but were grouped into "clam siphons (unident)." No discernable distribution pattern for clam siphons (unident) was observed by the authors. The complete list of all species found in this study is in Appendix A of Dethier and Schoch (2005).

Although high variability in abundance of particular species was observed at many spatial scales, some broader ecological patterns were observed. Species richness increased steadily with latitude as temperature, salinity, wave action, and substrate became more marine. This trend has been previously observed and appears linked to oceanographic variables (Constable 1999, Ysebaert and Herman 2002). In North Puget Sound, salinity was about 3 PSU higher, sea surface temperature was about 3°C lower, and wave energy and sediment size were somewhat higher than in the south Sound. Despite the positive correlation of species richness with latitude within Puget Sound, there were exceptions. For example, barnacles and grapsid crabs were abundant throughout the Sound and 20 taxa were patchily distributed with no obvious geographic trend. Additionally, some taxa were more abundant in South Puget Sound. These taxa tended to be either cultured directly (e.g., *Crassostrea gigas*) or associated with taxa cultured in this region (e.g., *Crepidula fornicata).*

Like many benthic invertebrates in Puget Sound, geoducks are patchily distributed (Goodwin and Pease 1991). Patchiness may be influenced by distribution of preferred

abiotic characteristics, or ecological associations, or both. In a study conducted in British Columbia, juvenile geoduck clams were found clustered around full-sized adult geoducks (Fyfe 1984). It is possible that adult conspecifics provide settlement cues for larval geoducks or that survival of juvenile geoducks is higher in microhabitats replete with adult geoducks. Goodwin and Pease (1991) used a subtidal transect methodology and determined that geoduck density was positively correlated with a number of taxa (based on non-parametric tests, not adjusted for multiple comparisons). The commonly co-occurring taxa include chaetopterid polychaetes (*Spiochaetopterus costarum* and *Phyllochaetopterus prolifica*), sea pen (*Ptilosarcus gurneyi*), horse clams (*Tresus* spp.), red rock crabs (*Cancer productus*), moon snails (*Polinices lewisii*), and laminarian kelp (*Laminaria* spp.). A positive correlation between chaetopterid polychaete density and density of various invertebrate taxa has been observed and suggests that these tube-building worms may facilitate the presence of other species (Morrisey et al. 1992). The correlation between red rock crabs/moon snails and geoducks is likely because these geoduck predators are attracted to areas of high geoduck density. Other positive correlations may be coincidental. Goodwin and Pease (1991) found only one negative correlation: Geoduck densities were considerably lower in quadrats containing red algae (Phylum Rhodophyta), one of the four most common phyla found by Dethier and Schoch (2005) in their survey of Puget Sound.

## OCEANOGRAPHY, WATER QUALITY, AND SEDIMENTS OF PUGET SOUND

Puget Sound is an estuarine fjord composed of a series of basins separated by sills. Water enters and leaves the Sound primarily through Admiralty Inlet, which is connected to the

Strait of Juan de Fuca. After Admiralty Inlet, Puget Sound consists of three major branches: the Main Basin/South Sound, Hood Canal in the southwest, and Whidbey Basin to the northeast. A sill (Tacoma Narrows) separates the deep Main Basin from the shallower South Sound, which is characterized by many branching inlets. Whidbey Basin has an additional outlet to the Strait of Juan de Fuca (Deception Pass), which is shallow and extremely narrow. The water in Puget Sound is about 90% oceanic and 10% fresh (Ebbesmeyer and Barnes 1980), with most of the fresh water provided by the Skagit, Stillaguamish, and Snohomish rivers (Babson et al. 2006). Circulation in Puget Sound is driven by tidal currents, riverine input, and density differences between river and marine water. Puget Sound is generally well oxygenated, with the exception of southern Hood Canal, where hypoxia has been associated with fish kills (Babson et al. 2006). Babson et al. (2006) used a modeling approach to examine seasonal and interannual variations in circulation and residence time in Puget Sound. At the seasonal scale, salinity in the Strait of Juan de Fuca had a larger affect on circulation than seasonal changes in river flow. However, at an interannual scale, changes in river flow had a larger effect than salinity. According to the model, the rate of circulation had high interannual variance, with residence times between 1992 and 2001 varying from 33 to 44 days in Whidbey Basin and 64 to 121 days in southern Hood Canal (Babson et al. 2006, Cox et al.1984) predicted residence times of > 9 mo in Hood Canal based on current records.

Human activity has heavily affected Puget Sound shorelines, water quality, and sediments. At least one-third of the Puget Sound shoreline has been extensively altered by activities including diking, filling, and devegetating (Rice 2006). A recent study

examining shoreline alteration found that light intensity, air temperature, and substrate temperature were considerably higher on altered beaches (without shoreline vegetation) than on vegetated beaches (Rice 2006). Rice (2006) also observed biological differences between the beaches; for example, smelt eggs containing live embryos were reduced by half on the altered beaches.

High levels of chemical contaminants including polychlorinated biphenyls (PCBs) have been documented in Puget Sound (Stein et al. 1993). PCBs have biological implications. For example, benthic flatfish in Puget Sound experience effects of contaminant exposure such as reproductive dysfunction, reduced immune function, and development of toxicopathic diseases (Johnson et al. 1998). Some evidence indicates that fish from urban areas of Puget Sound have increased levels of contaminant exposure and reduced survival compared with fish in less urban areas (Johnson et al. 1998). An extensive survey of sediment quality conducted at 300 locations in the greater Puget Sound (2,363 $km^2$) also indicated that urban areas had higher contaminant levels (Long et al. 2005). Sediments were classified as degraded, intermediate, or high-quality based on toxicity levels, exogenous chemical concentrations, and level of human perturbation. The authors found 1% degraded, 31% intermediate, and 68% high-quality sediments in the greater Puget Sound. Degraded conditions were associated with urbanization and industrial harbors, especially near the urban centers of Seattle, Tacoma, and Bremerton. Long et al. (2005) indicate that compared with other U.S. estuaries and marine bays, Puget Sound sediments showed minimal evidence of toxicant-induced degradation.

Biological toxins including paralytic shellfish poisoning toxin (PSP) and domoic acid (DA) are also present in Washington State. DA is a toxic amino acid produced by diatoms in the genus *Pseudonitzchia* (Bates et al. 1989) while PSP is produced by dinoflagellates from the genus *Alexandrium* (Curtis et al. 2000). Dungeness crab (*Cancer magister*) and razor clam (*Siliqua patula*) fisheries on Washington's outer coast have been periodically closed due to domoic acid (DA) since 1991 (Horner et al. 1993). DA is a particular challenge for razor clam fisherman, as razor clams can retain DA for up to a year (Trainer and Bill 2004). Low levels of DA and some *Pseudonitzchia* species have been observed in Puget Sound (Trainer et al. 2007) since 1991, and DA concentrations below the regulatory limit of 20 ppm have been detected in Puget Sound geoducks (Bill et al. 2006). No information is available on the retention time or depuration of DA by geoducks. Curtis et al. (2000) examined PSP in Puget Sound geoducks and found that toxin concentrations varied considerably among individual clams but that generally, geoducks in shallow water (7 m MLLW) contained higher concentrations of PSP toxin than deeper water (17 m MLLW). The toxin was concentrated in the gonadovisceral mass; toxin levels were below critical levels in mantle and siphon tissues, which were safe to consume even when the viscera were highly toxic.

## Recovery after Natural Disturbances

Levels of natural disturbance vary widely in Puget Sound, from calm, static areas to areas characterized by repetitive natural disturbance. To the authors' knowledge, no work has been published that examines disturbance patterns and recovery within Puget Sound.

Here, we briefly discuss the literature on recovery after natural disturbance, with a focus on sandy intertidal habitats.

Disturbance events vary widely on spatial, temporal, and intensity scales. Recolonization of benthic infauna also varies over space and time in ways dependant upon life-history characteristics, environmental conditions, and biotic interactions (Zajac and Whitlatch 2003). In deep subtidal habitats, larval settlement by opportunistic species is the primary method of recolonization, and succession proceeds in a somewhat predictable manner (McCall 1977, Rhoads et al. 1978). Following a major disturbance such as a storm, juveniles and adults are often important recolonizers (Dobbs and Vozarik 1983). In shallower habitats, the infaunal community is often dominated by opportunistic species. Here, larvae are the primary recolonizers after disturbance, but succession is unpredictable and endpoints vary widely (Zajac and Whitlatch 1982). In shallow and intertidal environments, recovery after disturbance is greatly influenced by hydrodynamic factors (Eckman 1983).

Many studies of sandy intertidal habitats have focused on how hydrodynamic factors influence recolonization (Norkko et al. 2001, Palmer 1988, Turner et al. 1995). Recolonization generally moves quickly in the sandy intertidal because in addition to larval settlement, adults and juveniles may actively burrow or be moved through bedload transport. For example, adult crustaceans colonized disturbed patches (897 $cm^2$) via passive dispersal within 24 days, with ambient densities attained approximately one month after disturbance (Grant 1981). In another experiment, researchers observed that

colonization mechanisms differed widely among infaunal polychaete species but that densities in disturbed areas (100 $cm^2$) returned to ambient levels within 20 days (Shull 1997). These experiments, however, were relatively small-scale and short-term. A recent experiment was conducted to determine whether the trend of quick recovery after disturbance in sand flats held true at larger spatial scales (1 $m^2$) over longer periods of time (4.5 months versus days; Zajac and Whitlatch 2003). The researchers examined population and community structure as well as sediment grain size as a measure of physical disturbance. Defaunated patches differed considerably in sediment grain size distribution, but this distribution returned to ambient levels after about two months. Population abundances of most species reached ambient levels two to three months after the sediment was defaunated, and the community structure returned to ambient conditions after four months. Published studies of recovery after disturbances in Puget Sound (e.g., geoduck harvest) are lacking.

## PREDATOR-PREY INTERACTIONS INTRODUCTION

Predation and competition play critical roles in regulating the distribution and abundance of benthic invertebrates (Peterson 1982, Virnstein 1977, Wilson 1990). Although the relative importance of pre- and post-settlement factors in structuring benthic communities is debated (Caley et al. 1996, Olafsson et al. 1994), predation is considered more important than competition in regulating invertebrate populations (Micheli 1997). Because very few peer-reviewed studies examining geoduck predator–prey interactions are available, we include literature on predator–prey interactions involving other infaunal bivalve species.

## PREDATION RISK AND GEODUCK LIFE-HISTORY STAGE

*Panopea abrupta* has a life cycle typical of many marine invertebrates, characterized by a planktonic larval stage and benthic juvenile and adult stages (Goodwin et al. 1979). Few studies have quantified predation on bivalve larvae, but theory dictates that species with type III life-history strategies, like geoducks, incur highest mortalities during the larval stage. We are not aware of any peer-reviewed literature that examines predation on geoduck larvae specifically, but ingestion of bivalve larvae has been documented in a wide range of taxa, including polychaetes (Johnson and Brink 1998), fish (Bullard et al. 1999, Young and Davis 1992), ctenophores (Purcell et al. 1991) and heterotrophic dinoflagellates (Johnson and Shanks 2003). A large body of literature also documents ingestion of bivalve larvae by bivalve adults (Andre et al. 1993, Lehane and Davenport 2004, 2006, Pechenik et al. 2004, Tamburri and ZimmerFaust 1996, Zeldis et al. 2004). Filter-feeding taxa including many annelids and mollusks are abundant in benthic habitats of Puget Sound (Dethier and Schoch 2005). Given that geoduck at 14°C spend 47 d as veligers (Goodwin et al. 1979), some proportion of geoduck larvae are probably ingested by filter feeders before settlement.

The population-level effects of filter feeders on bivalve larvae are difficult to quantify and are likely to be site- and species-specific. Some research has indicated that predation from filter-feeding bivalves has negative effects on bivalve recruitment (Andre et al. 1993, Andre and Rosenberg 1991). For example, researchers observed that 75% of common cockle (*Cerastoderma edule*) larvae were consumed when passing over high concentrations of adult conspecifics in laboratory experiments. Larvae in these

experiments had a mean survival time of 64 seconds and settlement was reduced by one-third in these areas of high adult concentrations (Andre et al. 1993). Other research, however, indicates that predation by filter feeding has little or no ecological effect (Black and Peterson 1988, Ertman and Jumars 1988). In an apparent paradox, some species of bivalve larvae appear to preferentially settle near conspecific or other bivalve filter feeders (Ahn et al. 1993, Snelgrove et al. 1999, Tamburri et al. 2007). Using laboratory flume experiments, Tamburri et al. (2007) found that although *Crassostrea gigas* larvae were attracted to a soluble cue from adult conspecifics, more than 95% of larvae settled without predation. Larvae that passed very close to the valve gape of an adult were ingested and suffered mortality but, owing to weak ciliary currents, as little as 1 mm distance afforded protection. In field surveys of oyster reefs in Washington State, the gape surface area was 5.2% of the plane surface area of the reef, suggesting that larvae passing over oyster reefs have a low probability of being ingested (Tamburri et al. 2007). After settlement, geoducks spend several weeks as postlarvae. At this stage, geoducks are active crawlers and have spines on their shells (Goodwin et al. 1979, Velasquez 1992) which may deter some predation. After two to four weeks as postlarvae, geoducks are 1.5 to 2 mm SL and have burrowed into the substrate (King 1986). Clam burial depth is directly related to shell and siphon length (Zwarts and Wanink 1989), as juvenile clams must remain shallowly buried in order to maintain contact with the water column. It has been shown that predation risk decreases with burial depth (Haddon et al. 1987, Holland et al. 1980, Virnstein 1977, Zaklan and Ydenberg 1997, Zwarts and Wanink 1989); thus, clams are most vulnerable to predation while they are small and shallowly buried. We include two examples to illustrate this point. Haddon et al. (1987) observed that predation

on intertidal green surf clams (*Paphies ventricosa*) by the paddle crab (*Ovalipes catharus*) declined linearly with increasing burial depth. Similarly, blue crabs (*Callinectes sapidus*) consumed considerably more soft-shelled clams (*Mya arenaria*) buried at 5 and 10 cm than those buried at 15 and 20 cm.

New Zealand pie crust crabs (*Cancer novaezelandiae*) and juvenile Dungeness crabs selectively forage on smaller sizes of soft-shelled clams (Creswell and McLay 1990, Palacios 1994), which may be due to burial depth but may also be directly related to size. Creswell and McLay (1990) documented that the New Zealand pie crust crab can crush smaller clams but must chip away at the shells of larger clams, thus increasing handling times. Given the lack of significant protection from their valves and extensive exposure of mantle and siphon tissues, juvenile and adult geoducks are likely to be extremely vulnerable to predation until they attain a depth refuge. However, as geoducks grow 20-30 mm SL per yr and bury deeper in the substrate during their first two to three years (Goodwin 1976), they may attain at least partial predation refuge relatively quickly. Adult geoducks are generally found at 50–60 cm burial depth (Goodwin 1976) although maximum depth is believed to be closer to 1 m (e.g. Zhang and Hand 2006). Predation on adult geoducks is generally considered rare (Anderson, 1971) but sea star predation on adult geoducks has been observed (Mauzey et al. 1968, Sloan and Robinson 1983, Anderson 1971). Natural mortality rate estimates of adult geoducks range from 0.0226–0.039 per year (Bradbury and Tagart 2000, Zhang and Campbell 2004). Additionally, geoducks of all size classes may be vulnerable to siphon cropping, which has been shown

to affect bivalve feeding and growth (Kamermans and Huitema 1994, Nakaoka 2000, Peterson and Quammen 1982).

## GEODUCK PREDATORS

Most studies on predation in marine soft-bottomed communities have focused on epibenthic predators although predatory infauna also appear to play an important role (Ambrose 1984). Research has documented predation on adult and juvenile soft-shelled clams (*Mya arenaria)* and the macoma clam (*Macoma baltica*) by infaunal organisms including the nemertean worm *Cerebratulus lacteus* (Bourque et al. 2001, Kalin 1984, Rowell and Woo 1990) and the polychaetes *Nereis virens* and *Arenicola marina* (Ambrose 1984, Hiddink et al. 2002). At least one species of carnivorous nemertean and many carnivorous polychaetes, including a congener to *Nereis virens*, are found in Puget Sound (Dethier and Schoch 2005). Juvenile geoducks likely experience predation from predatory infauna, but this has not been documented.

Common epibenthic bivalve predators include crabs, sea stars, gastropods, fish, birds, and mammals (Dame 1996). Research indicates that crabs influence clam distribution and abundance in soft-bottom habitats (Virnstein 1977). Crab predation on clams in general is discussed earlier. Common crabs in Puget Sound that prey on bivalves and are presumably capable of feeding on geoduck juveniles include the red rock crab (*Cancer productus*), the graceful crab (*C. gracilis*), and the Dungeness crab (Jensen 1995). Dungeness crab prey on juvenile *Mya arenaria* and field studies suggest that the distribution of this species may be limited to areas of low Dungeness crab density

(Palacios 1994). Stomach content analyses indicate that Dungeness crabs under one year (≤ 60 mm) consumed large quantities of bivalves (*Cryptomya californica*, *Macoma* sp. and *Tellina* sp.) in Grays Harbor, Washington (Stevens et al. 1982). Few studies have been done on feeding habits of the red rock crab or the graceful crab and no studies have been completed that examine crab predation on geoducks.

Many sea star species consume infaunal clams (Mauzey et al. 1968); sea stars at high densities have been shown to influence community structure and reduce bivalve population densities (Ross et al. 2002, Ross et al. 2004). The sea stars *Pisaster brevispinus* and *Pycnopodia helianthoides* have been observed consuming juvenile and adult geoduck clams in the Pacific Northwest (Mauzey et al. 1968, Sloan and Robinson 1983). *Pisaster brevispinus* is a large sea star, commonly found on soft-bottom sub-tidal habitats in Puget Sound (Mauzey et al. 1968) that preys efficiently on large, deeply buried bivalves by digging feeding pits (Van Veldhuizen and Phillips 1978). Sloan and Robinson (1983) reported that *P. brevispinus* in British Columbia fed preferentially on deeply buried clams, with geoduck making up one third of its diet. Mauzey et al. (1968) also observed *P. brevispinus* consuming geoducks, but noted that this occurred only occasionally at Alki Point, Washington. The feeding pits created by *P. brevispinus* averaged 11.6 cm deep, with the deepest pit reaching 18 cm (Sloan and Robinson 1983). The circumoral tube feet extended on average an additional 16.6 cm, with the longest measured being 23 cm (Sloan and Robinson 1983). These data suggest that *P. brevispinus* can prey on geoducks buried up to 40 cm. Adult geoducks at full burial depth are likely to be safe from *P. brevispinus* predation, but adult clams that are unable to

burrow through an impenetrable layer may be vulnerable. *Pycnopodia helianthoides* is another large Puget Sound sea star that can feed on infaunal clams by digging feeding pits (Mauzey et al. 1968). Large geoduck shells (95.8 mm average SL) have been found at *P. helianthoides* feeding-pits, suggesting that this species can excavate deeply buried clams (Sloan and Robinson 1983). It has been suggested that geoducks may account for up to one-third of the diet of *P. helianthoides* (Sloan and Robinson 1983).

Although gastropod predation on infaunal bivalves is well documented (Kingsley-Smith et al. 2003, Peitso et al. 1994, Savini and Occhipinti-Ambrogi 2006, Weissberger 1999), no published accounts of gastropod predation on geoducks exist. The moon snail (*Polinices lewisii*) is a predatory gastropod that is common in Puget Sound and has been observed feeding on bivalves including littleneck clams (*Protothaca stamina*) and surf clams (*Spisula solidisima*; Peitso et al. 1994). A congener, *Polinices pulchellus*, has also been observed feeding on the common cockle, *Cerastoderma edule* (Kingsley-Smith et al. 2003). Although not found in Puget Sound, *Rapana venosa* is another predatory gastropod that preys on mussels, oysters, and infaunal clams including northern quahog (*Mercenaria mercenaria*) and soft-shelled clams (*Mya arenaria*; Savini and Occhipinti-Ambrogi 2006). Although adult clams are likely to reach a depth refuge from gastropod predation, the impact of gastropod predation on juvenile geoducks should be examined. Juvenile clams are also preyed upon by many fish species. Whole juvenile bivalves have been found in fish stomachs, such as the English sole (*Parophrys vetulus*) and the staghorn sculpin (*Leptocottus armatus*) in Grays Harbor, Washington (Armstrong 1991, Williams 1994). The European flounder (*Platichthys flesus*) forages on juvenile soft shell

clams (*Mya arenaria*) up to 12 mm SL (Moller and Rosenberg 1983). Fishes and crustaceans can also exert non-lethal predation pressure on bivalve populations by siphon-cropping (Armstrong 1991, Kamermans and Huitema 1994, Peterson and Quammen 1982, Sandberg et al. 1996, Tomiyama and Omori 2007). In order to feed, infaunal bivalves extend their siphons above the sediment, which exposes this soft tissue to predators. Meyer and Byers (2005) conservatively estimated that 10% of the clams *Protothaca staminea* and *Venerupis philippinarum* exhibit cropped siphons on San Juan Island at any given time. Geoduck siphons have been found in the stomachs of fish including cabezon (*Scorpaenichthys marmoratus*) and spiny dogfish (*Squalus acanthias*) (Anderson 1971).

While siphon-cropping does not generally cause death, it may negatively affect bivalve growth (Irlandi and Mehlich 1996, Kamermans and Huitema 1994, Nakaoka 2000, Peterson and Quammen 1982) or result in decreased burial depth in *Macoma balthica* (de Goeij et al. 2001), *Protothaca staminea*, and *Venerupis philippinarum* (Meyer and Byers 2005). This decrease in burial depth may facilitate secondary predation (Zwarts and Wanink 1989). De Goeij et al. (2001) observed that *M. balthica* buried less deeply after siphon-cropping and became increasingly vulnerable to avian predators including oystercatchers and red knots. However, Meyer and Byers (2005) found that this result was species-specific. The authors removed the top 40% of the siphon to simulate cropping in *P. staminea* and *V. philippinarum*, and noted that cropped individuals burrowed 33–50% shallower than intact conspecifics. These clams were then used in a field experiment on San Juan Island, Washington, where clams experienced mortality

primarily from *Cancer* crabs. In *V. philippinarum*, cropped individuals experienced nearly double the mortality rate of intact individuals. In contrast, no significant increase in *P. staminea* mortality was observed (Meyer and Byers 2005). The authors attribute this difference to the fact that *P. staminea* has a longer siphon than *V. philippinarum*, and was able to remain buried at relatively safe depths even after cropping. Although siphon-cropping of geoducks has been noted (Anderson 1971), no published information is available indicating the extent, severity, affected size classes, tissue regeneration rates, and effects on burial depth.

Predation by birds can play a large role in structuring the intertidal marine invertebrate community (Clegg 1972, Cummings et al. 1997b). Although much of this work documents bird predation in rocky intertidal communities, recent studies have identified the importance of avian predators in marine soft-bottom communities (Lewis 2007, Richardson and Verbeek 1987, Szekely and Bamberger 1992, Thrush et al. 1994, Zharikov and Skilleter 2003). Two species of scoters—surf (*Melanitta perspicillata*) and white-winged (*M. fusca*)—are thought to play a large role in shaping community structure by consuming large quantities of clams while they overwinter in British Columbia (Lewis 2007). Manila clams (*Venerupis philippinarum*) and varnish clams (*Nuttallia obscurata*) were the primary prey items of both scoters, constituting 72–76% of their diet (Lewis 2007). Other birds are also capable of consuming clams; for example, northwestern crows (*Corvus caurinus*) have been observed digging and consuming Manila clams in British Columbia (Richardson and Verbeek 1987) and canvasbacks (*Aythya valisineria*) feed on multiple clam species including *Macoma balthica, Macoma*

*mitchelli, Mya arenaria*, and *Rangia cuneata* (Perry and Uhler 1988). All of these bird species spend at least some part of the year in Puget Sound, and could potentially be geoduck predators.

The sea otter, *Enhydra lutris,* has been well documented as a keystone predator in both rocky and soft bottom habitats throughout their range in the northeastern Pacific (Garshelis et al. 1986, Kvitek et al. 1998). Sea otters were hunted to extinction off the coast of Washington State early in the 20th century (Gerber et al. 2004). However, over the last decade, the Washington State sea otter population has expanded, from the initial translocation of 59 individuals from Alaska (Jameson et al. 1982) to at least 550 animals (Gerber et al. 2004, Kvitek et al. 1998). The prevailing view is that sea otter populations were never established east of the San Juan Islands or in Puget Sound and are not established there now (G. VanBlaricom, Univ. Washington, pers. comm.). Yet recent sightings of individual sea otters have been made at San Juan and Whidbey Islands, near Federal Way, off the Nisqually Delta, and in southern Puget Sound (unpublished observations verified by the Univ. Washington School of Aquatic and Fishery Sciences, the WDFW, and the Cascadia Research Collective; G. VanBlaricom, Univ. Washington, Seattle, pers. comm.). Sea otters exert a strong influence on infaunal prey communities in soft-sediment habitats (Kvitek et al. 1992). Direct observations of feeding sea otters at 11 sites in Southeast Alaska showed infaunal clams to be the primary prey utilized by otters throughout the region. Bivalve prey abundance, biomass, and size were inversely related to duration of sea otter occupancy (Kvitek et al. 1992). However, otter-cracked shells of the deep-burrowing clams *Tresus capax* and *Panopea abrupta* were rarely found, even at

otter foraging sites where these clams accounted for the majority of available prey biomass, suggesting that these species have a partial depth refuge from otter predation (Kvitek et al. 1993, Kvitek and Oliver 1992). It is important to note that otters have been observed excavating clams up to 0.5m deep (Hines and Loughlin 1980) and could certainly prey on juvenile or possibly adult geoducks. No research has been conducted on this topic specific to Puget Sound.

### ABIOTIC AND BIOTIC EFFECTS OF GEODUCK AQUACULTURE INTRODUCTION

Although geoducks have been cultured in Washington State for enhancement of wild stocks since 1991 (Beattie 1992) and on a commercial scale since 1996 (J.P. Davis, Taylor Resources, Inc., Quilcene, Washington, pers. comm.), little work has been done on the ecological impacts of these practices. Research that has been conducted comprises primarily pilot-scale studies that have not been subjected to formal peer-review. For this reason, we refer to the literature on aquaculture effects of other filter-feeding bivalves to provide a framework for considering the potential effects of geoduck aquaculture. Although there is a large body of literature on the environmental impacts of bivalve aquaculture, the majority of these studies have examined oyster and mussel culture (Crawford et al. 2003, Grant et al. 2007a, Lehane and Davenport 2004, 2006, Zeldis et al. 2004), while fewer have focused on clam culture (Jie et al. 2001, Munroe and McKinley 2007, Nizzoli et al. 2006, Spencer et al. 1997, Whiteley and Bendell-Young 2007). We have focused on clam culture whenever possible although we have taken examples from oyster and mussel culture when necessary. In these cases, it is important to note that many of these examples are from suspended or rack culture, which have a greater

potential to effect the environment because more animals may be cultured in a given area. There are many ways that aquaculture can disturb the environment, and these disturbance events vary on spatial, temporal, and intensity scales (Simenstad and Fresh 1995). In this section, we discuss the potential biotic and abiotic effects of geoduck aquaculture on water quality, substrate, community structure, and carrying capacity. The potential for disease transmission and genetic perturbation from cultured to wild stocks will be reviewed in later sections.

## WATER QUALITY

Many bivalve molluscs feed by filtering suspended particulate matter from the water column. Filtration rates have not been published for *Panopea abrupta*; however, rates can be estimated because bivalve filtration may be correlated with size (Powell et al. 1992, Winter 1978). If geoduck filtration is similar to that in other lamellibranchs of similar size, filtration rates could range from 7 to 20 L per hour per individual (Powell et al. 1992) as estimated from shell length in oysters. The veracity of this estimate is uncertain, however, since a geoduck at a given shell length is far more massive than an oyster of the same length. The range reported is due to the fact that even within a species and size class, the filtration rate varies depending on many environmental parameters plus the condition, health status, and satiation level of the individual.

Although geoduck filtration rates are not known, high densities of suspension-feeding bivalves clearly can impact water quality in a myriad of ways (Newell 2004). It has been suggested that high densities of bivalves in suspended culture could rapidly recycle

ingested organic matter back to the water as inorganic nutrients and could thus stimulate phytoplankton growth (Sorokin et al. 1999, Nizzoli et al. 2006). However, numerous studies have shown that filter-feeding bivalves can locally decrease phytoplankton abundance in both natural (Asmus and Asmus 1991, Cressman et al. 2003, Grizzle et al. 2006) and culture settings (Grizzle et al. 2006, Strohmeier et al. 2005). In tidal creeks in North Carolina, water upstream of oyster reefs contained an average of 25% more chlorophyll *a* than water downstream (Cressman et al. 2003). Phytoplankton depletion has also been documented in natural and farmed beds of blue mussels (*Mytilus edulis*). Phytoplankton biomass was reduced by 37% after passing over an intertidal mussel bed (Asmus and Asmus 1991) and the concentration of chlorophyll *a* decreased with increasing distance into a mussel farm in Norway, with more than 50% of the phytoplankton entering the farm depleted at the middle of the farm (30 m; Strohmeier et al. 2005). Evidence indicates that the northern quahog is also an efficient filter feeder: chlorophyll *a* was 62.3% lower downstream of a Virginia *Mercenaria* farm than it was upstream (Grizzle et al. 2006). Additionally, bivalve filter feeding may control plankton concentrations on a larger, ecosystem scale (Cloern 1982, Grant et al. 2007a). Cloern (1982) suggests that bivalve filter feeding is the principal mechanism controlling phytoplankton biomass in South San Francisco Bay. Using airborn remote sensing, Grant et al. (2007a) found reduced chlorophyll throughout an *M. edulis* farm in eastern Canada, with successive depletion of chlorophyll in the direction of flow through the farm. As well as reducing the concentration of phytoplankton, filter feeding bivalves may also change the composition of phytoplankton species by selective filtration (Shumway et al. 1985).

In addition to removing phytoplankton, bivalve filter feeding removes inorganic particles from the water column, reducing turbidity (Newell 2004). The reduced turbidity results in deeper light penetration, which can improve the condition for submerged aquatic vegetation (SAV), including sea grasses (Newell and Koch 2004). There are several cases of dramatic ecosystem changes attributed to the robust filtering ability of bivalves. The loss of historical oyster reefs in Chesapeake Bay, for example, has been associated with phytoplankton blooms, increased turbidity, and the loss of SAV (Jackson et al. 2001, Moore et al. 1996, Moore and Wetzel 2000). Introduced clams have also had a striking impact on several U.S. ecosystems. The introduction of the Asiatic clam (*Corbicula fluminea*) in the Potomac River estuary decreased turbidity and was linked to the reappearance of eelgrass in areas from which it had been absent for 50 years (Phelps 1994). Alternatively, invasive clams (*Potamocorbula amurensis*) introduced to the San Francisco Bay have altered food web dynamics via phytoplankton depletion to the detriment of native copepods (Kimmerer et al. 1994) It has also been shown that some mussel species ingest zooplankters (e.g. Davenport et al. 2000, Lehane and Davenport 2006, Zeldis et al. 2004).

Filter feeding also removes nitrogen and phosphorus from the water column, and these nutrients may ultimately be removed from the ecosystem via harvest of cultured bivalves. *Crassostrea virginica* meat and shell (dry weight) contain nitrogen (7% and 0.3%, respectively) and phosphorus (0.8% and 0.1%, respectively; Galtsoff 1964). Because of this nutrient removal ability, bivalve aquaculture can improve water quality and mitigate

eutrophication pressure in coastal systems (Lindahl et al. 2005, Newell 2004, Zhou et al. 2006) if the ecological carrying capacity (section 5.7) is not exceeded.

## SUBSTRATE

Many marine bivalves, like geoducks, filter particles from the water column and deposit them into the substrate, both with and without digestion (feces and psuedofeces respectively; together called biodeposits). Although geoduck biodeposition has not been examined, biodeposition in other species is well studied. Northern quahog (*Mercenaria mercenaria*) have a lower biodeposition rate than mussels or oysters (Tenore and Dunstan 1973), which may be due to differences in filtering behavior under conditions of excess phytoplankton. Oysters and mussels maintain high clearance rates and increase their biodeposition under high phytoplankton concentrations while clams including *Venerupis pullastra, M. mercenaria* and *Cerastoderma edule* reduce their clearance rates (Beiras et al. 1993, Grizzle et al. 2001, Hawkins et al. 1998). Bivalve biodeposits are high in carbon and nitrogen (Giles and Pilditch 2004, Kautsky and Evans 1987), show high microbial activity and may increase denitrification (Kaspar et al. 1985). Biodeposition increases the flow of particulate nutrients to the sediment, increases sediment oxygen demand, and may increase dissolved nutrients in the water column (Giles and Pilditch 2006). Biodeposition thus plays a key role in benthic-pelagic coupling (Kautsky and Evans 1987) and can have substantial ecological effects. For example, natural densities of the mussel (*Modiolus americanus*) notably increased productivity of turtle grass (*Thalassia testudinum*) in Florida (Peterson and Heck 2001). Increased growth was due to mussel biodeposition: mussels increased the nutrient content of the sediment and, when these

nutrients were taken up by the plants, the plants exhibited enhanced growth (Peterson and Heck 2001). A similar study examined interactions between eelgrass (*Zostera marina*) and an introduced mussel (*Musculita senhousia*) in California (Reusch and Williams 1998). This experiment demonstrated that mussel presence generally increased eelgrass productivity, although, at high densities, mussels inhibited eelgrass rhizome extension. Multiple field and laboratory studies have examined the effects of increased biodeposition resulting from high concentrations of bivalves in a culture setting. Biodeposition rates of a one year old scallop, *Chlamys farreri,* were 34-133 mg dry material per individual per day with mean rates of C, N, and P biodeposition of 4.00, 0.51, and 0.11 mg per individual per d, respectively (Zhou et al. 2006). Benthic respiration and sediment ammonia concentrations are well documented to be higher under longline mussel farms than at reference sites (Christensen et al. 2003, Giles et al. 2006, Hatcher et al. 1994, Kaspar et al. 1985). Prokaryotes in the sediment may also differ between cultured and control areas, with sulfate reducing and sulfur oxidizing bacteria more abundant in sediments under shellfish farms (Asami et al. 2005).

Changes in the sediment and water from *Venerupis philippinarum* culture have also been documented. Water at the control sites had five to nine times more particulate nitrogen and phosphorus while the culture sites, with 300 to 800 individuals per $m^2$, showed considerably more dissolved phosphorus and increased ammonia concentrations in the sediment (Nizzoli et al. 2006). Because biodeposition increases organic carbon levels and thus sediment oxygen demand (Giles and Pilditch 2006), high biodeposition rates may lead to anoxic conditions. The mechanism for anoxia was demonstrated at an oyster farm

in France (Castel et al. 1989). Oyster biodeposition elevated sediment carbon levels, which increased oxygen demand. These changes led to anoxia, which caused localized changes in benthic diversity: levels of meiofauna increased to 3-4 times their former abundance and macrofauna levels declined to approximately 50% of their former abundance (Castel et al. 1989). Contrary to these trends, however, a study examining longline subtidal oyster and mussel farms in Tasmania, Australia, found no differences in sediment deposition, sediment sulphide concentrations, organic carbon content, or water turbidity between farm and control sites. This may be due to low stocking densities used in Tasmanian shellfish farms (Crawford et al. 2003).

Many studies have shown that shellfish aquaculture can lead to increased sedimentation (Giles et al. 2006, Mallet et al. 2006, Zhou et al. 2006). For example, sedimentation was found to be nearly 2.5 times greater under scallop (*Chlamys farreri*) cultures than at reference sites in China (Zhou et al. 2006). However, these studies generally examine suspended or off-bottom aquaculture and may not be relevant to culture of infaunal organisms like geoducks.

Many clam species including geoducks are vulnerable to predation in the early stages of culture and are grown under protective netting (Spencer et al. 1992). This practice has been shown to increase survival of juvenile Manila clams (*Venerupis philippinarum*) in the UK and in Spain (Cigarria and Fernandez 2000, Spencer et al. 1992) as well as juvenile soft-shell clams (*Mya arenaria*) in eastern Maine (Beal and Kraus 2002). Netting has also been implicated in increased sedimentation (Goulletquer et al. 1999, Spencer et

al. 1996, Spencer et al. 1997). Spencer et al. (1996) found sedimentation four times higher on netted Manila clam plots than on non-netted Manila clam plots. Spencer et al. (1997) compared netted clam plots, netted plots without clams, and control plots without nets or clams, and found that it was the nets themselves rather than the Manila clams that caused increased sedimentation. In contrast, a recent study in British Columbia compared paired netted and non-netted Manila clam plots and found no significant differences in sedimentation or gravel accumulation (Munroe and McKinley 2007). It appears likely that the influence of predator exclusion netting on sedimentation is site-specific; these effects should be examined for geoduck aquaculture in Puget Sound.

### Effects of Tubes

There is no peer-reviewed information available on the ecological impacts of the mesh-covered polyvinylchloride (PVC) tubes currently used in geoduck aquaculture to protect seed from predation and dessication. As this system both appears unique to geoduck culture and is rapidly evolving, no data are available to review.

### Community Structure

The effects of shellfish aquaculture on the benthic faunal community are strongly debated as many contrasting effects have been reported: for example, Grant et al. (1995) reported increased species diversity due to mussel culture, Beadman et al. (2004) reported decreased species richness due to oyster culture, and Crawford et al. (2003) observed no significant differences in benthic infauna between mussel and oyster farms and reference

sites. As there are no data available for geoducks, we briefly review the literature from mussel and oyster aquaculture and discuss the available papers for clam culture. The impacts of clam harvest on the surrounding benthic community are covered in following text.

Crawford et al. (2003) compared the benthic environment under longline mussel and oyster farms in Australia and found that benthic community structure was not significantly different between farm and control sites. Greater differences in benthic infauna were found among farms than between farm and control sites, suggesting that local conditions may dictate how the benthic environment is affected by shellfish aquaculture. Grant et al. (1995) found relatively minor changes in benthic macrofauna between mussel culture and reference sites. Reference sites showed higher abundance of benthic macrofauna but lower biomass, and species diversity was higher at the farm sites. In contrast, Beadman et al. (2004) examined the infaunal benthic community under mussels cultured at four densities: 2, 3, 5, and 7.5 kg mussles per $m^2$ and found that infaunal communities comprised fewer individuals and species at the three highest mussel densities. Castel et al. (1989) compared an oyster farm in France to reference sites and observed dramatic changes to the benthic community: meiofauna levels were 3-4 times higher at the oyster farm while macrofauna levels were approximately 50% lower at the oyster farm (Castel et al. 1989). The benthic community under a New Zealand longline mussel farm experienced dramatic declines in species diversity, from a healthy and diverse complex of species to a community consisting entirely of infaunal polychaetes (Kaspar et al. 1985). A striking increase in predators under longline mussel

culture was also observed in New Zealand, with mean densities of the sea star *Coscinasterias muricata* up to 39 times greater at farm sites than reference sites (Inglis and Gust 2003). A decrease of suspension feeders and an increase of predators have also been noted beneath oyster farms (Dubois et al. 2007).

Whiteley and Bendell-Young (2007) examined the impact of Manila clam aquaculture on bivalve community structure. Aside from an increased abundance of Manila clams at farm sites, bivalve species composition did not differ significantly between farm and reference sites. However, farm sites showed higher similarity to one another than to reference sites, indicating a decline in regional distinctness. Spencer et al. (1997) found that the netting used to reduce Manila clam predation led to changes in benthic community composition consistent with organic enrichment, independent of the presence of clams. Particularly, they observed an increase in surface deposit feeding worms with the opportunistic *Pygospio elegans* dominating the fauna in the first six months of clam culture and other surface deposit-feeding worms dominating after one year. In the non-netted plots, the community dominant was a sub-surface deposit feeding worm, *Scoloplos armiger*. Spencer et al. (1997) suggest that competition from surface deposit-feeding worms on the netted plots may have excluded *S. armiger*. Powers et al. (2007) found that macroalgal and epifaunal growth on clam netting could also alter the community composition by enhancing nursery habitat for juvenile fishes and mobile invertebrates. They compared biomass and community structure at two clam lease sites—an eelgrass bed and an unstructured sand flat. Macrofaunal and epifaunal biomass at the aquaculture sites were significantly greater than on the sand flat but did not differ significantly from

the eelgrass bed. Similarly, significantly more mobile invertebrates and fishes were found at the culture sites than the unstructured sand flat, and community structure on the culture sites was more similar to that of the seagrass bed than to the unstructured sand flat.

Intertidal geoduck culture operations are sited in locations where birds forage at low tide. Although people have speculated about the effects of shellfish aquaculture on birds (Bendell-Young 2006, Kaiser et al. 1998), this interaction is not well studied. Zydelis et al. (2006) examined the effects of shellfish aquaculture on winter scoter populations in Baynes Sound, British Columbia. Baynes Sound is an area of extensive shellfish culture that produces approximately 50% of British Columbia's cultured shellfish (Ministry of Sustainable Resource Management 2002, as cited by Zydelis et al. 2006). More than 20% of the intertidal in Baynes Sound is used for shellfish cultivation and clam netting covers 2.9% of the intertidal area (Carswell et al. 2006). However, the authors found no correlation between shellfish aquaculture variables and scoter density, concluding that winter scoter populations and the current aquaculture practices were mutually sustainable. Similar conclusions were reached in a study looking at the impact of on-bottom mussel culture on bird assemblages (Caldow et al. 2003). Although bird assemblages changed after the mussels were placed, two key species increased and none decreased in abundance. At this site, the authors conclude that mussel aquaculture may have beneficial effects for birds owing to increased habitat complexity and changes to the benthic fauna. The effects of shellfish aquaculture on birds are likely to vary depending on location, bird species, and aquaculture methods. These effects should be examined for geoduck aquaculture in Puget Sound.

## EFFECTS OF HARVEST

Geoducks are harvested using pressurized water to quickly dig/liquefy the sediment. This may alter the abiotic condition of the sediment (grain size, oxygen, nutrient levels, etc.) as well as alter the community of organisms in the benthos. This method is unique to geoduck harvest and no peer-reviewed papers have been published which examine these questions. Here we review the available data on other forms of clam harvest, including dredging, hydraulic harvest, and hand raking. The breadth and depth of disturbances from these forms of harvest, while not directly comparable, may help elucidate effects of geoduck harvest.

The environmental effects of intertidal clam harvest have been examined in Europe and North America for species including the Manila clam, the common cockle (*Cerastoderma edule*), and the northern quahog (Badino et al. 2004, Hall and Harding 1997, Kaiser et al. 1996, Peterson et al. 1987, Spencer et al. 1998). In general, suction or mechanical harvest is a physical disturbance associated with sediment and benthic faunal changes. In most cases, mechanical harvest reduced the number of species present and their abundance. For example, the sediment and the benthic community were highly disturbed by mechanical harvest of Manila clams in Italy (Badino et al. 2004). A considerable decrease of benthic organisms was observed after harvest. Dredging also resuspended the top layer of sediment and brought deeper anoxic sediments up, which could potentially reduce the rate of recolonization. Harvesting clams by hand raking has also been documented to mix the sediment layers (Badino et al. 2004) and reduce infaunal species abundance and richness immediately following harvest (Brown and Wilson 1997).

However, Boese (2002) found that hand raking for cockles (*Clinocardium nuttalli*) and digging for gaper (*Tresus capax*) and butter clams (*Saxidomus giganteus*) in Yaquina Bay, Oregon, did not impact infaunal species number and abundance. Similarly, raking or dredging for northern quahogs did not appear to affect the species composition or density of small benthic macroinvertebrates in North Carolina (Peterson et al. 1987).

Recolonization rates of benthic fauna can range dramatically depending on physical conditions (sediment type and stablility, wave action, current), season, location, scale of disturbance, and whether recolonization occurs primarily through adult movement or larval settlement (Hall and Harding 1997, Kaiser et al. 1998). Hall and Harding (1997) found that immediately following intertidal cockle harvest, in Scotland, suction-dredged sites had an average of 30% fewer species and 50% fewer individuals. However, after 56 days, the faunal assemblages at these disturbed sites were not significantly different from control sites. A similar study in southeast England examined the sediment structure and benthic community immediately following and 7 months after suction-dredge harvesting for Manila clams at an aquaculture site (Kaiser et al. 1996). Harvest suspended the sandy layer but left the underlying clay substrate and substantively reduced both infaunal diversity and the mean number of individuals per sample. However, after 7 months, neither the sediment composition nor the benthic fauna were significantly different from control sites. The authors conclude that clam cultivation does not have long-term effects on the substrate or the benthic community at this location. The spatial scale of disturbance is likely to impact recovery and most studies have taken place on small scales. However, Hall and Harding (1997) found that the benthic fauna at harvested sites

were similar to control sites within 3 months of harvest, regardless of the scale of disturbance, which ranged from 225 $m^2$ to 2025 $m^2$. Although aquaculture harvest is likely to take place at a larger scale than that examined in the study, the authors emphasize that those areas might be patchily distributed and unlikely to further extend the trajectory of recovery. However, these results are very likely specific to site and harvest technology, and they need to be examined with geoduck culture in Puget Sound.

Clam harvest has also been shown to affect seagrass. Raking and light dredging to harvest northern quahogs caused a 25% decrease in seagrass biomass, but recovery was complete within one year (Peterson et al. 1987). Alternatively, heavy dredging caused a 65% decline in seagrass biomass and full recovery was not documented after four years (Peterson et al. 1987). A separate study showed that clam raking did not affect eelgrass (*Zostera marina* L.) cover or biomass, but digging clams individually reduced eelgrass cover and biomass, although no significant differences were observed after 10 months (Boese 2002). Individually digging clams was also shown to reduces shoot density and biomass of the seagrass *Zostera noltii* (Cabaco et al. 2005), although it is unclear how long these changes persisted because temporal change data were not included in this study.

## CARRYING CAPACITY

Before beginning a discussion on carrying capacity in bivalve aquaculture, this term needs to be defined. In an aquaculture context, three distinct definitions of carrying capacity are used. Production carrying capacity (PCC) is the level of culture at which

production is maximized without negatively affecting growth of the cultured species (Carver and Mallet 1990). Determining PCC for geoducks would be relatively simple in the field by simply expanding the density of cultured geoduck while monitoring growth rates. PCC is reached when growth rates begin to fall. However, significant ecological changes in the surrounding community are likely before PCC is reached, and this level of development may be unacceptable to many stakeholders.

Ecological carrying capacity (ECC) is the highest level of culture that can be undertaken without leading to significant changes in ecological processes, individual species, or communities in the surrounding habitat (Gibbs 2007); ECC is by definition lower than PCC. For example, Jiang and Gibbs (2005) predicted the carrying capacity of the greenshell mussel (*Perna canaliculus*) in the Tasman/ Golden Bay system in New Zealand using a steady, linear food-web model. The PCC was estimated at a mussel yield of 310 t per $km^2$ per yr. In contrast, the ECC was estimated at a mussel yield of 65 t per $km^2$ per yr, approximately 20% of PCC. The model indicated that introducing a mussel culture at production carrying capacity would lead to decreased mean trophic levels for the ecosystem as bivalves replaced zooplankton as the primary phytoplankton consumers (Jiang and Gibbs 2005).

A third definition of carrying capacity has recently been introduced in the aquaculture literature. Social carrying capacity (SCC) incorporates both PCC and ECC while taking into account demands of both the population (socioeconomic and cultural factors including employment, fisheries, and recreation) and the environment (McKindsey et al.

2006). No models have yet been developed that estimate SCC in an aquacultural setting. Determining ECC for geoducks in Puget Sound would be a challenging exercise although by determining ECC in multiple isolated embayments that vary substantially from one another, we could potentially estimate ECC for the whole Sound. Any discussion of carrying capacity for geoducks in Puget Sound should acknowledge that the commercial fishery has extracted large quantities of geoducks annually since 1970. This represents a net loss of geoduck even with the addition of geoduck culture at the present level. Studies on the environmental impacts of aquaculture often focus on the effects to the benthos under farms. This may be more appropriate for finfish culture where ecological carrying capacity is most often dictated by the benthic ability to absorb waste products. Carrying capacity in bivalve aquaculture is more often dictated by the amount and availability of food in the water column. As cultured bivalves compete with other filter feeders, bivalve aquaculture has the potential to displace other animals in the food web. For example, at the theoretical PCC, the food web collapses into a nutrient–phytoplankton–bivalve culture because the bivalve culture has out-competed zooplankton and benthic filter feeders (Gibbs 2004).

Estimating bivalve carrying capacity is not an easy task because increased bivalves in culture may alter nutrient cycling (as discussed previously); quantifying bivalve carrying capacity is an active area of research. Many nutrient–phytoplankton–zooplankton models have been developed that predict the carrying capacity of bivalves in coastal regions (Bacher et al. 1997, Duarte et al. 2003, Grant et al. 2007b, Smaal et al. 1997). These models however have been developed independently of one another and rarely

incorporate relationships parameterized in earlier models. It has been suggested that new "open source" ECC models be developed that allow free access to the mathematical code such that independent researchers could parameterize the models with their data and add sub-models of their specialties (Newell 2007). Alternative to modeling, performance indicators such as clearance efficiency or phytoplankton depletion footprints can be used to assess the impact of the culture in real time (Gibbs 2007). It should be noted that both approaches (models and performance indicators) rely heavily on filtration rate data, currently lacking for geoducks.

No peer-reviewed studies are available for geoduck carrying capacity or bivalve carrying capacity in Puget Sound. We have chosen not to review carrying capacity for different bivalves in different bodies of water because this would not add to our knowledge about geoduck culture in Puget Sound. However, we give one example to illustrate that location and model selection dramatically influence predictions. Sara and Mazzola (2004) used two models to assess the production carrying capacity of the mussel *Mytilus galloprovincialis* in two Italian Mediterranean locations. Numerous parameters, including current, filtration rate, and chlorophyll *a*, were measured and included in the model. The two regions differed widely with regard to current and phytoplankton availability and, thus, with regard to estimated carrying capacity. Using the original Incze model (Incze et al. 1981), the predicted PCC for the two regions was 2,034 t in the better locale, and 403 t in the poor locale. Using the Incze modification (Martincic 1998 as cited in Sara and Mazzola 2004), the predicted carrying capacity was 200 t in the better locale and 160 t in the poor locale (Sara and Mazzola 2004). Clearly, model selection is an important step,

and location may be highly influential in estimating carrying capacity and determining appropriate siting for a farm.

## Disease Introduction

Disease is an inherent part of all aquatic ecosystems; thus, it is important to explore the factors affecting its presence and severity. A complete understanding of the relationship between host, pathogen, and the environment, as well as the ecological impacts of disease in aquatic systems, is critical for proper management and prevention of infectious disease outbreaks in both aquaculture and natural settings. While many studies are dedicated to this topic, peer-reviewed research on diseases specific to geoduck clams is lacking for cultured animals and completely absent for wild stocks. However, Bower and Blackbourn (2003) conducted numerous surveys and experiments regarding wild geoduck health. Although not peer-reviewed, we feel the information presented in their studies is valuable. We discuss their work in this section in consideration of the fact that no peer-reviewed research has been conducted on disease in wild geoducks. In this section, we refer extensively to the web publication of Bower and Blackbourn (2003). We also discuss literature related to transmission, prevalence, and distribution of diseases in other marine bivalve species in Washington State and highlight preliminary work specific to geoducks.

## AQUACULTURE IMPACTS ON DISEASE PREVALENCE AND DISTRIBUTION IN THE PACIFIC NORTHWEST

Many pathogens that cause disease in shellfish are facultative forms and are ubiquitous in aquatic systems. In nature, a high percentage of apparently normal and healthy animals harbor potential pathogens without clinical signs or evidence of overt disease. The development of disease in aquaculture systems often occurs via disruption in the environment in which the animals are being reared. Unfavorable conditions, such as crowding, temperature fluctuations, inadequate dissolved oxygen, excessive handling, inadequate diets, or toxic substances may stress the animals; if the level of stress exceeds the ability to adjust, susceptibility to disease may occur (Meyer 1991). Contact between individuals greatly affects the dynamics of infectious disease. High host density increases contact rates between infected and uninfected individuals (May et al. 1981). For this reason, dense populations tend to have more parasites, meaning that some epizootics could be due to increasing host density as well as outside stressors (Arneberg 2001). Factors that determine the taxonomic range of hosts that can be infected by a specific pathogen are also of great importance. Host specificity relates to the co-evolution of host susceptibility and pathogen virulence, as well as to the factors underlying the emergence of new pathogens. How pathogens evolve and adapt to new hosts is crucial to understanding the fundamental basis for the origin of infectious diseases as well as the emergence of new pathogens.

Several factors underlie the recent increase in reported shellfish disease outbreaks. Transportation of stocks as well as climate change have been implicated in the expansion

of disease. For example, prevalence of Dermo, caused by *Perkinsus marinus*, and Delaware Bay Disease, also known as MSX (multinucleated sphere unknown) caused by *Haplosporidium nelsoni*, has increased among eastern oysters, *Crassostrea virginica*, because of higher water temperatures and the relocation of infected stocks along the eastern and southern U.S. coasts (Andrews 1996, Hofmann et al. 2001, Sweijd et al. 1998). Parasites have been introduced into new areas through increased shipment of host shellfish for aquaculture (Bustnes et al. 2000, Elston et al. 1986). These newly introduced animals may be susceptible to local pathogens (Ford et al. 2002). Many examples exist of species that have acted as vectors for the spread of hitch-hiking species that serve as predators, competitors, and pathogens to natives (Ruiz et al. 2000). Also, non-native species may serve as reservoirs for enzootic pathogens formerly at low abundance, facilitating their proliferation to levels that threaten native species (Bishop et al. 2006).

In addition to disease, shellfish fall prey to introduced predators. Two major predators were introduced with Pacific oyster seed over the years: the Japanese oyster drill (*Ocenebra japonica*) and the turbellarian flatworm (*Pseudostylochus ostreophagus*). These two species have become well established in various oyster-growing bays in the state of Washington as well as in Humboldt Bay in California (Chew 1991). Shellfish aquaculture, which has increased markedly over the past few decades, can also be a source of disease outbreaks caused by culture conditions themselves. The high densities under which animals are grown and the high temperatures sustained in hatcheries favor the proliferation and transmission of opportunistic pathogens (e.g. Elston 1990). For example, LeDeuff et al. (1996) found that cultured Pacific oyster larvae reared at 25–

26°C were more susceptible to herpes-like viral infections than those reared at 22–23°C. Elston and Wilkinson (1985) found that infection by an irido-like virus of larval Pacific oysters in high-density hatcheries resulted in oyster velar virus disease (OVVD). The etiological agent of withering syndrome, *Candidatus xenohaliotis californiensis*, may have also extended its geographic range in California via the outplanting of hatchery-reared abalone, suggesting a link between aquaculture and the present distribution of this pathogen (Friedman and Finley 2003).

Shellfish disease outbreaks have occurred in the Pacific Northwest in association with the introduction of non-native species and transfer of culture animals (Elston 1990). These outbreaks may have been exacerbated by intensive shellfish aquaculture. Bacterial diseases with low host specificity, such as *Vibrio* spp., as well as host-specific parasites, including *Bonamia ostreae* and *Mikrocytos mackini*, have impacted shellfish aquaculture. While a number of these diseases have become established in Puget Sound, it is important to note that none of the etiological agents discussed throughout this section have been observed in wild or cultured geoducks.

Summer mortality of the non-native Pacific oyster (*Crassostrea gigas*), which is the most commonly cultured species in the Pacific Northwest, stems from a combination of stress at or near spawning time and high summer temperatures. Summer mortality has also been associated with numerous bacteria, mostly species of the *Vibrio* and *Nocardia*, but it remains unclear whether these bacteria act as primary pathogens or as opportunists (Paillard et al. 2004). High but sporadic *C. gigas* spat mortality rates have been observed

during the summer in naturalized and cultured oysters. Summer mortality seems to have a complex etiology with several factors implicated, including environmental conditions, physiological and genetic host parameters, and infectious agents (Soletchnik et al. 1999). The two *Vibrio* strains that have been associated with summer mortality outbreaks, and which are potentially pathogenic for *C. gigas* spat as shown by experimental challenge, have been phenotypically and genotypically identified as *Vibrio splendidus biovar I* (Lacoste et al. 2001) and biovar II (Le Roux et al. 2002). Nocardiosis is a bacterial disease that is also an important component of summer mortality associated with *C. gigas* (Friedman et al. 1991). The disease causes yellow lesions on the body and, although *C. gigas* is the principal oyster affected, a few specimens of the European flat oyster (*Ostrea edulis*), cultivated near areas of infected Pacific oysters, have been reported to have a similar disease (Elston 1990). Nocardiosis originated in Japan and has since been reported in California, Washington, and British Columbia (Elston 1990, Friedman et al. 1991, Friedman and Hedrick 1991).

Denman Island disease is characterized by focal lesions of hemocyte infiltration (pustules) on the surface of the body and/or within the mantle, labial palps, and adductor muscle of *C. gigas* (Bower et al. 2005, Hervio et al. 1996, Hine et al. 2001). The etiological agent, *Mikrocytos mackini*, is a small intracellular parasite (Farley et al. 1988). The development of clinical disease upon infection with *M. mackini* requires three to four months of temperatures < 10°C (Hervio et al. 1996). In addition to *C. gigas*, *M. mackini* produces disease and mortalities in other species of economically important oysters such as *C. virginica*, *O. edulis*, and *O. conchaphila* during laboratory challenges (Bower et al.

1997). Preliminary evidence suggests that these alternate species may be more susceptible to infection and the resulting disease than the usual host *C. gigas*. To date, *M. mackini* has been detected on the west coast of North America from southern British Columbia to Washington State (Bower et al. 2005). In laboratory bath exposure experiments, a prevalence of infection approaching 100% and mortalities were observed in the small *C. gigas* (~18 mm in shell length). However, in the same laboratory exposure experiment, similar-aged geoduck clams (*Panopea abrupta*, ~8 mm in shell length) were resistant to infection (Bower et al. 2005).

Bonamiasis of the European flat oyster (*Ostrea edulis*) was first described in oysters from France in 1979 (Comps et al. 1980) and has since spread to other European countries associated with the transfer of oysters. Bonamiasis was transplanted to Washington from a California hatchery and remains an important disease in the Pacific Northwest (Elston 1990). It is caused by an intracellular haplosporidian parasite, *Bonamia ostreae*, that infects the blood cells of oysters, causing cumulative mortality rates $\leq$ 80% within 6 mo of introduction (Balouet et al. 1983). In laboratory experiments, *B. ostreae* was transmitted to uninfected oysters via the water column. However, close proximity to infected oysters is believed to be necessary for effective transmission (Elston et al. 1986). The export and juvenile transplant of live bivalves for aquaculture raises concerns about the vulnerability of the wild populations to disease and the ability of bivalves to harbor and transfer pathogens to new areas and species. Determining the risks of the inadvertent introduction of pathogens with the transfers of juvenile bivalves for grow-out and the marketing of live *Panopea abrupta* from areas within the current distribution of known

etiological agents requires that the susceptibility of *P. abrupta* to endemic and naturalized diseases be assessed.

## PARASITES AND DISEASES ASSOCIATED WITH GEODUCK AQUACULTURE

There is one peer-reviewed report of a protozoan parasite associated with disease and mortalities among cultured geoduck larvae at an experimental hatchery in Washington State. Kent et al (1987) identified the etiological agent as an *Isonema*-like flagellate that penetrates the mantle and proliferates within the coelom, ultimately resulting in the death of heavily infected geoduck larvae. This flagellate is not known to infect juvenile or adult geoduck clams, or oyster larvae grown in the same hatchery facility as infected larval geoduck clams (Elston 1990). No other reports of the invasive, pathogenic *Isonema* sp. affecting cultured geoduck larvae have been published and attempts to obtain infected larvae to perform transmission experiments were unsuccessful (Kent et al. 1987). This may suggest that crowded conditions within the culture system may have predisposed larvae to infection and the resulting mortalities.

In a preliminary study, cultured juvenile geoduck clams planted at four locations in the Strait of Georgia, British Columbia, were surveyed for infectious diseases (Bower and Blackbourn 2003). Upon histological examination, none of the 795 cultured geoducks showed signs of infectious diseases or pathogenic organisms. However, further research is required to characterize the distribution and effect of any pathogens or diseases impacting cultured and wild geoduck clams.

## PARASITES AND DISEASE ASSOCIATED WITH WILD GEODUCKS

Bower and Blackbourn (2003) conducted a disease survey of 146 wild adult geoducks clams that appeared abnormal when harvested by the commercial fishery along the coast of British Columbia. Abnormalities included dark periostracum, warts, inclusion bodies, and protozoan infections. The authors observed wild individuals with a dark, thickened periostracum on the siphon, and/or mantle that appeared brown, black, or rust colored. Histological examination determined that the underlying epithelium and musculature was intact and healthy, while surface discoloration and thickening were sometimes attributed to fungal infections, protozoan colonization, multiple layers of periostracum being secreted, and an unknown waxy acellular material. Preliminary transmission experiments were conducted to determine if the observed fungus was infectious. Healthy, cultured juvenile geoduck clams were used as potential fungal recipients. Attempts to transmit the fungus by prolonged contact and cohabitation were not successful after at least 82 d; attempts to isolate the fungus on aseptic culture media also failed. More sensitive methods of detecting and identifying the fungus (or fungi) are needed to fully assess involvement in geoduck integument abnormalities.

Bower and Blackbourn (2003) also noticed warts or regions of smooth, raised, gray-pink or creamy-colored lesions on the siphon and mantle of wild geoducks. The warts consisted of swellings of the periostracum filled with necrotic cells. Upon histological examination, Bower and Blackbourn (2003) observed no obvious etiological agent in conjunction with the warts; in order to determine whether the lesions were caused by an infectious organism, they inoculated, via syringe injection, healthy cultured juvenile

geoduck clams with warts collected from wild adult geoducks. Both control (injections without wart material) and experimental animals developed pustules reminiscent of warts found on the wild adults. The development of warts on control animals indicated the lesions may be a consequence of the response of the clam to foreign material or non-specific stimulus. The histopathology of the induced warts was similar to that observed in naturally infected wild geoducks; an etiological agent was not detected. Whether the warts result from a response to an invading infectious pathogen or to mechanical damage remains unresolved. Other geoduck gross abnormalities noted include blisters, scars, discoloration of internal tissues, and nodules associated with the inner valve surface, none of which appeared to be caused by an etiological agent.

A high prevalence of intracellular prokaryote microcolonies (inclusion bodies) in the epithelial cells of the gill filaments and palps of geoduck clams were observed by Bower and Blackbourn (2003). However, the infection intensity was very low, hindering the specific identification of these parasites believed to be *Rickettsia* or *Chlamydia*-like organisms. These bacteria are commonly observed in the tissues of a variety of wild bivalve mollusks throughout the world—including northern quahogs, soft clams, Eastern oysters, bay scallops (*Argopectin irradians*), Pacific razor clams, Manila clams, and Japanese scallops (*Patinopecten yessoensis*) (Elston 1986, Harshbarger et al. 1977, Meyers 1979, Morrison and Shum 1982)—and they occur in healthy animals without apparent detriment (Elston 1990). However, extensive mortalities in cultured giant clams (*Hippopus hippopus*) have been associated with heavy gill infections of *Rickettsia*-like organisms in the Philippines and Micronesia (Norton et al. 1993). It was suggested that

overcrowding and low exchange rates of water in land-based culture tanks predisposed *H. hippopus* to increased intensity of infection, clinical disease, and mortalities. A similar *Rickettsia*-like organism, *Candidatus xenohaliotis californiensis*, is the etiological agent of withering syndrome, a chronic wasting disease responsible for mass mortality in wild black abalone (*Haliotis cracherodii*) and responsible for extensive losses of cultured red abalone (*H. rufescens*) (Friedman et al. 2000, Haaker et al. 1992, Moore et al. 2001). Experiments illustrate that this pathogen can be transmitted via the water column, and that above normal temperatures have a synergistic effect on the disease (Friedman et al. 2002, Moore et al. 2001).

Two unidentified parasites are associated with geoduck clams. Bower and Blackbourn (2003) observed clam protozoan unknown (CLPX) in the wall of the gonad; in the musculature of the siphon, mantle and foot; and under the epithelial lining of the water channels and mantle cavity of geoduck clams. However, prevalence and intensity of infection was low. CLPX resembles an unidentified protozoan observed in the Pacific littleneck clam, which has been speculated to be the early developmental stage of a vermiform apicomplexan parasite (Desser and Bower 1997). The vermiform stage of the parasite commonly found in Pacific littleneck clams (70–100% of the clams were infected in some populations; Desser and Bower 1997) has not been found in geoduck clams and Bower and Blackbourn (2003) found no evidence of associated pathology. Bower and Blackbourn (2003) also observed another parasite, apicomplexan protozoan unknown (APX), in the palps, mantle, and gills of geoduck clams, again with infections occurring at very low prevalence and intensity. As of 2003, there was no evidence of

associated pathology (Bower and Blackbourn 2003). Parasitism by apicomplexans has been well documented in clams throughout the world including Pacific littleneck clams and Manila clams with no evidence of associated disease (Desser and Bower 1997, Marshall et al. 2003).

Wild geoducks have also been observed in commensal relationships with turbellarians (free living, flatworms) and small pea crabs (family Pinnotheridae); no evidence of pathology was found (Bower and Blackbourn 2003). Because commensal organisms are often not host-specific, precautions should be taken to prevent them from being introduced to non-indigenous areas to avoid transfer to other bivalves. With no known methods of control, transfers of commensal organisms could have negative environmental repercussions.

To stop the spread of infectious organisms among infected and uninfected individuals, stocks, or populations, the following is required: (a) accurate identification of the pathogens responsible for disease outbreaks, (b) sensitive detection of pathogens in sub-clinical carriers or abnormal hosts, and (c) accurate differentiation between benign and significant infectious organisms (Harvell et al. 2004). Although Bower and Blackbourn's preliminary work was initiated to address the health status of geoduck clams, the risks, distribution, prevention, and management of geoduck-related diseases need further exploration to develop an understanding of potential effects of geoduck disease on the ecosystem.

## GENETIC EFFECTS ON WILD CONSPECIFICS INTRODUCTION

Before beginning or expanding an aquaculture culture program, it is important to consider the genetic risks to the wild populations associated with these culture activities. Genetic risk is broadly defined as exposing a natural population to genetic change by human action (Currens and Busack 1995). With culture of a native species, such as geoduck in Puget Sound, these risks center on the potential loss of natural genetic variation, which serves to buffer the population against natural selective forces. In this section, we discuss potential adverse genetic effects of geoduck aquaculture on wild stocks and the level of risk as well as methods of risk reduction.

## GENETIC COMPARISON OF WILD AND CULTURED BIVALVE POPULATIONS

Hatchery-reared shellfish may differ genetically from their wild counterparts for multiple reasons. Broodstock may be collected from distant geographic points and thus be adapted to a different set of environmental conditions. Additionally, the selection processes in a shellfish hatchery are, by design, vastly different from the selection processes in the natural environment. Geoducks, like most broadcast spawning invertebrates, have type III survivorship, characterized by very high larval mortalities. In contrast, the hatchery environment is designed to minimize larval mortalities and thus relaxes many selective forces. Active artificial selection may also take place in geoduck hatcheries through breeding programs, culling of larval stocks, or changing environmental parameters such as temperature and salinity. Finally, the extremely high fecundity of geoducks, typical of many marine invertebrates, can reduce the genetic effective population size ($N_e$) in the hatchery because relatively few broodstock pairs may produce entire hatchery cohorts

(Hedgecock and Sly 1990). Ample evidence exists in the literature on cultured oysters that $N_e$ can be much lower in hatchery than in wild populations (Gaffney 1992, Hedgecock et al. 1992, Hedgecock and Sly 1990, Saavedra 1997). A reduced genetic effective population size can result in a drastic reduction of genetic variability in the progeny. Once outplanted, purifying selection will not necessarily purge effects of domestication in the same or subsequent generations, because the genes under selection in the hatchery will not necessarily be subjected to selection during adulthood or in subsequent generations

Hatchery shellfish have been found to be genetically distinct from their wild counterparts, which is often due to reduced genetic variability and genetic drift (Apte et al. 2003, Evans et al. 2004, Hedgecock et al. 1992, Li et al. 2007, Yu and Chu 2006). The Japanese scallop (*Patinopecten yessoensis*) has been cultured in China for two decades. Using six microsatellite loci, Li et al. (2007) documented that three hatchery populations of *P. yessoensis* in China were notably less variable than wild Japanese populations, with fewer alleles per locus and lower heterozygosities. Similarly, Apte (2003) used three classes of genetic markers (allozymes, mtDNA, and RAPDs) to document that cultured Greenshell mussels (*Perna canaliculus*) were genetically differentiated from wild populations. Also, cultured abalone (*Haliotis rubra* and *H. midae*) were shown to be genetically differentiated from wild abalone; the cultured abalone had fewer alleles per locus with approximately 40% of relatively infrequent microsatellite alleles present in wild collections 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. In pearl oysters (*Pinctada fucata*) from southern China, both wild and cultured populations showed a high proportion of polymorphic loci, but cultured populations had more fixed loci than the corresponding wild populations (Yu and Chu 2006). Although these studies suggest the possibility of genetic differentiation between hatchery and wild geoducks, this has not been investigated.

## GENETIC IMPLICATIONS CONCERNING WILD AND CULTURED GEODUCKS

In order to protect the genetic integrity of wild geoducks, we must understand the population structure of wild geoducks and determine whether hatchery populations are genetically differentiated from wild populations. In previous studies, little evidence of wild stock structure was found among Puget Sound geoduck collections via analyses of variation at allozyme and microsatellite loci (Miller et al. 2006, Vadopalas et al. 2004); thus, disruption of neutral genetic stock structure is not a primary concern. However, genetic variability at presumed neutral microsatellite loci is high in wild populations: of the 15 published microsatellite loci for geoduck clams (Kaukinen et al. 2004, Vadopalas and Bentzen 2000, Vadopalas et al. 2004), all expected heterozygosities exceed 0.90. This hypervariability is a strong indication that wild geoduck populations have high levels of genetic variability that could be perturbed by an influx of cultured genotypes. Minimizing gene flow between cultured and wild populations is the key to maintaining natural genetic variability in wild geoduck clams.

While we have a fairly good understanding of neutral genetic differentiation of wild geoduck aggregations (Miller et al. 2006, Vadopalas et al. 2004), work needs to be done to determine the effective population size of hatcheries and examine genetic differentiation among wild and hatchery geoducks. If hatchery and wild geoducks are genetically differentiated, genetic risks to wild geoduck populations may increase. Reasons why hatchery geoducks may differ from wild geoduck populations were discussed previously, while here we discuss potential implications of those differences. For example, broodstock may be collected from distant geographic points and thus be adapted to a different set of environmental conditions. If these animals breed with wild conspecifics, it may lead to outbreeding depression, a reduction in wild fitness that follows mating between members of distant populations (Allendorf et al. 2001, Allendorf and Ryman 1987, Lynch 1991). Outbreeding depression has been observed in a myriad of species including nematodes (Dolgin et al. 2007), partridges (Barilani et al. 2007), and copepods (Brown 1991a), and has been observed in crosses between wild and domesticated salmonids (Tymchuk et al. 2006, Tymchuk et al. 2007).

Even if broodstock are collected locally, hatchery populations may differ from wild populations owing to random genetic drift or different selective pressures in the hatchery. These differences may reduce the fitness of cultured geoduck and cultured-wild hybrids in the natural environment (Ford 2002, Lynch and O'Hely 2001). As the differentiation between wild and cultured populations increases, the potential for negative genetic interactions between wild and cultured populations increases. For example, faster growth in the intertidal environment may be selected for in the hatchery, but intraspecific

introgression of the same traits may be maladaptive for wild geoducks. Lynch and O'Hely (2001) modeled these dynamics and showed that if the captive population does not receive gene flow from the wild population, even low levels of gene flow from the captive to the wild population will likely shift the average phenotype of the wild population towards the average culture phenotype. If gene flow does occur from the wild population to the cultured population, this shift is less pronounced but may still occur (Lynch and O'Hely 2001). Thus, if differences exist between wild and cultured geoduck populations, minimizing gene flow from cultured to wild populations is vital to maintaining genetic integrity of wild populations.

One way to minimize gene flow between wild and cultured populations of geoduck clams is to harvest the geoducks before they mature. Cultured geoducks are outplanted for four to eight years before harvest (J.P. Davis, Taylor Resources, Inc., Quilcene, Washington, pers. comm.), but as was discussed in previously, age of reproductive maturity is currently unclear. If the Sloan and Robinson (1984) estimate is correct, geoducks do not mature during the four to eight year culture cycle and there is no need for concern about genetic interactions between cultured and wild geoducks. However, if the Campbell and Ming (2003) estimate is correct, geoducks mature and may spawn multiple times before harvest. Age at reproductive maturity also varies by location (Campbell and Ming 2003) and should be examined for intertidal geoducks at potential culture sites. Additionally, as was discussed previously, young geoduck show a highly skewed sex ratio with more than 90% of small (SL < 100 mm, Anderson 1971) or young individuals (< 11 yr, Sloan and Robinson 1984) identified as male. If such strongly skewed sex ratios remain among

commercially grown geoducks until harvest, the likelihood of reproductive success among cultured geoducks would be considerably reduced. As gamete age and density affect fertilization success (Hodgson et al. 2007, Kupriyanova 2006, Williams and Bentley 2002), skewed sex ratios will reduce reproductive success especially if the watercourse distance between cultured and wild geoduck aggregations is great enough to prevent downstream fertilization. However, deviations from a 1:1 sex ratio would also decrease effective population size of the cultured populations.

Cultured geoducks are typically planted in higher densities than the average density in the natural environment: densities in wild aggregations in Puget Sound average 1.7 geoducks per $m^2$ with a range of 0–22 geoducks per $m^2$ (Goodwin and Pease 1991), while intertidal culture densities average about 13.5 geoducks per $m^2$ (J.P. Davis, Taylor Resources, Inc., Quilcene, Washington, pers. comm.). Proximity and spawning synchrony are the strongest predictors of individual reproductive success, with the likelihood of gamete union increasing exponentially with proximity. Thus, if high-density aggregations of male and female cultured geoducks spawn in synchrony, fertilization success is likely to be much higher in cultured than in most wild populations. Under this scenario, most of the cultured–wild genetic interactions will occur between naturalized progeny and wild geoducks, rather than direct interaction between outplants and wild congeners.

## Risk Reduction

There are many ways to reduce the potential for genetic interactions between cultured and wild geoducks. The current practice is to collect broodstock each year from the wild

population with which their progeny will potentially interact. Collecting local, wild broodstock annually maintains population structure, preserves any local adaptations in the wild populations, helps maintain high levels of genetic variation in the progeny, reduces long-term domestication selection, and increases the hatchery $N_e$ over generations. Using large numbers of wild broodstock and ensuring roughly equal fertilization success also increases the hatchery $N_e$ and can help retain high levels of genetic variation in the offspring. The hatchery environment can also be designed to mimic the natural environment, so that the selection regimes are similar (Maynard et al. 1995). The most risk-averse strategy would use land-based aquaculture to completely isolate the cultured geoducks from wild populations. While this is possible in some species, it is currently not feasible for geoducks as culture methods are constrained to intertidal or subtidal outplants.

Sex control of cultured populations is an additional method of risk reduction that has been advocated to prevent genetic change to wild populations (Piferrer et al. 1993). The production of monosex populations for release has the most utility if used in exotics (Quillet et al. 1991, Thorgaard and Allen 1988). Sterility, however, prevents genetic interactions between the cultured population and wild populations, and may be very useful in the culture of geoduck clams. Sterility can be conferred on shellfish primarily via triploid induction. Triploid bivalves are produced either by crossing tetraploids and diploids (Guo et al. 1996) or by administering some form of shock to developing zygotes to suppress the first or second polar body extrusion (reviewed in Beaumont and Fairbrother 1991). Triploids have been used in aquaculture settings because they exhibit

reduced or absent gonadogenesis or gametogenesis, retain product quality during the spawning season, and often show increased growth (Brake et al. 2004, Mallia et al. 2006, Nell and Perkins 2005). Triploidy techniques have been developed for *Panopea abrupta* (Vadopalas and Davis 2004), but the efficacy of triploidy in conferring sterility in geoducks and the permanence of the triploid state must be verified prior to using this technique.

For commercial aquaculture, harvest management of geoducks may have some utility for risk reduction. While geoducks are outplanted for four to eight years (J.P. Davis, Taylor Resources, Inc., Quilcene, Washington, pers. comm.), they are not sexually mature during this whole culture cycle and they are harvested while still young. This truncation of the reproductive period reduces the chances of lifetime reproductive success in cultured geoducks. However, any avoidance of genetic risk via harvest management may be counteracted by the increased probability of individual fertilization success among cultured geoducks due to high culture densities. Using sterile outplants or managing harvest to preempt reproduction, or both, could mitigate risks by reducing cultured–wild interactions.

# Chapter V
# Reduced genetic variation and decreased effective number of breeders in five year classes of cultured geoduck clams (*Panopea generosa*)


## ABSTRACT

Aquaculture for the Pacific geoduck (*Panopea generosa*) is a small but expanding industry in Washington State, USA, where geoducks are native and genetic interactions between wild and cultured geoducks are likely. To examine the potential genetic implications of geoduck aquaculture, we compared genetic diversity and effective number of breeders ($N_b$) in five contiguous year classes of cultured geoducks to a wild population. Our results from five microsatellite loci indicate that the cultured year classes exhibited reduced allelic richness and $N_b$ and 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 comprised a large proportion of individuals unrelated to others at the full-sib level and nine to 25 full-sib groups. No clear pattern emerged regarding changes in genetic diversity over the five year time span of this study. Our results may aid in developing geoduck hatchery practices that reduce genetic risk to wild populations.


## INTRODUCTION

The culture of native taxa is often advocated as a way to reduce negative environmental impacts of aquaculture (e.g. De Silva et al. 2009, Naylor et al. 2001), however, culture of native species also carries risks, including genetic risks (Camara and Vadopalas 2009,

Hedgecock and Coykendall 2007, Utter and Epifanio 2002). If wild populations exhibit local adaptation, aquaculture may homogenize these groups and reduce overall fitness through outbreeding depression (e.g. Gilk et al. 2004, Roberge et al. 2008, Tymchuk et al. 2007). In addition, because cultured shellfish tend to exhibit lower genetic diversity than their wild counterparts (e.g. Evans et al. 2004, Lemay and Boulding 2009, Li et al. 2007, Lind et al. 2009) genetic introgression from cultured to wild conspecifics may reduce the genetic diversity of wild populations (Allendorf and Ryman 1987, Camara and Vadopalas 2009, Hedgecock and Coykendall 2007).

Aquaculture for geoduck clams (*Panopea generosa*; previously misidentified as *P. abrupta*; Vadopalas et al. 2010) is a small but expanding industry in Puget Sound, Washington, USA. Wild geoducks are common in this region where they support an economically valuable fishery (Hoffmann et al. 2000, Washington Department of Natural Resources 2000) and likely influence the ecosystem through filter feeding and biodeposition, as has been documented in other bivalves (Clavier and Chauvaud 2010, Newell 2004, Norling and Kautsky 2007). 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 two years (Campbell and Ming 2003) to eight years (Sloan and Robinson 1984), evidence from Puget Sound suggests that cultured geoducks mature and spawn during the typical five year culture cycle, with 50% maturity in both sexes occurring at age two years with concomitant

gamete release (B. Vadopalas et al. unpublished data). Preliminary evidence also suggests that geoduck culture is sited close enough to wild populations that cultured and wild gametes may directly interact (B. Vadopalas, unpublished data). Additionally, because geoduck larvae are pelagic for approximately six weeks (Goodwin 1976), larvae of cultured provenance may settle broadly within Puget Sound. If these larvae survive to maturity, their gametes may interact with those of wild geoducks. These data suggest that 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 collections of geoduck clams from Puget Sound (Miller et al. 2006, Vadopalas et al. 2004); Outbreeding depression and homogenization of populations are not of primary concern. However, these studies found very high microsatellite variation among geoduck clams. Although microsatellites are considered neutral markers, high microsatellite diversity may suggest high diversity in other genomic regions which 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 mid1990s; approximately 80 hectares under cultivation (Washington Department of Natural Resources 2009)) geoduck aquaculture industry affords us the opportunity to evaluate the potential for genetic risk of this activity. In this study, we examined whether cultured geoducks exhibit reduced genetic diversity

compared to wild conspecifics. Specifically, we used five microsatellite markers to compare allelic richness, heterozygosities, effective number of breeders ($N_b$), and relatedness in a wild population and five year classes of cultured geoducks. These geoducks, planted by the emerging geoduck aquaculture industry beginning in 1999, were sampled by our group one to five years later; our samples represent hatchery seed geoduck planted on a farm and surviving for several years. Genetic diversity found in our samples is thus representative of geoducks cultured during this time period. Our results provide insight into whether culture practices effectively maintain genetic diversity observed in wild geoduck; information that will be essential for sustainable management of this emerging industry.

## METHODS

*Tissue samples, DNA extraction, polymerase chain reaction, and genotyping*

In 2004, we collaborated with a geoduck farmer to collect 96 cultured geoduck from each of five contiguous year classes (age one to five years, comprising the 1999-2003 year classes) from Hartstine Island, Puget Sound, Washington, USA. 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 using the protocol developed by Ivanova et al. (2006) with vertebrate lysis buffer. Due to the slow speed of our centrifuge, the 5000 g centrifuge steps were

performed at 1928 g (top speed of our centrifuge) for 13 min (5 min Ivanova protocol) and 5 min (2 min Ivanova protocol). All DNA was diluted 1:20 with lo TE buffer before use in polymerase chain reaction (PCR). Five microsatellite loci were amplified in all individuals using PCR (Table 4). PCRs were conducted in 10 µl reactions containing 1 µl diluted template DNA, 5 µl SensiMix (Bioline, London, UK) with the final solution of 3mM $MgCl_2$ and 0.5 µM each primer (except for PCR of Pab3 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, USA). 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 30s, locus specific annealing temperature for 30s (see table 4) and 72 °C for 30s, followed by 35 cycles of 90 °C for 15s, locus specific annealing temperature for 15s (see table 4), and 72 °C for 30s, with a final extension step of 72 °C for 40 min.

Following amplification, 1 µl of PCR product was added to 3.9 µl Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 1 µl GeneScan 500 LIZ size standard (Applied Biosystems, Foster City, CA, USA) and 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 (Applied Biosystems, Foster City, CA, USA). Allele sizes were calculated using GeneMarker version 1.8 (SoftGenetics, State College, PA, USA). Each plate was run with three control samples of known genotype to enable quantification of genotyping error.

*Statistical Analysis*

The following analyses were performed on the wild geoduck collection and each of the five year classes of cultured geoducks. In addition, we analyzed our data examining three year old, four year old, and five year old geoducks together as a single group because these three year classes potentially interbreed during a typical five year geoduck culture cycle. This group is referred to as the breeding group. We used Microchecker version 2.2.3 (Van Oosterhout et al. 2004) to detect genotyping errors and calculate null allele frequencies. Expected and observed heterozygosities in each group were calculated using HW-Quickcheck (Kalinowski 2006) while 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 the program R (R Development 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 the program Colony version 2.0.0.1 (Wang and Santure 2009, Wang 2004). 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 version 1.3 (Peel et al. 2004). A sibship assignment based method (SA) was implemented in Colony version 2.0.0.1 (Wang and Santure 2009, Wang 2004) and the Parentage without Parents method (PwoP; Waples and Waples 2010) was implemented in Python version 2.6.4 (Python Software Foundation 2010) using relationship data generated in ML-Relate (Kalinowski et al. 2006).

## RESULTS

Over 95% of cultured and wild geoduck samples were genotyped at all five microsatellite loci. Individuals where amplification failed at any locus were removed from analysis. Average genotyping error was 1.1% but varied by locus, ranging from 0.00% in *Pab 101e* and *Pab 112e* to 3.1% in *Pab 6* (Table 4). Null allele rates varied per locus and population and are shown in Table 5. Analysis using Micro-Checker (Van Oosterhout et al. 2004) showed no evidence of scoring error due to stuttering or large allele dropout.

### *Genetic Diversity*

Number of alleles (A), allelic richness (AR), observed ($H_o$) and expected ($H_e$) heterozygosity for the Hardy-Weinberg equilibrium for each geoduck group at each locus are shown in Table 5. 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 higher 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 expected heterozygosity across the five loci was higher 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 $H_e = 0.88 \pm 0.021$; Kruskal-Wallis test; $p < 0.001$). The breeding group (mean $H_e = 0.93 \pm 0.011$) also showed significantly higher expected heterozygosity 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 allelic richness or expected heterozygosity were observed among the year classes of cultured geoducks (Kruskal-Wallis test, $p > 0.05$). Deviations from the Hardy-Weinberg expectations (HWE) were observed in both wild and cultured geoducks (Table 5). Depending on the locus, these deviations are characterized by either heterozygote deficiencies or heterozygote excess.

*Relatedness and Sibship*

Mean pairwise relatedness values were lower in the wild collection than in the cultured year classes of geoducks (Figure 13). The wild collection was characterized by a mean relatedness (mean ± 95% CI) of 0.041 ± 0.002 while the mean relatedness in the cultured

geoduck groups ranged from 0.066 ± 0.003 to 0.083 ± 0.004. The breeding group exhibited a higher degree of relatedness than the wild, but 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 families each comprising 2.1% of the population (Figure 14). 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 amongst 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 (Figure 14).

*Effective number of breeders ($N_b$)*

The effective number of breeders ($N_b$) estimated for each geoduck group using three different methods are shown in Table 6. $N_b$ estimates varied widely according to the method used. The LD (Hill, 1981) and PwoP (Waples and Waples 2010) 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 SA (Wang and Santure 2009, Wang 2004) method gave

lower estimates; mean $N_b$ across the five cultured year classes was 22.4 ± 3.8. The wild collection was characterized by substantially higher $N_b$ estimates using all three methods. The SA and PwoP estimates were similar ($N_b$ = 108 (77 – 152) and 120 (95% CI not provided by the program)), respectively, while the LD estimate was much larger ($N_b$ = 3241 (909 - ∞)).

## DISCUSSION

This study compared genetic diversity in five year classes of cultured geoduck clams with an adjacent wild population. We combined the three mature year classes into a group of potentially interbreeding geoducks as a more realistic approach to assessing the potential genetic impacts of geoduck aquaculture on wild conspecifics. Our results reveal that cultured geoduck exhibit decreased genetic diversity as evidenced by reduced allelic richness, increased relatedness, and reduced $N_b$ when compared with a wild collection.

The decline in genetic diversity is characterized by reduced allelic richness: the five cultured year classes exhibit an average of 32.6% fewer alleles than the wild aggregation (Table 5). A comparable reduction in allelic richness (28.2%) was also observed in the breeding group. Although each year class had significantly lower allelic richness 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. Comparable declines in

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

The effective number of breeders ($N_b$) 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 (Frankham et al. 2002, Wright 1931, 1938). Although estimates of $N_b$ varied widely depending on the method used (Table 6), $N_b$ estimates for the wild collection were substantially larger than those for the cultured year classes. Depending on the method used, $N_b$ estimates for the wild population were about two-fold (PwoP; (Waples and Waples 2010), five-fold (SA;(Wang and Santure 2009, Wang 2004), or 70-fold higher (LD; (Hill 1981) than 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 to understand the great variation in $N_b$ estimates and determine which is the most appropriate for predicting the genetic risks of geoduck aquaculture is needed.

Examination of relatedness indicates that the five year classes of cultured geoduck exhibited higher mean pairwise relatedness than the wild collection (Figure 13). Although mean relatedness values in the cultured year classes were 1.5 to 2 fold higher than that observed in the wild aggregation (0.041), relatedness values remained relatively low (0.066 to 0.083). These numbers are lower than have generally been reported for cultured shellfish. For example, while wild silver-lipped pearl oysters (*Pinctada maxima*) exhibit relatedness values of 0 to 0.01, cultured *P. maxima* show relatedness values ranging from 0.07 to 0.28, with all but one group over 0.15 (Lind et al. 2009). Abalone (*Haliotis rubra* and *H. midae*) relatedness in six cultured groups ranged from 0.16 to 0.44, although relatedness in a seventh cultured group was estimated to be 0 (Evans et al. 2004). Relatedness in cultured Pacific lion-paw scallops (*Nodipecten subnodosis*) ranged from 0.15 to 0.55 while wild conspecifics exhibited relatedness values ranging from 0 to 0.06 (Petersen et al. 2010).

Analysis of the sibgroup assignments (Figure 14) 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 geoduck were not related to a single other geoduck from our sample at the full-sib level (n = 92 to 94). These numbers are surprising given the extremely high fecundities of geoduck clams (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 (Boudry et al. 2002, Lemay and Boulding 2009, Lind et al. 2009, Selvamani et al. 2001). In contrast, the Washington State geoduck hatchery that produced these seed must have both spawned quite a large number of broodstock and successfully husbanded larvae and seed to ensure survival of many different families. Our sibgroup assignments bear this out. In addition to the large proportion of individuals unrelated to others at the full-sib level, nine to 25 full sib groups comprise each year class, with over 50% of these groups made up of only two individuals. No clear pattern emerged regarding changes in relatedness over the five year time span of this study. The most recent year class of this study (2003), exhibits a high proportion of unrelated individuals (54%) while also exhibiting among the among highest relatedness (0.078). This apparent contradiction is due to 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 that we observed as reduced allelic richness, increased relatedness, and reduced $N_b$ suggests that genetic introgression from cultured to wild geoducks may reduce the genetic diversity of wild populations (Allendorf and Ryman 1987b, Camara and Vadopalas 2009, Ford 2002, Hedgecock and Coykendall 2007, Lynch and O'Hely 2001, Ryman and Laikre 1991). As the differentiation between wild and cultured populations increases, the potential for negative genetic interactions between wild and cultured populations increases. 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 towards the average culture phenotype. Two strategies which may be useful in decreasing the genetic risk of geoduck aquaculture are: 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 may help to increase the genetic diversity of cultured geoducks. Currently, when hatchery personnel spawn geoducks, individual geoducks are removed from the spawning tank as they are observed releasing gametes. Females are placed into individual holding tanks while all spawning males are placed together into a larger tank. Oocytes from each female are then fertilized individually with pooled sperm. However, fertilizing with pooled sperm can increase the variance in reproductive success and decrease the effective number of breeders due to sperm competition (Campton 2004, Withler 1988, Withler and Beacham 1994). In oysters, Boudry et al (2002) estimated that this practice was responsible for a 20% decrease in effective population size (Boudry et al. 2002). Isolating both males and females in individual spawning tanks upon the commencement of gamete release would enable hatchery personnel to conduct factorial crosses rather than using pooled sperm to fertilize eggs. A complete factorial breeding scheme without equalizing family size comes closest to the goal of maintaining genetic diversity while maximizing progeny production (Busack and Knudsen 2007, Fiumera et al. 2004) but partial factorial designs as small as two by two provide many of the benefits of full-factorial mating schemes (Busack and 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 gametogenesis and often show increased growth (Brake et al. 2004, Mallia et al. 2006, Nell and Perkins 2005). Triploidy techniques have been developed for geoduck clams (Vadopalas and Davis 2004), but the efficacy of triploidy in conferring sterility in geoducks and the permanence of the triploid state must be verified prior to using this technique.

Table 4. Microsatellite markers used for genetic analysis of *P. generosa*

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

Table 5. Genetic diversity statistics for wild and cultured *P. generosa:* number of alleles (A), allelic richness (AR), expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), and null alleles (null). Bold text for $H_o$ indicates that the population is significantly out of the Hardy-Weinberg equilibrium at this locus.

| | 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 |

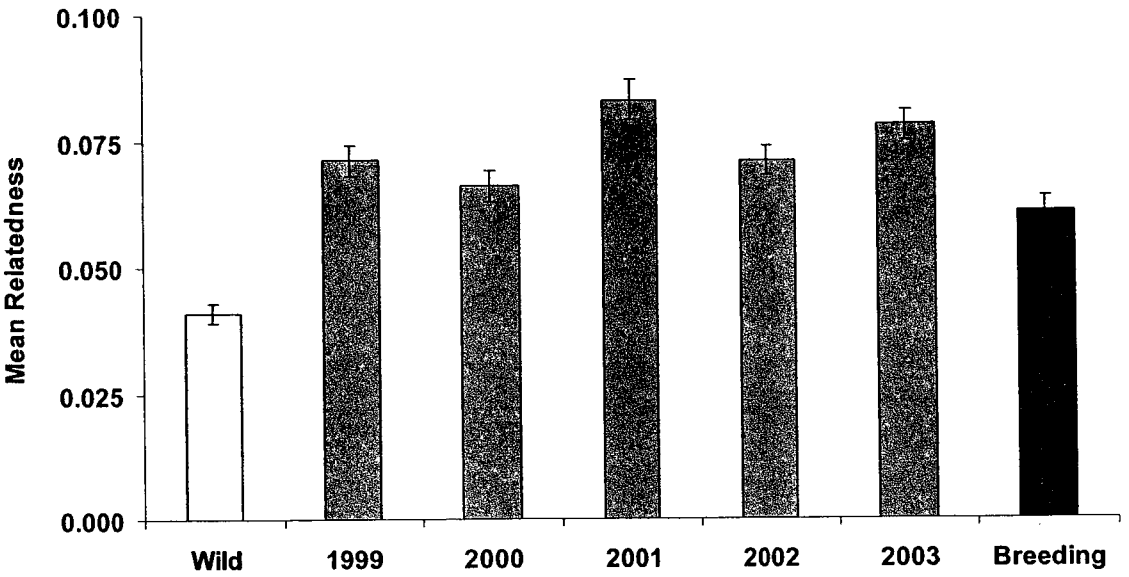



Fig 13. Relatedness values (mean ± 95% confidence interval) for wild and cultured geoduck (hatchery year classes 1999 - 2003 and the three year class breeding group). The wild geoduck collection is shown with a white bar, individual year classes are shown with grey bars, and the breeding group is shown with a black bar.

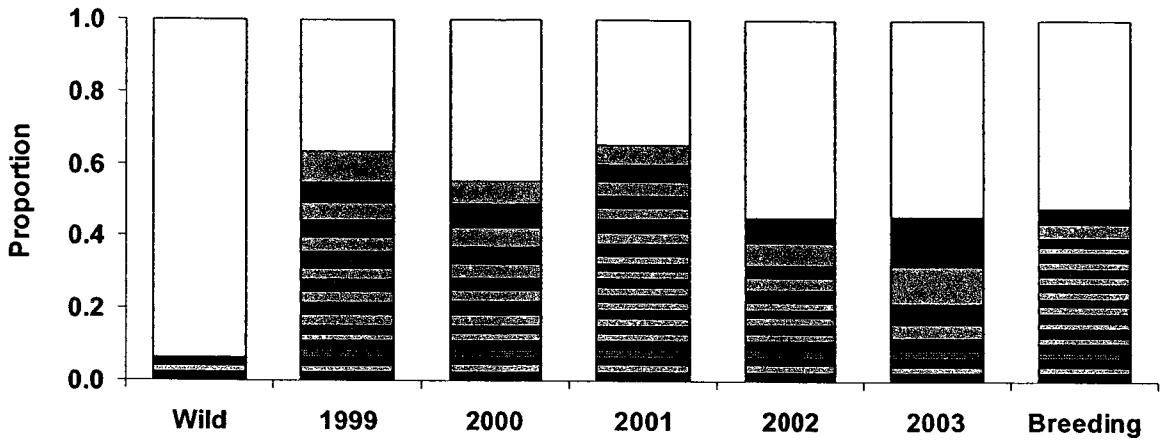



Figure 14. Full-sib assignment in wild and cultured geoduck groups. The proportion of individuals not related to any other individuals at the full sib-level is shown in white. The grey and black bars represent the proportion of individuals assigned to each different full-sib family.

Table 6. Effective number of breeders in wild and cultured *P. generosa* groups estimated using three methods: Parentage without Parents (Waples & Waples 2010), Linkage Disequilibrium (Hill 1981), and Sibship Assignment (Wang and Santure 2009; Wang 2004).

| | PwoP | | Linkage Disequilibrium | | Sibship Assignment | |
|---|---|---|---|---|---|---|
| Group | $N_b$ | 95% CI | $N_b$ | 95% CI | $N_b$ | 95% CI |
| Wild | 120.0 | | 3240.7 | 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 |

# Chapter VI
# Microsatellite parentage assignment indicates high variance in reproductive success and decreased genetic diversity in cultured geoduck, *Panopea generosa*


## ABSTRACT

Aquaculture for the Pacific geoduck (*Panopea generosa*) is a small but expanding industry in Washington State, USA, where geoducks are native and genetic interactions between wild and cultured geoducks are likely. To examine the potential genetic implications of geoduck aquaculture, we used five microsatellite loci to compare genetic diversity in wild geoducks and cultured geoducks produced in two hatcheries with different culture protocols. We estimated effective number of breeders ($N_b$) using two demographic and three genetic methods. Cultured populations were characterized by reduced heterozygosity, allelic richness, and $N_b$ and increased mean pairwise genetic relatedness. Parentage assignment revealed that genetic diversity and $N_b$ was affected by large variance in reproductive success. In one hatchery, many parents contributed to each seed cohort with the largest full-sib families comprising from 11% to 31% of the offspring. In contrast, the seed cohort from the second hatchery was dominated by a single full-sib family which comprised 94% of the offspring. Although both hatcheries produced geoduck seed with reduced genetic diversity compared to wild geoducks, this difference was far more pronounced in the second hatchery. Our results demonstrate that hatchery practices affect genetic diversity in their progeny and may aid in developing geoduck breeding practices that reduce risk to wild populations.

## INTRODUCTION

Preservation of genetic diversity within cultured populations is a key challenge currently facing the aquaculture industry. The successful maintenance of genetic variation both reduces potential negative environmental impacts of aquaculture and substantially benefits the aquaculture industry. From a conservation perspective, maintaining high levels of genetic diversity in cultured groups is of paramount importance because failure to do so may compromise the long term fitness of wild conspecifics if genetic interactions occur between cultured and wild groups (Allendorf and Ryman 1987, Ford 2002, Lynch and O'Hely 2001, Ryman and Laikre 1991). From an industry perspective, maintaining a high degree of genetic variation is valuable because such diversity promotes an efficient response to selection for commercially valuable traits and increases the ability of the population to withstand changing environmental conditions. Evidence suggests, however, that aquaculture practices do not adequately maintain the genetic diversity present in wild populations: examples abound of cultured populations with significantly lower genetic diversity and reduced effective population sizes than their wild counterparts (e.g. Evans et al. 2004, Hara and Sekino 2007, Hedgecock et al. 1992, Li et al. 2007). Substantial reductions in genetic diversity and effective population size may occur in a single generation, in situations where new wild broodstock are used every year (Lemay and Boulding 2009). Maintaining genetic diversity in an aquaculture setting may be more challenging in broadcast spawning molluscs which are characterized by extremely high fecundities (e.g. the Pacific Oyster, *Crassostrea gigas,* can release 55 million oocytes in a single spawn; Galtsoff 1930). In such cases, a few broodstock pairs may produce entire

hatchery cohorts (Boudry et al. 2002, Lemay and Boulding 2009, Lind et al. 2009, Selvamani et al. 2001).

The Pacific geoduck (*Panopea generosa;* previously misidentified as *P. abrupta* (Vadopalas et al. 2010) is an extremely large and long-lived hiatellid clam found in soft substrates from the low intertidal to depths of over 60 m (Goodwin 1976). Geoducks range from north Pacific Baja California, Mexico to S.E. Alaska, USA (Coan et al. 2000, Morris et al. 1980) and are very common in Puget Sound, WA, USA where they support a lucrative commercial fishery (Washington Department of Natural Resources 2000). Geoduck aquaculture is a small but growing industry along the west Coast of North America. In Washington State, geoducks are currently farmed on about 80 hectares of intertidal lands in Puget Sound (Washington Department of Natural Resources 2009).

We collaborated closely with a large commercial geoduck hatchery in Washington State (Hatchery A) to examine culture practices and determine how the genetic diversity and effective population size within the hatchery compared with that of wild geoduck aggregations. We were allowed unparalleled access to hatchery operations: we took tissue samples of all broodstock, worked directly with hatchery personnel during spawns to monitor culture practices and numbers of broodstock used, and were given subsamples of four separate seed cohorts upon which to do genetic analysis. We also purchased geoduck seed from a second Washington State geoduck hatchery (Hatchery B) to compare levels of genetic diversity across two hatcheries using different culture methods (Table 7). We used five polymorphic microsatellite markers to compare allelic richness, heterozygosity,

effective population size, relatedness, and to estimate parental contributions among seed in these two hatcheries and a wild group. In addition, we examined whether the number of broodstock believed to have contributed to each seed cohort was a good predictor of cohort genetic diversity. Our results provide insight into whether current culture practices effectively maintain geoduck genetic diversity; this information is essential for sustainable management of this emerging industry.

## METHODS

*Hatchery Procedures and sample collection*

A total of 351 geoduck broodstock were obtained in autumn 2007 from Puget Sound, Washington, USA and spawned in Hatchery A to produce seed sold in the spring and summer of 2008. These broodstock were comprised of four groups depending on collection origin: 86 individuals in broodstock group 1, 95 individuals in broodstock group 2, 98 individuals in broodstock group 3, and 72 individuals in broodstock group 4 (B1, B2, B3, and B4 respectively). All broodstock were individually tagged using laminated vinyl shellfish tags (Floy Tag, Seattle, WA, USA). A 2 mm biopsy punch was used to obtain siphon tissue nonlethally from each broodstock; these tissue samples were stored in 95% ethanol until genetic analyses.

Geoduck were volitionally spawned by hatchery personnel following standard production protocols (Vadopalas & Davis 2004). Broodstock groups were maintained separately in the hatchery so that only one group of geoduck was spawned per day on ten dates

between December 2007 and March 2008. Because geoduck sex is not evident from external morphology, males and females were induced to spawn in a single tank. Once an individual geoduck was observed releasing gametes, that animal was removed from the spawning tank and placed into either a) a small cylindrical tank designed to hold a single spawning female or b) a sperm collection tank that held all of the spawning males together. Tag numbers of all spawning geoducks were recorded. Tag number was also used to isolate known females before spawning in future spawning attempts. Upon completion of spawning, oocytes from each female were fertilized individually with the pooled sperm. Fertilized oocytes were mixed and hatchery personnel husbanded cohorts from these spawns separately until seed were released to be planted. Subsamples of 96 seed from four of these cohorts were collected (Seed groups S1-S4). An additional sample of 96 live seed was obtained from a second hatchery (Hatchery B; Seed group 5; S5); all seed were preserved whole in 95% ethanol until genetic analyses. Foot tissues from an archived collection of wild geoduck (n = 96) sampled in 1999 and stored in 95% ethanol were also examined.

*DNA extraction, polymerase chain reaction, and genotyping*

DNA was extracted from all ethanol preserved tissues using the protocol developed by Ivanova et al. (2006) with vertebrate lysis buffer. Due to the slow speed of our centrifuge, the 5000 g centrifuge steps were performed at 1928 g (top speed of our centrifuge) for 13 min (5 min Ivanova protocol) and 5 min (2 min Ivanova protocol). All DNA was diluted 1:20 with lo TE buffer before use in polymerase chain reaction (PCR). Five microsatellite loci were amplified in all individuals using PCR (Table 7). All PCRs were conducted in

10 µl reactions containing 1 µl diluted template DNA, 5 µl SensiMix (Bioline, London, UK) with the final solution of 3mM $MgCl_2$ and 0.5 µM each primer (except for PCR of Pab3 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, USA). Thermal cycling programs for all PCRs began with an initial denaturation step of 95 °C for 10 min followed by 5 cycles of 95 °C for 30s, locus specific annealing temperature for 30s (see Table 4) and 72 °C for 30s, followed by 35 cycles of 90 °C for 15s, locus specific annealing temperature for 15s (see Table 4), and 72 °C for 30s, with a final extension step of 72 °C for 40 min.

Following amplification, 1 µl of PCR product was added to 3.9 µl Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) and 1 µl GeneScan 500 LIZ size standard (Applied Biosystems, Foster City, CA, USA) and 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 (Applied Biosystems, Foster City, CA, USA). Allele sizes were calculated using GeneMarker version 1.8 (SoftGenetics, State College, PA, USA). Each plate was run with three samples of known genotype to quantify genotyping error.

*Statistical Analysis*

Before molecular work commenced, we used P-LOCI (Matson et al. 2008) to simulate seed assignment based on allele frequencies previously observed in wild geoducks (Miller et al. 2006, Vadopalas et al. 2004) and estimated that three to four loci would be

sufficient to correctly assign > 95% of offspring to parents. Analyses were performed on all geoduck groups examined: the wild collection, the four broodstock groups and the five seed cohorts. Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004) was used to detect genotyping errors and calculate null allele frequency in our data set. Expected and observed heterozygosities in each group were calculated using HW-Quickcheck (Kalinowski 2006) while both allele counts and allelic richness after rarefaction were estimated using HP-Rare (Kalinowski 2005). The nonparametric Mann-Whitney U test (Zar 1999) was used to test for differences in mean allelic richness and average expected heterozygosity between hatchery and wild samples using the program R (R Development Team 2010). Maximum likelihood pairwise estimates of relatedness were calculated using the program ML-Relate (Kalinowski et al. 2006); from these data, mean pairwise relatedness values and 95% confidence intervals were calculated using Microsoft Excel.

Individual geoduck seed from cohorts S1-S4 were assigned to parents using exclusion based methods, allowing for a single mismatch, as implemented by the program WhichParents version 1.0 (Hedgecock and Eichert 1999). In addition, Colony version 2.0.0.1 (Wang and Santure 2009, Wang 2004) was used to assign the most likely parent using a full maximum likelihood model incorporating both null allele and genotyping error frequency. 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. Seed cohort S5 was purchased from Hatchery B, thus we did not have access to broodstock tissue samples. For S5, we used Colony to assign seed to sibgroups based on allele sharing; we

used this information to reconstruct parental genotypes. After parental reconstruction, seed were assigned to parents using the exclusion based method described above.

Effective number of breeders ($N_b$) was calculated using both demographic and genetic approaches. Two different demographic models were used: sex ratio of breeding individuals (Wright 1938) was calculated using hatchery observations and a model incorporating both sex ratio (Wright 1938) and reproductive skew (Kimura and Crow 1963) as in Ballou and Foose (1995) was calculated using parentage assignment data. These demographic estimates were compared with three different genetic estimates of $N_b$. The linkage disequilibrium method (LD; Hill 1981) was implemented in NeEstimator version 1.3 (Peel et al. 2004). A sibship assignment based method (SA) was implemented in Colony version 2.0.0.1 (Wang and Santure 2009, Wang 2004) and the Parentage without Parents method (PwoP; Waples and Waples 2010) was conducted using relationship data from ML-Relate (Kalinowski et al. 2006).

## RESULTS

Over 95% of geoduck samples were genotyped at ≥ four of five loci. Mean genotyping error rate across all loci was 1.1% and ranged from 0.0% in *Pab 101e* and *Pab 112e* to 3.1% in *Pab 6* (Table 4). Null allele rates varied per locus and population and are shown in Table 8. Analysis using Micro-Checker (Van Oosterhout et al. 2004) showed no evidence of scoring error due large allele dropout. However, this analysis highlighted a significant shortage of genotypes with alleles of one repeat unit difference in *Pab 101e* in S5, suggesting scoring error due to stuttering at this locus in this population.

*Genetic Diversity*

Number of alleles (A), allelic richness (AR), observed ($H_o$) and expected ($H_e$) heterozygosity for the Hardy-Weinberg equilibrium for each geoduck group are shown in Table 4. All groups of geoduck were polymorphic at each locus but fewer alleles were observed in the seed cohorts than in the wild groups (wild collection and broodstock groups). Mean allelic richness across the five loci (mean ± 95% confidence interval (CI)) was 32.2 ± 3.8 in the wild groups, which was significantly higher than the 15.1 ± 2.4 observed in the hatchery seed cohorts (Mann-Whitney U test, $p < 0.001$). Many rare alleles present in the wild groups were not observed in the seed cohorts: Across all loci, a total of 82.4 alleles present in at least one wild group were lost in all seed cohorts. Wild groups of geoduck also showed significantly higher expected heterozygosity (0.95 ± 0.007) than the seed cohorts (0.84 ± 0.043; Mann-Whitney U test, $p < 0.001$). No differences in mean allelic richness or expected heterozygosity were observed among the wild geoduck groups or among the Hatchery A geoduck seed cohorts. The only cohort obtained from Hatchery B (S5) was characterized by significantly lower mean allelic richness and expected heterozygosity than the Hatchery A seed cohorts (S5 mean AR = 5.7 ± 1.2; Hatchery A mean AR= 17.5 ± 3.5; Mann-Whitney U test, $p < 0.001$; S5 mean $H_e = 0.65 \pm 0.086$, Hatchery A mean $H_e = 0.89 \pm 0.011$; Mann-Whitney U test, $p < 0.001$). However, when S5 is removed from analysis and the four Hatchery A seed cohorts are compared to the wild groups, the wild groups continue to display significantly higher mean allelic richness and expected heterozygosity than that observed in Hatchery A (wild mean AR = 32.2 ± 3.8, Hatchery A mean AR =17.5 ± 3.5; Mann-Whitney U test,

$p < 0.001$; wild mean $H_e = 0.95 \pm 0.007$, Hatchery A mean $H_e = 0.89 \pm 0.011$; Mann-Whitney U test, $p < 0.001$). Deviations from Hardy-Weinberg Expectations (HWE) were observed in both wild and seed geoduck (Table 4). These deviations are generally due to heterozygote deficiencies although heterozygote excesses are seen in S4 (locus *Pab 106e*) and S5 (loci *Pab 6* and *Pab 112e*).

*Relatedness and Parental Contributions*

Mean pairwise relatedness values were consistently lower in the wild groups than the seed cohorts (Figure 15). The range of mean relatedness values observed in the wild groups was $0.041 \pm 0.002$ to $0.046 \pm 0.003$ while that observed in the seed cohorts was $0.076 \pm 0.004$ to $0.196 \pm 0.005$. The single sample from Hatchery B (S5) was characterized by a mean relatedness value of $0.196 \pm 0.005$, more than twice as large as any observed in Hatchery A seed cohorts.

After parental genotype reconstruction, we were able to assign 94% of Hatchery B seed (S5) back to two parents. All of these seed assigned to the same two parents. The remaining 6% of seed assigned to one of those two parents but we did not have enough data to reconstruct the genotypes of the missing parents. We were able to assign > 95% of the 370 Hatchery A seed that amplified at $\geq 4$ loci back to two broodstock parents. On average, 20.3 fathers and 8.5 mothers contributed offspring to each of the four Hatchery A seed cohorts examined. However, the number of parents per cohort varied substantially; from nine to 25 fathers and from six to 13 mothers produced progeny (Table 9).

Reproductive success was also quite variable among parents (Figure 16). In S3, a single mother produced 64.2% of the seed. This mother produced progeny with seven different males; thus there were several small full-sib groups (2-5 individuals) as well as three larger full-sib groups comprising 9.5%, 21.1% and 20.0% of the S3 seed respectively. S1 shows similar results: 52.1% of the seed were produced by one female in combination with ten males, with a single full-sib family comprising 30.9% of the seed. In cohort S1, a second female was responsible for 33.0% of the seed with nine fathers. In all cohorts, many individuals produced one or two offspring while very few individuals were responsible for $\geq$ 10% of the seed.

In each of the Hatchery A seed cohorts, parental assignment revealed that individual geoduck not recorded as having spawned on that date actually contributed to the cohort (Table 9). In the most extreme example, broodstock group B2 was identified as the parents of cohorts S2 and S4, however none of our records indicate that group B2 was successfully spawned. Broodstock group B3 was confirmed to be the parents of S3. All B3 females recorded as having spawned successfully contributed progeny, but parentage assignment identified an additional three females as mothers of S3, including one female from the B2 group. Six of the 13 males recorded as having spawned on this date were successful at producing progeny and an additional three fathers (all B3) were identified using parentage assignment. Similarly, broodstock group B1 was confirmed to be the parents of S1. All five females recorded as having spawned successfully contributed offspring but two additional B1 females were identified as mothers using parentage

assignment. Twelve of the twenty males recorded as having spawned on this date produced progeny and an additional eleven fathers (all B1) were identified using parentage assignment.

*Effective Number of breeders ($N_b$)*

Estimates of $N_b$ based on two demographic and three genetic methods for the five hatchery cohorts and the wild control population are shown in Table 9. We estimated $N_b$ using hatchery records of the number of breeding individuals of each sex in two of our four Hatchery A cohorts (S1 and S3). Using this metric, $N_b$ for S1 and S3 was 13 and 16 respectively. We could not use this method for cohorts S2 and S4 because parental assignment data identified broodstock B2 as the parents of these cohorts and none of our records indicate a successful spawn for this broodstock group. We estimated $N_b$ from the parental assignment data in all five seed cohorts; estimates range from 2 (S5) to 13 (S2).

The SA estimates for the five seed cohorts are generally similar to the demographic estimates, ranging from ($N_b$ estimate with 95% CI) 5 (2 – 20; S3) to 25 (14 – 43; S5). Genetic estimates of the number of breeders in these populations calculated using the LD or PwoP methods are generally substantially larger than the demographic and SA estimates. The PwoP method (Waples and Waples 2010) generated Nb estimates without 95% confidence intervals; these estimates for the seed cohorts ranged from 35 (S5) to 54 (S2). The LD method (Hill 1981) seed cohort estimates of Nb range from 27 (25 – 30; S1) to 64 (44 – 102; S5). For the wild population, the SA and PwoP estimates are similar

(Nb = 108 (77 – 152) and 120), respectively while the LD estimate is much larger (Nb = 3241 (909 – ∞).

## DISCUSSION

Our results indicate that hatchery-reared seed geoducks show a substantial loss of genetic variation compared with wild groups. It appears that despite recommended hatchery practices in place at Hatchery A, including the use of new wild broodstock each year, the inclusion of many broodstock at each spawning and fertilizing the oocytes from each female separately, hatchery protocols fail to capture available genetic variability in wild geoducks. Significant differences in genetic diversity observed between hatcheries A and B strongly suggest that protocol differences can substantially affect genetic diversity in hatchery produced geoducks.

All five seed cohorts in this study demonstrate a significant loss of allelic richness when compared with wild geoduck groups. Across the five microsatellite loci, mean allelic richness was more than twice as high in the wild groups (32.2 ± 3.8) as in the hatchery seed cohorts (15.1 ± 2.4). When compared with wild groups, all seed cohorts show at least a 42% decline in allelic richness, with the seed cohort from Hatchery B (S5) exhibiting an 82% decline in allelic richness. In addition, many rare alleles present in at least one wild group of geoducks are absent from all of the seed cohorts. Across all loci, 36.2% of the alleles present in the wild groups are absent from all of the cultured groups. All geoduck broodstock used in both hatcheries were collected directly from the wild; these observed losses in allelic richness occurred in a single generation in culture.

Because reduced microsatellite genetic diversity may indicate reduced diversity in other genomic regions and thus imply reduced adaptive potential, such substantial losses of microsatellite alleles is cause for concern. However, comparable losses in genetic diversity have previously been documented in cultured invertebrates (Evans et al. 2004, Hara and Sekino 2007, Lemay and Boulding 2009, Lind et al. 2009). In addition to declines in allelic richness, geoduck seed cohorts showed significantly lower expected heterozygosities than the wild groups; similar declines have previously been documented in aquaculture settings (Hara and Sekino 2007, Lemay and Boulding 2009, Li 2004).

Examination of relatedness indicates that the five cultured geoduck seed cohorts exhibit increased mean pairwise relatedness when compared to the wild collections (Figure 15). Overall mean pairwise relatedness in the wild geoduck groups was 0.042. In contrast, the Hatchery A seed cohorts exhibited an overall mean pairwise relatedness of 0.082, approximately twice that observed in the wild groups, while the single seed cohort from Hatchery B (S5) exhibited a mean pairwise relatedness value of 0.196; nearly fivefold higher than that observed in the wild groups. Relatedness values observed in Hatchery A are lower than has generally been reported for cultured invertebrates while relatedness values obtained for Hatchery B are similar to previously published results (Evans et al. 2004, Lind et al. 2009, Petersen et al. 2010).

Estimates of $N_b$ vary widely depending on the method used (Table 9). The demographic $N_b$ estimates using the parental assignment data (Wright 1938) range from two to 13. In the two cohorts for which we also estimated $N_b$ based on hatchery observations (Ballou

and Foose 1995, Kimura and Crow 1963), the parentage assignment estimate is the smaller of the two demographic estimates. As this estimate takes into account differences in reproductive success in addition to the sex ratio of the parents, such differences are to be expected. Though limited, our hatchery observation data suggests that, in Hatchery A, the number of broodstock believed to have contributed to each seed cohort is a reasonably good predictor of cohort genetic diversity. Of the genetic estimates, the SA $N_b$ estimate (Wang and Santure 2009, Wang 2004) is the most similar to demographic estimates, while the LD (Hill 1981) and PwoP (Waples and Waples 2010) estimates are substantially larger than the demographic and SA estimates. It is important to note that both the SA and LD estimates indicate that cohort S5 has the highest $N_b$ of the seed cohorts, a result that contrasts with our demographic estimates and appears to conflict with the data. The seed from cohort S5 exhibited the highest relatedness and the lowest allelic richness; 94% of these seed comprised one full-sib group. It is hard to reconcile these data with a high $N_b$ estimate. For the wild population, the SA and PwoP estimates are similar (108 and 120, respectively) while the LD estimate is much larger (3241) with an upper confidence limit that includes infinity. Few studies have included both demographic and genetic estimates of $N_b$. The discrepancies observed highlight the fact that none of the models adequately describes the relationship among all of the parameters determining $N_b$.

Results from parental assignment indicate the number of parents contributing to each cohort varied greatly between the two hatcheries. In hatchery B, 94% of the seed examined comprised one full-sib group. The remaining 6% of the seed (five individuals)

were identified as half-sibs to this group; however, our data lack sufficient power to reconstruct the genotypes of the missing parents. At most, there were five additional parents and a maximum of seven parents contributed to the S5 cohort. Because Hatchery B produced seed from only one spawn in 2008 and larvae were reared in communal tanks (Table 7), it is likely that any shellfish grower purchasing seed from Hatchery B in 2008 received geoducks genetically similar to the seed we examined.

In contrast, our data suggest that Hatchery A culture protocols effectively ensure that many parents contribute to each seed cohort. An average of 29 parents (20.3 males and 8.8 females) produced progeny in each seed cohort (Table 8). Because some broodstock contributed offspring to several seed cohorts, a total of 98 geoduck broodstock (71 males and 27 females) produced progeny in the four Hatchery A cohorts examined. These four seed cohorts represent about 50% of Hatchery A production during 2008; it may be reasonable to estimate that 200 geoduck broodstock contributed genetic material to the seed produced by this hatchery in 2008. These large numbers of broodstock would suggest that genetic diversity within Hatchery A remains very high; however, results indicating significant declines in allelic richness and expected heterozygosity and substantial increases in relatedness do not bear this out.

Closer examination of the parental assignment data suggests that a high variance in reproductive success among the Hatchery A broodstock may be responsible for the observed reductions in genetic diversity. Although a large number of parents contributed to each seed cohort, there was substantial variation in family size, with most cohorts

dominated by a few very large half-sib groups (Figure 16). For example, in S1, two females were responsible for over 80% of the progeny. Although these offspring were sired by 16 different fathers, a single full-sib group comprised 30% of the seed. Such differential reproductive success has previously been observed in broadcast spawning species in an aquaculture setting (Boudry et al. 2002, Frost et al. 2006, Lemay and Boulding 2009, Li et al. 2009, Lind et al. 2009, Selvamani et al. 2001).

Understanding the processes responsible for variation in reproductive success is an important step towards ameliorating the problem and increasing genetic diversity in geoduck hatcheries. Our data indicate that female geoducks exhibited great variance in reproductive success; this is likely due in part to the large variation in oocyte quantity and quality among females. Gamete age may also contribute to differential reproductive success as sperm viability declines quickly with time after release in broadcast spawning invertebrates (Babcock and Keesing 1999, Encena et al. 1998, Levitan 1995). Differential family survival in communal rearing tanks has also been observed in culture settings (Boudry et al. 2002, Frost et al. 2006, Taris et al. 2006), and likely contributed to the differential reproductive success we observed. Differences in gamete quantity are not currently controlled in commercial geoduck hatcheries; all oocytes spawned are fertilized. Hatchery A fertilizes oocytes with pooled sperm, a practice which increases the variance in reproductive success and decreases the effective population size due to sperm competition (Campton 2004, Withler 1988, Withler and Beacham 1994). In oysters, Boudry et al. 2002 estimated that sperm competition was responsible for a 20% decrease in effective population size (Boudry et al. 2002). In contrast, Hatchery B fertilizes

oocytes from each female with the sperm of one male, a practice which, all else being equal, will lead to higher effective population sizes than are obtained when using pooled sperm (Boudry et al. 2002). That seed from Hatchery B exhibited lower genetic diversity points to other protocol differences between the hatcheries; in particular, Hatchery B used far fewer broodstock (Table 7) and we do not know how many of these individuals actually spawned. Although simply increasing the number of broodstock does not necessarily increase genetic diversity because a few broodstock may dominate hatchery cohorts (Lallias et al. 2010, Lind et al. 2009), using more broodstock would likely be a good start towards ameliorating the low genetic diversity and high relatedness observed in Hatchery B.

More broadly, aquaculture methods need to be developed that maximize hatchery production while ensuring that the genetic composition of the seed reflects the genetic composition of the wild-collected broodstock. A more controlled fertilization protocol in hatcheries may help maintain genetic diversity in cultured geoducks. As a first step, male and female geoducks should be isolated in individual spawning tanks once gamete release commences. This protocol change would enable hatchery personnel to conduct factorial crosses rather than using pooled sperm to fertilize oocytes. A complete factorial breeding scheme without equalizing family size comes closest to the goal of maximizing the progeny production while maintaining genetic diversity (Busack and Knudsen 2007, Fiumera et al. 2004). Partial factorial designs as small as two by two provide many of the benefits of full-factorial mating schemes (Busack and Knudsen 2007) and may be more manageable for hatchery personnel to conduct. Alternatively, a hatchery might pool all of

the oocytes then divide them equally into separate groups. To preclude sperm competition, each group of oocytes would be fertilized by sperm from a single male. Although equalizing the number of oocytes fertilized may decrease the variance in reproductive success, this would challenge an industry that needs to maximize seed production to be profitable. Likewise, rearing each family separately and equalizing family sizes before releasing seed to be planted would be the most genetically risk-averse approach, but due to space and financial considerations, this may not be feasible within the logistical constraints of a production-scale hatchery.

Table 7. Geoduck culture protocols at two different geoduck hatcheries in Washington State, USA.

| | Hatchery A | Hatchery B |
|---|---|---|
| broodstock origin | local wild | local wild |
| # broodstock used (2008) | 351 | 8-12 |
| # successful spawns (2008) | ≥ 9 | 1 |
| mating design | oocytes of each female fertilized by pooled sperm | single pair mating |
| larval rearing | larvae from different families reared in communal tanks | larvae from different families reared in communal tanks |

Table 8. Genetic diversity statistics for wild broodstock and seed cohorts of *P. generosa:* number of individuals genotyped (n), number of alleles (A), allelic richness (AR), expected heterozygosity ($H_e$), observed heterozygosity ($H_o$), and null allele frequency (null). Bold text for $H_o$ indicates that the population is significantly out of the Hardy-Weinberg equilibrium at this locus.

| | Wild Geoduck | | | | | Hatchery Seed Geoduck | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wild | B1 | B2 | B3 | B4 | S1 | S2 | S3 | S4 | S5 |
| *Pab 3* | | | | | | | | | | |
| n | 96 | 82 | 86 | 90 | 70 | 96 | 83 | 92 | 96 | 96 |
| A | 31 | 26 | 30 | 31 | 30 | 19 | 17 | 15 | 20 | 4 |
| AR | 29.0 | 25.1 | 28.9 | 29.0 | 30.0 | 17.9 | 16.7 | 14.5 | 18.5 | 3.7 |
| $H_e$ | 0.95 | 0.95 | 0.96 | 0.95 | 0.95 | 0.88 | 0.90 | 0.85 | 0.86 | 0.65 |
| $H_o$ | **0.75** | **0.76** | **0.76** | **0.78** | **0.79** | **0.54** | **0.72** | **0.66** | **0.77** | **0.45** |
| null | 0.104 | 0.096 | 0.103 | 0.089 | 0.085 | 0.191 | 0.090 | 0.119 | 0.045 | 0.158 |
| *Pab 6* | | | | | | | | | | |
| n | 96 | 81 | 87 | 94 | 72 | 78 | 76 | 89 | 96 | 96 |
| A | 33 | 26 | 27 | 32 | 23 | 15 | 14 | 14 | 14 | 7 |
| AR | 29.5 | 24.6 | 25.4 | 28.8 | 23.0 | 14.8 | 13.8 | 13.3 | 13.7 | 6.1 |
| $H_e$ | 0.93 | 0.93 | 0.93 | 0.93 | 0.93 | 0.89 | 0.87 | 0.86 | 0.87 | 0.66 |
| $H_o$ | 0.90 | 0.88 | 0.90 | 0.88 | **0.86** | 0.90 | 0.89 | 0.84 | 0.92 | **0.79** |
| null | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *Pab 101e* | | | | | | | | | | |
| n | 96 | 84 | 89 | 95 | 72 | 95 | 91 | 94 | 96 | 96 |
| A | 21 | 21 | 22 | 22 | 20 | 15 | 14 | 13 | 14 | 6 |
| AR | 20.1 | 20.1 | 20.8 | 20.9 | 20.0 | 14.4 | 13.8 | 12.7 | 13.7 | 5.4 |
| $H_e$ | 0.94 | 0.93 | 0.93 | 0.94 | 0.93 | 0.90 | 0.90 | 0.88 | 0.91 | 0.68 |
| $H_o$ | **0.81** | **0.82** | **0.76** | **0.80** | **0.85** | 0.91 | **0.59** | **0.69** | 0.85 | **0.54** |
| null | 0.065 | 0.055 | 0.086 | 0.072 | 0.045 | 0.000 | 0.169 | 0.102 | 0.000 | 0.101 |
| *Pab 106e* | | | | | | | | | | |
| n | 96 | 84 | 89 | 96 | 71 | 93 | 81 | 93 | 96 | 96 |
| A | 44 | 43 | 41 | 39 | 40 | 20 | 27 | 20 | 25 | 9 |
| AR | 40.9 | 40.7 | 39.0 | 37.4 | 40.0 | 19.1 | 26.0 | 18.6 | 23.5 | 7.5 |
| $H_e$ | 0.96 | 0.96 | 0.96 | 0.97 | 0.96 | 0.91 | 0.94 | 0.89 | 0.93 | 0.49 |
| $H_o$ | 0.95 | 0.93 | 0.93 | **0.89** | 0.96 | **0.78** | **0.77** | **0.78** | **0.98** | 0.47 |
| null | 0.000 | 0.000 | 0.000 | 0.041 | 0.000 | 0.064 | 0.084 | 0.052 | 0.000 | 0.000 |
| *Pab 112e* | | | | | | | | | | |
| n | 96 | 84 | 88 | 94 | 72 | 91 | 86 | 94 | 96 | 96 |
| A | 51 | 51 | 48 | 50 | 49 | 25 | 23 | 18 | 24 | 6 |
| AR | 45.9 | 47.7 | 44.0 | 45.7 | 49.0 | 23.4 | 21.4 | 17.2 | 22.2 | 5.6 |
| $H_e$ | 0.97 | 0.97 | 0.97 | 0.97 | 0.98 | 0.93 | 0.89 | 0.9 | 0.88 | 0.76 |
| $H_o$ | 0.97 | 0.98 | **0.86** | 0.96 | 0.97 | **0.71** | **0.77** | 0.87 | 0.85 | **1.00** |
| null | 0.000 | 0.000 | 0.053 | 0.000 | 0.000 | 0.111 | 0.061 | 0.000 | 0.000 | 0.000 |
| *Mean* | | | | | | | | | | |
| A | 36 | 33.4 | 33.6 | 34.8 | 32.4 | 18.8 | 19.0 | 16.0 | 19.4 | 6.4 |
| AR | 33.1 | 31.6 | 31.6 | 32.4 | 32.4 | 17.6 | 16.4 | 14.4 | 17.0 | 5.2 |
| $H_e$ | 0.95 | 0.948 | 0.95 | 0.952 | 0.95 | 0.90 | 0.90 | 0.88 | 0.89 | 0.65 |
| $H_o$ | 0.88 | 0.87 | 0.84 | 0.86 | 0.89 | 0.77 | 0.75 | 0.77 | 0.87 | 0.65 |
| null | 0.034 | 0.03 | 0.048 | 0.04 | 0.026 | 0.073 | 0.081 | 0.054 | 0.009 | 0.052 |

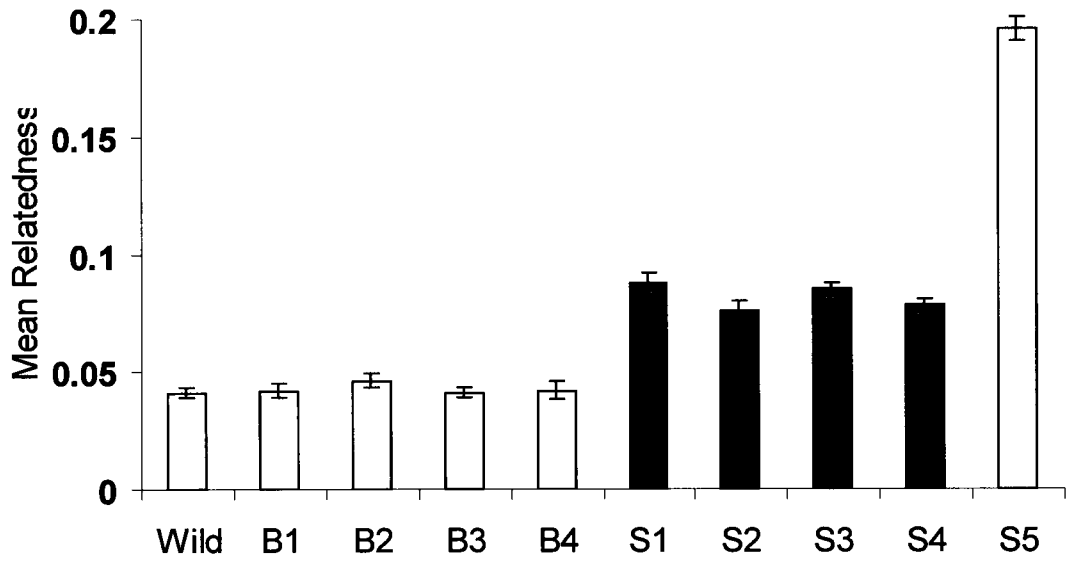



Fig 15. Relatedness values (mean ± 95% confidence interval) for wild and broodstock geoduck (Wild, B1-B4) and seed cohorts (S1-S5). Seed cohorts from Hatchery A are shown in black while the single seed cohort from Hatchery B (S5) is shown in grey.

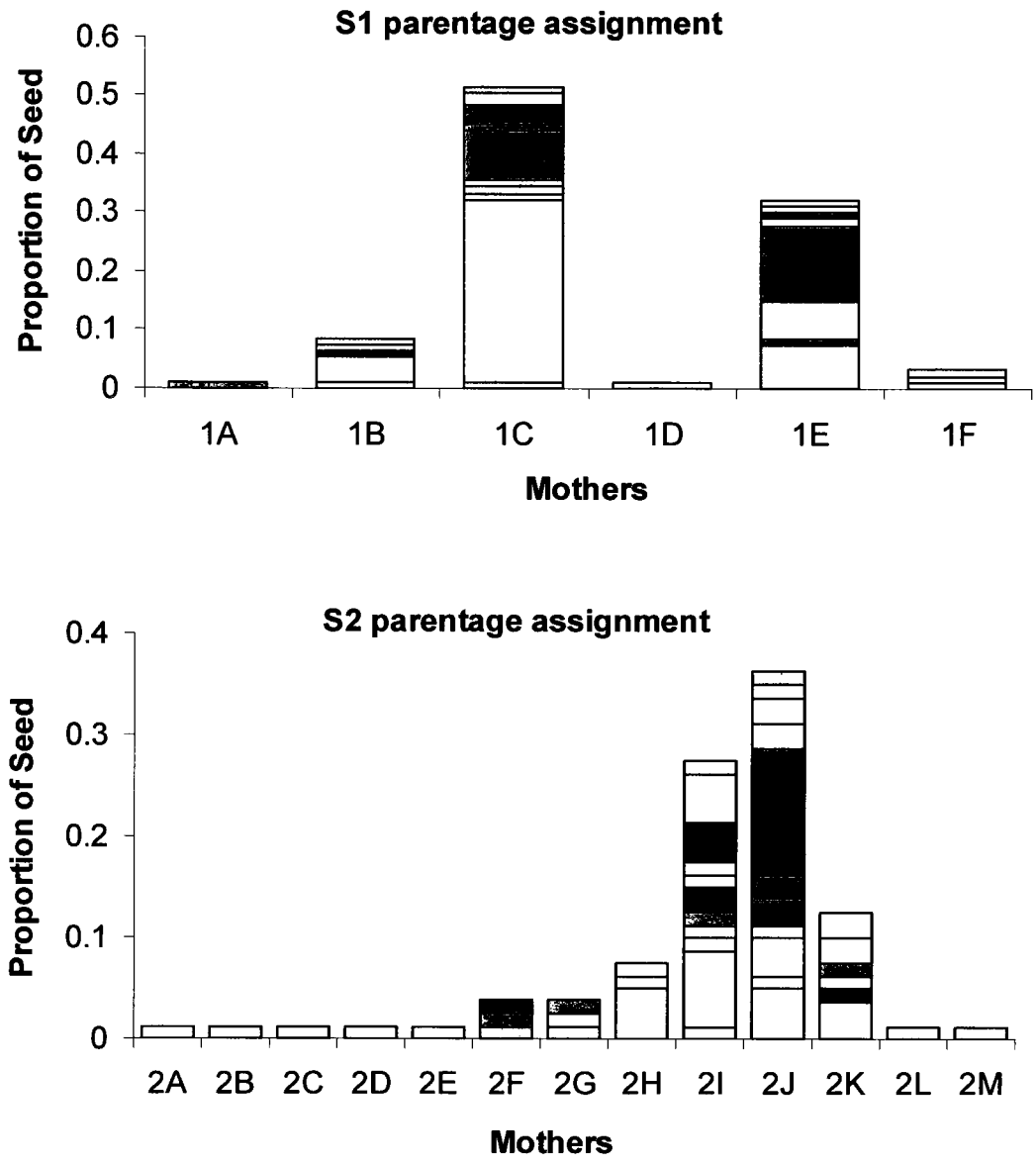
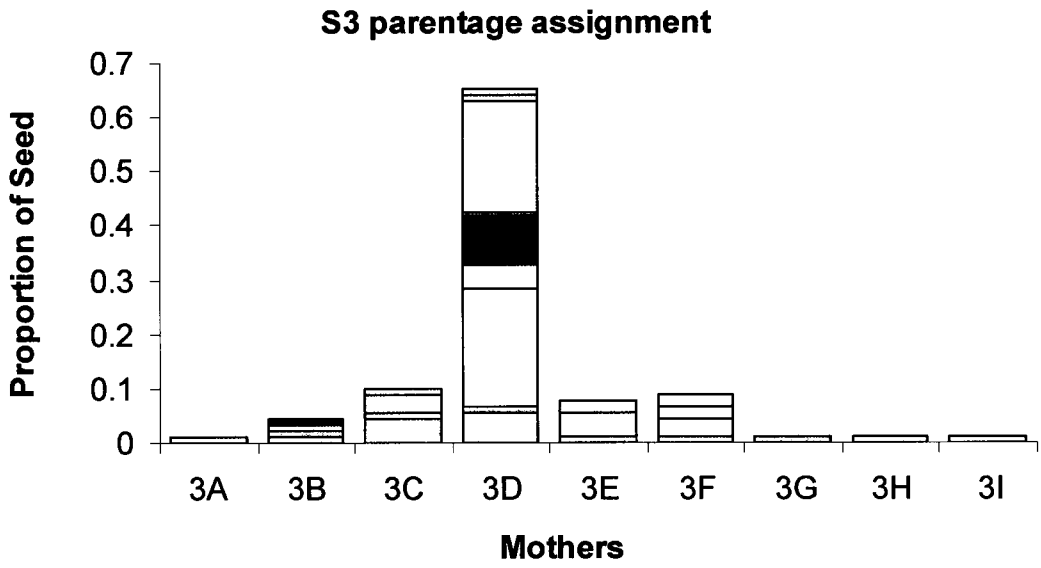



Figure 16. Geoduck parentage assignment in the four seed cohorts from Hatchery A. Mothers are shown along the X axis while fathers are shown as different colors within the stacked bars. In each panel, the same color across several bars indicates the same father mating with several females.


S3 parentage assignment
Proportion of Seed
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3A
3B
3C
3D
3E
3F
3G
3H
3I
Mothers

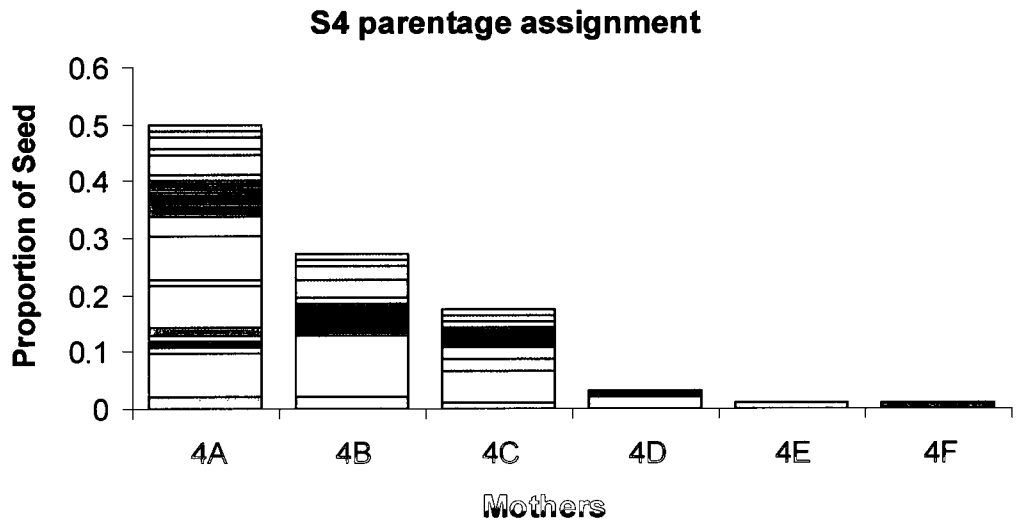

S4 parentage assignment
Proportion of Seed
0.6
0.5
0.4
0.3
0.2
0.1
0
4A
4B
4C
4D
4E
4F
Mothers

Table 9. Number of geoduck thought to contribute to each seed cohort (hatchery observations) vs. the number of parents actually contributing to each seed cohort (genetics data).

| | Hatchery Observations | | Genetic Data | | |
|---|---|---|---|---|---|
| seed cohort | # spawning males | # spawning females | # fathers | # mothers | total # parents |
| S1 | 20 | 4 | 23 | 6 | 29 |
| S2 | NA | NA | 25 | 13 | 38 |
| S3 | 13 | 6 | 9 | 9 | 18 |
| S4 | NA | NA | 24 | 6 | 30 |

Table 10. Effective population sizes of wild and cultured *P. generosa* populations based on demographic and genetic estimates.

| | Demographic | | Genetic | | | | |
|---|---|---|---|---|---|---|---|
| | | | | Linkage | | Sibship | |
| Population | Hatchery Observation | Parentage Assignment | PwoP | $N_e$ | 95% CI | $N_e$ | 95% CI |
| Wild | NA | NA | 120 | 3241 | 909 - ∞ | 108 | 77 - 152 |
| S1 | 13 | 7 | 42 | 27 | 25 - 30 | 7 | 3 - 21 |
| S2 | NA | 13 | 54 | 48 | 42 - 55 | 9 | 4 - 24 |
| S3 | 16 | 6 | 50 | 33 | 30 - 38 | 5 | 2 - 20 |
| S4 | NA | 9 | 53 | 39 | 35 - 43 | 10 | 5 - 23 |
| S5 | NA | 2 | 35 | 64 | 44 - 102 | 25 | 14 - 43 |

# Literature Cited

**Ahn, I.Y., Malouf, R., and Lopez, G. 1993.** Enhanced laraval settlement of the hard clam *Mercenaria mercenaria* by the gem clam *Gemma gemma*. Marine Ecology-Progress Series **99**(1-2): 51-59.

**Aitchison, J. 1986.** The statistical analysis of compositional data. Chapman and Hall, London.

**Allee, W.C., Emerson, A.E., Park, O., Park, T., and Schmidt, K.P. 1949.** Principles of animal ecology. Saunders, Philadelphia.

**Allendorf, F.W. 1986.** Genetic drift and the loss of alleles versus heterozygosity. Zoological Biology **5**: 181-190.

**Allendorf, F.W., Leary, R.F., Spruell, P., and Wenburg, J.K. 2001.** The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution **16**(11): 613-622.

**Allendorf, F.W., and Ryman, N. 1987.** Genetic management of hatchery stocks. *In* Population Genetics and Fishery Management. *Edited by* N.U. Ryman and F.M. Utter. University of Washington Press, Seattle. pp. 141-159.

**Altstatt, J.M., Ambrose, R.F., Engle, J.M., Haaker, P.L., Lafferty, K.D., and Raimondi, P.T. 1996.** Recent declines of black abalone *Haliotis cracherodii* on the mainland coast of central California. Marine Ecology Progress Series **142**(1-3): 185-192.

**Ambrose Jr, W.G. 1984.** Role of predatory infauna in structuring marine soft-bottom communities. Marine Ecology-Progress Series **17**: 109-115.

**Anderson, A.M.J. 1971.** Spawning, growth, and spatial distribution of the geoduck clam, *Panope generosa* (Gould) in Hood Canal, Washington. Ph. D. Dissertation, School of Fisheries, University of Washington, Seattle.

**Andre, C., Jonsson, P.R., and Lindegarth, M. 1993.** Predation on settling bivalve larvae by benthic suspension feeders—the role of hydrodynamics and larval behavior. Marine Ecology-Progress Series **97**(2): 183-192.

**Andre, C., and Rosenberg, R. 1991.** Adult-larval interactions in the suspension feeding bivalves *Cerastoderma edule* and *Mya arenaria*. Marine Ecology Progress Series **71**(3): 227-234.

**Andres, J.A., Maroja, L.S., and Harrison, R.G. 2008.** Searching for candidate speciation genes using a proteomic approach: seminal proteins in field crickets. Proceedings of the Royal Society B-Biological Sciences **275**(1646): 1975-1983.

**Andrews, J.D. 1996.** History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950-1984. Journal of Shellfish Research **15**(1): 13-16.

**Apte, S., Star, B., and Gardner, J.P.A. 2003.** A comparison of genetic diversity between cultured and wild populations, and a test for genetic introgression in the New Zealand greenshell mussel *Perna canaliculus* (Gmelin 1791). Aquaculture **219**(1-4): 193-220.

**Araki, H., Ardren, W.R., Olsen, E., Cooper, B., and Blouin, M.S. 2007.** Reproductive success of captive-bred steelhead trout in the wild: Evaluation of three hatchery programs in the Hood River. Conservation Biology **21**(1): 181-190.

**Armstrong, J.L. 1991.** Food habits of staghorn sculpin (*Leptocottus armatus*) and their role as predators of juvenile Dungeness crab (*Cancer magister*), in the Grays Harbor estuary, Washington. Masters, School of Aquatic and Fishery Sciences, University of Washington, Seattle.

**Arneberg, P. 2001.** An ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. Ecography **24**(3): 352-358.

**Arnold, W.S. 2008.** Application of larval release for restocking and stock enhancement of coastal marine bivalve populations. Reviews in Fisheries Science **16**(1-3): 65-71.

**Asami, H., Aida, M., and Watanabe, K. 2005.** Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture. Applications in Environmental Microbiology **71**(6): 2925-2933.

**Asmus, R.M., and Asmus, H. 1991.** Mussel beds—limiting or promoting phytoplankton. Journal of Experimental Marine Biology and Ecology **148**(2): 215-232.

**Ayre, D.J., and Hughes, T.P. 2000.** Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution **54**(5): 1590-1605.

**Babcock, R., and Keesing, J. 1999.** Fertilization biology of the abalone *Haliotis laevigata*: laboratory and field studies. Canadian Journal of Fisheries and Aquatic Sciences **56**(9): 1668-1678.

**Babson, A.L., Kawase, A., and MacCready, P. 2006.** Seasonal and interannual variability in the circulation of Puget Sound, Washington: A box model study. Atmosphere-Ocean **44**(1): 29-45.

**Bacher, C., Duarte, P., Ferreira, J.G., Héral, M., and Raillard, O. 1997.** Assessment and comparison of the Marennes-Oléron Bay (France) and Carlingford Lough (Ireland) carrying capacity with ecosystem models. Aquatic Ecology **31**(4): 379-394.

**Badino, G., Bona, F., Maffiotti, A., Giovanardi, O., and Pranovi, F. 2004.** Impact of mechanical clam harvesting on a benthic habitat: evaluation by means of sediment profile imaging. Aquatic Conservation-Marine and Freshwater Ecosystems **14**: S59-S67.

**Bain, R.H., Lathrop, A., Murphy, R.W., Orlov, N.L., and Cuc, H.T. 2003.** Cryptic species of a cascade frog from Southeast Asia: Taxonomic revisions and descriptions of six new species. American Museum Novitates **3417**: 1-60.

**Ballou, J.D., and Foose, T.J. 1995.** Demographic and genetic management of captive populations. *In* Wild Mammals in Captivity. *Edited by* D.G. Kleinman, S. Lumpkin, M. Allen, H. Harris and K. Thompson. University of Chicago Press, Chicago. pp. 263-283.

**Balouet, G., Poder, M., and Cahour, A. 1983.** Hemocytic parasitosis – morphology and pathology of lesions in the French flat oyster, *Ostrea edulis*. Aquaculture **34**(1-2): 1-14.

**Barilani, M., Bernard-Laurent, A., Mucci, N., Tabarroni, C., Kark, S., Garrido, J.A.P., and Randi, E. 2007.** Hybridisation with introduced chukars (*Alectoris chukar*) threatens the gene pool integrity of native rock (*A. graeca*) and red-legged (*A. rufa*) partridge populations. Biological Conservation **137**(1): 57-69.

**Bates, S.S., Bird, C.J., Defreitas, A.S.W., Foxall, R., Gilgan, M., Hanic, L.A., Johnson, G.R., McCulloch, A.W., Odense, P., Pocklington, R., Quilliam, M.A., Sim, P.G., Smith, J.C., Rao, D.V.S., Todd, E.C.D., Walter, J.A., and Wright, J.L.C. 1989.** Pennate diatom *Nitschia pungens* as the primary source of domoic acid, a toxin in shellfish from Eastern Prince Edward Island, Canada. Canadian Journal of Fisheries and Aquatic Sciences **46**(7): 1203-1215.

**Beadman, H.A., Kaiser, M.J., Galanidi, M., Shucksmith, R., and Willows, R.I. 2004.** Changes in species richness with stocking density of marine bivalves. Journal of Applied Ecology **41**: 464-475.

**Beal, B.F., and Kraus, M.G. 2002.** Interactive effects of initial size, stocking density, and type of predator deterrent netting on survival and growth of cultured juveniles of the soft-shell clam, *Mya arenaria L.*, in eastern Maine. Aquaculture **208**(1-2): 81-111.

**Beattie, J.H. 1992.** Geoduck enhancement in Washington State. Bulletin of the Aquaculture Association of Canada **92-4**: 18-24.

**Beaumont, A.R., and Fairbrother, J.E. 1991.** Ploidy manipulation in molluscan shellfish: A review. Journal of Shellfish Research **10**(1): 1-17.

**Beiras, R., Perezcamacho, A., and Albentosa, M. 1993.** Influence of food concentration on energy-balance and growth-performance of *Venerupis pullastra* seed reared in an open flow system. Aquaculture **116**(4): 353-365.

**Bell, J.D., Bartley, D.M., Lorenzen, K., and Loneragan, N.R. 2006.** Restocking and stock enhancement of coastal fisheries: Potential, problems and progress. Fisheries Research **80**(1): 1-8.

**Bell, J.D., Leber, K.M., Blankenship, H.L., Loneragan, N.R., and Masuda, R. 2008.** A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. Reviews in Fisheries Science **16**(1-3): 1-9.

**Bell, J.D., Purcell, S.W., Nash, W.J. 2008.** Restoring small-scale fisheries for tropical sea cucumbers. Ocean & Coastal Management **51**: 589-593.

**Bell, J.D., Rothlisberg, P.C., Munro, J.L., Loneragan, N.R., Nash, W.J., Ward, R.D., and Andrew, N.L. 2005.** Restocking and stock enhancement of marine invertebrate fisheries. *In* Restocking and Stock Enhancement of Marine Invertebrate Fisheries. pp. 1-+.

**Bendell-Young, L.I. 2006.** Contrasting the community structure and select geochemical characteristics of three intertidal regions in relation to shellfish farming. Environmental Conservation **33**(1): 21-27.

**Berejikian, B.A., Tezak, E.P., Flagg, T.A., LaRae, A.L., Kummerow, E., and Mahnken, C.V.W. 2000.** Social dominance, growth, and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environments. Canadian Journal of Fisheries and Aquatic Sciences **57**(3): 628-636.

**Bertness, M.D., and Gaines, S.D. 1993.** Larval dispersal and local adaptation in acorn barnacles. Evolution **47**(1): 316-320.

**Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., and Das, I. 2007.** Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution **22**(3): 148-155.

**Bill, B.D., Cox, F.H., Horner, R.A., Borchert, J.A., and Trainer, V.L. 2006.** The first closure of shellfish harvesting due to domoic acid in Puget Sound, Washington, USA. African Journal of Marine Science **28**(2): 435-440.

**Bishop, M.J., Carnegie, R.B., Stokes, N.A., Peterson, C.H., and Burreson, E.M. 2006.** Complications of a non-native oyster introduction: facilitation of a local parasite. Mar Ecol Prog Ser **325**: 145–152.

**Black, R., and Peterson, C.H. 1988.** Absence of preemption and interference competition for space between large suspension-feeding bivalves and smaller infaunal macroinvertebrates. Journal of Experimental Marine Biology and Ecology **120**(3): 183-198.

**Boese, B.L. 2002.** Effects of recreational clam harvesting on eelgrass (*Zostera marina*) and associated infaunal invertebrates: in situ manipulative experiments. Aquatic Botany **73**(1): 63-74.

**Bohonak, A.J. 1999.** Dispersal, gene flow, and population structure. Quarterly Review of Biology **74**(1): 21-45.

**Boudry, P., Collet, B., Cornette, F., Hervouet, V., and Bonhomme, F. 2002.** High variance in reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage analysis of multifactorial crosses. Aquaculture **204**(3-4): 283-296.

**Bouma, J.V. 2007.** Early life history dynamics of pinto abalone (*Haliotis kamtschatkana*) and implications for recovery in the San Juan Archipelago, Washington State. M. Sc, School of Aquatic and Fishery Sciences, University of Washington, Seattle.

**Bourque, D., Miron, G., and Landry, T. 2001.** Predation on soft-shell clams (*Mya arenaria*) by the nemertean *Cerebratulus lacteus* in Atlantic Canada: implications for control measures. Hydrobiologia **456**(1): 33-44.

**Bower, S.M., Bate, K., and Meyer, G.R. 2005.** Susceptibility of juvenile *Crassostrea gigas* and resistance of *Panope abrupta* to *Mikrocytos mackini*. Journal of Invertebrate Pathology **88**(2): 95-99.

**Bower, S.M., and Blackbourn, J. 2003.** Geoduck clam (*Panopea abrupta*): Anatomy, Histology, Development, Pathology, Parasites and Symbionts. Available from http://www-sci.pac.dfo-mpo.gc.ca/geoduck/title_e.htm

**Bower, S.M., Hervio, D., and Meyer, G.R. 1997.** Infectivity of *Mikrocytos mackini* the causative agent of Denman island disease in Pacific oysters *Crassostrea gigas*, to various species of oysters. Diseases of Aquatic Organisms **29**(2): 111-116.

**Bradbury, A., and Tagart, J.V. 2000.** Modeling geoduck, *Panopea abrupta* (Conrad, 1849) population dynamics. II. Natural mortality and equilibrium yield. Journal of Shellfish Research **19**(1): 63-70.

**Bradshaw, A.D., Caspari, E.W., and Thoday, J.M. 1965.** Evolutionary significance of phenotypic plasticity in plants. *In* Advances in Genetics. Academic Press. pp. 115-155.

**Brake, J., Davidson, J., and Davis, J. 2004.** Field observations on growth, gametogenesis, and sex ratio of triploid and diploid *Mytilus edulis*. Aquaculture **236**(1-4): 179-191.

**Breen, P.A., and Shields, T.L. 1983.** Age and size structure in five populations of geoduck clams (*Panope generosa*) in British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences **1169**: 62.

**Brown, A.F. 1991.** Outbreeding depression as a cost of dispersal in the harpacticoid copepod, *Tigriopus californicus*. Biological Bulletin **181**(1): 123-126.

**Brown, B., and Wilson, W. 1997.** The role of commercial digging of mudflats as an agent for change of infaunal intertidal populations. Journal of Experimental Marine Biology and Ecology **218**(1): 49-61.

**Brown, C., Davidson, T., and Laland, K. 2003.** Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. Journal of Fish Biology **63**: 187-196.

**Brown, L.D. 1991.** Genetic variation and population structure in the blacklip abalone, *Haliotis rubra* Australian Journal of Marine and Freshwater Research **42**(1): 77-90.

**Brown, L.D., and Murray, N.D. 1992.** Population genetics, gene flow, and stock structure in *Haliotis rubra* and *Haliotis laevigata*. *In* Abalone of the World, Biology, Fisheries and Culture. *Edited by* S.A. Shepherd, M.J. Tegner and S.A. Guzman del Proo. Blackwell Scientific, Oxford. pp. 24-33.

**Bullard, S.G., Lindquist, N.L., and Hay, M.E. 1999.** Susceptibility of invertebrate larvae to predators: how common are post-capture larval defenses? Marine Ecology Progress Series **191**: 153-161.

**Bureau, D., Hajas, W., Surry, N.W., Hand, C.M., Dovey, G., and Campbell, A. 2002.** Age, size structure, and growth parameters of geoducks (*Panopea abrupta* Conrad, 1849) from 34 locations in British Columbia sampled between 1993 and 2000. Canadian Technical Report of Fisheries and Aquatic Sciences **2413**.

**Burreson, E.M., Stokes, N.A., and Friedman, C.S. 2000.** Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster, *Crassostrea virginica*. Journal of Aquatic Animal Health **12**(1): 1-8.

**Burton, R.S., and Tegner, M.J. 2000.** Enhancement of red abalone *Haliotis rufescens* stocks at San Miguel Island: reassessing a success story. Marine Ecology Progress Series **202**: 303-308.

**Busack, C., and Knudsen, C.M. 2007.** Using factorial mating designs to increase the effective number of breeders in fish hatcheries. Aquaculture **273**(1): 24-32.

**Bustnes, J.O., Galaktionov, K.V., and Irwin, S.W.B. 2000.** Potential threats to littoral biodiversity: is increased parasitism a consequence of human activity? Oikos **90**(1): 189-190.

**Cabaco, S., Alexandre, A., and Santos, R. 2005.** Population-level effects of clam harvesting on the seagrass *Zostera noltii*. Marine Ecology Progress Series **298**: 123-129.

**Caldow, R.W.G., Beadman, H.A., McGrorty, S., Kaiser, M.J., Goss-Custard, J.D., Mould, K., and Wilson, A. 2003.** Effects of intertidal mussel cultivation on bird assemblages. Marine Ecology Progress Series **259**: 173-183.

**Caley, M.J., Carr, M.H., Hixon, M.A., Hughes, T.P., Jones, G.P., and Menge, B.A. 1996.** Recruitment and the local dynamics of open marine populations. Annual Review of Ecology and Systematics **27**: 477-500.

**Camara, M.D., and Vadopalas, B. 2009.** Genetic aspects of restoring Olympia oysters and other native bivalves: balancing the need for action, good intentions, and the risks of making things worse. Journal of Shellfish Research **28**(1): 121-145.

**Campbell, A. 2000.** Review of northern abalone, *Haliotis kamtschatkana*, stock status in British Columbia. Canadian Special Publication of Fisheries and Aquatic Science **130**: 41-50.

**Campbell, A., Harbo, R.M., and Hand, C.M. 1998.** Harvesting and distribution of Pacific geoduck clams, *Panopea abrupta*, in British Columbia. *In* Proceedings of the North Pacific Symposium on Invertebrate Stock Assessment and Management. *Edited by* G.S. Jamieson and A. Campbell. National Research Council of Canada Research Press, Ottawa. pp. 349-358.

**Campbell, A., Lessard, J., and Jamieson, G.S. 2003.** Fecundity and seasonal reproduction of northern abalone, *Haliotis kamtschatkana*, in Barkley Sound, Canada. Journal of Shellfish Research **22**(3): 811-818.

**Campbell, A., and Ming, M.D. 2003.** Maturity and growth of the pacific geoduck clam, *Panopea abrupta*, in Southern British Columbia, Canada. Journal of Shellfish Research **22**(1): 85-90.

**Campbell, A., Yeung, C.W., Dovey, G., and Zhang, Z. 2004.** Population biology of the Pacific geoduck clam, *Panopea abrupta*, in experimental plots, southern British Columbia, Canada. Journal of Shellfish Research **23**(3): 661-673.

**Campton, D.E. 2004.** Sperm competition in salmon hatcheries: The need to institutionalize genetically benign spawning protocols. Transactions of the American Fisheries Society **133**(5): 1277-1289.

**Carriker, M.R. 1967.** Ecology of estuarine benthic invertebrates; a perspective. American Association for the Advancement of Science, Washington, D.C.

**Carswell, B., Cheesman, S., and Anderson, J. 2006.** The use of spatial analysis for environmental assessment of shellfish aquaculture in Baynes Sound, Vancouver Island, British Columbia, Canada. Aquaculture **253**(1-4): 408-414.

**Carver, C.E.A., and Mallet, A.L. 1990.** Estimating the carrying capacity of a coastal inlet for mussel culture. Aquaculture **88**(1): 39-53.

**Castel, J., Labourg, P.J., Escaravage, V., Auby, I., and Garcia, M. 1989.** Influence of seagrass beds and oyster parks on the abundance and biomass patterns of meio- and macrobenthos in tidal flats. Estuarine, Coastal Shelf Science **28**(71-85).

**Castro, K.M., and Cobb, J.S. 2005.** Behaviour of hatchery-reared and wild-caught 4th and 5th stage American lobsters, *Homarus americanus*. New Zealand Journal of Marine and Freshwater Research **39**(4): 963-972.

**Chambers, M.D., VanBlaricom, G.R., Hauser, L., Utter, F., and Friedman, C.S. 2006.** Genetic structure of black abalone *(Haliotis cracherodii)* populations in the California islands and central California coast: Impacts of larval dispersal and decimation from withering syndrome. Journal of Experimental Marine Biology and Ecology **331**(2): 173-185.

**Chew, K.K. 1991.** Shoalwater Bay Indian Reservation: characterization of intertidal habitats, changes, and potential for shellfish culture. University of Washington, School of Fisheries, Seattle.

**Christensen, P.B., Glud, R.N., Dalsgaard, T., and Gillespie, P. 2003.** Impacts of longline mussel farming on oxygen and nitrogen dynamics and biological communities of coastal sediments. Aquaculture **218**(1-4): 567-588.

**Cigarria, J., and Fernandez, J.M. 2000.** Management of Manila clam beds I. Influence of seed size, type of substratum and protection on initial mortality. Aquaculture **182**(1-2): 173-182.

**Clark, W.G., and Hare, S.R. 2002.** Effects of climate and stock size on recruitment and growth of Pacific halibut. North American Journal of Fisheries Management **22**(3): 852-862.

**Clarke, K.R., and Green, R.H. 1988.** Statistical design and analysis for a biological effects study. Marine Ecology-Progress Series **46**(1-3): 213-226.

**Clavier, J., and Chauvaud, L. 2010.** Biodeposit production by *Bractechlamys vexillum* (bivalve: pectinidae) in a tropical lagoon. Journal of Sea Research **64**(3): 231-240.

**Clegg, M. 1972.** Carrion crows feeding on marine mollusks and taking fish. Bird Study **19**(4): 249-250.

**Cloern, J.E. 1982.** Does the benthos control phytoplankton biomass in South San Francisco Bay. Marine Ecology-Progress Series **9**(2): 191-202.

**Coan, E.V., Scott, P.H., and Bernard, F.R. 2000.** Bivalve seashells of western North America. Santa Barbara Museum of Natural History Publication, Santa Barbara.

**Coe, W.R. 1943.** Sexual Differentiation in Mollusks. I. Pelecypods. The Quarterly Review of Biology **18**(2): 154-164.

**Coleman, A.W., and Vacquier, V.D. 2002.** Exploring the phylogenetic utility of ITS sequences for animals: A test case for abalone (*Haliotis*). Journal of Molecular Evolution **54**(2): 246-257.

**Coleman, N., Cuff, W., Moverley, J., Gason, A.S.H., and Heislers, S. 2007.** Depth, sediment type, biogeography and high species richness in shallow-water benthos. Marine and Freshwater Research **58**(3): 293-305.

**Collin, R. 2001.** The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda : Calyptraeidae). Molecular Ecology **10**(9): 2249-2262.

**Comps, M., Tige, G., and Grizel, H. 1980.** Ultrastructural study of a protistan parasite of the flat oyster *Ostera edulis* L. Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie D **290**(4): 383-384.

**Constable, A.J. 1999.** Ecology of benthic macro-invertebrates in soft-sediment environments: A review of progress towards quantitative models and predictions. Australian Journal of Ecology **24**(4): 452-476.

**COSEWIC. 2009.** COSEWIC assessment and update status report on the Northern Abalone *Haliotis kamtschatkana* in Canada, Committee on the Status of Endangered Wildlife in Canada, Ottawa.

**Costa, F.O., deWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R.T., Hajibabaei, M., and Hebert, P.D.N. 2007.** Biological identifications through DNA barcodes: the case of the Crustacea. Canadian Journal of Fisheries and Aquatic Sciences **64**(2): 272-295.

**Cox, J.M., Ebbesmeyer, C.C., Coomes, C.A., Helseth, J.M., Hinchey, L.R., Cannon, G.A., and Barnes, C.J. 1984.** Circulation in Puget Sound: an interpretation based on historical records of currents. *In* Synthesis of Current Measurements in Puget Sound. U.S. Dept. of Commerce, NOAA Technical Memorandum 84-159, Rockville, MD. pp. 1-73.

**Crandall, K.A., Bininda-Emonds, O.R.P., Mace, G.M., and Wayne, R.K. 2000.** Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution **15**(7): 290-295.

**Crawford, C.M., Macleod, C.K.A., and Mitchell, I.M. 2003.** Effects of shellfish farming on the benthic environment. Aquaculture **224**(1-4): 117-140.

**Crawley, M.J. 2002.** Statistical computing: an introduction to data analysis using S-Plus. Wiley, Chichester, West Sussex, England.

**Crawley, M.J. 2007.** The R book. Wiley, Chichester, U.K.

**Creese, R.G., and Underwood, A.J. 1976.** Observations on biology of trochid gastrod *Austrocochlea constricta* Lamarck (Prosobranchia). 1. Factors affecting shell banding pattern. Journal of Experimental Marine Biology and Ecology **23**(3): 211-228.

**Cressman, K.A., Posey, M.H., Mallin, M.A., Leonard, L.A., and Alphin, T.D. 2003.** Effects of oyster reefs on water quality in a tidal creek estuary. Journal of Shellfish Research **22**(3): 753-762.

**Creswell, P.D., and McLay, C.L. 1990.** Handling times, prey size and species selection by *Cancer novaezelandidae* (Jacquinot, 1853) feeding on molluscan prey. Journal of Experimental Marine Biology and Ecology **140**(1-2): 13-28.

**Culliton, T.J. 1998.** Population: Distribution, Density, and Growth, National Oceanic and Atmospheric Administration Silver Spring, MD.

**Cummings, S.A., Brannon, E.L., Adams, K.J., and Thorgaard, G.H. 1997a.** Genetic analyses to establish captive breeding priorities for endangered snake river sockeye salmon. Conservation Biology **11**(3): 662-669.

**Cummings, V.J., Schneider, D.C., and Wilkinson, M.R. 1997b.** Multiscale experimental analysis of aggregative responses of mobile predators to infaunal prey. Journal of Experimental Marine Biology and Ecology **216**(1-2): 211-227.

**Currens, K.P., and Busack, C.A. 1995.** A framework for assessing genetic vulnerability. Fisheries **20**(12): 24-31.

**Curtis, K.M., Trainer, V.L., and Shumway, S.E. 2000.** Paralytic shellfish toxins in geoduck clams (*Panope abrupta*): Variability, anatomical distribution, and comparison of two toxin detection methods. Journal of Shellfish Research **19**(1): 313-319.

**Dame, R.F. 1996.** Ecology of marine bivalves: An ecosystem approach. CRC Press Inc., Boca Raton, Florida.

**Davenport, J., Smith, R., and Packer, M. 2000.** Mussels *Mytilus edulis*: significant consumers and destroyers of mesozooplankton. Marine Ecology Progress Series **198**: 131-137.

**Davis, G.E. 1995.** Recruitment of juvenile abalone (*Haliotis* spp) measured in artificial habitats. Marine and Freshwater Research **46**(3): 549-554.

**Davis, J.L.D., Young-Williams, A.C., Aguilar, R., Carswell, B.L., Goodison, M.R., Hines, A.H., Kramer, M.A., Zohar, Y., and Zmora, O. 2004.** Differences between hatchery-raised and wild blue crabs: Implications for stock enhancement potential. Transactions of the American Fisheries Society **133**(1): 1-14.

**de Goeij, P., Luttikhuizen, P.C., van der Meer, J., and Piersma, T. 2001.** Facilitation on an intertidal mudflat: the effect of siphon nipping by flatfish on burying depth of the bivalve *Macoma balthica*. Oecologia **126**(4): 500-506.

**De Silva, S.S., Nguyen, T.T.T., Turchini, G.M., Amarasinghe, U.S., and Abery, N.W. 2009.** Alien Species in Aquaculture and Biodiversity: A Paradox in Food Production. Ambio **38**(1): 24-28.

**De Waal, S., and Cook, P. 2001.** Quantifying the physical and biological attributes of successful ocean seeding sites for farm-reared juvenile abalone (*Haliotis midae*). Journal of Shellfish Research **20**: 857-861.

**De Wolf, H., Verhagen, R., and Backeljau, T. 2000.** Large scale population structure and gene flow in the planktonic developing periwinkle, *Littorina striata*, in Macaronesia (Mollusca : Gastropoda). Journal of Experimental Marine Biology and Ecology **246**(1): 69-83.

**Degnan, S.A., Imron, Geiger, D.L., and Degnan, B.M. 2006.** Evolution in temperate and tropical seas: Disparate patterns in southern hemisphere abalone (Mollusca : Vetigastropoda : Haliotidae). Molecular Phylogenetics and Evolution **41**(1): 249-256.

**DeHaan, P.W., Jordan, G.R., and Ardren, W.R. 2008.** Use of genetic tags to identify captive-bred pallid sturgeon (*Scaphirhynchus albus*) in the wild: improving abundance estimates for an endangered species. Conservation Genetics **9**(3): 691-697.

**Desser, S.S., and Bower, S.M. 1997.** The distribution, prevalence, and morphological features of the cystic stage of an apicomplexan parasite of native littleneck clams (*Protothaca staminea*) in British Columbia. The Journal of Parasitology **83**(4): 642-646.

**Dethier, M.N., and Schoch, G.C. 2005.** The consequences of scale: assessing the distribution of benthic populations in a complex estuarine fjord. Estuarine, Coastal and Shelf Science **62**(1-2): 253-270.

**Dixon, C.D., Day, R.W., Huchette, S.M.H., and Shepherd, S.A. 2006.** Successful seeding of hatchery-produced juvenile greenlip abalone to restore wild stocks. Fisheries Research **78**(2-3): 179-185.

**Dobbs, F.C., and Vozarik, J.M. 1983.** Immediate effects of a storm on coastal infauna. Marine Ecology Progress Series **11**(3): 273-279.

**Dolgin, E.S., Charlesworth, B., Baird, S.E., and Cutter, A.D. 2007.** Inbreeding and outbreeding depression in caenorhabditis nematodes. Evolution **61**(6): 1339-1352.

**Dolgov, L.V. 1991.** Sex expression and environmental stress in a mollusk, *Pinctada margaritifera*. Invertebrate Reproduction & Development **20**(2): 121-124.

**Duarte, P., Meneses, R., Hawkins, A.J.S., Zhu, M., Fang, J., and Grant, J. 2003.** Mathematical modeling to assess the carrying capacity for multi-species culture within coastal waters. Ecological Modeling **168**(1-2): 109-143.

**Dubois, S., Marin-Leal, J.C., Ropert, M., and Lefebvre, S. 2007.** Effects of oyster farming on macrofaunal assemblages associated with *Lanice conchilega* tubeworm populations: A trophic analysis using natural stable isotopes. Aquaculture **271**(1-4): 336-349.

**Ebbesmeyer, C.C., and Barnes, C.A. 1980.** Control of a Fjord Basin's Dynamics by Tidal Mixing in Embracing Sill Zones. Estuarine and Coastal Marine Science **11**(3): 311-330.

**Ebbesmeyer, C.C., Cayan, D.R., Milan, D.R., Nichols, F.H., Peterson, D.H., and Redmond, K.T. 1991.** 1976 step in the Pacific climate: forty environmental changes between 1968-75 and 1977-84.

**Eckman, J.E. 1983.** Hydrodynamic processes affecting benthic recruitment. Limnology and Oceanography **28**(2): 241-257.

**Edmands, S., Moberg, P.E., and Burton, R.S. 1996.** Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. Marine Biology **126**: 443-450.

**Elston, R. 1986.** Occurrence of branchial rickettsiales-like infections in two bivalve molluscs, *Tapes japonica* and *Patinopecten yessoensis*, with comments on their significance. Journal of Fish Diseases **9**(1): 69-71.

**Elston, R.A. 1990.** Mollusc diseases: guide for the shellfish farmer. University of Washington Press, Seattle.

**Elston, R.A., Farley, C.A., and Kent, M.L. 1986.** Occurrence and significance of Bonamiasis in European flat oysters *Ostrea edulis* in North America. Diseases of Aquatic Organisms **2**(1): 49-54.

**Elston, R.A., and Wilkinson, M.T. 1985.** Pathology, management and diagnosis of oyster velar virus disease (OVVD). Aquaculture **48**(1): 189-210.

**Encena, V.C., Capinpin, E.C., and Bayona, N.C. 1998.** Optimal sperm concentration and time for fertilization of the tropical abalone, *Haliotis asinina* Linne 1758. Aquaculture **165**(3-4): 347-352.

**Ertman, S.C., and Jumars, P.A. 1988.** Effects of bivalve siphonal currents on the settlement of inert particles and larvae. Journal of Marine Research **46**(4): 797-813.

**Evans, B., Bartlett, J., Sweijd, N., Cook, P., and Elliott, N.G. 2004.** Loss of genetic variation at microsatellite loci in hatchery produced abalone in Australia (*Haliotis rubra*) and South Africa (*Haliotis midae*). Aquaculture **233**(1-4): 109-127.

**Eversole, A.G., Michener, W.K., and Eldridge, P.J. 1980.** Reproductive cycle of *Mercenaria Mercenaria* in a South Carolina estuary. Proceedings of the National Shellfisheries Association **70**: 22-30.

**Farley, C., Wolf, P., and Elston, R. 1988.** A long term study of 'microcell' disease in oysters with a description of a new genus, *Mikrocytos* (g. n.), and two new species, *Mikrocytos mackini* (sp. n.) and *Mikrocytos roughleyi* (sp. n.). Fishery Bulletin **86**: 581-593.

**Fegley, S. 2001.** Demography and dynamics of hard clam populations. *In* Biology of the Hard Clam. *Edited by* J. Kraeuter and M. Castagna. Elsevier, New York. pp. 383-422.

**Felsenstein, J. 1985.** Confidence limits on phylogenies - an approach using the bootstrap. Evolution **39**(4): 783-791.

**Fiumera, A.C., Porter, B.A., Looney, G., Asmussen, M.A., and Avise, J.C. 2004.** Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. Conservation Biology **18**(1): 94-101.

**Ford, J.S., and Myers, R.A. 2008.** A global assessment of salmon aquaculture impacts on wild salmonids. Plos Biology **6**(2): 411-417.

**Ford, M.J. 2002.** Selection in captivity during supportive breeding may reduce fitness in the wild. Conservation Biology **16**(3): 815-825.

**Ford, S.E., Kraeuter, J.N., Barber, R.D., and Mathis, G. 2002.** Aquaculture-associated factors in QPX disease of hard clams: density and seed source. Aquaculture **208**(1-2): 23-38.

**Francis, R.C., Hare, S.R., Hollowed, A.B., and Wooster, W.S. 1998.** Effects of interdecadal climate variability on the oceanic ecosystems of the NE Pacific. Fisheries Oceanography **7**(1): 1-21.

**Frankham, R., Ballou, J., and Briscoe, D.A. 2002.** Introduction to Conservation Genetics. University Press, Cambridge.

**Frankham, R., and Ralls, K. 1998.** Conservation biology - Inbreeding leads to extinction. Nature **392**(6675): 441-442.

**Fraser, D.A., Gaydos, J.K., Karlsen, E., and Rylko, M.S. 2006.** Collaborative science, policy development and program implementation in the transboundary Georgia Basin/Puget Sound ecosystem. Environmental Monitoring and Assessment **113**(1-3): 49-69.

**Friedman, C.S., Andree, K.B., Beauchamp, K.A., Moore, J.D., Robbins, T.T., Shields, J.D., and Hedrick, R.P. 2000.** 'Candidatus Xenohaliotis californiensis', a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. International Journal of Systematic and Evolutionary Microbiology **50**: 847-855.

**Friedman, C.S., Beattie, J.H., Elston, R.A., and Hedrick, R.P. 1991b.** Investigation of the relationship between the presence of a gram-positive bacterial-infection and summer mortality of the Pacific oyster, *Crassostrea gigas* Thunberg. Aquaculture **94**(1): 1-15.

**Friedman, C.S., Biggs, W., Shields, J.D., and Hedrick, R.P. 2002.** Transmission of withering syndrome in black abalone, *Haliotis cracherodii* leach. Journal of Shellfish Research **21**(2): 817-824.

**Friedman, C.S., and Finley, C.A. 2003.** Anthropogenic introduction of the etiological agent of withering syndrome into northern California abalone populations via conservation efforts. Canadian Journal of Fisheries and Aquatic Sciences **60**(11): 1424-1431.

**Friedman, C.S., and Hedrick, R.P. 1991.** Pacific oyster nocardiosis – isolation of the bacterium and induction of laboratory infections. Journal of Invertebrate Pathology **57**(1): 109-120.

**Frost, L.A., Evans, B.S., and Jerry, D.R. 2006.** Loss of genetic diversity due to hatchery culture practices in barramundi (*Lates calcarifer*). Aquaculture **261**(3): 1056-1064.

**Fyfe, D.A. 1984.** The effect of conspecific association on growth and dispersion of the geoduck clam, *Panope generosa*. Masters, Simon Frazer University.

**Gaffney, P.M. 1992.** Assessment of drift and selection in hatchery populations of oysters (*Crassostrea virginica*). Aquaculture **105**(1): 1-20.

**Gaffney, P.M. 2006.** The role of genetics in shellfish restoration. Aquatic Living Resources **19**(3): 277-282.

**Galtsoff, P.S. 1930.** The fecundity of the oyster. Science **72**: 97-98.

**Galtsoff, P.S. 1964.** The American Oyster. Fisheries Bulletin **64**: 1-480.

**Garshelis, D.L., Garshelis, J.A., and Kimker, A.T. 1986.** Sea otter time budgets and prey relationships in Alaska. Journal of Wildlife Management **50**(4): 637-647.

**Gartner-Kepkay, K.E., Zouros, E., Dickie, L.M., and Freeman, K.R. 1983.** Genetic differentiation in the face of gene flow - A study of mussel populations from a single Nova Scotian embayment. Canadian Journal of Fisheries and Aquatic Sciences **40**(4): 443-451.

**Geiger, D.L. 1998.** Recent genera and species of the family Haliotidae Rafinesque, 1815 (Gastropoda : Vetigastropoda). Nautilus **111**(3): 85-116.

**Geiger, D.L. 1999.** A total evidence cladistic analysis of the family Haliotidae (Gastropoda: Vetigastropoda), University of Southern California, Los Angeles.

**Geiger, D.L. 2000.** Distribution and biogeography of the recent Haliotidae (Gaastropoda: Vetigastropoda) world-wide. Bollettino Malacologico **35**: 5-12.

**Gerber, L.R., Buenau, K.E., and Vanblaricom, G.R. 2004.** Density dependence and risk of extinction in a small population of sea otters. Biodiversity and Conservation **13**(14): 2741-2757.

**Gibbs, M.T. 2004.** Interactions between bivalve shellfish farms and fishery resources. Aquaculture **240**(1-4): 267-296.

**Gibbs, M.T. 2007.** Sustainability performance indicators for suspended bivalve aquaculture activities. Ecological Indicators **7**(1): 94-107.

**Giles, H., and Pilditch, C. 2006.** Effects of mussel (*Perna canaliculus*) biodeposit decomposition on benthic respiration and nutrient fluxes. Marine Biology **150**(2): 261-271.

**Giles, H., and Pilditch, C.A. 2004.** Effects of diet on sinking rates and erosion thresholds of mussel *Perna canaliculus* biodeposits. Marine Ecology-Progress Series **282**: 205-219.

**Giles, H., Pilditch, C.A., and Bell, D.G. 2006.** Sedimentation from mussel (*Perna canaliculus*) culture in the Firth of Thames, New Zealand: Impacts on sediment oxygen and nutrient fluxes. Aquaculture **261**(1): 125-140.

**Gilk, S.E., Wang, I.A., Hoover, C.L., Smoker, W.W., Taylor, S.G., Gray, A.K., and Gharrett, A.J. 2004.** Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. Environmental Biology of Fishes **69**(1-4): 287-297.

**Goodsell, P.J., Underwood, A.J., Chapman, M.G., and Heasman, M.P. 2006.** Seeding small numbers of cultured black-lip abalone (*Haliotis rubra* Leach) to match natural densities of wild populations. Marine and Freshwater Research **57**(7): 747-756.

**Goodwin, C.L. 1976.** Observations of spawnings and growth of subtidal geoducks (*Panope generosa,* Gould). Proceedings of the National Shellfisheries Association **65**: 49-58.

**Goodwin, C.L. 1977.** The effect of season on visual and photographic assessment of subtidal geoduck clam (*Panope generosa* Gould) populations. Veliger **20**: 155-158.

**Goodwin, C.L., and Pease, B.C. 1991.** Geoduck, *Panopea abrupta* (Conrad, 1849) size, density, and quality as related to various environmental parameters in Puget Sound, Washington. Journal of Shellfish Research **10**(1): 65-77.

**Goodwin, C.L., Shaul, W., and Budd, C. 1979.** Larval development of the geoduck clam (*Panopea generosa,* Gould). Proceedings of the National Shellfisheries Association **69**: 73-76.

**Goodwin, L., and Shaul, W. 1984.** Age, recruitment and growth of the geoduck clam (*Panope generosa*, Gould) in Puget Sound, Washington. Washington Department of Fisheries Progress Report **#215**.

**Gosselin, L.A. 2004.** Localized synchronous spawning of *Mytilus californianus* conrad in Barkley Sound, British Columbia, Canada. Journal of Shellfish Research **23**(2): 529-533.

**Goulletquer, P., Robert, R., and Trut, G. 1999.** Manila clam *Tapes philippinarum* culture: Sediment-clam interactions. Aquatic Living Resources **12**(1): 45-56.

**Govind, C.K., and Kent, K.S. 1982.** Transformation of fast fibers to slow prevented by lack of activity in developing lobster muscle. Nature **298**(5876): 755-757.

**Grant, J. 1981.** Sediment transport and disturbance on an intertidal sandflat – infaunal distribution and recolonization. Marine Ecology Progress Series **6**(3): 249-255.

**Grant, J., Bugden, G., Horne, E., Archambault, M.C., and Carreau, M. 2007a.** Remote sensing of particle depletion by coastal suspension-feeders. Canadian Journal of Fisheries and Aquatic Sciences **64**(3): 387-390.

**Grant, J., Curran, K.J., Guyondet, T.L., Tita, G., Bacher, C., Koutitonsky, V., and Dowd, M. 2007b.** A box model of carrying capacity for suspended mussel aquaculture in Lagune de la Grande-Entree, Iles-de-la-Madeleine, Quebec. Ecological Modeling **200**(1-2): 193-206.

**Grant, J., Hatcher, A., Scott, D.B., Pocklington, P., Schafer, C.T., and Winters, G.V. 1995.** A multidisciplinary approach to evaluating impacts of shellfish aquaculture on benthic communities. Estuaries **18**(1A): 124-144.

**Gray, J.S. 1974.** Animal-sediment relationships. Oceanography and Marine Biology: An Annual Review **12**: 223-261.

**Grefsrud, E.S., and Strand, O. 2006.** Comparison of shell strength in wild and cultured scallops (*Pecten maximus*). Aquaculture **251**(2-4): 306-313.

**Gribben, P.E., and Creese, R.G. 2003.** Protandry in the New Zealand geoduck, *Panopea zelandica* (Mollusca, Bivalvia). Invertebrate Reproduction & Development **44**(2-3): 119-129.

**Griffiths, A.M., and Gosselin, L.A. 2008.** Ontogenetic shift in susceptibility to predators in juvenile northern abalone, *Haliotis kamtschatkana*. Journal of Experimental Marine Biology and Ecology **360**(2): 85-93.

**Grizzle, R.E., Bricelj, V.M., and Shumway, S.E. 2001.** Physiological Ecology of *Mercenaria mercenaria*. *In* Biology of the Hard Clam. *Edited by* K.J. N. and C. M. Elsevier, New York. pp. 305-382.

**Grizzle, R.E., Greene, J.K., Luckenbach, M.W., and Coen, L.D. 2006.** A new in situ method for measuring seston uptake by suspension-feeding bivalve molluscs. Journal of Shellfish Research **25**(2): 643-647.

**Groom, M.J., Meffe, G.K., and Carroll, C.R. 2006.** Principles of Conservation Biology. Sinauer Associates, Inc., Sunderland, MA.

**Gruenthal, K.M., Acheson, L.K., and Burton, R.S. 2007.** Genetic structure of natural populations of California red abalone (*Haliotis rufescens*) using multiple genetic markers. Marine Biology **152**(6): 1237-1248.

**Gruenthal, K.M., and Burton, R.S. 2005.** Genetic diversity and species identification in the endangered white abalone (*Haliotis sorenseni*). Conservation Genetics **6**(6): 929-939.

**Gruenthal, K.M., and Burton, R.S. 2008.** Genetic structure of natural populations of the California black abalone (*Haliotis cracherodii* Leach, 1814), a candidate for endangered species status. Journal of Experimental Marine Biology and Ecology **355**(1): 47-58.

**Guo, X., DeBrosse, G.A., and Allen, S.K. 1996.** All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture **52**: 394-402.

**Guo, X.M., Hedgecock, D., Hershberger, W.K., Cooper, K., and Allen, S.K. 1998.** Genetic determinants of protandric sex in the Pacific oyster, *Crassostrea gigas* Thunberg. Evolution **52**(2): 394-402.

**Guzman del Proo, S.A., Carillo-Laguna, J., Belmar-Perez, J., Carreon-Palau, L., and Castro, A. 2004.** Transplanting of wild and cultivated juveniles of green abalone (*Haliotis fulgens* phillipi 1845): growth and survival. Journal of Shellfish Research **23**: 855-860.

**Haaker, P.L., Henderson, K.C., and Parker, D.O. 1986.** Marine Resource Leaflet, State of California, Resource Agency, Department of Fish and Game.

**Haaker, P.L., Parker, D.O., Togstad, H., Richards, D.V., Davis, G.E., and Friedman, C.S. 1992a.** Mass mortality and withering syndrome in black abalone, *Haliotis cracherodii*, in California. Fishing News Books, Oxford.

**Haaker, P.L., Richards, D.V., Friedman, C.S., Davis, G.E., Parker, D.O., and Togstad, H.A. 1992b.** Mass mortality and withering syndrome in black abalone, *Haliotis cracherodii*, in California. *In* Abalone of the World. *Edited by* S.A. Shepherd, M.J. Tegner and S.A. Guzman del Proo. Blackwell Scientific Publications, Oxford, Engand. pp. 214-224.

**Haddon, M., Wear, R.G., and Packer, H.A. 1987.** Depth and density of burial by the bivalve *Paphies ventricosa* as refuges from predation by the crab *Ovalipes catharus*. Marine Biology **94**(1): 25-30.

**Hall, S.J., and Harding, M.J.C. 1997.** Physical disturbance and marine benthic communities: The effects of mechanical harvesting of cockles on non-target benthic infauna. The Journal of Applied Ecology **34**(2): 497-517.

**Hall, T.A. 1999.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**: 95-98.

**Hamm, D.E., and Burton, R.S. 2000.** Population genetics of black abalone, *Haliotis cracherodii*, along the central California coast. Journal of Experimental Marine Biology and Ecology **254**(2): 235-247.

**Hara, M., and Sekino, M. 2007.** Genetic differences between hatchery stocks and natural populations in pacific abalone (*Haliotis discus*) estimated using microsatellite DNA markers. Marine Biotechnology **9**(1): 74-81.

**Hare, S.R., and Mantua, N.J. 2000.** Empirical evidence for North Pacific regime shifts in 1977 and 1989. Progress In Oceanography **47**(2-4): 103-145.

**Harshbarger, J.C., Chang, S.C., and Otto, S.V. 1977.** Chlamydiae (with phages), mycoplasmas, and tickettsiae in Chesapeake Bay bivalves. Science **196**(4290): 666-668.

**Harvell, D., Aronson, R., Baron, N., Connell, J., Dobson, A., Ellner, S., Gerber, L., Kim, K., Kuris, A., McCallum, H., Lafferty, K., McKay, B., Porter, J., Pascual, M., Smith, G., Sutherland, K., and Ward, J. 2004.** The rising tide of ocean diseases: unsolved problems and research priorities. Frontiers in Ecology and the Environment **2**(7): 375-382.

**Hatcher, A., Grant, J., and Schofield, B. 1994.** Effects of suspended mussel culture (*Mytilus* spp) on sedimentation, benthic respiration and sediment nutrient dynamics in a coastal bay. Marine Ecology Progress Series **115**(3): 219-235.

**Hawken, P., Lovins, A.B., and Lovins, L.H. 1999.** Natural capitalism: creating the next industrial revolution. Little, Brown and Co., Boston.

**Hawkins, A.J.S., Bayne, B.L., Bougrier, S., Heral, M., Iglesias, J.I.P., Navarro, E., Smith, R.F.M., and Urrutia, M.B. 1998.** Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. Journal of Experimental Marine Biology and Ecology **219**(1-2): 87-103.

**Hebert, P.D.N., Cywinska, A., Ball, S.L., and DeWaard, J.R. 2003a.** Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B-Biological Sciences **270**(1512): 313-321.

**Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., and Hallwachs, W. 2004.** Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America **101**(41): 14812-14817.

**Hebert, P.D.N., Ratnasingham, S., and deWaard, J.R. 2003b.** Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London Series B-Biological Sciences **270**: S96-S99.

**Hedgecock, D., Chow, V., and Waples, R.S. 1992.** Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. Aquaculture **108**(3-4): 215-232.

**Hedgecock, D., and Coykendall, K. 2007.** Genetic risks of hatchery enhancement: the good, the bad, and the unknown. *In* Ecological and genetic implications of aquaculture activities. *Edited by* T.M. Bert. Kluwer Academic Press, Netherlands. pp. 85-101.

**Hedgecock, D., and Eichert, W. 1999.** WhichParents (version 1.0): a windows application for determining the most likely parents of offspring using multilocus genotype data.

**Hedgecock, D., and Sly, F. 1990.** Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific Oyster, *Crassotrea gigas*. Aquaculture **88**(1): 21-38.

**Hemphill, H. 1881.** On the habits and distribution of the geoduck, a clam of the Pacific (*Glycimeris generosa*, Gld.), with suggestions as to its introduction into the Atlantic coast of the U.S. Bulletin of the Untied States Fish Commission**1**: 200-202.

**Henderson, K.C., D.O. Parker, and P.L. Haaker. 1988.** The survival and growth of transplanted adult pink abalone, *Haliotis corrugata*, at Santa Catalina Island. California Fish and Game **74**(2): 82-86.

**Herlinveaux, R.H., and Tully, J.P. 1961.** Oceanography of Juan de Fuca Strait. Journal of the Fisheries Research Board Canada **18**(6): 1027-1071.

**Hervio, D., Bower, S.M., and Meyer, G.R. 1996.** Detection, isolation, and experimental transmission of *Mikrocytos mackini*, a microcell parasite of Pacific oysters *Crassostrea gigas* (Thunberg). Journal of Invertebrate Pathology **67**(1): 72-79.

**Hiddink, J.G., ter Hofstede, R., and Wolff, W.J. 2002.** Predation of intertidal infauna on juveniles of the bivalve *Macoma balthica*. Journal of Sea Research **47**(2): 141-159.

**Hilborn, R., and Eggers, D. 2001.** A review of the hatchery programs for pink salmon in Prince William Sound and Kodiak Island, Alaska: Response to comment. Transactions of the American Fisheries Society **130**(4): 720-724.

**Hill, W.G. 1981.** Estimation of effective population size from data on linkage disequilibrium. Genetical Research **38**(3): 209-216.

**Hine, P.M., Bower, S.M., Meyer, G.R., Cochennec-Laureau, N., and Berthe, F.C.J. 2001.** Ultrastructure of *Mikrocytos mackini,* the cause of Denman Island disease in oysters Crassostrea spp. and Ostrea spp. in British Columbia, Canada. Diseases of Aquatic Organisms **45**(3): 215-227.

**Hines, A.H., and Loughlin, T.R. 1980.** Observations of sea otters digging for clams at Monterey Harbor, California. Fish. Bull. **78**: 159-163.

**Hobday, A.J., Tegner, M.J., and Haaker, P.L. 2001.** Over-exploitation of a broadcast spawning marine invertebrate: Decline of the white abalone. Reviews in Fish Biology and Fisheries **10**(4): 493-514.

**Hodgson, A.N., Le Quesne, W.J.F., Hawkins, S.J., and Bishop, J.D.D. 2007.** Factors affecting fertilization success in two species of patellid limpet (Mollusca : Gastropoda) and development of fertilization kinetics models. Marine Biology **150**(3): 415-426.

**Hoffman, J.I., Peck, L.S., Hillyard, G., Zieritz, A., and Clark, M.S. 2010.** No evidence for genetic differentiation between Antarctic limpet *Nacella concinna* morphotypes. Marine Biology **157**(4): 765-778.

**Hoffmann, A., Bradbury, A., and Goodwin, C.L. 2000.** Modeling geoduck, *Panopea abrupta* (Conrad, 1849) population dynamics. I. Growth. Journal of Shellfish Research **19**(1): 57-62.

**Hofmann, E., Ford, S., Powell, E., and Klinck, J. 2001.** Modeling studies of the effect of climate variability on MSX disease in eastern oyster (*Crassostrea virginica*) populations. Hydrobiologia **460**: 195-212.

**Holgate, P. 1966.** A mathematical study of founder principle of evolutionary genetics Journal of Applied Probability **3**(1): 115-&.

**Holland, A.F., Mountford, N.K., Hiegel, M.H., Kaumeyer, K.R., and Mihursky, J.A. 1980.** Influence of predation on infaunal abundance in upper Chesapeake Bay, USA. Marine Biology **57**(3): 221-235.

**Horner, R.A., Kusske, M.B., Moynihan, B.P., Skinner, R.N., and Wekell, J.C. 1993.** Retention of domoic acid by Pacific razor clams, *Siliqua patula* (Dixon, 1789) – Preliminary study. Journal of Shellfish Research **12**(2): 451-456.

**Houston, A.I., and McNamara, J.M. 1992.** Phenotypic plasticity as a state-dependent life history decision. Evolutionary Ecology **6**(3): 243-253.

**Huang, B.X., Peakall, R., and Hanna, P.J. 2000.** Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. Marine Biology **136**(2): 207-216.

**Incze, L.S., Lutz, R.A., and True, E. 1981.** Modeling carrying capacities for bivalve molluscs in open, suspended-culture systems. Journal of the World Mariculture Society **12**: 143-155

**Inglis, G.J., and Gust, N. 2003.** Potential indirect effects of shellfish culture on the reproductive success of benthic predators. Journal of Applied Ecology **40**(6): 1077-1089.

**Ino, T. 1966.** The abalone science and its propagation in Japan. Nippon Suison Shigen Hogo Kyokai. Suisan Zoyoshoku Sosho **11**: 104.

**Irlandi, E.A., and Mehlich, M.E. 1996.** The effect of tissue cropping and disturbance by browsing fishes on growth of two species of suspension-feeding bivalves. Journal of Experimental Marine Biology and Ecology **197**(2): 279-293.

**International Union for the Conservation of Nature 2010.** IUCN Redlist. Available from http://www.iucnredlist.org/ [accessed 03/28/2010].

**Ivanova, N.V., Dewaard, J.R., and Hebert, P.D.N. 2006.** An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes **6**(4): 998-1002.

**Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J., Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange, C.B., Lenihan, H.S., Pandolfi, J.M., Peterson, C.H., Steneck, R.S., Tegner, M.J., and Warner, R.R. 2001.** Historical overfishing and the recent collapse of coastal ecosystems. Science **293**(5530): 629-638.

**Jameson, R., Kenyona, K., Johnson, A., and Wight, H. 1982.** History and status of translocated sea otter populations in North America. Wildlife Society Bulletin **10**(100-107).

**Jamieson, G.S. 2001.** Review of the status of the Northern Abalone, *Haliotis kamtschatkana*, in Canada. Canadian Field-Naturalist **115**(4): 555-563.

**Jamison, D., Heggen, R., and Lukes, J. 1984.** Underwater video in a regional benthos survey. *In* Proc. Pacific Congress on Marine Technology. Marine Technology Society, Honolulu, Hawaii. pp. 13-15.

**Jensen, G.C. 1995.** Pacific Coast Crabs and Shrimps. Sea Challengers, Monterey, CA.

**Jiang, L., Wu, W.L., and Huang, P.C. 1995.** The mitochondrial DNA of taiwan abalone *Haliotis diversicolor* Reeve, 1846 (Gastropoda: Archaeogastropoda: Haliotidae). Molecular Marine Biology and Biotechnology **4**(4): 353-364.

**Jie, H., Zhinan, Z., Zishan, Y., and Widdows, J. 2001.** Differences in the benthic-pelagic particle flux (biodeposition and sediment erosion) at intertidal sites with and without clam (*Ruditapes philippinarum*) cultivation in eastern China. Journal of Experimental Marine Biology and Ecology **261**: 245-261.

**Johnson, J.E., and Jensen, B.L. 1991.** Hatcheries for endangered freshwater fish. *In* Battle against extinction. *Edited by* W.L. Minckley and J.E. Deacon. University of Arizona Press, Tucson. pp. 199-217.

**Johnson, K.B., and Brink, L.A. 1998.** Predation on bivalve veligers by polychaete larvae. Biological Bulletin **194**(3): 297-303.

**Johnson, K.B., and Shanks, A.L. 2003.** Low rates of predation on planktonic marine invertebrate larvae. Marine Ecology Progress Series **248**: 125-139.

**Johnson, L.L., Landahl, J.T., Kubin, L.A., Horness, B.H., Myers, M.S., Collier, T.K., and Stein, J.E. 1998.** Assessing the effects of anthropogenic stressors on Puget Sound flatfish populations. Journal of Sea Research **39**(1-2): 125-137.

**Johnson, M.S., and Black, R. 1984.** Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution **38**: 1371-1383.

**Kaiser, M.J., Edwards, D.B., and Spencer, B.E. 1996.** Infaunal community changes as a result of commercial clam cultivation and harvesting. Aquatic Living Resources **9**(1): 57-63.

**Kaiser, M.J., Laing, I., Utting, S.D., and Burnell, G.M. 1998.** Environmental impacts of bivalve mariculture. Journal of Shellfish Research **17**(1): 59-66.

**Kalin, R.J. 1984.** Observations of a feeding method of the Atlantic riboon workm, *Cerebratulus lacteus*. Estuaries **7**(2): 179-180.

**Kalinowski, S.T. 2005.** HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes **5**(1): 187-189.

**Kalinowski, S.T. 2006.** HW-QUICKCHECK: an easy-to-use computer program for checking genotypes for agreement with Hardy-Weinberg expectations. Molecular Ecology Notes **6**(4): 974-979.

**Kalinowski, S.T., Wagner, A.P., and Taper, M.L. 2006.** ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes **6**(2): 576-579.

**Kamermans, P., and Huitema, H.J. 1994.** Shrimp (*Crangon crangon*) browsing upon siphon tips inhibits feeding and growth in the bivalve *Macoma balthica*. Journal of Experimental Marine Biology and Ecology **175**(1): 59-75.

**Karl, S.A., and Avise, J.C. 1992.** Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science **256**: 100-102.

**Kaspar, H.F., Gillespie, P.A., Boyer, I.C., and Mackenzie, A.L. 1985.** Effects of mussel aquaculture on the nitrogen cycle and benthic communities in Kenepuru Sound, Marlborough Sounds, New Zealand. Marine Biology **85**(2): 127-136.

**Kaukinen, K.H., Supernault, K.J., and Miller, K.M. 2004.** Enrichment of tetranucleotide microsatellite loci from invertebrate species. Journal of Shellfish Research **23**(2): 621-626.

**Kautsky, N., and Evans, S. 1987.** Role of biodeposition by *Mytilus edulis* in the circulation of matter and nutrients in a Baltic coastal ecosystem. Marine Ecology Progress Series **38**(3): 201-212.

**Kellison, G.T., Eggleston, D.B., and Burke, J.S. 2000.** Comparative behaviour and survival of hatchery-reared versus wild summer flounder (*Paralichthys dentatus*). Canadian Journal of Fisheries and Aquatic Sciences **57**(9): 1870-1877.

**Kennish, M.J., Haag, S.M., Sakowicz, G.P., and Durand, J.B. 2004.** Benthic macrofaunal community structure along a well-defined salinity gradient in the Mullica River Great Bay estuary. Journal of Coastal Research: 209-226.

**Kent, M.L., Elston, R.A., Nerad, T.A., and Sawyer, T.K. 1987.** An isonema-like flagellate (protozoa, mastigophora) infection in larval geoduck clams, *Panope abrupta*. Journal of Invertebrate Pathology **50**(3): 221-229.

**Kimmerer, W.J., Gartside, E., and Orsi, J.J. 1994.** Predation by an introduction clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. Marine Ecology Progress Series **113**(1-2): 81-93.

**Kimura, M., and Crow, J.F. 1963.** Measurement of effective population number. Evolution **17**(3): 279-&.

**King, J.J. 1986.** Juvenile feeding ontogeny of the geoduck *Panope abrupta* (Bivalvia saxicavacea) and comparative ontogeny and evolution of feeding in bivalves, M. Sc. Thesis. University of Victoria, Victoria.

**Kingsley-Smith, P.R., Richardson, C.A., and Seed, R. 2003.** Stereotypic and size-selective predation in *Polinices pulchellus* (Gastropoda : Naticidae) Risso 1826. Journal of Experimental Marine Biology and Ecology **295**(2): 173-190.

**Kirby, V.L., Villa, R., and Powers, D.A. 1998.** Identification of microsatellites in the California red abalone, *Haliotis rufescens*. Journal of Shellfish Research **17**(3): 801-804.

**Kiyomoto, S., and Yamasaki, M. 1999.** Size dependent changes in habitat, distribution and food habit of juvenile disc abalone *Haliotis discus discus* on the coast of the Nagasaki Prefecture, southwest Japan. Bulletin of the Tohoku National Fisheries Research Institute **62**: 71-81.

**Knowlton, N. 1993.** Sibling species in the sea. Annual Review of Ecology and Systematics **24**: 189-216.

**Knowlton, N. 2000.** Molecular genetic analyses of species boundaries in the sea. Hydrobiologia **420**: 73-90.

**Koehn, R.K., Milkman, R., and Mitton, J.B. 1976.** Population genetics of marine pelecypods. 4. Selection, migration, and genetic differentiation in the blue mussel *Mytilus edulis*. Evolution **30**(1): 2-32.

**Kojima, H. 1995.** Evaluation of abalone stock enhancement through the release of hatchery-reared seeds. Marine and Freshwater Research **46**(3): 689-695.

**Kong, L.F., and Li, Q. 2007.** Genetic comparison of cultured and wild populations of the clam *Coelomactra antiquata* (Spengler) in China using AFLP markers. Aquaculture **271**(1-4): 152-161.

**Krost, P., Chrzan, T., Schomann, H., and Rosenthal, H. 1994.** Effects of a floating fish farm in Kiel Fjord on the sediment. Journal of Applied Ichthyology **10**(4): 353-361.

**Kumar, S., Nei, M., Dudley, J., and Tamura, K. 2008.** MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics **9**(4): 299-306.

**Kupriyanova, E.K. 2006.** Fertilization success in *Galeolaria caespitosa* (Polychaeta : Serpulidae): gamete characteristics, role of sperm dilution, gamete age, and contact time. Scientia Marina **70**: 309-317.

**Kvitek, R.G., Bowlby, C.E., and Staedler, M. 1993.** Diet and foraging behavior of sea otters in Southeast Alaska. Marine Mammal Science **9**(2): 168-181.

**Kvitek, R.G., Iampietro, P., and Bowlby, C.E. 1998.** Sea otters and benthic prey communities: A direct test of the sea otter as keystone predator in Washington State. Marine Mammal Science **14**(4): 895-902.

**Kvitek, R.G., and Oliver, J.S. 1992.** Influence of sea otters on soft-bottom prey communities in Southeast Alaska. Marine Ecology Progress Series **82**(2): 103-113.

**Kvitek, R.G., Oliver, J.S., Degange, A.R., and Anderson, B.S. 1992.** Changes in Alaskan soft-bottom prey communities along a gradient in sea otter predation. Ecology **73**(2): 413-428.

**Lacoste, A., Jalabert, F., Malham, S., Cueff, A., Gelebart, F., Cordevant, C., Lange, M., and Poulet, S.A. 2001.** A *Vibrio splendidus* strain is associated with summer mortality of juvenile oysters *Crassostrea gigas* in the Bay of Morlaix (North Brittany, France). Diseases of Aquatic Organisms **46**(2): 139-145.

**Laerm, J., Avise, J.C., Patton, J.C., and Lansman, R.A. 1982.** Genetic determination of the status of an endangered species of pocket gopher in Georgia. Journal of Wildlife Management **46**(2): 513-518.

**Lallias, D., Taris, N., Boudry, P., Bonhomme, F., and Lapegue, S. 2010.** Variance in the reproductive success of flat oyster *Ostrea edulis* L. assessed by parentage analyses in natural and experimental conditions. Genetics Research **92**(3): 175-187.

**Le Roux, F., Gay, M., Lambert, C., Waechter, M., Poubalanne, S., Chollet, B., Nicolas, J.L., and Berthe, F. 2002.** Comparative analysis of Vibrio splendidus-related strains isolated during Crassostrea gigas mortality events. Aquatic Living Resources **15**(4): 251-258.

**Leber, K.M., Cantrell, R.N., and Leung, P.S. 2005.** Optimizing cost-effectiveness of size at release in stock enhancement programs. North American Journal of Fisheries Management **25**(4): 1596-1608.

**LeDeuff, R.M., Renault, T., and Gerard, A. 1996.** Effects of temperature on herpes-like virus detection among hatchery-reared larval Pacific oyster *Crassostrea gigas*. Diseases of Aquatic Organisms **24**(1): 149-157.

**Lee, J.S.F., and Berejikian, B.A. 2009.** Structural complexity in relation to the habitat preferences, territoriality, and hatchery rearing of juvenile China rockfish (*Sebastes nebulosus*). Environmental Biology of Fishes **84**(4): 411-419.

**Lee, Y., and Vacquier, V.D. 1992.** The divergence of species-specific abalone sperm lysin is promoted by positive Darwinian selection. Biological Bulletin **182**(1): 97-104.

**Lehane, C., and Davenport, J. 2004.** Ingestion of bivalve larvae by *Mytilus edulis*: experimental and field demonstrations of larviphagy in farmed blue mussels. Marine Biology **145**(1): 101-107.

**Lehane, C., and Davenport, J. 2006.** A 15-month study of zooplankton ingestion by farmed mussels (*Mytilus edulis*) in Bantry Bay, Southwest Ireland. Estuarine Coastal and Shelf Science **67**(4): 645-652.

**Leighton, D.L. 2000.** The biology and culture of the California abalones. Dorrance Publishing Co. Inc., Pittsburgh, Pennyslvania.

**Lemay, M.A., and Boulding, E.G. 2009.** Microsatellite pedigree analysis reveals high variance in reproductive success and reduced genetic diversity in hatchery-spawned northern abalone. Aquaculture **295**(1-2): 22-29.

**Levitan, D. 1995.** The ecology of fertilization in free-spawning invertebrates. *In* Ecology of Marine Invertebrate Larvae. *Edited by* L.R. McEdward. CRC Press, Boca Raton, Florida. pp. 123-156.

**Levitan, D., Sewell, M., and Chia, F. 1992.** How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. Ecology **73**: 248-254.

**Lewis, T.L., Daniel Esler, W. Sean Boyd. 2007.** Effects of predation by sea ducks on clam abundance in soft-bottom intertidal habitats. Marine Ecology Progress Series **329**: 131–144.

**Li, Q. 2004.** Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (*Haliotis discus hannai*). Aquaculture **235**: 207-222.

**Li, Q., Xu, K., and Yu, R. 2007.** Genetic variation in Chinese hatchery populations of the Japanese scallop (*Patinopecten yessoensis*) inferred from microsatellite data. Aquaculture **269**(1-4): 211-219.

**Li, R.H., L, Q., and Yu, R.H. 2009.** Parentage determination and effective population size estimation in mass spawning Pacific Oyster, *Crassostrea gigas*, based on microsatellite analysis. Journal of the World Aquaculture Society **40**(5): 667-677.

**Lind, C.E., Evans, B.S., Knauer, J., Taylor, J.J.U., and Jerry, D.R. 2009.** Decreased genetic diversity and a reduced effective population size in cultured silver-upped pearl oysters (*Pinctada maxima*). Aquaculture **286**(1-2): 12-19.

**Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.O., Olrog, L., Rehnstam-Holm, A.S., Svensson, J., Svensson, S., and Syversen, U. 2005.** Improving marine water quality by mussel farming: A profitable solution for Swedish society. Ambio **34**(2): 131-138.

**Lindberg, D.R., and Pearse, J.S. 1990.** Experimental manipulation of shell color and morphology of the limpets *Lottia asmi* (Middendorff) and *Lottia digitalis* (Rathke) (Mollusca, patellogastropoda). Journal of Experimental Marine Biology and Ecology **140**(3): 173-185.

**Liu, M., and de Mitcheson, Y.S. 2008.** Profile of a fishery collapse: why mariculture failed to save the large yellow croaker. Fish and Fisheries **9**(3): 219-242.

**Long, E., Dutch, M., Aasen, S., Welch, K., and Hameedi, M. 2005.** Spatial extent of degraded sediment quality in Puget Sound (Washington State, U.S.A.) based upon measures of the sediment quality triad. Environmental Monitoring and Assessment **111**(1): 173-222.

**Longo, F.J. 1987.** Fertilization. Chapman and Hall, New York.

**Lynch, M. 1991.** The genetic interpretation of inbreeding depression and outbreeding depression. Evolution **45**(3): 622-629.

**Lynch, M., and O'Hely, M. 2001.** Captive breeding and the genetic fitness of natural populations. Conservation Genetics **2**(4): 363-378.

**Mallet, A.L., Carver, C.E., and Landry, T. 2006.** Impact of suspended and off-bottom Eastern oyster culture on the benthic environment in eastern Canada. Aquaculture **255**(1-4): 362-373.

**Mallia, J.V., Muthiah, P., and Thomas, P.C. 2006.** Growth of triploid oyster, *Crassostrea madrasensis* (Preston). Aquaculture Research **37**(7): 718-724.

**Manriquez, P.H., Lagos, N.A., Jara, M.E., and Castilla, J.C. 2009.** Adaptive shell color plasticity during the early ontogeny of an intertidal keystone snail. Proceedings of the National Academy of Sciences of the United States of America **106**(38): 16298-16303.

**Mariani, S., Ketmaier, V., and de Matthaeis, E. 2002.** Genetic structuring and gene flow in *Cerastoderma glaucum* (Bivalvia: Cardiidae): evidence from allozyme variation at different geographic scales. Marine Biology **140**: 687-697.

**Marshall, D.J., Monro, K., Bode, M., Keough, M.J., and Swearer, S. 2010.** Phenotype-environment mismatches reduce connectivity in the sea. Ecology Letters **13**(1): 128-140.

**Marshall, W.L., Bower, S.M., and Meyer, G.R. 2003.** A comparison of the parasite and symbiont fauna of cohabiting native (*Protothaca staminea*) and introduced (*Venerupis philippinarum* and *Nuttalia obscurata*) clams in British Columbia. Journal of Shellfish Research **22**(1): 185-192.

**Martincic, B. 1998.** Modello di carrying capacity applicato alle mitilicolture in sospensione., University of Florence, Italy, Florence.

**Matson, S.E., Camara, M.D., Elchert, W., and Banks, M.A. 2008.** P-LOCI: a computer program for choosing the most efficient set of loci for parentage assignment. Molecular Ecology Resources **8**(4): 765-768.

**Matsui, M., Ito, H., Shimada, T., Ota, H., Saidapur, S.K., Khonsue, W., Tanaka-Ueno, T., and Wu, G.F. 2005.** Taxonomic relationships within the Pan-Oriental narrow-mouth toad *Microhyla ornata* as revealed by mtDNA analysis (Amphibia, Anura, Microhylidae). Zoological Science **22**(4): 489-495.

**Mauzey, K.P., Birkeland, C., and Dayton, P.K. 1968.** Feeding behavior of asteroids and escape responses of their prey in the Puget Sound region. Ecology **49**: 603-619.

**May, R.M., Hassell, M.P., Anderson, R.M., and Tonkyn, D.W. 1981.** Density dependence in host-parasitoid models. Journal of Animal Ecology **50**(3): 855-865.

**Maynard, D.J., Flagg, T.A., and Mahnken, C.V.W. 1995.** A review of seminatural culture strategies for enhancing the post-release survival of anadromous salmonids. Uses and Effects of Cultured Fishes in Aquatic Ecosystems **15**: 307-314.

**Mayr, E. 1942.** Systematics and the origin of species, from the viewpoint of a zoologist. Columbia University Press, New York.

**McCall, P.L. 1977.** Community patterns and adaptive strategies of infaunal benthos of Long Island Sound. Journal of Marine Research **35**(2): 221-266.

**McCormick, T.B. 2000.** Abalone (*Haliotis* spp.) aquaculture: present status and a stock enhancement tool. *In* Workshop on rebuilding abalone stocks in British Columbia. *Edited by* A. Campbell. Canadian Special Publication on Fisheries and Aquatic Science.

**McCormick, T.B., and Brogan, J.L. 2003.** Early reproduction in hatchery-raised white abalone, *Haliotis sorenseni*, Bartsch, 1940. Journal of Shellfish Research **22**(3): 825-829.

**McCormick, T.B., Herbinson, K., Mill, T.S., and Altick, J. 1994.** A review of abalone seeding, possible significance and a new seeding device Bulletin of Marine Science **55**(2-3): 680-693.

**McEachron, L.W., Colura, R.L., Bumguardner, B.W., and Ward, R. 1998.** Survival of stocked red drum in Texas. Bulletin of Marine Science **62**(2): 359-368.

**McKindsey, C.W., Thetmeyer, H., Landry, T., and Silvert, W. 2006.** Review of recent carrying capacity models for bivalve culture and recommendations for research and management. Aquaculture **261**(2): 451-462.

**McLean, J.H. 1966.** West American prosobranch and gastropoda: Superfamilies Patellacae, Pleurotomariacea, and Fissurellacea, Stanford University, Palo Alto.

**Meretsky, V.J., Snyder, N.F.R., Beissinger, S.R., Clendenen, D.A., and Wiley, J.W. 2000.** Demography of the California Condor: Implications for reestablishment. Conservation Biology **14**(4): 957-967.

**Metz, E.C., Robles-Sikisaka, R., and Vacquier, V.D. 1998.** Nonsynonymous substitution in abalone sperm fertilization genes exceeds substitution in introns and mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America **95**(18): 10676-10681.

**Meyer, F.P. 1991.** Aquaculture disease and health management. Journal of Animal Science **69**: 4201-4208.

**Meyer, J.J., and Byers, J.E. 2005.** As good as dead? Sublethal predation facilitates lethal predation on an intertidal clam. Ecology Letters **8**(2): 160-166.

**Meyers, T.R. 1979.** Preliminary studies on a chlamydial agent in the digestive diverticular epithelium of hard clams, *Mercenaria mercenaria* (L) from great South Bay, New York. Journal of Fish Diseases **2**(3): 179-189.

**Micheli, F. 1997.** Effects of predator foraging behavior on patterns of prey mortality in marine soft bottoms. Ecological Monographs **67**(2): 203-224.

**Miller, K.M., Supernault, K.J., Li, S.R., and Withler, R.E. 2006.** Population structure in two marine invertebrate species (*Panopea abrupta* and *Strongylocentrotus franciscanus*) targeted for aquaculture and enhancement in British Columbia. Journal of Shellfish Research **25**(1): 33-42.

**Miner, C.M., Altstatt, J.M., Raimondi, P.T., and Minchinton, T.E. 2006.** Recruitment failure and shifts in community structure following mass mortality limit recovery prospects of black abalone. Marine Ecology-Progress Series **327**: 107-117.

**Moberg, P.E., and Burton, R.S. 2000.** Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. Marine Biology **136**(5): 773-784.

**Moller, P., and Rosenberg, R. 1983.** Recruitment, abundance and production of *Mya arenaria* and *Cardium edule* in marine shallow waters, Western Sweden. Ophelia **22**(1): 33-55.

**Moore, J.D., Finley, C.A., Robbins, T.T., and Friedman, C.S. 2002.** Withering syndrome and restoration of southern California abalone populations. California Cooperative Oceanic Fisheries Investigations Reports **43**: 112-117.

**Moore, J.D., Robbins, T.T., Hedrick, R.P., and Friedman, C.S. 2001.** Transmission of the Rickettsiales-like prokaryote "*Candidatus* xenohaliotis californiensis" and its role in Withering syndrome of California abalone, *Haliotis* spp. Journal of Shellfish Research **20**(2): 867-874.

**Moore, K.A., Neckles, H.A., and Orth, R.J. 1996.** *Zostera marina* (eelgrass) growth and survival along a gradient of nutrients and turbidity in the lower Chesapeake Bay. Marine Ecology Progress Series **142**: 247-259.

**Moore, K.A., and Wetzel, R.L. 2000.** Seasonal variations in eelgrass (*Zostera marina L.*) responses to nutrient enrichment and reduced light availability in experimental ecosystems. Journal of Experimental Marine Biology and Ecology **244**: 1-28.

**Morris, R., Abbott, D., and Haderlie, E. 1980.** Intertidal Invertebrates of California. Stanford University Press, Stanford, Califonia.

**Morrisey, D.J., Howitt, L., Underwood, A.J., and Stark, J.S. 1992.** Spatial variation in soft-sediment benthos. Marine Ecology Progress Series **81**(2): 197-204.

**Morrison, C., and Shum, G. 1982.** Chlamydia-like organisms in the digestive diverticula of the bay scallop, *Argopecten irradians*. Journal of Fish Diseases **5**(3): 173-184.

**Morrison, D.F. 1967.** Multivariate statistical methods. McGraw-Hill, New York.

**Munroe, D., and McKinley, R.S. 2007.** Commercial Manila clam (*Tapes philippinarum*) culture in British Columbia, Canada: The effects of predator netting on intertidal sediment characteristics. Estuarine, Coastal and Shelf Science **72**(1-2): 319-328.

**Muse, B. 1998.** Management of the British Columbia Geoduck Fishery, Alaska Commercial Fisheries Entry Commission, Juneau, Alaska.

**Nakano, T., and Spencer, H.G. 2007.** Simultaneous polyphenism and cryptic species in an intertidal limpet from New Zealand. Molecular Phylogenetics and Evolution **45**(2): 470-479.

**Nakaoka, M. 2000.** Nonlethal effects of predators on prey populations: predator-mediated change in bivalve growth. Ecology **81**(4): 1031-1045.

**National Oceanic and Atmospheric Administration 2009.** NOAA Fisheries Office of Protected Resources: Species of Concern. Available from http://www.nmfs.noaa.gov/pr/species/concern/#list [accessed April 26 2009].

**Naylor, R.L., Williams, S.L., and Strong, D.R. 2001.** Ecology - Aquaculture - A gateway for exotic species. Science **294**(5547): 1655-1656.

**Nei, M., Maruyama, T., and Chakraborty, R. 1975.** The bottleneck effect and genetic variability in populations. Evolution **29**: 1-10.

**Nell, J.A., and Perkins, B. 2005.** Studies on triploid oysters in Australia: Farming potential of all-triploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Port Stephens, New South Wales, Australia. Aquaculture Research **36**(6): 530-536.

**Newell, R.C. 2007.** A framework for developing "ecological carrying capacity" mathematical models for bivalve mollusk aquaculture. Bulletin of the Fisheries Research Agency **19**: 41-51.

**Newell, R.I.E. 2004.** Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. Journal of Shellfish Research **23**(1): 51-61.

**Newell, R.I.E., and Koch, E.W. 2004.** Modeling seagrass density and distribution in response to changes in turbidity stemming from bivalve filtration and seagrass sediment stabilization. Estuaries **27**(5): 793-806.

**Nizzoli, D., Bartoli, M., and Viaroli, P. 2006.** Nitrogen and phosphorous budgets during a farming cycle of the Manila clam *Ruditapes philippinarum*: An in situ experiment. Aquaculture **261**(1): 98-108.

**Noakes, D.J., and Campbell, A. 1992.** Use of geoduck clams to indicate changes in the marine environment of Ladysmith Harbor, British Columbia. Environmetrics **3**(1): 81-97.

**Norkko, A., Cummings, V.J., Thrush, S.F., Hewitt, J.E., and Hume, T. 2001.** Local dispersal of juvenile bivalves: implications for sandflat ecology. Marine Ecology-Progress Series **212**: 131-144.

**Norling, P., and Kautsky, N. 2007.** Structural and functional effects of *Mytilus edulis* on diversity of associated species and ecosystem functioning. Marine Ecology Progress Series **351**: 163-175.

**Norton, J.H., Shepherd, M.A., Abdonnaguit, M.R., and Lindsay, S. 1993.** Mortalities in the giant clam *Hippopus hippopus* associated with rickettsiales like organisms. Journal of Invertebrate Pathology **62**(2): 207-209.

**Olafsson, E.B., Peterson, C.H., and Ambrose, W.G. 1994.** Does recruitment limitation structure populations and communities of macroinvertebrates in marine soft sediments – the relative significance of presettlement and postsettlement processes. *In* Oceanography and Marine Biology, Vol 32. pp. 65-109.

**Olla, B.L., Davis, M.W., and Ryer, C.H. 1998.** Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. Bulletin of Marine Science **62**(2): 531-550.

**Orensanz, J.M. 1989.** Studies on growth and population dynamics of a stable native littleneck clam (*Protothaca staminea*) population (Garrison Bay, North Puget Sound) and its cancrid crab predators (*Cancer* spp). Ph.D., School of Aquatic and Fishery Sciences, University of Washington, Seattle.

**Orensanz, J.M., Hand, C.M., Parma, A.M., Valero, J., and Hilborn, R. 2004.** Precaution in the harvest of Methuselah's clams - the difficulty of getting timely feedback from slow-paced dynamics. Canadian Journal of Fisheries and Aquatic Sciences **61**(8): 1355-1372.

**Paillard, C., Le Roux, F., and Borreg, J.J. 2004.** Bacterial disease in marine bivalves, a review of recent studies: Trends and evolution. Aquatic Living Resources **17**(4): 477-498.

**Palacios, R. 1994.** Individual growth and dynamics of living and extinct soft shell clam (*Mya arenaria*) populations in Grays Harbor, Washington, and ecological interactions with Dungeness crab (*Cancer magister*). Ph.D., School of Aquatic and Fishery Sciences, University of Washington, Seattle.

**Palmer, M.A. 1988.** Dispersal of marine meiofauna – a review and conceptual model explaining passive transport and active emergence with implications for recruitment. Marine Ecology Progress Series **48**(1): 81-91.

**Parker, M.S., Jumars, P.A., and Leclair, L.L. 2003.** Population genetics of two bivalve species (*Protothaca staminea* and *Macoma balthica*) in Puget Sound, Washington. Journal of Shellfish Research **22**(3): 681-688.

**Pearman, P.B. 2001.** Conservation value of independently evolving units: Sacred cow or testable hypothesis? Conservation Biology **15**(3): 780-783.

**Pechenik, J.A., Blanchard, M., and Rotjan, R. 2004.** Susceptibility of larval *Crepidula fornicata* to predation by suspension-feeding adults. Journal of Experimental Marine Biology and Ecology **306**(1): 75-94.

**Peel, D., Ovenden, J.R., and Peel, S.L. 2004.** NeEstimator: software for estimating effective population size, Version 1.3. Queensland Government, Department of Primary Industries and Fisheries.

**Peitso, E., Hui, E., Hartwick, B., and Bourne, N. 1994.** Predation by the naticid gastropod *Polinices Lewisii* (Gould) on littleneck clams *Protothaca staminea* (Conrad) in British Columbia. Canadian Journal of Zoology-Revue Canadienne De Zoologie **72**(2): 319-325.

**Perry, M.C., and Uhler, F.M. 1988.** Food habits and distribution of wintering canvasbacks, *Aythya valisineria*, on Chesapeake Bay. Estuaries **11**: 57-67.

**Petersen, J.L., Ibarra, A.M., and May, B. 2010.** Nuclear and mtDNA lineage diversity in wild and cultured Pacific lion-paw scallop, *Nodipecten subnodosus* (Baja California Peninsula, Mexico). Marine Biology **157**(12): 2751-2767.

**Peterson, B.J., and Heck, K.L. 2001.** An experimental test of the mechanism by which suspension feeding bivalves elevate seagrass productivity. Marine Ecology-Progress Series **218**: 115-125.

**Peterson, C.H. 1982.** The importance of predation and intra-and interspecific competition in the population biology of two infaunal suspension-feeding bivalves, *Prototahca staminea* and *Chione undatella*. Ecological Monographs **52**: 437-475.

**Peterson, C.H., and Quammen, M.L. 1982.** Siphon nipping – its importance to small fishes and its impact on growth of the bivalve *Protothaca staminea* (Conrad). Journal of Experimental Marine Biology and Ecology **63**(3): 249-268.

**Peterson, C.H., Summerson, H.C., and Fegley, S.R. 1987.** Ecological consequences of mechanical harvesting of clams. Fishery Bulletin (Washington DC) **85**(2): 281-298.

**Phelps, H.L. 1994.** The Asiatic clam (*Corbicula fluminea*) invasion and system level ecological change in the Potomoc River estuary near Washington DC. Estuaries **17**(3): 614-621.

**Piferrer, F., Baker, I.J., and Donaldson, E.M. 1993.** Effects of natural, synthetic, aromatizable, and nonaromatizable androgens in inducing male sex-differentiation in genotypic female chinook salmon (*Oncorhynchus tshawtscha)*. General and Comparative Endocrinology **91**(1): 59-65.

**Posada, D. 2008.** jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution **25**(7): 1253-1256.

**Powell, N.E., Hofmann, E.E., Klinck, J.M., and Ray, S.M. 1992.** Modeling oyster populations I. A commentary on filtration rate. Is faster always better? Journal of Shellfish Research **11**(2): 387-398.

**Powers, M.J., Peterson, C.H., Summerson, H.C., and Powers, S.P. 2007.** Macroalgal growth on bivalve aquaculture netting enhances nursery habitat for mobile invertebrates and juvenile fishes. Marine Ecology-Progress Series **339**: 109-122.

**Preston, S.J., Keys, A., and Roberts, D. 2007.** Culturing freshwater pearl mussel *Margaritifera margaritifera*: a breakthrough in the conservation of an endangered species. Aquatic Conservation-Marine and Freshwater Ecosystems **17**(5): 539-549.

**Prezant, R., Rollins, H., and Toll, R. 1990.** Dispersal of adult hard clams as an adjunct to larval recruitment. American Zoologist **30**: 89.

**Purcell, J.E., Cresswell, F.P., Cargo, D.G., and Kennedy, V.S. 1991.** Differential ingestion and digestion of bivalve larvae by the scphozoan *Chrysaora quinquecirrha* and the ctenophore *Mnemiopsis leidyi*. Biological Bulletin **180**(1): 103-111.

**Python Software Foundation. 2010.** IDLE: Python's integrated development environment. Available from http://www.python.org/psf/ [accessed December 12 2010].

**Quillet, E., Foisil, L., Chevassus, B., Chourrout, D., and Liu, F. 1991.** Production of all-triploid and all-female brown trout for aquaculture. Aquatic Living Resources **4**(1): 27-32.

**R Development Team. 2010.** The R project for statistical computing. Available from http://www.r-project.org/ [accessed April 12th 2010].

**Redford, K., and Sanjayan, M.A. 2003.** Retiring Cassandra. Conservation Biology **17**(6): 1473-1474.

**Reed, D.H., and Frankham, R. 2001.** How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution **55**(6): 1095–1103.

**Reusch, T.B.H., and Williams, S.L. 1998.** Variable responses of native eelgrass *Zostera marina* to a non-indigenous bivalve *Musculista senhousia*. Oecologia **113**(3): 428-441.

**Rhoads, D.C., McCall, P.L., and Yingst, J.Y. 1978.** Disturbance and production on estuarine seafloor. American Scientist **66**(5): 577-586.

**Rhymer, J.M., and Simberloff, D. 1996.** Extinction by hybridization and introgression. Annual Review of Ecology and Systematics **27**: 83-109.

**Rice, C.A. 2006.** Effects of shoreline modification on a northern Puget Sound beach: Microclimate and embryo mortality in surf smelt (*Hypomesus pretiosus*). Estuaries and Coasts **29**(1): 63-71.

**Richards, D.V., and Davis, G.E. 1993.** Early warnings of modern population collapse in black abalone *Haliotis cracherodii*, Leach, 1814 at the California Channel Islands. Journal of Shellfish Research **12**(2): 189-194.

**Richardson, H., and Verbeek, N.A.M. 1987.** Diet selection by yearling Northwestern Crows (*Corvus caurinus*) feeding on littleneck clams (*Venerupis japonica*). Auk **104**(2): 263-269.

**Riffell, J.A., Krug, P.J., and Zimmer, R.K. 2004.** The ecological and evolutionary consequences of sperm chemoattraction. Proceedings of the National Academy of Sciences of the United States of America **101**(13): 4501-4506.

**Riginos, C., and Cunningham, C.W. 2005.** Local adaptation and species segregation in two mussel (*Mytilus edulis* x *Mytilus trossulus*) hybrid zones. Molecular Ecology **14**(2): 381-400.

**Ripley, B. 1998.** Life history traits and population processes in marine bivalve molluscs. Ph.D. Dissertation, Massachusetts Institute of Technology, Cambridge.

**Roberge, C., Normandeau, E., Einum, S., Guderley, H., and Bernatchez, L. 2008.** Genetic consequences of interbreeding between farmed and wild Atlantic salmon: insights from the transcriptome. Molecular Ecology **17**(1): 314-324.

**Rogers-Bennett, L. 2007.** Is climate change contributing to range reductions and localized extinctions in northern (*Haliotis kamtschatkana*) and flat (*Haliotis walallensis*) abalones? Bulletin of Marine Science **81**(2): 283-296.

**Rogers-Bennett, L., and Pearse, J.S. 1998.** Experimental seeding of hatchery-reared juvenile red abalone in Northern California. Journal of Shellfish Research **17**(3): 877-880.

**Rogers-Bennett, L., and Pearse, J.S. 2001.** Indirect benefits of marine protected areas for juvenile abalone. Conservation Biology **15**(3): 642-647.

**Ross, D.J., Johnson, C.R., and Hewitt, C.L. 2002.** Impact of introduced seastars *Asterias amurensis* on survivorship of juvenile commercial bivalves *Fulvia tenuicostata*. Marine Ecology-Progress Series **241**: 99-112.

**Ross, D.J., Johnson, C.R., Hewitt, C.L., and Ruiz, G.M. 2004.** Interaction and impacts of two introduced species on a soft-sediment marine assemblage in SE Tasmania. Marine Biology **144**(4): 747-756.

**Rothaus, D.P., Vadopalas, B., and Friedman, C.S. 2008.** Precipitous declines in pinto abalone (*Haliotis kamtschatkana kamtschatkana*) abundance in the San Juan Archipelago, Washington, USA, despite statewide fishery closure. Canadian Journal of Fisheries and Aquatic Sciences **65**(12): 2703-2711.

**Rowell, T.W., and Woo, P. 1990.** Predation by the nemertean worm, *Cerebratulus lacteus* Verrill, on the soft-shell clam, *Mya arenarial* Linnaeus, 1758, and its apparent role in the destruction of a clam flat. Journal of Shellfish Research **9**: 291-297.

**Ruiz, G., Fofonoff, P., Carlton, J., Wonham, M., and Hines, A. 2000.** Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. Annual Review of Ecology and Systematics **31**(481–531).

**Ryman, N., and Laikre, L. 1991.** Effects of supportive breeding on the genetically effective population size. Conservation Biology **5**(3): 325-329.

**Saavedra, C. 1997.** Low effective sizes in hatchery populations of the European oyster (*Ostrea edulis*): Implications for the management of genetic resources. Journal of Shellfish Research **16**(2): 441-446.

**Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W., and Hanski, I. 1998.** Inbreeding and extinction in a butterfly metapopulation. Nature **392**(6675): 491-494.

**Saito, K. 1984.** Ocean ranching of abalones and scallops in Northern Japan. Aquaculture **39**(1-4): 361-373.

**Saitou, N., and Nei, M. 1987.** The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4**(4): 406-425.

**Salvanes, A.G.V., and Braithwaite, V. 2005a.** The need to understand the behaviour of fish reared for mariculture or restocking. ICES Journal of Marine Science **63**: 345-354.

**Salvanes, A.G.V., and Braithwaite, V.A. 2005b.** Exposure to variable spatial information in the early rearing environment generates asymmetries in social

interactions in cod (*Gadus morhua*). Behavioral Ecology and Sociobiology **59**(2): 250-257.

**Sandberg, E., Tallqvist, M., and Bonsdorff, E. 1996.** The effects of reduced oxygen content on predation and siphon cropping by the brown shrimp, *Crangon crangon.* Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I **17**(1-3): 411-423.

**Sanderson, E.W., Jaiteh, M., Levy, M.A., Redford, K.H., Wannebo, A.V., and Woolmer, G. 2002.** The human footprint and the last of the wild. Bioscience **52**(10): 891-904.

**Sara, G., and Mazzola, A. 2004.** The carrying capacity for Mediterranean bivalve suspension feeders: evidence from analysis of food availability and hydrodynamics and their integration into a local model. Ecological Modeling **179**(3): 281-296.

**Sastry, A. 1979.** Pelecypoda (Excluding Ostreidae). *In* Reproduction of Marine Invertebrates, Vol V. *Edited by* A. Giese and J. Pearse. Plenum Press, New York. pp. 113-292.

**Savini, D., and Occhipinti-Ambrogi, A. 2006.** Consumption rates and prey preference of the invasive gastropod *Rapana venosa* in the Northern Adriatic Sea. Helgoland Marine Research **60**(2): 153-159.

**Schiel, D.R. 1992.** The enhancement of paua (*Haliotis iris* Martyn) populations in New Zealand. *In* Abalone of the world: their biology, fisheries and culture. *Edited by* S.A. Shepherd, M.J. Tegner and S.A. Guzman del Proo. Blackwell Scientific, Oxford, UK.

**Schiel, D.R., and Welden, B.C. 1987.** Responses to predators of cultured and wild red abalone, *Haliotis rufescens* in laboratory experiments. Aquaculture **60**(3-4): 173-188.

**Sekino, M., Saido, T., Fujita, T., Kobayashi, T., and Takami, H. 2005.** Microsatellite DNA markers of Ezo abalone (*Haliotis discus hannai*): a preliminary assessment of natural populations sampled from heavily stocked areas. Aquaculture **243**(1-4): 33-47.

**Selvamani, M.J.P., Degnan, S.M., and Degnan, B.N. 2001.** Microsatellite genotyping of individual abalone larvae: Parentage assignment in aquaculture. Marine Biotechnology 3(5): 478-485.

**Shaklee, J.B., and Bentzen, P. 1998.** Genetic identification of stocks of marine fish and shellfish. Bulletin of Marine Science **62**(2): 589-621.

**Shanks, A.L., and Brink, L. 2005.** Upwelling, downwelling, and cross-shelf transport of bivalve larvae: test of a hypothesis. Marine Ecology Progress Series **302**(1): 1-12.

**Shapiro, S.S., and Wilk, M.B. 1965.** An analysis of variance test for normality (complete samples). Biometrika **52**: 591-&.

**Shaul, W., and Goodwin, L. 1982.** Geoduck (*Panope generosa*, bivalvia) age as determined by internal growth lines in the shell. Canadian Journal of Fisheries and Aquatic Sciences **39**(4): 632-636.

**Shepherd, S.A. 1973.** Studies on Southern Australian abalone (genus *Haliotis*). 1. Ecology of 5 sympatric species Australian Journal of Marine and Freshwater Research **24**(3): 217-257.

**Shepherd, S.A., and Brown, L.D. 1993.** What is an abalone stock--implications for the role of refugia in conservation. Canadian Journal of Fisheries and Aquatic Sciences **50**(9): 2001-2009.

**Shepherd, S.A., Rodda, K.R., and Vargas, K.M. 2001.** A chronicle of collapse in two abalone stocks with proposals for precautionary management. Journal of Shellfish Research **20**(2): 843-856.

**Shull, D.H. 1997.** Mechanisms of infaunal polychaete dispersal and colonization in an intertidal sandflat. Journal of Marine Research **55**(1): 153-179.

**Shumway, S.E., Cucci, T.L., Newell, R.C., and Yentch, T.M. 1985.** Particle selection, ingestion, and absorption in filter-feeding bivalves. Journal of Experimental Marine Biology and Ecology **91**: 77-92.

**Simenstad, C.A., and Fresh, K.L. 1995.** Influence of intertidal aquaculture on benthic communities in Pacific Northwest estuaries: scales of disturbance. Estuaries **18**: 43-70.

**Skalamera, J.-P., Renaud, F., Raymond, M., and de Meeus, T. 1999.** No Evidence for genetic differentiation of the mussel *Mytilus galloprovincialis* between lagoons and the seaside. Marine Ecology Progress Series **178**: 251-258.

**Sloan, N.A., and Breen, P.A. 1988.** Northern abalone, *Haliotis kamtschatkana*, in British Columbia: fisheries and synopsis of life history information. Canadian Special Publication of Fisheries and Aquatic Science **103**: 46.

**Sloan, N.A., and Robinson, M.C. 1983.** Winter feeding by asteroids on a subtidal sandbed in British Columbia. Ophelia **22**: 125-140.

**Sloan, N.A., and Robinson, S.M.C. 1984.** Age and gonad development in the geoduck clam *Panope abrupta* (Conrad) from southern British Columbia, Canada. Journal of Shellfish Research **4**(2): 131-137.

**Smaal, A.C., Prins, T.C., Dankers, N., and Ball, B. 1997.** Minimum requirements for modeling bivalve carrying capacity. Aquatic Ecology **31**(4): 423-428.

**Smith, F., and Witman, J.D. 1999.** Species diversity in subtidal landscapes: Maintenance by physical processes and larval recruitment. Ecology **80**(1): 51-69.

**Smith, M.E., and Fuiman, L.A. 2004.** Behavioral performance of wild-caught and laboratory-reared red drum *Sciaenops ocellatus* (Linnaeus) larvae. Journal of Experimental Marine Biology and Ecology **302**(1): 17-33.

**Snelgrove, P.V.R., Grant, J., and Pilditch, C.A. 1999.** Habitat selection and adult-larvae interactions in settling larvae of soft-shell clam *Mya arenaria*. Marine Ecology Progress Series **182**: 149-159.

**Soletchnik, P., Le Moine, O., Faury, N., Razet, D., Geairon, P., and Goulletquer, P. 1999.** Summer mortality of the oyster in the Bay Marennes-Oleron: Spatial variability of environment and biology using a geographical information system (GIS). Aquatic Living Resources **12**(2): 131-143.

**Sorokin, II, Giovanardi, O., Pranovi, F., and Sorokin, P.I. 1999.** Need for restricting bivalve culture in the southern basin of the Lagoon of Venice. Hydrobiologia **400**: 141-148.

**Sotka, E.E., Wares, J.P., Barth, J.A., Grosberg, R.K., and Palumbi, S.R. 2004.** Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. Molecular Ecology **13**(8): 2143-2156.

**Soule, M.E. 1985**. What is conservation biology? Bioscience **35**(11): 727-734.

**Spencer, B.E., Edwards, D.B., and Millican, P.F. 1992**. Protecting Manila clam (*Tapes philippinarum*) beds with plastic netting. Aquaculture **105**(3-4): 251-268.

**Spencer, B.E., Kaiser, M.J., and Edwards, D.B. 1996.** The effect of Manila clam cultivation on an intertidal benthic community: the early cultivation phase. Aquaculture Research **27**: 261-276.

**Spencer, B.E., Kaiser, M.J., and Edwards, D.B. 1997.** Ecological effects of intertidal Manila clam cultivation: observations at the end of the cultivation phase. The Journal of Applied Ecology **34**(2): 444-452.

**Spencer, B.E., Kaiser, M.J., and Edwards, D.B. 1998.** Intertidal clam harvesting: benthic community change and recovery. Aquaculture Research **29**(6): 429-437.

**Steffens, W. 2008.** Significance of aquaculture for the conservation and restoration of sturgeon populations. Bulgarian Journal of Agricultural Science **14**(2): 155-164.

**Stein, J.E., Collier, T.K., Reichert, W.L., Casillas, E., Hom, T., and Varanasi, U. 1993.** Bioindicators of contaminant exposure and sublethal effects in benthic fish from Puget Sound, WA, USA. Marine Environmental Research **35**(1-2): 95-100.

**Stevens, B.G., Armstrong, D.A., and Cusimano, R. 1982.** Feeding habits of the Dungeness crab *Cancer magister* as determined by the index of relative importance. Marine Biology **72**(2): 135-145.

**Stoner, A.W., and Davis, M. 1994.** Experimental outplanting of juvenile Queen Conch, *Strombus gigas* - comparison of wild and hatchery-reared stocks Fishery Bulletin **92**(2): 390-411.

**Stoner, A.W., and Ray-Culp, M. 2000.** Evidence for Allee effects in an over-harvested marine gastropod: density-dependent mating and egg production. Marine Ecology-Progress Series **202**: 297-302.

**Storfer, A. 1996.** Quantitative genetics: A promising approach for the assessment of genetic variation in endangered species. Trends in Ecology & Evolution **11**(8): 343-348.

**Strohmeier, T., Aure, J., Duinker, A., Castberg, T., Svardal, A., and Strand, O. 2005.** Flow reduction, seston depletion, meat content and distribution of diarrhetic shellfish toxins in a long-line blue mussel (*Mytilus edulis*) farm. **24**(1): 15(19).

**Strom, A., Francis, R.C., Mantua, N.J., Miles, E.L., and Peterson, D.L. 2004.** North Pacific climate recorded in growth rings of geoduck clams: A new tool for paleoenvironmental reconstruction. Geophysical Research Letters **31**(6).

**Stuart, B.L., Inger, R.F., and Voris, H.K. 2006.** High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. Biology Letters **2**(3): 470-474.

**Stunz, G.W., and Minello, T.J. 2001.** Habitat-related predation on juvenile wild-caught and hatchery-reared red drum *Sciaenops ocellatus* (Linnaeus). Journal of Experimental Marine Biology and Ecology **260**(1): 13-25.

**Supernault, K., Demsky, A., Campbell, A., Ming, T., Miller, K., and Withler, R. 2009.** Forensic genetic identification of abalone (*Haliotis* spp.) of the northeastern Pacific Ocean. Conservation Genetics.

**Sweijd, N., Snethlage, Q., Harvey, D., and Cook, P. 1998.** Experimental abalone (*Haliotis midae*) seeding in South Africa. Journal of Shellfish Research **17**(3): 897-904.

**Szekely, T., and Bamberger, Z. 1992.** Predation of waders (Charadrii) on prey populations – An exclosure experiment. Journal of Animal Ecology **61**(2): 447-456.

**Tamburri, M.N., Zimmer, R.K., and Zimmer, C.A. 2007.** Mechanisms reconciling gregarious larval settlement with adult cannibalism. Ecological Monographs **77**(2): 255-268.

**Tamburri, M.N., and ZimmerFaust, R.K. 1996.** Suspension feeding: Basic mechanisms controlling recognition and ingestion of larvae. Limnology and Oceanography **41**(6): 1188-1197.

**Tamura, K. 1992.** Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C content biases. Molecular Biology and Evolution **9**(4): 678-687.

**Tamura, K., and Nei, M. 1993.** Estimation of the number of nucelotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution **10**(3): 512-526.

**Taris, N., Ernande, B., McCombie, H., and Boudry, P. 2006.** Phenotypic and genetic consequences of size selection at the larval stage in the Pacific oyster (*Crassostrea gigas*). Journal of Experimental Marine Biology and Ecology **333**(1): 147-158.

**Tarr, R.J.Q. 2000.** The South African abalone (*Haliotis midae*) fishery: a decade of challenges and change. *In* Workshop on rebuilding abalone stocks in British Columbia. Canadian Special Publication on Fisheries and Aquatic Science. *Edited by* A. Campbell. pp. 32-40.

**Tarr, R.J.Q., Williams, P.V.G., and Mackenzie, A.J. 1996.** Abalone, sea urchins and rock lobster: A possible ecological shift that may affect traditional fisheries. South African Journal of Marine Science-Suid-Afrikaanse Tydskrif Vir Seewetenskap **17**: 319-323.

**Taylor, M.S., and Hellberg, M.E. 2003.** Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science **299**: 107-109.

**Tegner, M.J. 1992.** Brood stock transplants as an approach to abalone stock enhancement. *In* Abalone of the World: biology, fisheries and culture. *Edited by* S.A. Shepherd, M.J. Tegner, and S.A. Guzman del Proo. Fishing News Books, Blackwell Science Ltd, Oxford, U.K. pp. 461-473.

**Tegner, M.J. 1993.** Southern California abalones - can stocks be rebuilt using marine harvest refugia? Canadian Journal of Fisheries and Aquatic Sciences **50**(9): 2010-2018.

**Tegner, M.J., and Butler, R.A. 1985a.** Drift-tube study of the dispersal potential of green abalone (*Haliotis fulgens*) larvae in the Southern California bight - implications for recovery of depleted populations. Marine Ecology Progress Series **26**(1-2): 73-84.

**Tegner, M.J., and Butler, R.A. 1985b.** The survival and mortality of seeded and native red abalones, *Haliotis rufescens*, on the Palos Verdes peninsula. California Fish and Game Bulletin **71**: 150-153.

**Tegner, M.J., and Butler, R.A. 1989.** Abalone seeding. *In* Handbook on the culture of abalone and other marine gastropods. *Edited by* K.O. Hahn. CRC Press, Boca Raton, FL. pp. 157-182.

**Tegner, M.J., and Dayton, P.K. 1977.** Sea-urchin recruitment patterns and implications of commercial fishing. Science **196**(4287): 324-326.

**Tegner, M.J., Haaker, P.L., Riser, K.L., and Vilchis, L.I. 2001.** Climate variability, kelp forests, and the Southern California red abalone fishery. Journal of Shellfish Research **20**(2): 755-763.

**Templeton, A.R., Crandall, K.A., and Sing, C.F. 1992.** A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics **132**(2): 619-633.

**Tenore, K.R., and Dunstan, W.M. 1973.** Comparison of feeding and biodeposition of 3 bivalves at different food levels. Marine Biology **21**(3): 190-195.

**Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997.** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research **25**(24): 4876-4882.

**Thorgaard, G.H., and Allen, S.K. 1988.** Environmental impacts of inbred, hybrid, and polyploid aquatic species. Journal of Shellfish Research **7**: 556.

**Thrush, S.F., Pridmore, R.D., Hewitt, J.E., and Cummings, V.J. 1994.** The importance of predators on a sandflat – interplay between seasonal changes in prey densities and predator effects. Marine Ecology Progress Series **107**(3): 211-222.

**Tissot, B.N. 1995.** Recruitment, growth, and survivorship of black abalone on Santa Cruz Island following mass mortality. Bulletin of the Southern California Academy of Sciences **94**: 179-189.

**Tolimieri, N., and Levin, P. 2004.** Differences in responses of chinook salmon to climate shifts: implications for conservation. Environmental Biology of Fishes **70**(2): 155-167.

**Tomascik, T., and Holmes, H. 2003.** Distribution and abundance of *Haliotis kamtschatkana* in relation to habitat, competitors and predators in the Broken Group Islands, Pacific RIM National Park reserve of Canada. Journal of Shellfish Research **22**(3): 831-838.

**Tomiyama, T., and Omori, M. 2007.** Interactive effects of sublethal predation and body size on siphon production of the bivalve *Nuttallia olivacea*. Journal of Experimental Marine Biology and Ecology **341**(1): 102-109.

**Trainer, V.L., and Bill, B.D. 2004.** Characterization of a domoic acid binding site from Pacific razor clam. Aquatic Toxicology **69**(2): 125-132.

**Trainer, V.L., Cochlan, W.P., Erickson, A., Bill, B.D., Cox, F.H., Borchert, J.A., and Lefebvre, K.A. 2007.** Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. Harmful Algae **6**(3): 449-459.

**Turner, S.J., Thrush, S.F., Pridmore, R.D., Hewitt, J.E., Cummings, V.J., and Maskery, M. 1995.** Are soft-sediment communities stable – an example from a windy harbor. Marine Ecology Progress Series **120**(1-3): 219-230.

**Tymchuk, W.E., Biagi, C., Withler, R., and Devlin, R.H. 2006.** Growth and behavioral consequences of introgression of a domesticated aquaculture genotype into a native strain of Coho salmon. Transactions of the American Fisheries Society **135**(2): 442-455.

**Tymchuk, W.E., Sundstrom, L.F., and Devlin, R.H. 2007.** Growth and survival trade-offs and outbreeding depression in rainbow trout (*Oncorhynchus mykiss*). Evolution **61**(5): 1225-1237.

**United Nations Population Division 2000.** The world at six billion. Available from http://www.un.org/esa/population/publications/sixbillion/sixbilpart1.pdf [accessed 3/29/2010].

**United States Census Bureau. 2010.** U.S. & world population clocks. Available from http://www.census.gov/main/www/popclock.html [accessed 03/29/2010].

**Utter, F. 1998.** Genetic problems of hatchery-reared progeny released into the wild, and how to deal with them. Bulletin of Marine Science **62**(2): 623-640.

**Utter, F., and Epifanio, J. 2002.** Marine aquaculture: Genetic potentialities and pitfalls. Reviews in Fish Biology and Fisheries **12**(1): 59-77.

**Vacquier, V.D., Carner, K.R., and Stout, C.D. 1990.** Species-specific sequences of abalone lysin, the sperm protein that creates a hole in the egg envelope Proceedings of the National Academy of Sciences of the United States of America **87**(15): 5792-5796.

**Vadopalas, B., and Bentzen, P. 2000.** Isolation and characterization of di- and tetranucleotide microsatellite loci in geoduck clams, *Panopea abrupta*. Molecular Ecology **9**(9): 1435-1436.

**Vadopalas, B., and Davis, J. 2004.** Optimal chemical triploid induction in geoduck clams, *Panopea abrupta* (Conrad, 1849), by 6-dimethylaminopurine. Aquaculture **230**(1-4): 29-40.

**Vadopalas, B., Leclair, L.L., and Bentzen, P. 2004.** Microsatellite and allozyme analyses reveal few genetic differences among spatially distinct aggregations of geoduck clams (*Panopea abrupta*, Conrad 1849). Journal of Shellfish Research **23**(3): 693-706.

**Vadopalas, B., Pietsch, T.W., and Friedman, C.S. 2010.** The proper name for the geoduck: resurrection of *Panopea generosa* Gould, 1850, from the synonymy of

*Panopea abrupta* (Conrad, 1849) (Bivalvia: Myoida: Hiatellidae). Malacologia **52**(1): 169-173.

**Vadopalas, B.A. 1999.** Development and optimization of triploid indicution techniques in the geoduck clam, *Panopea abrupta*. Master's, School of Aquatic and Fishery Sciences, University of Washington, Seattle.

**Vadopalas, B.A. 2003.** Population genetics of the geoduck clam, *Panopea abrupta* (Conrad, 1849) in Puget Sound, Washington. Ph. D., School of Aquatic and Fishery Sciences, University of Washington, Seattle.

**Valero, J.L., Hand, C., Orensanz, J.M., Parma, A.M., Armstrong, D., and Hilborn, R. 2004.** Geoduck (*Panopea abrupta*) recruitment in the Pacific Northwest: Long-term changes in relation to climate. California Cooperative Oceanic Fisheries Investigations Reports **45**: 80-86.

**Van der Meeren, G.I., and Uksnoy, L.E. 2000.** A comparison of claw morphology and dominance between wild and cultivated male European lobster. Aquaculture International **8**(1): 77-94.

**Van Koeveringe, M.A.H. 1998.** Molecular population genetics of British Columbia geoduck clams, *Panope abrupta*, based on mitochondrial DNA sequences. M. Sc. Thesis, Simon Fraser University, Vancouver.

**Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004.** MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4**(3): 535-538.

**Van Veldhuizen, H.D., and Phillips, D.W. 1978.** Prey capture by *Pisaster brevispinus* (Asteroidea - echinodermata) on soft substrate. Marine Biology **48**(1): 89-97.

**VanBlaricom, G.R., Ruediger, J.L., Friedman, C.S., Woodard, D.D., and Hedrick, R.P. 1993.** Discovery of withering syndrome among black abalone *Haliotis cracherodii* Leach, 1814, populations at San Nicolas Island, California. Journal of Shellfish Research **12**(2): 185-188.

**Velasquez, D.E. 1992.** Shell structure and fragility in nursery-reared juvenile geoducks *Panopea abrupta* (Conrad). M.Sc. Thesis, School of Fisheries, University of Washington, Seattle.

**Vilchis, L.I., Tegner, M.J., Moore, J.D., Friedman, C.S., Riser, K.L., Robbins, T.T., and Dayton, P.K. 2005.** Ocean warming effects on growth, reproduction, and

survivorship of Southern California abalone. Ecological Applications **15**(2): 469-480.

**Virnstein, R.W. 1977.** The importance of predation by crabs and fishes on benthic infauna in Chesapeake Bay. Ecology **58**: 1199-1217.

**Wang, J., and Santure, A.W. 2009.** Parentage and Sibship Inference From Multilocus Genotype Data Under Polygamy. Genetics **181**(4): 1579-1594.

**Wang, J.L. 2004.** Sibship reconstruction from genetic data with typing errors. Genetics **166**(4): 1963-1979.

**Waples, R.S. 1987.** A Multispecies approach to the analysis of gene flow in marine shore fishes. Evolution **41**(2): 385-400.

**Waples, R.S. 1991.** Genetic interactions between hatchery and wild salmonids - lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences **48**: 124-133.

**Waples, R.S., and Waples, R.K. 2010.** Inbreeding effective population size and parentage analysis without parents. Molecular Ecology Resources **in press**.

**Ward, R.D. 1990.** Biochemical genetic variation in the genus Littorina (Prosobranchia, Mollusca). Hydrobiologia **193**: 53-69.

**Washington Department of Fish and Wildlife. 2009a.** Federally ESA listed fish species for Washington State. Available from http://wdfw.wa.gov/fish/management/esa/federally_listed_esa_fish.pdf [accessed December 13 2009].

**Washington Department of Fish and Wildlife. 2009b.** Species of Concern in Washington State. Available from http://wdfw.wa.gov/wlm/diversty/soc/soc.htm [accessed April 26, 2009.

**Washington Department of Natural Resources. 2000.** The Puget Sound Commercial Geoduck Fishery Management Plan.

**Washington Department of Natural Resources. 2009.** Geoduck Aquaculture Program. Available from http://www.dnr.wa.gov/BusinessPermits/Topics/ShellfishAquaticLeasing/Pages/aqr_aqua_geoduck_aquaculture.aspx [accessed January 13, 2009.

**Weber, E.D., and Fausch, K.D. 2005.** Competition between hatchery-reared and wild juvenile Chinook salmon in enclosures in the Sacramento River, California. Transactions of the American Fisheries Society **134**(1): 44-58.

**Weissberger, E.J. 1999.** Additive interactions between the moon snail *Euspira heros* and the sea star *Asterias forbesi*, two predators of the surfclam *Spisula solidissima*. Oecologia **119**(3): 461-466.

**Whiteley, J., and Bendell-Young, L. 2007.** Ecological implications of intertidal mariculture: observed differences in bivalve community structure between farm and reference sites. Journal of Applied Ecology **44**(3): 495-505.

**Wilke, T., and Falniowski, A. 2001.** The genus *Adriohydrobia* (Hydrobiidae : Gastropoda): polytypic species or polymorphic populations? Journal of Zoological Systematics and Evolutionary Research **39**(4): 227-234.

**Williams, G.D. 1994.** Effect of habitat modification on distribution and diets of intertidal fishes in Grays Harbor estuary, Washington. M. Sc. School of Aquatic and Fishery Sciences, University of Washington, Seattle.

**Williams, M.E., and Bentley, M.G. 2002.** Fertilization success in marine invertebrates: The influence of gamete age. Biological Bulletin **202**(1): 34-42.

**Wilson, W.H. 1990.** Competition and predation in marine soft-sediment communities. Annual Review of Ecology & Systematics **21**: 221-241.

**Winter, J.E. 1978.** A review of the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. Aquaculture **13**: 1-33.

**Withler, R.E. 1988.** Genetic consequences of fertilizing chinook salmon (*Oncorhynchus-tsawtscha*) eggs with pooled milt. Aquaculture **68**(1): 15-25.

**Withler, R.E., and Beacham, T.D. 1994.** Genetic consequences of the simultaneous or sequential addition of semen from multiple males during hatchery spawning of chinook salmon (*Oncorhynchus tsawtscha*). Aquaculture **126**(1-2): 11-23.

**Withler, R.E., Campbell, A., Li, S.R., Brouwer, D., Supernault, K.J., and Miller, K.M. 2003.** Implications of high levels of genetic diversity and weak population structure for the rebuilding of northern abalone in British Columbia, Canada. Journal of Shellfish Research **22**(3): 839-847.

**Wright, S. 1931.** Evolution in Mendelian populations. Genetics **16**(2): 0097-0159.

**Wright, S. 1938.** Size of population and breeding structure in relation to evolution. Science **87**(2263): 430-431.

**Yanick, J.F., Heath, J.W., and Heath, D.D. 2003.** Survival and growth of local and transplanted blue mussels (*Mytilus trossulus,* Lamark). Aquaculture Research **34**(10): 869-875.

**Yonge, C., and Thompson, T. 1976.** Living Marine Molluscs. Collins, London.

**Young, J.W., and Davis, T.L.O. 1992.** Feeding ecology and interannual variations in diet of larval jack mackerel, *Trachurus declivis* (pisces, carangidae), from coastal waters of eastern Tasmania. Marine Biology **113**(1): 11-20.

**Ysebaert, T., and Herman, P.M.J. 2002.** Spatial and temporal variation in benthic macrofauna and relationships with environmental variables in an estuarine, intertidal soft-sediment environment. Marine Ecology Progress Series **244**: 105-124.

**Yu, D.H., and Chu, K.H. 2006.** Genetic variation in wild and cultured populations of the pearl oyster *Pinctada fucata* from southern China. Aquaculture **258**(1-4): 220-227.

**Zacherl, D.C. 2005.** Spatial and temporal variation in statolith and protoconch trace elements as natural tags to track larval dispersal. Marine Ecology Progress Series **290**: 145-163.

**Zajac, R.N., and Whitlatch, R.B. 1982.** Responses of estuarine infauna to disturbance. 1. Spatial and temporal variation of initial recolonization. Marine Ecology-Progress Series **10**(1): 1-14.

**Zajac, R.N., and Whitlatch, R.B. 2003.** Community and population-level responses to disturbance in a sandflat community. Journal of Experimental Marine Biology and Ecology **294**(1): 101-125.

**Zaklan, S.D., and Ydenberg, R. 1997.** The body size burial depth relationship in the infaunal clam *Mya arenaria*. Journal of Experimental Marine Biology and Ecology **215**(1): 1-17.

**Zar, J.H. 1999.** Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.

**Zeldis, J., Robinson, K., Ross, A., and Hayden, B. 2004.** First observations of predation by New Zealand Greenshell mussels (*Perna canaliculus*) on

zooplankton. Journal of Experimental Marine Biology and Ecology **311**(2): 287-299.

**Zhang, Z., and Campbell, A. 2004.** Natural mortality and recruitment rates of the Pacific geoduck clam (*Panopea abrupta*) in experimental plots. Journal of Shellfish Research **23**(3): 675-682.

**Zhang, Z., and Hand, C. 2006.** Recruitment patterns and precautionary exploitation rates for geoduck (*Panopea abrupta*) populations in British Columbia. Journal of Shellfish Research **25**(2): 445-453.

**Zharikov, Y., and Skilleter, G.A. 2003.** Depletion of benthic invertebrates by bar-tailed godwits *Limosa lapponica* in a subtropical estuary. Marine Ecology Progress Series **254**: 151-162.

**Zhou, Y., Yang, H.S., Zhang, T., Liu, S.L., Zhang, S.M., Liu, Q., Xiang, J.H., and Zhang, F.S. 2006.** Influence of filtering and biodeposition by the cultured scallop *Chlamys farreri* on benthic-pelagic coupling in a eutrophic bay in China. Marine Ecology Progress Series **317**: 127-141.

**Zwarts, L., and Wanink, J. 1989.** Siphon size and burying depth in deposit- and suspension-feeding benthic bivalves. Marine Biology **100**(2): 227-240.

**Zydelis, R., Esler, D., Boyd, W.S., LaCroix, D.L., and Kirk, M. 2006.** Habitat use by wintering surf and white-winged scoters: Effects of environmental attributes and shellfish aquaculture. Journal of Wildlife Management **70**(6): 1754-1762.