# WASHINGTON SEA GRANT PROGRESS REPORT

for the period 2/11/2007 – 1/31/2008

WSG Project Number: **R/A-85**
Project Title: Culturing native marine shellfish: effects of life history parameters on sustainability (Geoduck)

Principal Investigator(s) and Affiliation:
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## 1. ABSTRACT ELEMENTS

### OBJECTIVES

While the culture of native species reduces risks often associated with exotic species, in natives, reproduction between cultured and wild (cultured-wild interactions, CWI) may decrease fitness of wild populations. Geoduck clam (clam) aquaculture is proliferating proximate to wild clam populations that provide both vital ecosystem services and a valuable ($20 million) fishery resource in Washington. CWI may place wild stocks at risk if genetic differences exist between cultured and wild clams, cultured individuals are reproductively successful, and progeny carrying cultured genotypes settle within wild beds. To assess potential CWIs, we plan to 1) characterize genetic differentiation between cultured cohorts and wild; 2) examine cultured clams for evidence of spawning; 3) compare maturation, fecundity and progeny survival in cultured vs wild clams, and 4) use "artificial" geoducks to measure fertilization success at cultured and wild densities. These data will be used to evaluate risks associated with CWI involving ecologically important wild geoduck populations.

### METHODS

1) We will use the PCR to amplify fluorescently labeled microsatellite loci in geoduck and analyze allele frequency variation to characterize differentiation between cultured and wild clams and estimate annual hatchery $N_e$.
2) Are cultured geoduck reproductive and in synchrony with wild populations? Monthly during spawning season (May, June, July), we will sample 10 geoduck each from two cultured year classes (2 and 4 year olds) at 5 intertidal culture sites. Proximate to each site, subtidal wild geoduck will be sampled contemporaneously at three depths (3, 12, and 21 m MLLW). Histological examinations will determine the generality of our finding and determine reproductive synchrony between cultured and wild.
3) Are fecundities and survivorship of progeny different between cultured and wild? Using established hatchery methods, we will induce spawning in 4 year classes of cultured geoduck and wild counterparts and conduct single pair matings. We will measure average fecundity using a Coulter particle counter, assess fertilization rates, and assess survivorship in progeny from both cultured and wild crosses.
4) How long do gametes remain viable? We will hold male and female gametes for intervals of 1-12 hours before fertilization, and assess survivorship at 3 days post-fertilization.

5) What is the difference in reproductive success at cultured (12 m-2) and wild (0.5-3.0 m-2) bed densities? PVC structures with modified Niskin bottles filled with sperm representing males and unfertilized eggs in Nytex screen cages representing females will comprise our "artificial" geoducks. Divers will deploy and "spawn" artificial geoducks arrayed in situ to represent three bed densities to measure egg fertilization rates under high and low flow conditions.

**RATIONALE**

An emerging trend, including in Washington State, is the culture of native species, which is thought to reduce the potential impacts on native ecosystems. However, the culture of native shellfish species is not without risks and raises issues distinct from those involving the culture of non-native species. As native geoduck aquaculture proliferates in the State, genetic interactions between native stocks and cultured genotypes becomes a critical concern; with annual fecundities of over 10 million, it is conceivable that a few broodstock pairs could produce entire hatchery cohorts. Genetic changes associated with low $N_e$, domestication or inbreeding may decrease long-term population viability in ecologically valuable wild populations. Understanding critical aspects of geoduck culture and biology is imperative to determine whether negative effects of CWI are heightened (e.g. low hatchery $N_e$, increased reproductive success at hatchery outplant densities) or attenuated (e.g. immaturity/low fecundity due to truncated life span, inhibited reproductive success from inbreeding). Our data will enable state and tribal resource managers to proactively manage for wild geoduck resource sustainability.

## 2. ACCOMPLISHMENTS AND OUTCOMES

1) Primary activities consisted of training and preparation for genotyping, scheduled to occur in late 2008. We have modified methods for DNA isolation. We used P-LOCI (Matson et al. in press) to simulate the broodstock to offspring ratio and estimate that 3-4 extant loci will be sufficient to correctly assign >95% of offspring to parents. Five candidate microsatellite loci are currently undergoing multiplex optimization.

2) Clams (2, 3, 4 and 5 years) were induced to spawn. No females spawned and the male:female ratio was 9:1. Because of the likely lack of spawning females, we plan to adapt our experiment to yield additional data (Fig. 1) on sperm quantity and are developing a novel technique for sperm quantification using fluorescent intercalating dyes. Unless we can successfully induce spawning in cultured females, we will be unable to compare survivorship in veliger larvae from cultured and wild crosses.

3) Chilling gametes extends viability (Fig. 2); sperm viability is more limiting than egg viability.

4) Geoduck clams begin reproduction during their first year and release gametes as early as year 2 (Fig. 3), thereby suggesting the potential for five spawns in a 6yr cycle.

5) A high degree of maturation synchronicity existed among farm year classes and wild conspecifics (Fig. 3), clearly demonstrating the potential for genetic interactions among wild and cultured geoducks.

6) We developed novel systems for both sperm release and egg containment.

7) Egg fertilization rates appear similar for farmed and wild geoduck.

## 3. IMPACTS

**Sea Grant promotes research focused on minimizing environmental effects of aquaculture.** Shellfish aquaculture ecological impacts can vary from minimal to high. In Puget Sound, the culture of native shellfish may cause genetic effects on wild conspecifics. WSG-supported researchers have found that geoduck clams begin reproduction during their first year and release gametes as early as year 2 of a six year culture cycle, and a high degree of maturation synchronicity among farmed and wild conspecifics. Our field fertilization study conducted to date suggests that male densities employed did not influence egg fertilization rates and suggests that current farm densities may not result in higher fertilization rates than is expected from wild aggregations. **Impact:** Washington State resource managers (Depts. Of Ecology, Natural Resources, and Fish and Wildlife), stakeholders (shellfish growers, property owners, tribes), and the Shellfish Aquaculture Regulatory Committee (SARC) can use this information to help manage geoduck aquaculture practices.

## 4. PERFORMANCE MEASURES

### Measure 1: Economic and societal benefits derived from the discovery and application of new sustainable coastal, ocean, and Great Lakes products from the sea.

*Actual (reporting period covered by this report):*

*Anticipated (12-month period following this reporting period):*
2 benefits: Hatcheries can hold geoduck gametes at 6ºC to extend their viability, thereby providing a mechanism to increase the number of broodstock used in a spawn, and for gamete exchange among hatcheries to enhance genetic variability.

### Measure 2: Cumulative number of coastal, marine, and Great Lakes issue-based forecast capabilities developed and used for management.

*Actual (reporting period covered by this report):*

*Anticipated (12-month period following this reporting period):*

### Measure 3: Percentage/number of tools, technologies, and information services that are used by managers (NOAA and/or its partners and customers) to improve ecosystem-based management.

*Actual (reporting period covered by this report):*
8 new facts about geoduck reproduction (age at maturation, spawning synchrony, effects of gamete age on viability, effects of depth and locale on maturation and spawning, and fertilization success rates at farmed and wild densities) used by the shellfish industry, resource agencies and committees to improve farm management and wild resource stewardship.

*Anticipated (12-month period following this reporting period):*
5 new facts about the genetic relationship of hatchery and wild geoduck (differences in allele frequency, rare alleles, private alleles, heterozygosity, and estimated effective number of breeders).

5 new facts about geoduck reproduction (fertilization success rates at varied distances between individuals and effects of depth, locale, age, and provenance on gonad occupation rates) used by the shellfish industry, resource agencies and committees to improve farm management and wild resource stewardship.

## 5. PUBLICATIONS

**None during this period**

## 6. PRESENTATIONS - inc. Conference (Poster or Oral), Seminar & Public

Vadopalas B, Ma CSY, Wight NA, Davis J P, and Friedman CS (2007) Avoiding genetic effects of geoduck clam Panopea abrupta aquaculture on wild stocks: proactive steps toward sustainability. 99th Annual Meeting of the National Shellfisheries Association, San Antonio, Texas, Feb 26- March 2 (oral)

Vadopalas B, Ma CSY, Wight NA, Davis J P, and Friedman CS (2007) Potential genetic effects of geoduck aquaculture on wild stocks: current and future research. 15th Conference for Shellfish Growers, Washington Sea Grant Program. Shelton, Washington, March 5-6 (oral)

Vadopalas, B (2007) Geoduck genetics: what we know, what we don't know, and why it matters. Northwest Workshop on Bivalve Aquaculture and the Environment, Talaris Conference Center, Seattle Washington, September 13-14 (oral)

Vadopalas B, Santacruz A, Wight N, Jackels C, Davis, J, and Friedman CS (2007) Maturation and maturation control in geoduck clams (*Panopea abrupta*). 61st Joint Annual Meeting of the National Shellfisheries Association, Pacific Coast Section, Pacific Coast Shellfish Growers Association. Welches, OR. October 10-12 (oral)

Straus K, Vadopalas B, Davis J, and Friedman C (2007) Genetic Diversity of Cultured and Wild Geoduck. 61st Joint Annual Meeting of the National Shellfisheries Association, Pacific Coast Section, Pacific Coast Shellfish Growers Association. Welches, OR. October 10-12 (oral)

## 9. LIST ALL STUDENTS SUPPORTED BY OR AFFILIATED WITH THIS PROJECT

Student Name: Kristina M. Straus
Department: School of Aquatic and Fishery Sciences
Major/Degree field:
Major Professor: C. Friedman
Student Type : Ph.D.
Dissertation/Thesis title: Conservation of native shellfish in Washington state
Date of graduation (actual or anticipated): December 2009
Total support or affiliation period (e.g., Jan – June 2005): Feb-July 2008
Type of support (RA, research costs, conferences – list all that apply): RA
Current employment if applicable: Same

## 10. INTERACTIONS

We advise Washington Depts. of Natural Resources and Fish and Wildlife re. geoduck genetic integrity.

## 11. OUTREACH AND INFORMATION/TECHNOLOGY TRANSFER

Participated in the Northwest Workshop on Bivalve Aquaculture and the Environment, Talaris Conference Center, Seattle Washington, September 13-14.
Consulted for the Monterey Bay Aquarium Seafood Watch geoduck report and for an article on geoducks written for submission to The Smithsonian Magazine.

## 12. FUTURE ACTIVITIES

We intend to characterize contemporary trends in geoduck hatchery $N_e$, and pursue funding to both characterize local adaptation in geoduck and evaluate triploidy for avoidance of genetic interaction between cultured and wild geoduck.

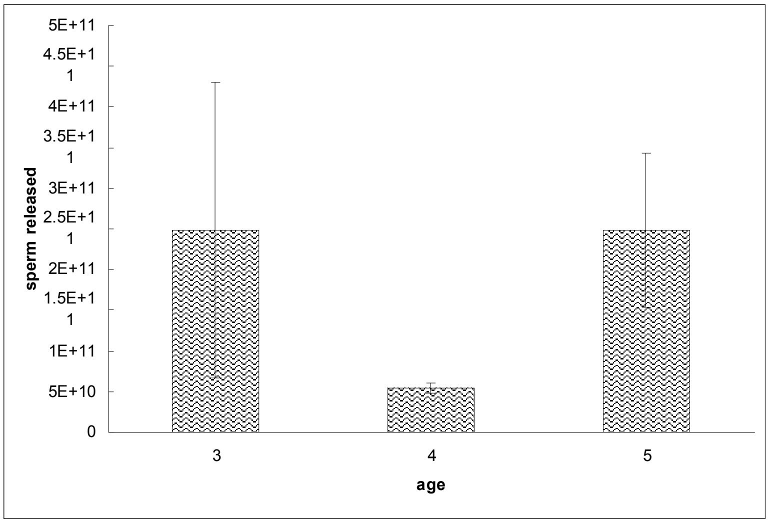

Fig. 1. Sperm counts for single induced spawns of three year classes of farmed male geoduck. Bars indicate range.

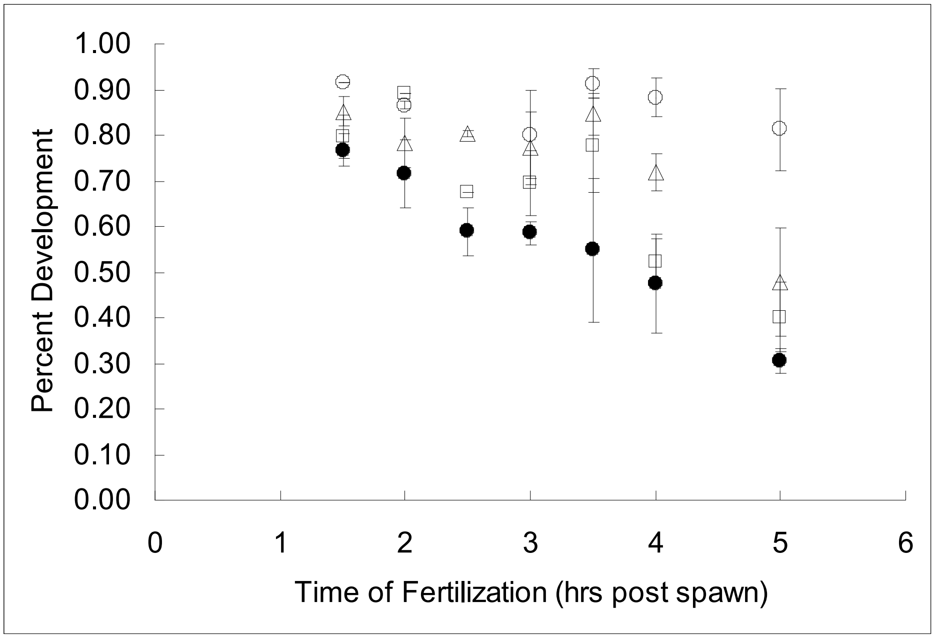


Fig. 2. Percent development in delayed fertilizations using geoduck sperm and eggs both held at 14°C (□), both at 6°C (□), sperm at 14°C and eggs at 6°C (□) and sperm at 6°C and eggs at 14°C (Δ).

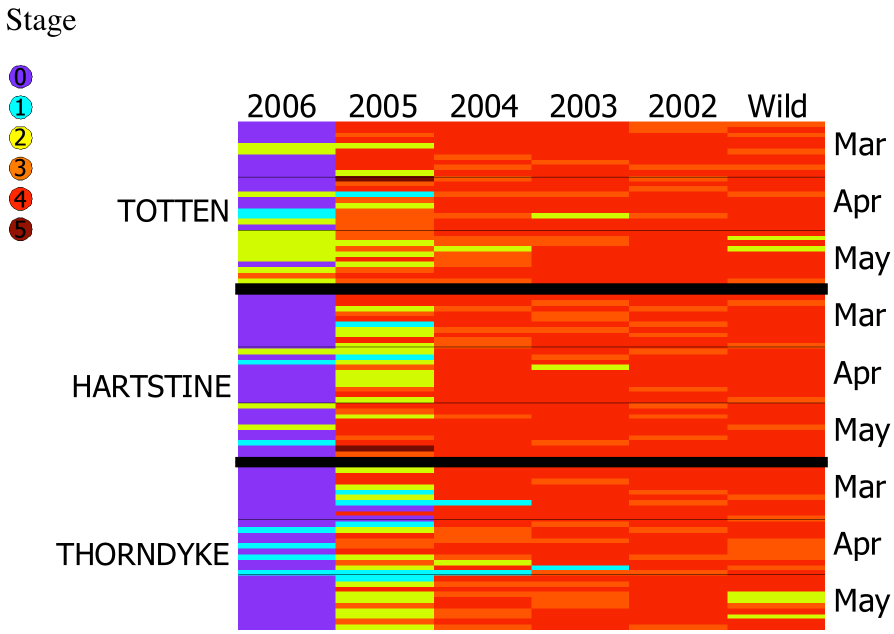


Fig. 3. Maturation stages in wild and five year classes of farmed geoduck (2002-2006) from three sites (left y axis) over three months (right y axis). Color coded stages are undifferentiated (0), early active (1), late active (2), ripe (3), partially spawned (4), or spent/resorbed (5).