IDENTIFICATION OF RESEARCH PRIORITIES RELEVANT TO GEODUCK (*PANOPEA ABRUPTA*) AQUACULTURE ENVIRONMENTAL IMPACTS

Prepared for

Washington State Department of Natural Resources

by

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WDNR PHASE II GEODUCK PROPOSAL

General Background and Overview

Introduction

The Washington Department of Natural Resources (WNDR) is initiating a long-term and extensive development program in aquaculture of the geoduck clam (*Panopea abrupta* Conrad, 1849 [*Panope generosa* Gould, 1850]). Commercial scale hatchery rearing and cultivation of this clam began nearly ten years ago. Initial results from these plantings and studies completed by the private sector, the University of Washington, and Washington State Department of Fish and Wildlife (WDFW) lead to the conclusion that the geoduck possesses characteristics that make it an exceptional candidate for commercial culture on both public and private lands. These include: 1) large size at maturity -- one of the largest burrowing clams in the world; 2) fairly rapid growth in relation to other shellfish -- likely to reach market size in 4 to 5 years; 3) naturally occurring -- broodstock are available; and 4) good success when cultivated on privately owned tidelands. In addition, there are strong markets and demand for geoduck, especially in Asia, where they currently sell for up to $10 per pound.

Significant impediments remain, however, to full commercialization of geoduck aquaculture on state lands. While practical considerations of handling and husbandry during hatchery, seeding and intertidal growout phases of culture are relatively well understood by private growers, there is a fundamental lack of understanding of the ecological effects of geoduck culture, and the inter-relationships of cultured and wild-stock animals.

The overarching goal of Phase II geoduck research is to address the community and ecosystem impacts of geoduck aquaculture. These impacts can be categorized into two groups: those that directly affect the geoduck resource such as recruitment, disease, and genetic impacts, and those that affect the environment such as habitat, infauna, and water column impacts. These two categories are not distinct, but are interrelated and equally important in our view. Within these broad categories, we have defined four specific tasks we are proposing as the work plan for Phase II. Again, the tasks, described below, are multifariously interconnected, but have been separated to delineate involvement of personnel. The critical tasks that must be completed before full-scale development can proceed include:

1) *Determine benthic and water column effects of planting, predator protection and harvest, and associated effects of differing planting densities;*
2) *Characterize gametogenesis and reproductive capacity;*
3) *Establish a baseline sample set of disease status in wild populations; and*
4) *Initiate triploid performance and reversion trials*

Many of these tasks will necessitate a study period that covers an entire culture cycle, from hatchery to harvest. A 5-7 year timeline for some of the topics will require funding for more than one biennium. Projected timelines and associated costs for each task are outlined in Table 1.

Table 1. Costs and Timeline for Geoduck Research Tasks

Task #	Task	Yr 1 cost ($1000)	Yr 2 cost ($1000)	Total biennial Cost ($1000)	Projected Duration (years)
1	Community effects	178.0	179.0	357.0	6
2	Genetic Effects	90.0	70.0	160.0	4
3	Disease	15.0	0.0	15.0	3
4	Triploids	8.7	7.6	16.3	7
	Project coordinator (UW)	15.0	15.0	30.0	
TOTALS		**306.7**	**271.6**	**578.3**	

In addition, there are longer-term issues that should be addressed in future funding cycles, once some of the initial questions are answered. These include:

1) *Continue monitoring of effects of geoduck aquaculture on water column and community;*
2) *Conduct genetic analyses to determine the degree of genetic differentiation between cultured and wild geoducks;*
3) *Genotype newly settled geoduck to determine provenance;*
4) *In triploid geoducks, assess gametogenesis, gonad reversion from triploid to diploid, and survivorship in outplanted triploid geoducks;*
5) *Screen wild and cultured geoducks for disease to establish baseline; and*
6) *Conduct controlled releases of geoduck larvae to investigate dispersal potential.*

It must be stressed that to fully address these continuing issues, additional support will be required in the future.

The proposed Phase II research encompasses key facets of the four critical tasks listed above (the longer-term issues will be fully addressed in later phases of this study). These proposed tasks also directly address or touch upon many of the questions posed in the preliminary identification of research needs under Section 9 of Deliverable 1 to WDNR (Phase I), and are reproduced in modified form below.

Reproduction, recruitment, and genetic interactions

- What factors influence settlement (biotic, abiotic, conspecific)?
- What effect does commercial harvest have on subsequent settlement?
- How long do spawned gametes remain viable?
- At what age do geoducks of each sex mature? What are the sex ratios of geoducks during the first 10 years? Are geoducks gonochoristic or protandrous hermaphrodites? This information is necessary to understand the level of genetic risk associated with intertidal or subtidal geoduck aquaculture.

Predator-prey interactions

- What are the densities and distributions of geoduck predators and how do they vary temporally and spatially within Puget Sound?
- How does the spatial distribution of geoducks occurring naturally and in out-planted beds affect the densities and distributions of predators?
- Do predator densities, and thus predation risk to geoducks, vary with respect to tidal elevation?

Community and ecosystem interactions

Suspension feeding activities and water column effects

- Do high densities of geoducks locally deplete phytoplankton and other suspended materials that in turn impact other suspension feeding animals?
- At what spatial or temporal scales do these possible water column effects operate? How do changes in geoduck density affect these interactions?
- Do high densities of geoducks affect water column clarity and light penetration and therefore increase potential for submerged aquatic vegetation? If so, at what spatial scales do these interactions operate?
- How do geoducks affect dissolved oxygen (DO) and to what extent has low DO affected geoduck distribution?

Sediment interactions

- How are sediment properties changed by material processing by geoducks?
- Does alteration of the sediment by adult geoducks influence the recruitment of conspecifics or other species (e.g. tube-building polychaetes) that may in turn help to recruit conspecifics to existing adult geoduck aggregations?
- How do planted geoducks affect species diversity and abundance of other benthic organisms?

The proposed activities are essential for WDNR to progress in the leasing of state lands for the purpose of commercial geoduck culture. They will provide a significant opportunity for expansion of geoduck shellfish culture in subtidal regions where traditional aquaculture practices are not yet possible. The research will also greatly enhance and expand understanding of the biology and community effects of this important shellfish species. While four “tasks” are recommended with this report, they are in no sense intended to be read as independent activities, but are rather arrayed separately for clarity and as help to guide decisions by participants regarding priorities that reflect budgets and logistics. The tasks can be coarsely grouped into two primary categories, and the resultant data are complimentary for intent to help guide DNR’s decisions about leasing and overall management of the resource:

1. Geoduck Life History: Included in this category are gametogenesis, fertilization and larval viability, disease, and feasibility of developing triploid geoducks for commercial production. The research described in these tasks fills voids in information essential to understand maturation dynamics of cultured stocks, build predictive models that assess genetic risk to wild populations; establishes a disease baseline for wild stocks; and initiates a study to characterize both the commercial performance and the reproductive potential of triploid geoducks over the growout cycle.
2. Ecosystem/community Impacts: Tasks within this group include predator-prey dynamics, effects/changes in benthic communities on and adjacent to intensely cultivated lease tracks, and water quality and phytoplankton changes. These topics will be used to access the regional effects of commercial culture on both wild stocks and the broader marine community as means to portray the relative spatial scale of any changes deemed adverse.

Background Information

The geoduck clam is the largest species of clam in the U.S. West Coast, reaching a shell length of at least 8 1/2 inches and a live weight of over 6 pounds. It is found in marine waters from the lower intertidal to depths below 350 feet along the West Coast of North America from Alaska to Baja California. The geoduck has a very high meat yield and has been prized by subsistence and recreational clam harvesters throughout modern history. Within the last ten years commercial interests have burgeoned and retail prices have risen ten-fold to the current price of $10 per pound.

Wild harvests in Washington State and British Columbia each average several million pounds per year, and smaller quantities are taken in Alaska. This makes the geoduck fishery the largest clam fishery on the West Coast, both in terms of value and number of pounds harvested.

The commercial fishery for geoducks in the State of Washington is co-managed by the Departments of Natural Resources (WDNR), Fish and Wildlife (WDFW), and Puget Sound Treaty Tribes. These agencies offer the right to harvest subtidal geoduck clam tracts to Washington owned purchasing companies who hire commercial divers to take the clams one at a time, using a water jet to loosen the clams from the substrate. The commercial and tribal fisheries are constrained by the sustainable yield quotas. When harvesting wild geoduck from subtidal tracts, prospective harvesters bid competitively for the tract and then pay a premium stumpage to the state for the clams harvested as well as an extraction tax. Annual production has been held at or below sustainable levels for the last several years, down from a peak harvest of nearly 9 million pounds in the late 1970's. There are similar harvest management programs in place in British Columbia and Alaska.

Initially, the dominant market for the geoduck clam was for export to Japan where the siphon was sliced for sashimi. A changing Japanese economy reduced that demand and now geoducks are predominantly exported to China, Hong Kong and Singapore, still for use in sashimi and other high value dishes. Domestically, some geoduck are marketed to the Asian communities in major U.S. cities.

Added value coupled with increasing domestic and export demand has led a number of private shellfish farmers in Washington to cultivate geoducks and produce hatchery-reared seed. The WDFW Point Whitney Shellfish Hatchery pioneered the early work to develop a consistent source of geoduck seed with a goal of augmenting natural production. While early efforts to increase geoduck abundance in state waters by out-planting hatchery-produced seed proved unsuccessful, the hatchery technology was a valuable contribution to the understanding of geoduck culture. Recently, commercial aquaculture businesses in Washington State and British Columbia have adapted this technology in commercial plantings made on privately owned or leased, and largely intertidal shellfish grounds. The total area now in production is approximately 100 acres. Initial harvests in Southern Puget Sound indicate a 2 pound geoduck can be raised in 4 to 6 years at harvest densities of up to 100,000 pounds per acre.

Relationship with Future Research or Research and Development

The studies proposed herein address those aspects of geoduck clam culture that we believe are critical to assess prior to initiating commercialization of this species on state-owned intertidal and subtidal lands. This research is designed to evaluate areas of specific importance in the siting and permitting of these activities. In addition, Phase II activities will also address environmentally sensitive planting, husbandry and harvest procedures.

Environmental, Economic, Social, and other Benefits to the State and to Users of the Results

We envision that results gleaned from the proposed studies will be used broadly. The project is developing at a time when the export market for geoducks is very strong and growing and will be of direct benefit to geoduck aquaculture and harvesting enterprises in Washington State. In addition to the direct application of results, it will also benefit the public, as new revenues will be generated from taxes on increased product, and in some cases, lease payments from state-owned marine lands. Key benefits to user groups include:

- Minimize negative long term impacts to the environment
- Minimize negative genetic impacts to wild geoduck stocks
- Increased capability for production of a high value food type having high demand and food value
- Eventual employment growth in both primary and secondary sectors (production, marketing, materials, supply and manufacturing, etc.)

Executive Summary

Geoduck Aquaculture Phase II Proposed Tasks

Task 1: Quantify the effects of geoduck culture practices (planting, predator protection, growout, and harvest) on benthic community structure and water column properties—D. Armstrong, G. Van Blaricom (UW); D. Cheney, J. Davis (PSI)

During the past decade there has been a rapid expansion of intertidal geoduck aquaculture in Washington State and in the use of alternative and innovative methods for planting, cultivation, and harvest of these valuable shellfish. Yet little research has been conducted to assess the ecological impacts of geoduck cultivation practices on benthic habitats, sediment and water column properties, and associated flora and fauna. In this task, we will document and quantify the ecological effects of geoduck farming practices through an entire production cycle (planting, predator protection tubes, grow-out, and harvest) on benthic community structure, fish usage and water column properties. Commercial geoduck farms will be surveyed for infauna, macro-epifauna, large mobile species (shrimps, crabs, finfish), submerged aquatic vegetation (SAV), sediment properties, and water quality parameters to provide a temporal and spatial comparison across habitat types (various stages of geoduck culture) on and off commercial and experimental beds. In addition to monitoring existing geoduck farms, we will use a manipulative experimental approach on state-owned land to gauge the responses of selected parameters to predator protection and changes in geoduck biomass and density at various points in the culture cycle. These data will assist WDNR in evaluating environmental impacts and technical issues that surround geoduck aquaculture.

Task 2: Geoduck gametogensis, fecundity, and larval viability—C. Friedman (UW, and J. Davis (PSI)

While the culture of a native species reduces risks often associated with exotic species, it is important to investigate potential interactions with wild counterparts. Should geoducks cultured in the field become reproductively mature and spawn during the 5-7 year culture cycle, the potential arises for the introduction of cultured genotypes into wild populations. An on-going pilot investigation at the School of Aquatic and Fishery Sciences, University of Washington, seeks to evaluate the reproductive capacity and sex ratio of cultured intertidal geoducks. This pilot project involves, on a monthly basis, the sampling of 12 individuals from each of 5 year classes outplanted intertidally on northeast Hartstene Island in south Puget Sound. Individuals are weighed, measured, and analyzed histologically to calculate gonadal somatic index, sex ratio, and rank the progression of maturation in these young geoduck clams. The most important findings of this pilot study are a) that both males and females are present in young geoducks and b) that maturation begins earlier in cultured intertidal geoducks than suggested in the literature for wild subtidal geoducks. An imperative next step is to verify the generality of these results that bear directly on the genetic risk to wild geoducks from geoduck culture. To this end, it is necessary to extend our investigations at additional intertidal sites. Alongside sampling for gametogenesis, as it may become important to further address concerns regarding genetic effects of geoduck culture on wild populations (see Task 6), we will also separately bank tissue samples for possible future DNA extraction and genetic analyses.

To further evaluate the potential for the introduction of cultured genotypes into wild populations, we will also investigate the reproductive capacity of cultured intertidal geoducks. We will collect geoducks and for subsequent spawning trials in the Taylor Resources Inc. bivalve hatchery to compare reproductive output of cultured geoducks to that of wild geoducks, along with other life history parameters described below. If their reproductive output is low, or cultured geoducks are spatially separated from wild populations, the genetic risk is likely to be low, however if large assemblages of farmed geoducks coexist within close proximity to wild populations, the probability of interactions between wild and cultured populations may be significant. A primary objective of the tasks described below is to obtain specific life history parameters for cultured geoducks, with an eye toward developing hatchery practices that maximize genetic diversity in farmed stocks prior to leasing public tidelands and bedlands for commercial geoduck aquaculture.

Four specific tasks comparing cultured geoducks to their wild counterparts are proposed to address these questions. For both groups, we will:

1. characterize the level of spawning synchrony;
2. quantify egg and sperm production per year class;
3. assess the effects of gamete age on viability; and
4. determine larval viability within and between cultured and wild broodstocks.

Task3: Baseline health and disease assessment—C. Friedman (UW) and R. Elston (PSI)

Although diseases are a natural component of ecosystems, anthropogenic and natural perturbations have resulted in an increase in emerging diseases have been in a variety of species globally, including marine invertebrates, over the past few decades (Harvell et al. 1999). The culture of geoducks in Washington State includes high density plantings where disease transmission might readily occur, both within cultured populations and between wild and cultured populations.

Consensus among human and animal health experts is that a lack of baseline data exists on the health of species impacted by a disease. Without prior knowledge on the typical symbiotic organisms (including parasites), it is difficult to predict potential health problems, such as a disease outbreak (epidemic) or to determine the source of infection during an epidemic when large numbers of a species are impacted and dying. Approaches to successful health management of any species, wild or cultured, is predicated on the basis of what is normal. We intend to establish baseline health information on wild and cultured geoducks. This will provide us with information on existing parasites that may become problematic under certain stressful conditions (e.g. El Nino event or high density culture conditions) and provide crucial information should a health problem arise in either wild or cultured populations. As little information exists on normal flora, parasites and diseases of geoducks, this study will provide valuable baseline data for successful management of both wild and cultured geoduck populations.

Alongside sampling for diseases, as it may become important to further address concerns regarding genetic effects of geoduck culture on wild populations (see Task 6), we will also separately bank tissue samples for possible future DNA extraction and analyses.

Task4: Triploid geoduck field performance—J. Davis (PSI)

Reproductively sterile triploid geoducks may offer a viable alternative to growing diploid geoducks on WDNR leased tidelands and bedlands in Washington State, to address concerns over possible genetic interactions between native clams and farmed geoducks. Research to date suggests that hatchery production of triploid seed is feasible and relatively inexpensive to produce using chemical induction methods. We are proposing to initiate pilot scale studies to investigate the rate of growth, survivorship, gametogenesis and other biological parameters associated with growing triploid geoducks on three intertidal sites in Puget Sound. Triploid and diploid geoducks have been produced from the same group of broodstock and are currently being reared at the Taylor Resources hatchery facility in Quilcene. Diploid and triploid seed would be planted using conventional farming techniques and monitored for two years for rate of growth (size at age), survivorship and gametogenic activity (in the second year) at three sites in Washington State. In addition, clams would be retained at each of the test sites for gametogenic studies in later years.

TASK 1: Community Effects

Effects of geoduck culture practices (planting, predator protection, growout, and harvest) on benthic community structure and water column properties

PRINCIPAL INVESTIGATORS: ***D. Armstrong,*** **G.** ***VanBlaricom (UW); D. Cheney, J. Davis (PSI)***

Task Summary

During the past decade there has been a rapid expansion of intertidal geoduck aquaculture in Washington State and in the use of alternative and innovative methods for planting, cultivation, and harvest of these valuable shellfish. Yet little research has been conducted to assess the ecological impacts of geoduck cultivation practices on benthic habitats, sediment and water column properties, and associated flora and fauna. In this task, we will document and quantify the ecological effects of geoduck farming practices through an entire production cycle (planting, predator protection tubes, grow-out, and harvest) on benthic community structure, fish usage and water column properties. Commercial geoduck farms will be surveyed for infauna, macro-epifauna, large mobile species (shrimps, crabs, finfish), submerged aquatic vegetation (SAV), sediment properties, and water quality parameters to provide a temporal and spatial comparison across habitat types (various stages of geoduck culture) on and off commercial and experimental beds. In addition to monitoring existing geoduck farms, we will use a manipulative experimental approach on state-owned land to gauge the responses of selected parameters to predator protection and changes in geoduck biomass and density at various points in the culture cycle. These data will assist WDNR in evaluating environmental impacts and technical issues that surround geoduck aquaculture.

Background and Justification

During the past decade there has been a rapid expansion of intertidal geoduck aquaculture in Washington State and in the use of alternative and innovative methods for planting, cultivation, and harvest of these valuable shellfish.

Research has been and is being conducted by various companies and institutions in Washington State. A two-phase Small Business Innovation Research (SBIR) project was funded by the U.S. Department of Agriculture from 1999 to 2002. In Phase I, team members, Taylor Shellfish Farms and PSI, identified and tested various factors that were important in the success of planting geoduck seed in specific geographical areas in Puget Sound. In Phase II, commercial scale production methods and equipment were employed at farm sites in south Puget Sound and Hood Canal and tested to determine optimum production efficiencies and yields. Methods tested included a range of geoduck seed sizes and condition, and specific out-planting tidal heights. Various predator protection devices were also tested to assess their efficacy in excluding a wide range of predators over time.

More recently, the Western Regional Aquaculture Center (WRAC) funded a project led by Jennifer Ruesink (University of Washington. Biology Dept) to assess the potential sediment nutrient fertilization of eelgrass beds by suspension-feeding geoducks. Specifically, this project is examining the effects of geoducks on sediment grain size and organic content, pore-water nutrient concentration, and eelgrass growth and density. This project is part of a larger effort to understand the interactions between shellfish aquaculture and eelgrass in Washington State.

Currently, the Hood Canal Salmon Enhancement Group is assessing the feasibility of using natural or cultivated stocks of geoducks to offset the effects of increasing eutrophication in Hood Canal. This organization is gathering data on geoduck filtration rates utilizing seed money from the Washington State Department of Ecology (DOE).

Other than the aforementioned studies, little information is available on the effects of geoduck aquaculture practices on the surrounding environment. This task workplan describes research needs related to critical benthic, fisheries and water column environmental issues associated with the development of intertidal and subtidal geoduck aquaculture on state lands. Conceptually, we believe the following environmental effects are possible over the course of culture operations: 1) altered/enhanced recruitment and growth of eelgrass/macroalgae; 2) increased biodiversity and production on site of infauna and mobile macro-fauna including fish and shellfish; 3) changes in essential fish habitat as result of 1 & 2; and 4) and near-scale or localized changes in water quality and sediment composition. We believe these and other possible effects must be fully explored and documented on and near cultured beds before the State leases public lands for large-scale geoduck farming operations.

The information developed in Task 1 will assist WDNR in responding to the environmental and technical issues affecting their proposed operations with the most accurate and relevant data available. However, solving critical issues addressed in this project requires a level of scientific expertise and facilities that are beyond the scope of existing state agencies. The extensive involvement in the project by the University of Washington School of Aquatic and Fisheries Sciences and collaboration with the Pacific Shellfish Institute will ensure the highest level of performance in this study.

Proposed Research

Objectives

Specific objectives of this project task are as follows:

a) Conduct experiments within existing intertidal geoduck beds in Years 1 and 2 to:

    1) Assess operational effects -- Quantify and compare sediment characteristics and water column parameters, macroalgae, benthic infauna and epifauna, shrimp, crab, and finfish diversity, density, size distribution, and biomass at various phases of geoduck aquaculture (pre-planting, newly planted with predator protection, grow-out, and after harvest) across a gradient of farm to control sites to assess long-term species-specific and community-level responses to culture conditions;

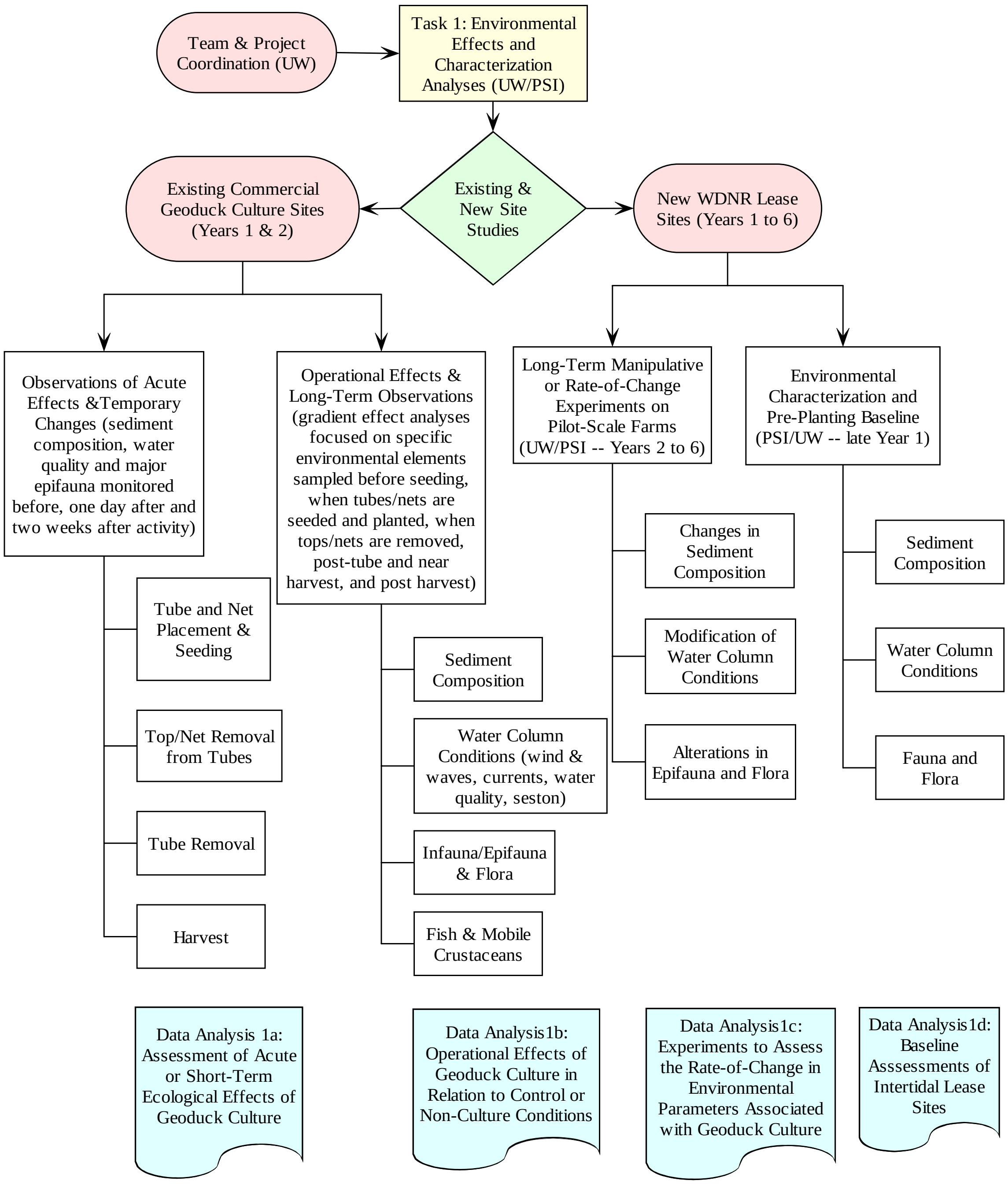
    2) Assess acute or temporary effects -- Quantify selected acute or short-term effects associated with the tube placement and seeding, net removal, tube

removal and harvest, and compare these most critical and intensive phases of geoduck culture to control sites.

b) Conduct manipulative field experiments -- following establishment of geoduck tracts at pilot-scale farm sites and on state lands, examine the nature of community response and rate of environmental change attributable to predator protection methods, and increasing geoduck biomass and density of macroalgae, infauna, epifauna, and sediment properties over an entire culture cycle (ca. 6 yr).

The flow chart in Figure 1 illustrates the primary organizational components and deliverables of Task 1.

Figure 1. WDNR Geoduck Task 1 Organization



Study Locations

We will take a collaborative approach to this project in order to utilize available field resources from selected south Puget Sound commercial geoduck farms on private lands, as well as pilot-scale intertidal farm sites on State lands. Data identified under Objective 1 will be collected from three existing intertidal shellfish farming locations. Existing farm sites will be selected to encompass the primary culture conditions of planting/predator protection, late stage growout, and harvest. Experimental manipulations identified under Objective 2 will be conducted on state-owned land consisting of three intertidal sites. We will work with DNR and other participants to decide what mix of studies on ongoing commercial beds and new activities on DNR land; there seem advantages in both.

Plan of Work

Subtask 1.1. Conduct sampling and experiments within existing intertidal commercial geoduck beds.

This subtask will be designed to assess and quantify the effects of geoduck aquaculture on the marine communities within and adjacent to existing farm sites. We assume large-scale geoduck culture will have a wide-range of effects. Some should be relatively short duration responses to initial planting or final harvest. Others will occur at varying levels of intensity over the life of the farming cycle. In this subtask we will determine the extent and degree of environmental changes associated with farm activities and operations, and will assess the effects of these changes on marine habitats and biota. These studies will allow the development of predictive tools to objectively address the consequences of establishing similar large-scale geoduck farms on state lands.

Subtask 1.1.1. Assess operational effects across a gradient of farm to control sites to assess long-term species-specific and community-level responses to culture conditions.

This subtask will focus on measuring a gradient of change for selected environmental parameters from farm sites to off-site locations during geoduck grow-out. Replicated sampling will be conducted at each of the three farm sites or combination of sites before seeding, when tubes/nets are seeded and planted, when tops/nets are removed, and after tubes are removed and near harvest. The following environmental parameters will be monitored:

1. Current speed and direction – assessed over a 2-week period at each farm site using a Sontek acoustic doppler profiler (ADP).
2. Culture site and adjacent near-bottom water quality characteristics -- monitored during tidal immersion and subtidally *in situ* in all study areas using YSI 6600 data logging sondes or similar instruments (logging temperature, dissolved oxygen, pH, salinity, turbidity and chlorophyll).
3. Ambient food and nutrient concentrations over and adjacent to culture sites – discrete samples taken near the end of growout cycle and analyzed for chlorophyll-a and nutrient levels.

4. Sediments – small core sampling to determine grain size, pore-water nutrients, organic carbon content and surface characteristics.
5. Geoduck hole densities – quadrat counts of geoduck abundance at sample site.
6. Macroalgae and eelgrass – quadrat sampling to determine dry weight, percent cover and species of macroalgae.
7. Macro-infauna – large core samples to measure the presence of polychaete worms, bivalves other than geoduck, and certain small crustaceans.
8. Epibenthic fauna – sampling to measure the abundance of key taxa and important salmonid prey species.
9. Large epifauna – sampling and observations to assess presence of large or motile organisms such as crab, shrimp, barnacles, moon-snails, and starfish, etc.
10. Finfish – observations and sampling on presence or absence of finfish species, including salmonids.

At each farm site a detailed bathymetric map will be obtained with the location of the culture units, their size and density, date and method of stocking, and stocking densities illustrated on the map. From this information we will prepare a sampling strategy to assess all of the above parameters (with the exception of 1, above) across a gradient to assess on-farm, near-field and far-field effects. In most cases this will involve a single linear transect paralleling the shoreline and at approximately 0-ft MLLW. However, two or more linear transects may be required in tracts spanning wide bottom elevation ranges (i.e. +1 to -1m). These transects will be sampled with replication when appropriate (n = 5±) inside the farm site, approximately 2 m, 25 m, 50 m and >100 m from one edge (perpendicular to the shoreline) of the site. These data will allow the effects of farm operations to be distinguished from ambient or non-farm conditions, such as shown in the sample graphic in Figure 2 for harpacticoid copepod and large polychaete abundances (hypothetical trend).

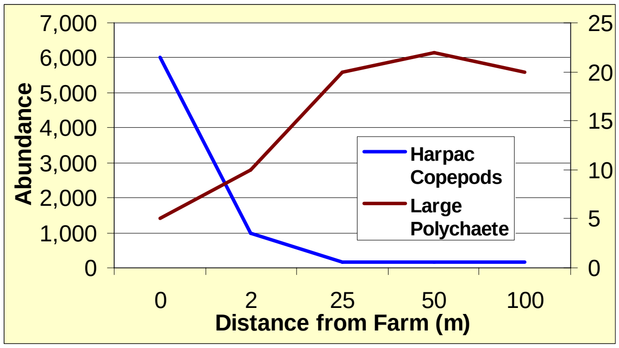


Figure 2. Sample graphic of abundance gradients

Additional sampling details

Sample timing and processing: All samples and observations will be taken beginning in July 2005 near the periods noted above. We anticipate use of minus tides for most sampling of infauna, and work later in each day to measure mobile species on beds during flood tides. Depending on length of time to take all samples on a given bed, proximity of beds to each other, and overall logisitics including field personnel, a full suite of sampling will be done long-term either once or twice per month across a 5-6 month period in spring and summer. These sampling times approximate peak levels of abundance of the target organisms or concentrations of the measured parameter. Sorting, taxonomic identifications, and counts of infaunal and epifaunal invertebrates will be made by the Pacific Shellfish Institute (PSI) and University of Washington (UW) staff. All samples will be analyzed for species richness, abundance, size distribution, diversity, and biomass. Graphical presentations and statistical tests will be used to test for differences in species composition and biomass among geoduck culture sites and culture conditions.

Ambient food concentrations. A Sea-Bird 16-Plus CTD with fluorescence probe or similar instrument will be used to profile chlorophyll-a prior to harvest at maximum geoduck biomass to estimate the effects of the cultured shellfish on ambient food concentrations. Water samples will also be taken to analyze chlorophyll-a inside and outside of the farm system to investigate rates of consumption by the shellfish. Water sampling will consist of two types of samples: long-term (25 hours/ 2 tidal cycles) composite samples, and short-term discreet grab samples. The long-term samples will be taken with two ISCO automated samplers, one with its intake inside the culture unit, i.e. oyster bag; and the other one at a control site at the same sampling height off the bottom where there are no shellfish. The units will be programmed to take samples every hour at the shellfish and control sites. Samples will be analyzed for chlorophyll-a, particulate organic carbon (POC), particulate organic nitrogen (PON), total particulate matter (TPM) and particulate inorganic matter (PIM) [note: this phase of the work could be combined with an on-going National Marine Aquaculture Initiative funded PSI project to use Flow-3D® software to model flow and chlorophyll concentrations for at least one site to determine the effect of geoduck farms on flow/seston depletion].

Sediments. Sediments will be characterized by taking small core samples. Gross characteristics of the core sampled surficial sediment will include: texture and color, biological structures (live shellfish, polychaete tubes, macrophytes), presence of debris (shell fragments, wood chips, etc.), presence of bacterial mats, odor (hydrocarbons or hydrogen sulfide), presence and depth of the redox potential discontinuity (RPD), and pH. Grain size from core samples will be determined by sieve analysis. Oxidation-reduction potential (ORP) analysis will be conducted in the field using a combination Redox/ORP probe, or similar, procedure.

Finfish and crab. Utilization of farm sites and adjacent areas by major species of finfish and crab will be based on quantified observations using a combination of net-sampling methods, diving and underwater video. A high resolution color underwater time-lapse video will be employed to obtain digital data over discrete periods of time on all culture sites and control sites. Approximately 12 hours of video imagery will be taken at three or more of the sites during March through May, which is juvenile salmon out-migration period, and July, during submerged aquatic vegetation (SAV) production. Video images will be quantified according to protocol developed in similar intertidal crab and fish studies of habitat utilization in Willapa Bay (species, count, size frequency by light/dark intervals and tidal stage).

Subtask 1.1.2. Quantify selected acute or short-term effects

Geoduck farming practices are similar to other types of farming activities in which there are short-term and sometimes intense disturbances followed by long period of little or no human intervention. These practices may be characterized by sediment disturbance, elevated rates of surface runoff, and other changes. It is proposed to carry out observations of acute effects and temporary changes in sediment composition, water quality and major epifauna immediately before, during, one day after and two weeks after the activity. The primary activities to be assessed will be: 1) tube and net placement, and seeding, 2) net and net-top removal, 3) tube removal, and 4) harvest. These effects will be assessed and compared to control sites using the procedures described in Subtask 1.1.1. for these most critical and intensive phases of geoduck culture. The data will also be incorporated into BMP guidance practices to enhance BMPs prepared in Phase I studies.

Subtask 1.2. Conduct manipulative field experiments at pilot-scale farm sites on state lands

Culture activities for geoducks in intertidal habitats will bring physical alterations to the intertidal ecosystem, possibly affecting the densities, population structures, and distributions of sessile or weakly mobile epifauna and infauna. Such alterations may also affect utilization of culture areas by more mobile epifauna such as crabs, large gastropods, or sea stars. Placement of structures, such as tubes to protect individual cultured clams, may alter patterns of water movement and sedimentation or erosion. Exposed structures can also serve as attractants for mobile epifauna, as settlement locations for algae and sessile or weakly mobile invertebrates, and as sites for attachment by macroalgae. All of these factors have the potential for modifying infaunal and epifaunal community structure and function as compared to sites without culture activities.

The effects of predator protection and changed geoduck biomass and density on sediment characteristics, macroalgae and selected benthic species and community parameters will be measured in long-term manipulative field experiments conducted on previously uncultured DNR intertidal beds. These manipulative experiments will be particularly helpful in determining the response and compensation of the ecological community to the increasing geoduck biomass as the animals age. Replicated intertidal experimental arrays will be established at three pilot-scale farm sites within Puget Sound. Each array will contain the following treatments: a) unfarmed controls; b) newly seeded and net-covered predator protection tubes; and c) seed ground with tube-supported predator netting.

Plot size, blocking, and replication will be determined based on findings from Subtask 1.1 and site characterization sampling conducted within and among the pilot farm sites (see Figure 1). All plots will be sampled immediately prior to and following application of treatments as well as throughout the geoduck growout and harvest cycle. Under this grant period (2005-2006), we will be able to assess the effects of different treatments on biotic and abiotic response variables listed under Subtask 1.1.1 up through tube removal on intertidal beds, which occurs between 8 and 16 months following the addition of seed to the tubes. Renewal of funding through 2010 (two additional biennia) will enable us to examine the rate of ecological change through the entire geoduck culture cycle.

TASK 2: Genetic Effects

Geoduck Gametogenesis, fecundity, and larval viability

PRINCIPAL INVESTIGATORS: ***C. Friedman (UW) and J. Davis (PSI)***

Task Summary

While the culture of a native species reduces risks often associated with exotic species, it is important to investigate potential interactions with wild counterparts. Should geoducks cultured in the field become reproductively mature and spawn during the 5-7 year culture cycle, the potential arises for the introduction of cultured genotypes into wild populations. An on-going pilot investigation at the School of Aquatic and Fishery Sciences, University of Washington, seeks to evaluate the reproductive capacity and sex ratio of cultured intertidal geoducks. This pilot project involves the monthly sampling of 12 individuals from each of 5 year classes outplanted intertidally on northeast Hartstene Island in south Puget Sound. Individuals are weighed, measured, and analyzed histologically to calculate gonadal somatic index, sex ratio, and rank the progression of maturation in these young geoduck clams. The most important findings of this pilot study are a) that both males and females are present in young geoducks and b) that maturation begins earlier in cultured intertidal geoducks than suggested in the literature for wild subtidal geoducks. An imperative next step is to verify the generality of these results that bear directly on the genetic risk to wild geoducks from geoduck culture. To this end, it is necessary to extend our investigations at additional intertidal sites. Alongside sampling for gametogenesis, as it may become important to further address concerns regarding genetic effects of geoduck culture on wild populations, we will also separately bank tissue samples for possible future DNA extraction and analyses.

To further evaluate the potential for the introduction of cultured genotypes into wild populations, we will investigate the reproductive capacity of cultured intertidal geoducks. We will collect geoducks and condition them for subsequent spawning trials in the Taylor Resources Inc. bivalve hatchery to compare reproductive output of cultured geoducks to that of wild geoducks, along with other life history parameters described below.

At issue is not only whether cultured geoducks spawn, but also the potential contribution of gametes and/or larvae from farmed to wild populations. If their reproductive output is low, or cultured geoducks are spatially separated from wild populations, the genetic risk is likely to be low, however if large assemblages of farmed geoducks coexist within close proximity to wild populations, the probability of interactions between wild and cultured populations may be significant. A primary objective of the tasks described below is to obtain specific life history parameters for cultured geoducks that may affect interactions with wild stocks.

Even though the existence of genetic interactions between cultured and wild geoducks is unclear, it is prudent to implement broodstock management practices that maximize genetic diversity in farmed stocks prior to leasing public tidelands and bedlands for commercial geoduck aquaculture.

Four specific tasks comparing cultured geoducks to their wild counterparts are proposed to address these questions. For both groups, we will:

1. characterize the level of spawning synchrony;
2. quantify egg and sperm production per year class;
3. assess the effects of gamete age on viability; and
4. determine larval viability.

Differences in any or all of these parameters are likely to have a significant effect on the potential for genetic interaction of cultured and wild geoducks. We will also, where possible, collect preliminary data to begin to characterize larval dispersal patterns under temperature stratifications and daily light cycles.

Background and Justification

1) Current state of knowledge:

The level of maturation in young geoducks has primarily been addressed using wild geoducks. The suggested ages at 100% maturity for male and female subtidal geoducks vary among studies, but in general are 5 and 6 years old, respectively. However, young geoducks are often significantly under-represented in wild survey samples. In addition, a paucity of information exists on geoducks in the intertidal, where most geoduck culture activity exists. In Puget Sound, intertidal geoducks are relatively uncommon except in the low intertidal zone, where they are typically found in low densities. Cultured geoducks, on the other hand, are planted from approximately +1 to -2 ft MLLW at densities of up to 20 clams per square meter.

Preliminary results of the SAFS investigation on gametogenesis in cultured intertidal geoducks suggest that gametogenesis, or reproductive maturation, is initiated in the second year. Preliminary results also indicate both male and female mature gametes are developed in three year old geoducks. In our preliminary monthly survey, we observed a shift from fully mature gonads to partially spawned or spent reproductive follicles, suggesting that in some 3-5 year old individuals gametes had clearly been released between June and July, 2004. In addition, in some age classes geoduck gonadal follicles changed from fully ripe (filled with mature gametes) to spent (few or no gametes present) within one month, illustrating that more frequent samplings are needed during the spring and summer months to further elucidate the proportion of clams that spawn relative to those that resorb their gametes. If, in the majority of young geoducks, gametes are resorbed and not spawned, reproductive and genetic concerns are minimized for these age classes. It is important to understand if young geoducks typically do not spawn, or necessary spawning cues are somehow impaired in the intertidal environment.

We have two important objectives in advancing this project. First, we plan to increase the sampling from monthly to bimonthly during the spawning season to increase the resolution of the information on the extent of spawning vs resorbtion. Second, generalizing these results requires the inclusion of additional Puget Sound locations so we can more fully understand the effects of location, size, and age on maturation. While data exist on gametogenesis in wild Puget Sound geoducks, very few geoducks known to be under age seven have been examined histologically for level of maturation.

Due to the relative youth of the culture industry, little is known about the reproductive capacity of young geoducks. It is not known, for example, how long gametes remain viable in the environment once spawned, the age specific production of gametes, and whether there are differences in reproductive success between cultured and wild geoducks. Ideally, we would investigate these parameters directly in the natural environment, but it would be both highly impractical and cost prohibitive to do so. Instead, we propose to conduct these investigations at the Taylor Resources bivalve hatchery, where experiments can be conducted in a controlled environment.

The proposed research will provide data for describing life history parameters pertaining to gametogenesis and reproductive output for geoducks within the range of ages farmed for aquaculture production. This information is critical for understanding potential impacts of geoduck culture on wild stocks, and for designing best management practices to avoid negative impacts.

Geoducks are broadcast spawners; they release gametes directly into the water column. Successful fertilization depends on a number of factors:

- Males and females must be in close proximity; at low densities, diffusion greatly decreases the probability of gamete contact and fertilization.
- Spawning must be synchronous. If individuals release gametes at different times or on different days, the effort is wasted due either to gamete age or diffusion.
- Individuals must release large quantities of gametes. Due to diffusion, fertilization in the water column depends on the quantity of eggs and sperm released by each individual.
- Gametes must be viable. The duration of viability must account for variation in proximity and synchrony.

Thus, greatest fertilization success will be achieved in dense aggregations that spawn large quantities of viable gametes at the same time.

In Washington, average wild geoduck densities on commercial beds vary from 0.2 to 1.7 geoducks/m^2; cultured intertidal bed densities are 9 to 20 geoducks/ m^2, roughly an order of magnitude greater. Since cultured geoducks are typically planted at densities much higher than found in the wild, we know that proximity should not be an impediment to successful fertilization.

Spawning synchrony in geoducks has not been directly investigated. However, indirect evidence suggests that among cultured intertidal geoducks, gametogenesis is more synchronous than in the wild population. Suggesting a high level of developmental synchrony, 80% of four and five year old cultured geoducks were ripe one month and

100% spent the next; wild individuals, on the other hand, were found at all levels of development during each month. Whether the synchrony among cultured geoduck is a function of age, tidal elevation, relatedness, density, or other culture factors is unknown. It is also not clear whether developmental synchrony is correlated to spawning synchrony. We propose to investigate the level of synchrony among groups of cultured and wild geoducks by conducting the experiment described below.

Fecundity, or number of gametes produced by farmed geoducks, has also not been investigated. Fecundity is associated with both size and age in many invertebrates and it is likely that fecundity in geoducks is a function of both size and age. We know that wild geoducks brought into the hatchery will spawn 15,000,000 eggs on average, and are capable of doing so several times over the course of the spawning season (December to May in the hatchery environment). Fecundity of males is not currently known. We propose to compare fecundities of cultured geoducks ages 1-5 with those of wild geoduck as controls.

Because viability decreases with gamete age, we will also investigate gamete quality indirectly by assessing the effects of gamete age on successful fertilization.

An additional requirement for successful reproduction is the viability of larvae. Whether larvae produced from cultured parents exhibit different survivorship from those produced from wild parents is an important consideration: if low in cultured groups, the risk to wild populations would be reduced. The converse, of course, would increase the risk. We propose, therefore, to measure survivorship differences between cultured and wild larvae as described below.

2) Contributions to the goals of the state and DNR: This study will provide critical information on the generality of preliminary results that suggest that cultured geoducks mature and spawn before harvest. Whether maturation or spawning rates are correlated with size or tidal elevation is essential information for management of genetic risks to wild populations, and this information will bear directly on Best Management Practices for geoduck culture on state-owned intertidal and subtidal land. This study will also provide information critical to understanding the potential for any genetic interaction of cultured and wild geoducks.

3) Contributions toward improving geoduck aquaculture: Understanding and characterizing the maturation rate of cultured geoducks will not directly improve geoduck aquaculture, but will provide valuable information for avoidance of potentially negative genetic and competitive effects of geoduck aquaculture on wild populations. Information on spawning synchrony, effects of gamete age on viability, and effects of age on larval viability will be used directly in the geoduck culture industry to improve hatchery performance. These data, coupled with fecundity at age, will be used to define Best Management Practices for the geoduck culture industry.

4) Anticipated users of the research findings: The information gleaned from this study will be used by state and tribal resource managers, aquaculturists, members of the fishing industry, and the academic community. Due to the involvement of the University of Washington in this project, this work will provide additional benefit by training both undergraduate and graduate students in geoduck life history and conservation genetics.

Proposed Research

Goals:

A. To determine the age at maturation of cultured geoduck clams.

Objectives:

1. To characterize level of maturation for five year classes of cultured geoducks from additional Puget Sound locations;
2. To characterize sex ratio of young geoducks;
3. To quantify proportion gonad area in histological sections and examine correlations with specimen length and weight;
4. To assess correlations of maturation with age and size in young geoduck; and
5. To quantify proportions of spawned vs resorbed gametes at each of five year classes across different environments.

B. To assess the potential for either gametes or larvae to be produced by cultured geoducks.

Objectives:

1. To quantify fecundity (egg production) and sperm output for clams of different age classes over a discrete spawning period;
2. To characterize the level of spawning synchrony in cultured and wild groups;
3. To assess the effects of gamete age on viability; and
4. To quantify the viability of larvae produced from cultured geoducks.
5. To begin characterization of larval behaviors, varying temperature stratifications and daylength.

Methods:

A. Gametogenesis

Geoduck sampling will be initiated one month prior to the onset of gametogenesis, as determined from the pilot study at Hartstene Island. We will initiate sampling of geoducks from two additional sites, such as the Taylor Farms in South Sound and the Lummi reservation. We will also sample from at least two other intertidal sites in South Puget Sound during the month we predict spawning to occur. All sites will be selected in further planning with DNR and across other tasks to best coordinate field personnel and logistics. For the intertidal sites, sampling will be done during low tide series each month. In January, February, March, and August, samples will be taken once. During April, May, June, and July, samples will be taken twice monthly. Preliminary data suggest that during September-December, gametogenesis is only in the earliest stages. From each sample, geoducks will be labeled to identify individual and year class. Valve length and width and wet weight measurements will be taken for each geoduck. A small tissue sample from the foot will be placed in 95% ethanol for future characterization of genetic diversity within year classes. Two transverse sections of the gonad-viseral complex will be fixed in Davidson's invertebrate fixative for 24 hours, stored in 70% ethanol, embedded in paraffin, slide mounted, and stained with hematoxylin-eosin. Using light microscopy, the sections will be examined and ranked according to established gametogenic criteria. Digital photographs of each slide will be demarcated to calculate gonad area. Data will be analyzed using contingency tables and bootstrapped resampling techniques.

B. Fecundity and gamete viability

The control group will consist of wild geoducks harvested for broodstock from southern Puget Sound, and the treatment groups will consist of 20 individuals from each of 5 year classes outplanted intertidally on northeast Hartstene Island in south Puget Sound (Seattle Shellfish). Individuals for both control and experimental groups will be weighed, measured, and labeled with a numbered plastic tag. Two sets of experiments will be conducted.

Set 1 will determine the maximum reproductive output from each year class by exposing the geoducks to artificial conditioning. For Set 1, the clams will be harvested in the fall. Each age class will be maintained under the same environmental conditions to stimulate gametogenesis at the Taylor Resources Bivalve Hatchery using conventional methods. During the 4-6 week conditioning period, geoducks will have access to high ration algal live algal diets, consisting of *Isochrisis galbana, Chatoceros calcetrans, and Chaetoceros mulleri.*

Set 2 will approximate reproductive output under more natural conditions. Geoducks sampled from the same site as Set 1, but will be harvested in the late spring when a high proportion of geoducks are known, from studies conducted in A (above) to be in the ripe state. These geoducks will be held in the hatchery for acclimatization for only one week, and fed a standard food ration.

For both Set 1 and 2, spawning will be induced by elevating the temperature of the inflowing seawater from 12-15 ºC, increasing the algal density by an order of magnitude, and alternating between static and high flow-through conditions. Spawning geoducks will be removed to individual seawater containers as spawning commences. Study components include:

- The time that each clam is actively expelling gametes will be recorded to measure spawning synchrony.

- Once spawning ceases, gametes will be collected and counted. Counts for each age group will be compared to counts from the control group. These data will establish a baseline mean fecundity per year class.

- Effects of gamete age on viability will be assessed by holding replicate vessels of unfertilized male or female gametes and fertilizing at specified time points over a time period of 6 hours.

- The proportion of normal:abnormal veliger larvae for each treatment will be measured to assess effects of geoduck age on gamete viability over time. These data will help determine dispersal distances for viable gametes.

- We will perform crosses between pairs of cultured and pairs of wild geoducks to assess differences in relative reproductive success. Larvae from cultured crosses will be held in five replicate tanks alongside five wild cross control groups. Data will be taken at discrete embryonic, larval and post-larval developmental points and include, percent fertilization, size at age and percent to veliger larvae (prodisoconch I), size at age and percent to the umbone stage (prodisoconch II), size at age and percent to pediveliger stage and size at age and percent to the post settlement early juvenile stage.

- When possible, we will make preliminary observations of larval behaviors at different temperature stratifications and diel cycles.

Personnel:
Aside from the principal investigators, project personnel will include undergraduate research technicians and at least one graduate student; the data will comprise undergraduate and graduate student theses.

TASK 3: Disease

Baseline health and disease assessment

PRINCIPAL INVESTIGATORS: ***C. Friedman* (*UW*) *and R. Elston (PSI)***

Task Summary

Although diseases are a natural component of ecosystems, anthropogenic and natural perturbations have resulted in a global increase in emerging diseases in a variety of species, including marine invertebrates, over the past few decades (Harvell et al. 1999). The culture of geoducks in Washington State includes high density plantings where disease transmission could be facilitated, both within cultured populations and between wild and cultured populations. A lack of baseline information on geoduck health and condition hinders their management similar to the lack of baseline data for other animal and human diseases. Without prior knowledge of the typical symbiotic organisms (including parasites), it is difficult to predict potential health problems, such as a disease outbreak (epidemic) or to determine the source of infection during an epidemic when large numbers of a species are impacted and dying. Approaches to successful health management of any species, wild or cultured, is predicated on the basis of what is normal. We intend to establish baseline health information on wild and cultured geoducks. This will provide us with information on existing parasites and disease conditions that may become problematic under certain stressful conditions (e.g. El Nino event or high density culture conditions) and provide information crucial for management should a health problem arise in either wild or cultured populations. As little information exists on normal flora, parasites and diseases of geoducks, this study will provide valuable baseline data for successful management of both wild and cultured geoduck populations.

Background and Justification

1) Current state of knowledge: Little is known about diseases of geoducks. The only known disease of geoducks associated with mortalities is an amoeboflagellate, protistan parasite infecting cultured larval geoduck clams (Kent et al. 1987, Elston 1990). Mortalities were first observed with this *Isonema*-like protozoan parasite in larval geoduck clams (*Panopea abrupta*) in the 1980s. The continued sporadic occurrence of this disease in cultured larval geoducks in Washington state and British Columbia suggests that continued management is required to exclude the disease and prevent its deleterious effects on larval cultures. No juvenile or adult geoducks or other species have been reported affected in geoduck hatcheries, although a similar disease has been observed in eastern hard clam larvae on the east coast of the United States (Kent et al. 1987, Elston 1990, Elston, in preparation).

Given the interest in geoduck aquaculture in Canada and recognizing the importance of baseline health information, Canadian researchers have begun to assess the health of both wild and cultured geoducks in British Columbia, Canada (B.C.; Bower 2002, Bower and Blackbourn 2003). During surveys of key fishery areas in British Columbia, low prevalences of two protozoa of unknown taxonomic affinity (Clam Protozoan Unknown or CLPX and *Nematopsis*-like apicomplexan spores), a Rickettsiales-like bacterium and turbellarian worms were observed in geoducks in British Columbia, Canada; the parasites did not evoke a host response and were not associated with losses (Bower 2002, Bower and Blackbourn 2003). These researchers also examined abnormal geoducks harvested by the commercial fishery. Several abnormalities were observed on the siphon and mantle including warts, blisters and scars, and pustules, respectively. No infectious agents were observed in association with these lesions. Individuals from experimental geoduck plantings from two different year classes (spawned in either 1995 or 1996) and planted at four locations in the Strait of Georgia were surveyed for diseases. No evidence of any infectious diseases was observed in these experimental animals (Bower 2002). However, it is recognized that further work is required to assess if diseases from either wild congeners or low levels of potential pathogens with the planted geoducks could present a health risks to wild or cultured clams in the Strait of Georgia. Bower (2002) indicated that the "absence of disease during geoduck culture trials in the Strait of Georgia is most encouraging for the industry but also brings complacency. Nevertheless, we must be on our guard concerning indiscriminate transplantation of geoduck clams around the province," recognizing the importance of acquiring baseline health information of both wild and cultured animals.

During 2001 and 2002, evaluations were made of the health and condition of a limited number of geoduck broodstock harvested from wild populations, in conjunction with a project to commercialize culture methods for the species. During that study, abnormalities of the siphon integument were observed in about 5% of individuals but could not be linked to an infectious agent, based on histological examination. Rickettsiales-like organisms were also found in Washington geoduck broodstock.

2) Contributions that the study task will make to the goals of the state and DNR: This study will provide invaluable information on the health and condition of wild and cultured geoducks in key fishery and aquaculture areas of Washington State as described below in section 3.

3) Contributions that the study task will make toward improving geoduck aquaculture: Successful culture of any species requires knowledge of potential problems, especially diseases that may influence the success and sustainability of the culture operation, minimizing its impact on the nearby ecosystem and public acceptance of the activity. Knowledge of 'normal' flora associated with this clam species will provide key baseline data for comparison should a disease emerge. In addition, this information will provide insight into the presence of potential disease-causing parasites, such as the rickettsiales-like bacterium infecting the gills of B.C. geoduck clams, thereby arming the geoduck culturists and resource managers with important information to incorporate into management strategies. Successful management of recently emerging infectious diseases, such as the rickettsial pathogen of cultured and wild abalone in California (Friedman et al. 2000), are routinely hampered by a lack of baseline information on the distribution and prevalence of endosymbiotic organisms (e.g. parasites). Recent popular (e.g. news paper and magazine articles) and scientific press articles have suggested that movement and culture of aquatic species, particularly marine bivalves, are key vectors of disease (Harvell et al. 1999, Burreson et al. 2000, Naylor et al. 2001). Ultimately, the success of a culture operation requires public acceptance of the activity and, due to current concerns about environmental quality and conservation, knowledge that measures are being taken to reduce the risk of disease introductions or transfers via aquaculture (often coined as "biological pollution") will enhance public support and the success of geoduck aquaculture in Washington State.

4) Anticipated users of the research findings: The information gleaned from this study will be used by state and tribal resource managers, aquaculturists, members of the fishing industry, and academic community. Due to the involvement of the University of Washington in this project, this work will provide additional benefit by training both undergraduate and graduate students in geoduck health issues.

Proposed Research

Objectives: We plan to characterize endosymbiotic organisms associated with geoduck clams in at least two wild populations and 2-3 cultured populations (n=60 each) at least twice per year for two years to provide baseline health/disease information. The latter task will be conducted in conjunction with the gametogenesis portion of this proposal (additional tissues will be collected to assess the health of these individuals). For this biennium, we will collect and process samples. Histological preparation and analyses described below will be conducted pending sufficient future funding.

Methods: <u>Wild geoducks</u>: In conjunction with WDFW annual wild geoduck collections, we will sample a range of sizes (ages) of clams twice during year one of this study with a goal of collecting one sample in the Spring and one in late Summer. In each collection period we plan to target populations in south Puget Sound, north Puget Sound, Hood Canal, and the Strait of Juan de Fuca. For each collection, sixty animals will be measured (length and weight) and 3-5 mm cross sections will be removed that contain siphon, gills, mantle, foot, digestive organs, and gonads. Any gross lesions will be recorded and pieces of observed lesions will also be removed for histological processing or other standard pathology/microbiological methods (e.g. bacteriology). Samples will be preserved in Davidson's solution for 24 hr, and then stored in 70% ethanol for future processing for routine paraffin histology. A small piece of siphon or mantle will also be excised and stored in 95% ethanol for future characterization of genetic diversity within the collected populations.

<u>Cultured geoducks</u>: In conjunction with our monthly surveys of cultured geoduck gametogenesis, we will collect additional tissues (siphon, gill and foot) to complement the mantle, digestive organs and gonads collected during our gametogenesis study. We will target two age classes: one year olds and four year olds; samples will be processed as described above.

<u>Analysis of current data</u>: We will incorporate currently available data on health and condition and gametogenesis into the database developed for this project. This data consists of unpublished information collected during the Taylor Resources - Pacific

Shellfish Institute USDA funded study on the development of geoduck culture methods in Washington State.

Personnel: This research will be conducted in the Friedman laboratory in collaboration with Dr. Elston who will participate in slide interpretation and analyses. The study will also involve both technical staff and students.

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TASK 4: Triploids

Triploid Geoduck Pilot Growout Studies in Puget Sound

PRINCIPAL INVESTIGATOR: ***J. Davis (PSI.)***

Task Summary

Reproductively sterile triploid geoducks may offer a viable alternative to growing diploid geoducks on WDNR leased tidelands and bedlands in Washington State where concerns over possible genetic interactions between native clams and farmed geoducks may occur. Research to date suggests that hatchery production of triploid seed is feasible and relatively inexpensive to produce using chemical induction methods. We are proposing to conduct pilot scale studies to investigate the rate of growth, survivorship, gametogenesis and other biological parameters associated with growing triploid geoducks on three intertidal sites in Puget Sound. Triploid and diploid geoducks have been produced from the same group of broodstock and are currently being reared at the Taylor Resources hatchery facility in Quilcene. For this project, diploid and triploid seed will be planted using conventional farming techniques and monitored for two years for rate of growth (size at age), survivorship and gametogenic activity (in the second year) at three sites in Washington State. In addition, clams will be retained at each of the test sites for gametogenic studies in later years.

Background and Justification

The development of large-scale hatchery based culture methods (Beattie 1992) enabled shellfish growers in Washington State to initiate geoduck clam culture on intertidal beds. Farmed geoduck production in Puget Sound comprises a developing aquaculture sector with three to four million seed clams planted annually in intertidal beds. Adult geoducks utilized in the aquaculture industry for broodstock are typically collected from wild subtidal aggregations in established commercial beds identified by the WDFW. Voluntary actions by hatchery seed producers have resulted in most seed geoducks planted in recent years originating from the same general geographic area as broodstock, thus contributing to the preservation of local genetic variability in outplants. However, reduced genetic diversity in hatchery outplants may still occur as limited numbers of broodstock may still be contributing to hatchery stock transfers; as a result, low effective population size as well as domestication selection in hatchery stocks could disrupt natural patterns of genetic structure. Thus, risk of introgression may exist, dependent on the potential for genetic interaction between hatchery and wild stocks. Development of techniques to confer sterility on hatchery outplants via the use of triploids could help to mitigate potential genetic risk.

Induced triploidy has been utilized by the shellfish aquaculture industry since the mid-1980's, in some cases to confer sterility (for a review see Beaumont and Fairbrother 1991). In a number of studies, triploid manila clams were produced in an attempt to avoid genetic introgression of an introduced species (e.g. Beaumont and Contaris 1988, Laing and Utting 1994). For the same reason, triploid oysters *Crassostrea ariakensis* are being contemplated for widespread outplanting into Chesapeake Bay, where *C. virginica* is the native oyster (Thorgaard and Allen 1988). Methods to produce commercial quantities of triploid geoducks have been developed through a Washington Sea Grant funded project (Vadopalas and Davis 2004), and an experimental cohort of triploid geoducks has recently been produced by Taylor Resources, Inc. for use in evaluating the commercial viability of triploids.

In addition to the possible benefits afforded by induced sterility, triploid geoduck clams may also exhibit better growth performance compared to diploid clams. In numerous studies considering comparing triploid and diploid bivalves, a growth advantage in triploids has been often noted (e.g. Downing and Allen 1987; Guo et al. 1996). The mechanism for growth advantage in triploid bivalves may be due to energy reallocated from reproduction to somatic growth (Barber et al. 1992; Hawkins et al. 1994; Hand et al. 1998), or the effects on growth due to larger average cell size in triploids (Guo and Allen 1994), The combined effects of sterility and enhanced growth due to triploidy may therefore offer a significant advantage over cultured diploid stocks.

An attempt to assess the potential for triploid geoduck production in three growout environments was made several years ago with Washington Sea Grant funding by JP Davis with inconclusive results. Trends for growth and survivorship in triploids compared to diploid clams was reduced in all three sites differing in annual productivity, however sample sizes were very small and the differences were not statistically significant. The question of whether triploid clams are fully or partially sterile has also not been fully addressed. As a result, it is proposed here to examine more fully triploid performance and gametogenesis compared to a diploid cohort under commercial growout conditions over a six year period at three sites in Washington State.

This study will provide current information on the utility of triploid geoducks for potential use in commercial aquaculture. Information on the size and age that possible full or partial sterility may be conferred to triploids will be a cornerstone of the proposed research. In addition, the question of triploid reversion in part or full to the diploid state will be examined in geoduck as has been seen in other molluscan species. Information on triploidy in geoducks will directly benefit the goals of the state and DNR by providing information on the use of triploid geoducks in a commercial setting. If triploid geoducks demonstrate satisfactory growout performance as well as partial or full sterility, then the use of triploid geoducks for aquaculture production may offer an important tool to help reduce genetic risk to wild geoduck populations.

Proposed Research

A cohort of diploid and triploid geoduck seed has been produced at the Taylor Resources bivalve hatchery and is currently being maintained in a nursery system. Triploids were produced by incubating fertilized embryos in a water bath containing 6-dimethylaminopurine (6DMAP). Subsequent analysis of the larvae by flow cytometry indicated that over 75% of the treated embryos were triploids. A diploid cohort of seed was produced from the same parents as the triploids so that a matched cohort of diploid and triploid geoducks will be available for research purposes early in 2005.

There are three goals of the proposed research:

1. Deploy diploid and triploid seed in three locations to be determined, including DNR leased properties differing in overall productivity and other characteristics and evaluate early seed growth and survivorship over the two-year period covered by this grant. Seed will be planted in quantity to enable monthly sampling for production in concert with the collection of environmental information on food availability, temperature, salinity and other water quality parameters. In addition, enough seed will be deployed to enable further evaluation of growth, survivorship and gametogenesis over an additional four years to fully characterize triploids over a full production cycle, contingent on additional funding.
2. Gametogenesis in diploid geoducks has been observed in clams only two years old. A major focus of this study beginning in the second year of the project will be to conduct intensive sampling for gametogenesis in triploid geoducks (as compared to diploids) in order to later evaluate the onset of partial or total sterility in triploid geoducks. Sampling for gametogenesis would commence during the second year of growout in two year old geoducks.
3. The question of partial or full reversion of triploid bivalves (including selected tissues) to the diploid state has not received very much attention. A focus of the proposed research will be to conduct a full analysis of the potential for triploid reversion in triploid geoducks by analyzing tissues for ploidy at regular intervals.

Methods for accomplishing these research goals include the establishment of plots of diploid and triploid seed at selected locations. Seed will be planted using conventional methods in 4” diameter tubes (two seed per tube) covered with mesh to reduce predation. Three hundred diploid and triploid seed (600 seed total) will be randomly assigned to tubes that will be established at the 0 to +1 tidal height (MLLW) at three sites to be determined. Tubes will be arranged in three rectangular blocks of 100 tubes each to enable statistical blocking for treatment effects. Seed will be planted in the spring of 2005 and monitored for growth and survivorship at monthly intervals for two years. Monitoring will include the removal of a sample of clams, return of samples to the laboratory for ploidy analyses (including specific body tissues) utilizing standard flow cytometeric techniques, measurements for size at age (live weight, shell dimensions). Survivorship will be determined in the field by evaluating the number of diploid and triploid clams recovered at each sampling interval.

Beginning in the spring of the second year, geoducks will be sampled at monthly intervals for gametogenic development. Clams will be returned to the Taylor Resources laboratory and a sample of gonadal tissue excised and fixed in Davidson’s solution, transferred to 70% ethanol for storage and later submission to a histolology laboratory for embedding and production of slides for microscopic examination of gametogenesis. At least 12 diploid and 12 triploid geoducks will be sampled for gametogenic development and diploid reversion (triploids only) each month.

Sites for evaluating triploids will be determined in consultation with WDNR, PSI and the University of Washington research teams. We propose to utilize sites for triploid evaluation that will be part of the overall research studies on ecological effects of geoduck culture to enable coordination between studies. This approach will enable data to be taken on water quality, current flow and food (seston) availability for each site and help to characterize the different growing environments. In addition, travel, labor and other sampling costs can be shared among projects over the course of the two year study.

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