ALLEVIATING REGULATORY IMPEDIMENTS TO NATIVE SHELLFISH AQUACULTURE

Environmentally and economically sustainable shellfish aquaculture is of great importance to commercial and restoration goals. Filter-feeding bivalves provide beneficial ecosystem services including nutrient sequestration and essential habitat (Coen and Luckenbach 2000). In addition, shellfish aquaculture in the United States provides domestic food, valuable coastal community jobs, and significant private, state, and export revenues.

The aquaculture industry is growing rapidly, necessitating a continued focus on the interactions between aquaculture and the environment. The culture of native species, for restoration or commercial purposes, is frequently touted as a way to reduce or avoid harmful ecological-level interactions between cultured exotics and native species (*e.g.* Naylor et al., 2001). However, the culture of native shellfish can impact nearby ecological systems and wild conspecifics by creating opportunities for genetic impacts on native populations (Hoftyzer et al. 2008; Camara and Vadopalas 2009). *If wild populations are genetically adapted to local environmental conditions, interbreeding with cultured conspecifics from other locales may disrupt patterns of local adaptation*, potentially jeopardizing wild populations by decreasing their adaptive potential. *On the other hand, the addition of genetically diverse cultured organisms may enhance genetically depauperate populations.* This enhancement is likely to occur in populations where genetic discontinuities exist as a result of population fragmentation from anthropogenic disturbances, rather than naturally occurring restrictions to gene flow.

A significant impediment to sustainable aquaculture is the lack of proper information to predict the impacts of culturing native shellfish species for restoration and commercial production. ***As a result, expansion and growth of domestic aquaculture is constrained and may be halted by management directives that restrict distribution of hatchery derived native shellfish until the potential interactions are better understood.*** Evidence of this is seen in attached letters of support: J. Hetrick- Alutiiq Pride Shellfish Hatchery, Alaska; K. Toy- Jamestown S'Kallam Tribe; R. Childers- Washington Department of Fish and Wildlife. The central goal of the proposed research is to identify and characterize the phenomena (*i.e.* local adaptation) responsible for structure in wild shellfish populations, specifically in cases where the potential for restoration aquaculture activity exists.

Background: Population Structure and Local Adaptation

In many marine bivalves, observations at neutral molecular markers of weak genetic structure, or even panmixia, indicate significant gene flow and may be considered suggestive of a lack of adaptive differentiation. On the other hand, the large populations and substantial within-population genetic variation provides plenty of opportunity for natural selection to occur in different ecological niches. Recent studies of species hypothesized to have high gene flow over large spatial scales have demonstrated the occurrence of local adaptation (e.g. Atlantic cod, Bradbury et al. 2010, Atlantic herring, Gaggiotti et al. 2009). In marine invertebrates, the preconception of little adaptive differentiation has likewise been recently challenged (*e.g.* Palumbi 2004 and references therein; Levin 2006 and references therein). The basis for these preconceptions lies in both the biological characteristics (high fecundity, broadcast spawning, and pelagic larval propagules) and the preponderance of genetic studies using neutral genetic markers (Bohonak 1999), including the concept that relatively few migrants are necessary to maintain genetic homogeneity among subpopulations. However, selection can increase survival of locally adapted populations, as measured by markers associated with adaptive genes (Marshall et al. 2010). For example, in *Strongylocentrotus purpuratus*, the purple sea urchin, Pespeni et al.

(2012) observed significant differentiation at functional genes between populations at distinct locales. Riginos and Cunningham (2005) provide strong evidence for local adaptation in the *Mytilus* spp. complex, which has been observed even on small spatial scales Yanick et al. (2003), and Sanford and Worth (2010) used reciprocal transplants to demonstrate local adaptation in the snail *Nucella canaliculata*.

If wild populations are genetically adapted to local environmental conditions, interbreeding with shellfish from other locales might disrupt patterns of local adaptation. Local adaptation can arise from a complex of parameters, such as diseases, temperatures, and salinities, at a particular locale. To characterize local adaptation, three requirements must be met (Savolainen et al. 2007). First, individuals from potentially divergent populations must be evaluated in both their home sites and in sites with different environmental conditions. Second, transferred and home site individuals must be directly compared. And third, data collection must include phenotypic fitness traits. Local adaptation is indicated if populations enjoy a "home field advantage" (Figure 1 A). In addition, even if there is no clear "home field advantage," local populations may be "internally adapted" if natural selection has favored different combinations of interacting alleles (*i.e.* co-adapted epistatic gene complexes) in different populations (*e.g.* Fenster et al. 1997). Under this scenario, interbreeding between different populations would disrupt these favorable multi-locus gene complexes resulting in outbreeding depression even in the absence of clear phenotypic differences between populations (Lynch, 1991; Templeton, 1986).

There are two facets of population differentiation to consider in restoration: 1) reproductive isolation and 2) adaptive divergence. Crandall et al. (2000) use the terms genetic exchangeability and ecological exchangeability to refer to these two facets. Genetically exchangeable populations are populations connected by ample gene flow, whereas ecologically exchangeable populations show no evidence of local adaptation. In addition, they argue that these two aspects of population distinctiveness should ideally be understood in both recent and historical time frames. If wild populations are not locally adapted, in many cases they can be treated as a single population, even when restricted gene flow is evident (Crandall et al 2000), as ecological exchangeability may obviate conservation of populations. Arguably the best diagnostic gauge of local adaptation in a reciprocal transplant experiment is the local versus foreign criterion (Kawecki and Ebert 2004), because it directly addresses differential selection among habitats (Figure 1).

In species with high gene flow, adaptive genetic differentiation can occur if post-settlement selection is strong. Referred to as balanced polymorphism (Grosberg and Cunningham 2001) such genetic differentiation can be distinguished from true local adaption using molecular tools (Sanford and Kelly 2011) as we describe below. This distinction between local adaptation and balanced polymorphism is important because with the latter, all genotypes in the population are available via gene flow for selection to act upon every generation, yielding more overall population resiliency than when differentiation arises due to low gene flow.

Combined with the growing need for conservation aquaculture to aid in species restoration, these studies highlight the necessity of understanding local adaptation. As more species are in jeopardy of listing by state and federal agencies, regulatory impediments to restoration aquaculture are likely to increase as we have observed in Washington state (e.g. letter from R. Childers- Washington Department of Fish and Wildlife).

Research Approach

For the current proposal we will investigate local adaptation by carrying out a transplant experiment as described. To complement the ecological component, we will also characterize molecular (genetic and epigenetic) factors using high-throughput sequencing technology to perform a comprehensive analysis that goes well beyond the conventional use of neutral markers. Our integrated approach will 1) provide important information on factors that underpin results from the transplant experiment and 2) provide scientifically sound, dense, and informative data on genetics and epigenetics of shellfish.

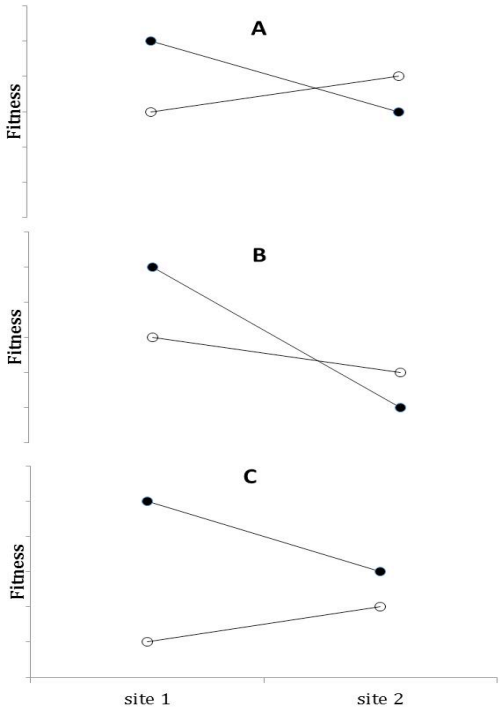


Figure 1. Evidence for local versus foreign adaptation for site 1 population (solid circles) over site 2 population (open circles) is illustrated in panels A (best overall performance at local sites) and B (better relative performance at local sites). Panel C illustrates overall better performance for the site 1 population at both sites, with local adaptation possibly confounded by site quality differences or epigenetic differences (figure adapted from Kawecki and Ebert, 2004).

Previous work has shown analyses of population divergence at neutral markers cannot satisfactorily address the issue of adaption in the presence of gene flow (Storfer 1996; Pearman 2001; Reed and Frankham 2001). For example, Savolainen et al. (2007) recently reviewed the evidence for local adaptation in forest trees using data from transplantation experiments and comparisons of population divergence for neutral molecular markers with respect to fitness traits. They concluded that local adaptation is common, and that the extent of local adaptation is determined by the balance between gene flow and selection. In voles, variation in major histocompatibility complex (MHC) allele frequencies is related to changes in population size and ecological interactions whereas variation at neutral microsatellite loci are not (Bryja et al. 2007). Several recent studies of fish populations have found that despite little or no differentiation at selectively neutral microsatellite markers, candidate loci involved in stress tolerance (Hemmer-Hansen et al. 2007) and the timing of migration and spawning (O'Malley et al. 2007) provide evidence for adaptive population divergence. These studies provide further evidence of the necessity of examining functional responses to discern the presence or absence of local adaptation or adaptive potential.

The molecular approaches in combination with the ecological approaches taken here will provide important insights into selection, fitness, and local adaptation. High-resolution genetic analyses can provide important information on phenomena driving population structure that may not be immediately apparent from transplant procedures (*i.e.* Figure 1 C). For example, if fitness proves similar among populations, our molecular approach will facilitate the determination of whether mortality is genetically random or if certain genotypes (both within and across populations) are more susceptible to mortality. Furthermore, these data will provide some of the first information on the molecular diversity, genotype-specific survival, growth, and fecundity in

shellfish production in a commercial aquaculture setting.

Restriction-site Associated DNA sequencing (RAD-seq) will be used for genotyping of individuals at thousands of single nucleotide polymorphism (SNP) loci (*see Research Plan for details*). While some markers will be neutral, the high density of markers will also produce a large suite of non-neutral SNPs. Furthermore, epigenetic markers may be informative via their close association with the environmental conditions and phenotype. The influence of epigenetics is increasingly being recognized as playing a significant role in ecology (Bossdorf et al. 2008) and livestock production (González-Recio 2012). Epigenetics refers to processes capable of inducing changes in genetic activity without altering the underlying DNA sequence (Jablonka and Lamb, 2002). In other words, epigenetic processes do not modify nucleotides but can alter phenotype. Arguably the most studied epigenetic mark and the focus in this proposal is DNA methylation, which refers to the addition of a methyl group to a cytosine residue. These epigenetic marks are sometimes inherited (Anway and Skinner, 2006), and can be independent of genotype (Liu et al 2010). As epigenetic signatures contribute to a phenotype, these markers (*e.g.* DNA methylation patterns) can be selected upon in a manner similar to genotypes.

Our recent work suggests that DNA methylation significantly affects Pacific oyster (*Crassostrea gigas*) response to different environments (Roberts and Gavery 2012). Bossdorf et al. (2010) has described similar results in *Arabidopsis*. In ongoing work, we are empirically demonstrating epigenetic stability and degree of inheritance in Pacific oysters (Gavery, 2012). As the relatively new field of epigenetics progresses, it is increasingly evident that epigenetic properties must be considered in conservation and management of natural resources. This component of the environment-phenotype interaction, largely ignored in the past due to technological limitations, may significantly change how we consider population structure and local adaptation.

The Olympia oyster

The research proposed here will focus on the aquaculture production and restoration of the Olympia oyster, *Ostrea lurida*. The Olympia oyster is grown for both commercial and restoration purposes on the Pacific coast due to its economic and ecological importance. Commercially, Olympia oysters command a high price as a specialty product. FAO production statistics are sparse, but the price has increased steadily over the last two decades (Figure 2). This species is commercially produced in Washington by at least two major shellfish growers (Taylor Shellfish Co. and Olympia Oyster Co.) as well as a number of smaller growers. As an iconic native species that declined dramatically during the first part of the 1900s (Steele 1957; Baker 1995; White et al. 2009), restoration activities have been advancing significantly since the 1990s (McGraw 2009). In part via NOAA Restoration Center funding, restoration specialists have applied a variety of strategies to increase Olympia oyster populations, including improving water quality, supplying suitable substrates for settlement, translocating naturally seeded cultch, and producing seed in hatcheries (Camara and Vadopalas 2009).

Olympia oyster hatchery and nursery techniques are now being used to supplement remnant and reestablish locally extinct populations, and for continued commercial production. For both of these enterprises, resource managers, cognizant of the potential genetic risks to wild populations, are reluctant to approve stock transfers beyond restricted locales. Through our collaborative approach, we propose to directly address this regulatory information bottleneck. We plan to develop an integrative framework to evaluate the compatibility of hatchery derived Olympia oysters with remnant wild stocks. We will accomplish our goal by a reciprocal

transplant field experiment coupled with genetic and epigenetic characterizations. In Olympia oysters, gene flow appears constrained by both biotic (pelagic, migratory phase is limited for brooded larvae) and abiotic (complex hydrodynamics in Puget Sound) factors (Stick 2012). This restriction of gene flow is an important precondition for adaptive differentiation (Sanford and Kelly 2011).

The Washington Department of Fish and Wildlife (WDFW) has asked for clear information on population differences at the adaptive level for permitting restoration using hatchery produced seed. Without scientifically sound data, as will be generated as part of this project, resource managers across the United States are reluctant to remove restrictions from commercial and restoration aquaculture activities (see letters of support: R. Childers- Washington Department of Fish and Wildlife; S. Geiger- Florida Fish and Wildlife Conservation Commission; S. Rumrill- Oregon Department of Fish and Wildlife). As co-PI, Brady Blake, WDFW native oyster restoration biologist, will insure our results provide the critical information, in usable form, necessary for regulatory decisions regarding preservation of adaptive stock structure, should it exist.

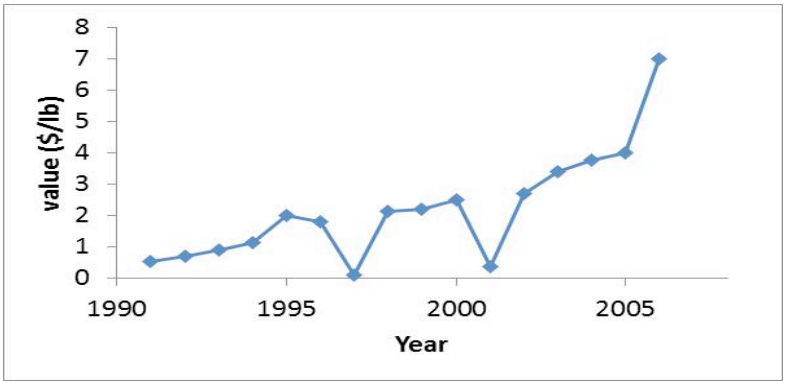


Figure 2. Olympia oyster value, 1991-2006 (FAO.org accessed 04-10-2012)

While this proposal will focus on the Olympia oyster, the underlying ecological processes and scenarios take place in aquaculture sectors across the US. The results generated from this project will not only shed light on the nuances of genetic structure as it pertains to aquaculture considerations, but because of the interdisciplinary framework of transplant experiments coupled with state of the art genomic approaches, will also provide an elegant model for informed sustainable culture of other native species (see letters of support: R. Goetz- National Oceanic and Atmospheric Administration; J. Hetrick- Alutiiq Pride Shellfish Hatchery, Alaska; S. Geiger- Florida Fish and Wildlife Conservation Commission).

Contribution to Sea Grant Strategic Plans and Initiatives

The proposed project directly contributes to addressing the problems and issues identified as priorities by NOAA and Sea Grant. Washington Sea Grant had identified Living Marine Ecosystems as a priority and specifically indicates the importance of "Understanding the marine environment and conserving marine resources while providing for sustainable use and ensuring healthy populations in the future." This research directly addresses this goal by providing scientifically sound information necessary for aquaculture activities to advance in a sustainable manner while preserving native species. Another specific Washington Sea Grant goal is to "Improve ocean literacy and interest in the marine sciences among students and educators, including those in tribal and under-represented communities". As described in the Outreach Plan (below), we have developed a multifaceted approach with our partners to educate and engage the public and resource managers via real-time sharing of data and research activities in a dedicated online portal as well the development of a citizen science effort, "*The Hunt for the Great*

Olympia Oyster". This public engagement will not only inform resource agencies about local populations of oysters, but will provide a direct means to interact with the public concerning our ecological and molecular research. During year 2, a workshop will be held by the Puget Sound Restoration Fund where stakeholders, students, educators, and the public will be invited to learn about our research results and engage in discussion about aquaculture, restoration, and local adaptation. Shellfish restoration and aquaculture are of great importance to local tribal groups (see letter of support: J. Sparkman- Squaxin Island Tribe). Tribes will not only be a part of the workshop, but are partners in our activities throughout the duration (see letter of support; J. Barber- Swinomish Tribe) and support this work as important in removing regulatory impediments (see letter of support: K. Toy- Jamestown S'Klallam Tribe).

This project directly fulfills the Cross-Cutting Goal of the National Sea Grant Program to produce sound scientific information to advance understanding of the nature and value of our coastal resources, to identify new ways to conserve and use these resources, and to support evaluation of the environmental impacts. Similarly, one of the primary goals of this project is to help inform the public to facilitate their understanding of the value and vulnerability of our natural marine resources. Once informed, we predict that the public will demand science-based decisions about the conservation, use, and management of these resources.

In December 2011, the Washington Shellfish Initiative became the first local implementation of the NOAA National Shellfish Initiative. The Washington Shellfish Initiative supports the long-term goal of abundant shellfish resources for tribal and non-tribal Washington residents, as well as a thriving and healthy commercial shellfish aquaculture industry. Our proposed project directly aligns with Section III-1B and III-2A of the Washington Shellfish Initiative by continuing vital shellfish aquaculture research partnerships and promoting Olympia oyster restoration, respectively. An example of this is our partnership with NOAA's Manchester Research Station, which was recently awarded $155,000 to construct a shellfish hatchery for Olympia oyster restoration activities (see letter of support: R. Goetz- National Oceanic and Atmospheric Administration).

RESEARCH WORK PLAN

Our approach is to simultaneously address local adaptation in three genetically differentiated populations of Olympia oysters (Stick 2012) by evaluating genotype-by-environment interactions. We will reciprocally transplant seed produced from wild parents collected from contrasting environments (Fig 3) into all environments. This very large reciprocal transplant experiment can test for a home field advantage in survival, maturation and growth in Olympia oysters. The overall goals of this project are to increase our knowledge of local adaptation in Olympia oysters to address concerns that interbreeding between potentially maladapted cultured and wild stocks could negatively impact wild populations. Accordingly, in order to attain these goals, this project has two specific objectives:

1) Evaluate fitness components and performance of seed from different origins in a reciprocal transplant experiment.

2) Characterize genetic and epigenetic markers associated with oysters from different origins in a reciprocal transplant experiment.

Research Objective 1*: Evaluate fitness components and performance of seed from different origins in a reciprocal transplant experiment.*

To investigate adaptation potential in Olympia oysters, we will conduct a reciprocal transplant experiment using seed produced from wild broodstock procured from three distinct Puget Sound locales in South Sound (Oyster Bay), Hood Canal (Dabob Bay) and North Sound (Fidalgo Bay) (Figure 3). The broodstock (n= ~600) from these three populations will be acclimated, ripened, and bred in common conditions at the Taylor Shellfish hatchery. Their progeny will also be reared initially under common conditions at the hatchery. At ~5 mm shell length (SL), 600 seed from each population will be placed into six 1 m^2 trays covered with 5 mm mesh screen at the three broodstock provenance locations (Figure 3). Each population will thus be represented by replicate trays, each containing 100 seed, in randomized blocks at growout sites at each of the three original broodstock collection locations. Field husbandry will follow standard commercial protocols for Olympia oysters.

Seed will also be placed in a fourth common environment (Clam Bay), near the NOAA Manchester Research Station. When these F1s mature, we will use the new Manchester shellfish hatchery to produce F2s for use in future work to back-truth outcomes of the research proposed herein (see letter of support: R. Goetz- National Oceanic and Atmospheric Administration).

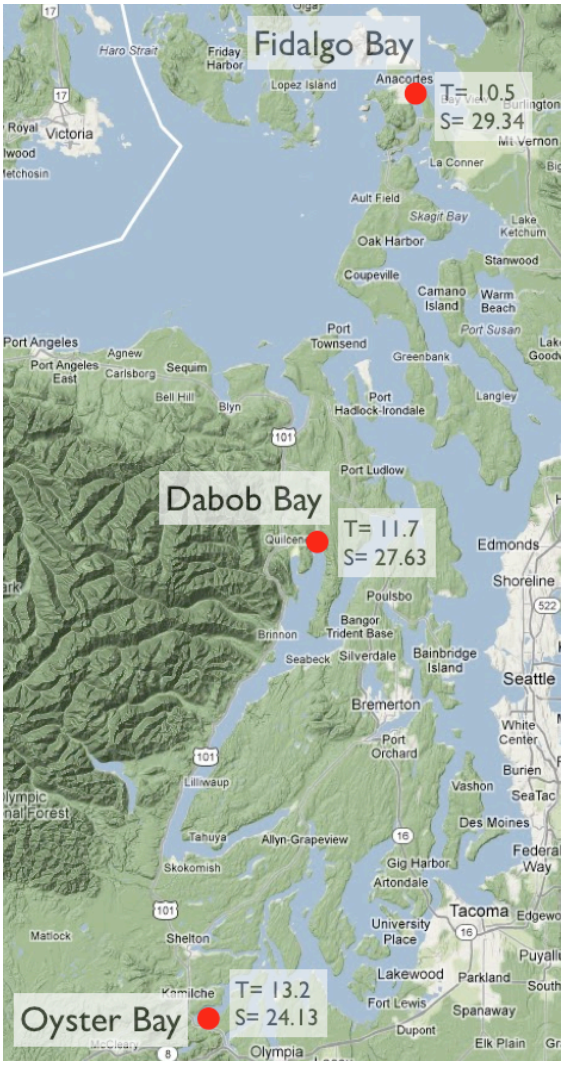


Figure 3. Map indicating three sites where Olympia oysters will be characterized for both phenotypic and molecular traits following a reciprocal transplant experiment. Mean annual temperature - ˚C (T) and mean salinty - *PSU* (S) are shown.

Phenotypic Traits

Perhaps the most important consideration for evaluating local adaptation is which phenotypic traits to measure. While it is obvious that the most ecologically relevant trait is fitness itself, measuring fitness is extremely complicated in that it incorporates many facets of the overall phenotype through the entire life cycle, including the combination of survival, growth, and fecundity. Further, because the vast majority of mortality in marine mollusks occurs during the larval planktonic stage, we recognize that a comprehensive assessment of survival would ideally include larval viability. However, there is no reason to expect that larval survival and growth under hatchery conditions (high food availability, elevated temperature, absence of salinity gradients, dissolved oxygen gradients, advection to unsuitable environments, no predators) are in any way correlated with larval performance under natural conditions. Absent a

full life cycle assessment of fitness, measurement of individual parameters can be substituted. Direct measurement of fitness components (survival, fecundity) enables insights into whether differential performance in novel environments is a plastic response or genetically based. We will focus on survival and fecundity in the field to address the question of local adaptation because these characters are key components of fitness. Any population by site interaction (Figure 1- A and B) is a strong indicator of adaptive difference. Growth, a potential fitness correlate, will also yield valuable information to the aquaculture industry.

Hatchery, nursery, and field protocols

We will procure wild broodstock from three Washington locales with extremely different characteristics: Strait of Juan de Fuca (Fidalgo Bay), Hood Canal (Dabob Bay), and South Puget Sound (Oyster Bay) (Figure 3). Broodstock will be held in a common environment prior to annual maturation for at least one month prior to conditioning for maturation and spawning. Larvae captured after release from brooding Olympia oyster females will be reared using standard hatchery protocols for the 15 day standard larval period. Pediveligers will be collected from each of the culture vessels when larvae are competent to settle and metamorphose. Pediveligers will be added to specially designed settling units utilizing a gentle water flow through a screened silo (downwell unit). Oysters will generally settle and metamorphose within 2-3 days. Following 3 weeks of culture in this primary nursery oysters will be moved to a nursery system. Following 4-6 weeks in this system, culture units will be transferred to the three origination field sites for growout.

At each field site, we will plant three experimental blocks, each containing two trays of 100 oysters from each of the three seed groups. (600 individuals/block x 3 blocks = 1800 individuals/site). Tidal height for establishing replicate blocks will be the same for all sites (approximately -1 to -2 ft mean tidal elevation). All sites are suitable for intertidal Olympia oyster culture and will be co-located with existing conspecific aggregations. Sites are characterized as having semi-permanently wet locations adjacent to tidal channels, pools, or saltwater seeps with shell or pebble-based substrates.

Survival and Fecundity

At the time of outplant and at monthly intervals, we will assess mortality and growth for all trays. In the Spring of Year 1 and Year 2, we will assess fecundity. Survival will be measured approximately monthly, depending on tidal access. Each oyster will be examined, and mortalities will be counted and removed.

Oysters will be relaxed via immersion in 50 $g \cdot L^{-1}$ $MgSO_4$ for 3-5 hrs to identify gravid females. In Year 1, we will use light microscopy to make direct counts of larvae after gently washing the ripening brood from female oysters into 100 mL of seawater. We will collect morphometric data (shell length, shell width, total weight, and buoyant weight) from oysters examined to allow us to follow growth rates and calculate fecundity relative to the size of the brooding oyster. As we expect only ~10-12% of Olympia oysters are brooding at any time (Friedman, personal observation), we can repeat this method with oysters during sequential low tides to assess the reproductive potential of the population over the entire reproduction period. In Year 2, we will assess fecundity as above. In addition, we will excise a 3 mm cross section that contains gonad, mantle digestive gland and ctenidia from each of 25 oysters per population per site. Sections will be fixed in Davidson's solution (Shaw and Battle 1957) for 24 hours and preserved in 70% ethanol until processed by routine paraffin histology (Luna 1968). We will

take high-resolution digital photos of each histology slide, from which we will calculate the gonadal somatic index (GSI=gonad area/total area), determine the sex and stage of maturation, and, if present, measure and count larvae entrained in the ctenidia of each animal. GSI and larval size will be quantified using ImageJ (Version 1.34s; NIH 2005).

Morphometric measurements & growth

For every oyster, shell length and shell width will be measured using Vernier or digital calipers and obtain whole live weights using a digital balance on a monthly basis. We will also take high-resolution digital photos of each oyster, and use ImageJ (Version 1.34s; NIH 2005) to produce the same measurements. If ImageJ analysis produces suitable data after comparison with those produced by calipers, we will exclusively use digital imagery.

Statistical Analysis

A suite of mixed effects models will be used to evaluate response variables with respect to the population x environment interactions. Survival and maturation will be analyzed as bivariate responses, while fecundity and growth will be analyzed as univariate responses. All models will include initial size as a covariate to enable inferences regarding maternal effects. All models will be evaluated for parsimony using the deviance information criterion (DIC, Gaussian responses) or the Akaike information criterion (AIC, binomial responses). Significant interaction between source population and site indicates local adaptation (Figure 1). We will also use Aster analysis (Shaw et al. 2008), a likelihood-based approach that can account for interdependent variables with different underlying distributions, to compare average fitness among sites and populations.

Research Objective 2: Characterize genetic and epigenetic markers associated with oysters from different origins in a reciprocal transplant experiment.

A central question we are addressing in the proposed work is the role of natural selection in structuring populations. This information is critical for resource managers to make informed decisions concerning commercial and restoration aquaculture production and placement. The focus of Research Objective 2 is to characterize population structure at the molecular level. This will 1) provide important information for correct interpretation of results from the transplant experiment and 2) provide scientifically sound information on genetics and epigenetics of the Olympia oyster. The latter will serve as a foundation for biological characterizations and be invaluable in supporting improved aquaculture production (*e.g.* marker assisted selection).

Molecular Sampling and Analysis

Oyster samples will be taken at two time points over the course of the project. The first sampling will occur at the initiation of the grow-out trial when hatchery produced oyster seed progeny from three separate broodstock populations (Oyster Bay, Dabob Bay, and Fidalgo Bay) are outplanted. At deployment, 100 oyster seed mantle (for RAD-seq) and ctenidia tissue (for epigenetic characterization) from each of the three source populations will be immediately frozen for DNA extraction.

RAD-seq genotyping methods will be used similar to those previously described (Baird et al 2008, Etter et al 2011). For subsequent sequencing we plan to multiplex 50 individuals per lane and will barcode samples accordingly. The RAD-seq approach uses a restriction enzyme along with size selection to reduce genomic representation of a sample to include common regions across individuals. Sequencing will be carried out by the High-throughput Genomics

Unit at the University of Washington on the Illumina Hi-Seq platform. We predict that we will be able to score approximately 10,000 loci using this approach. Loci will include single nucleotide polymorphisms (SNPs) as well as nucleotide insertions and deletions (INDELs). Loci will be evaluated and genotyping performed using STACKS software, which is installed on our computing cluster at the University of Washington. Dr. Jim Seeb has significant experience using this approach in salmonid population genomics studies and will ensure appropriate procedures and analyses are performed.

Epigenetic characterization will also be carried on these same individuals. The general approach is similar, except that DNA is digested by the methylation-insensitive restriction enzyme MspI to generate short fragments that contain CpG dinucleotides at the ends. Appropriate adaptors are added to the DNA fragments prior to being size-selected (40–220 bp) and subjected to bisulfite conversion. The products are PCR amplified and single-end sequenced on the Illumina Hi-Seq platform at the High-throughput Genomics Unit at the University of Washington. Fifty individual samples (ctenidia tissue) will be barcoded and analyzed for each of the three populations. Bisulfite sequencing requires specific alignment and data processing software to analyze DNA methylation patterns. For this effort BSMAP software will be utilized as it includes a version specifically targeted at reduced representation bisulfite data (Xi & Li 2009). The investigators have significant experience using bisulfite sequencing and analysis in the Pacific oyster (Roberts and Gavery 2012).

The second sampling for molecular analysis will occur in Year 2. The second sampling is designed specifically to evaluate the molecular traits associated with evaluating fitness components and performance of seed from different origins in a reciprocal transplant experiment (Research Objective 1). Specifically this will involve A) ***sampling oysters derived from one broodstock population at all three sites*** and B) ***sampling oysters derived from all three broodstock populations at a single site***. The former will provide important insight into the environmental response given a common genetic background; the latter will help in determining if similar or disparate mechanisms are associated with performance across populations within one location. Given budgetary considerations we will not comprehensively sample all oysters for molecular analysis, but rather will select a single population cohort with respect to point 'A' and a single site with respect to point 'B'. This decision will be made in Year 2 based on empirical data from ecological sampling. For point 'A', the genotypes of oysters derived from the selected cohort at each locale will be compared to genotypes sampled at outplanting. Based on experience we know that mortality will occur, (independent of performance). By comparing genotypes (and epigenotypes) of oyster samples we will determine whether the mortality was genotype dependent. If specific alleles can be identified as associated with increased survival, fecundity, or growth, this information will be used in future efforts in broodstock selection practices in both restoration and commercial settings.

Characterizing all oysters (originating from three broodstock populations) at a single site (point 'B') will allow us to assess how selection relates on a functional basis across populations. For instance, assuming that some form of non-random mortality occurs at a single site in all populations, we can compare the distribution of molecular markers across populations to see if selected loci are functionally related. This approach will enable us to elucidate the genetic basis underlying phenotypic responses to different environments.

Genotyping will be performed as described with two lanes on the Illumina Hi-Seq (each lane containing 50 individuals barcoded) for each of the five groups of samples in Year 2 (oysters derived from one broodstock population at all three sites = 3; plus oysters derived from

all three broodstock populations at a single site; = 2 more). Likewise, epigenetic analysis will be performed as described on 50 individuals from each group of samples.

Alignment with Program Priorities

The research plan outline above will provide essential information needed for regulatory decisions that impact commercial and restoration aquaculture. Brady Blake (a Co-PI on the current proposal; also see letter of support from R. Childers, Washington Dept. Fish and Wildlife) has asked for clear information on population differences at the adaptive level for permitting restoration using hatchery produced seed. Without the information generated herein detailing to what degree (if any) population structure is dictated by local adaptation, resource managers are challenged to make the proper regulatory decisions to ensure sustainable production and harvest of native shellfish. For instance, if population characteristics are unique but not advantageous (result of anthropogenic activity), propagation of that cohort might be detrimental to the ecosystem. On the other hand, if genetically divergent oysters are used to restore a population that is locally adapted, outbreeding depression could have negative impacts on the population as a whole. Not only will the proposed project address current and regulatory "standstills" related to restoration of native shellfish in the Washington, it also proactively provides a foundational strategy to address similar concerns nationwide, as clearly indicated by resource managers across the country (see letters of support: R. Childers- Washington Department of Fish and Wildlife; S. Geiger- Florida Fish and Wildlife Conservation Commission; S. Rumrill- Oregon Department of Fish and Wildlife).

ROLE OF PROJECT PERSONNEL

Overall project management and coordination will be carried out by Steven Roberts (UW). Roberts will also be involved in graduate student mentoring and will be integral to the molecular analysis (Research Objective 2), particularly the epigenetic characterization. Brent Vadopalas (UW) will coordinate research and conduct analyses associated with Research Objective 1 (field sites) working closely with Joth Davis (Taylor Shellfish, PSRF). Carolyn Friedman (UW) will be involved in student education and outreach, as well as be responsible for the histological analysis conducted during both years of the proposed project. Jim Seeb (UW) will oversee RAD-seq genotyping, which will include mentoring students and other scientists. Rick Goetz (NOAA) will facilitate work performed at the Manchester Research Station as well as providing guidance on molecular data analysis. Brady Blake (WDFW) will be involved in sampling and data analysis, and critical in providing continual feedback on the information required to inform regulatory decisions.

All investigators will be involved in outreach and education (see Outreach Plan section below), however specific persons will responsible for each component of the multi-faceted plan. Roberts will maintain the online portal and will implement surveys required to quantify project impact. Teri King (Washington Sea Grant) will administer the citizen science effort with Janice McNeal (Washington Sea Grant) assisting in a technical and logistical capacity. Vadopalas will spearhead the professional community outreach that includes presentations at the annual Genetics and Breeding Workshop. Betsy Peabody (Puget Sound Restoration Fund) will design and convene the workshop focused on local adaptation in cultured native marine molluscs.

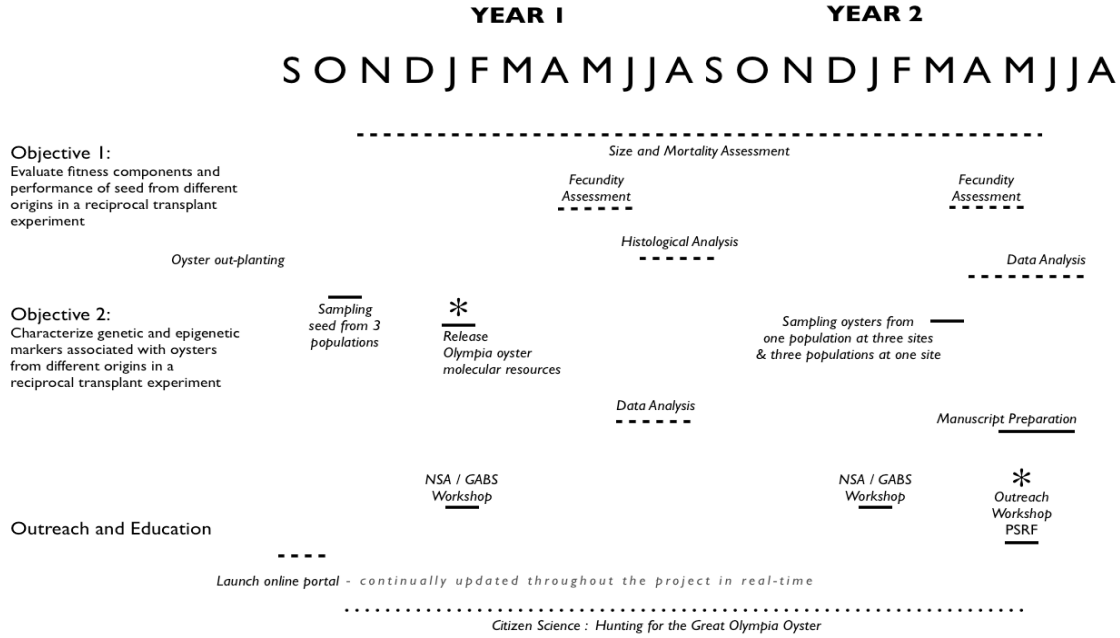
PERFORMANCE MEASURES

Our collaborative project directly involves tribal and non-tribal shellfish farmers, resource managers, and restoration practitioners. Based on our results, these stakeholders will be able to modify practices to increase aquaculture sustainability and resolve regulatory impediments to the successful expansion of domestic aquaculture. Culturists can incorporate marker-assisted protocols to ensure compatibility and inform managers responsible for making regulatory decisions. We anticipate that two commercial hatcheries and one restoration hatchery will use our results to modify practices to increase sustainable production. We also anticipate at least one resource management agency responsible for Olympia oysters will modify their requirements based on the extent of adaptive population structure in Puget Sound. The framework itself, including the integration of genomic approaches with reciprocal transplants, can be readily transferred to other species. The information we produce will identify biological impacts of shellfish aquaculture necessary for the development of sustainable policies, allow culturists to obtain permits, and contribute to the retention and creation of jobs in the shellfish aquaculture industry.

MILESTONES

In order to start this project in September 2012, oyster broodstock have already been obtained from the three sites (Fidalgo Bay, Dabob Bay, and Oyster Bay) and spawned at the Taylor Shellfish hatchery. Before seed are out planted, samples will be taken for initial genetic and epigenetic analysis. A major milestone in Year 1 will be the genetic and epigenetic resources for Olympia oysters generated from library construction and high-throughput sequencing (Illumina Hi-Seq). Phenotypic traits will immediately be characterized in the field, including fecundity analysis during the Spring of Year 1.

Ecological and morphometric analysis will continue throughout Year 2 with a second molecular sampling occurring late in Year 2. The coupling of these data with the initial molecular sampling will allow us to directly assess the underpinnings of population structure, local adaptation, and performance traits. Year 2 will include continued analysis and integration of ecological and molecular data in collaboration with all project partners. At the end of Year 2 we expect to have submitted at least two manuscripts for publication in the peer-reviewed literature. The project will culminate in a workshop hosted by Puget Sound Restoration Fund.



OUTCOMES

In the short-term, our collaborative approach will advance shellfish aquaculture by providing information on adaptive differentiation coupled with genomic resources on a native oyster species, *Ostrea lurida,* to improve restoration and commercial aquaculture production. These data alone will provide key information on genetic structure, genetic diversity, epigenetic diversity, and on the relationship between genetic and epigenetic variation. Specifically, molecular markers with adaptive significance will be made immediately available to assess and maintain appropriate genetic differences (*see Milestone Chart*). Furthermore, molecular markers may be used to aid in selection of commercially important traits. In the medium-term this project will offer an integrative, efficient framework for assessing and improving genomic compatibility of hatchery reared native shellfish. ***If*** both genomic and ecological metrics demonstrate the absence of negative impacts on remnant wild stocks, ***then*** production and distribution can be increased. On the other hand, ***if*** negative impacts are identified, ***then*** we will have developed the tools necessary to alter hatchery practices to increase sustainable production. Our novel, interdisciplinary approach will also serve as an important model for the evaluation of other US species being cultured alongside wild conspecifics.

OUTREACH PLAN

The importance of educating the public is difficult to overstate, as sharing the concepts of our integrative project and interpreting the results to a broad range of stakeholders is a critical part of our proposed research. Our multifaceted approach to this objective has four key parts including an online portal, citizen science effort, direct connection with the aquaculture community, and a targeted workshop. Each component is described in detail below.

Online Portal

A central component of our outreach plan is to document the activity and results in real-time on a dedicated online, open access portal, with all lab personnel maintaining publicly accessible notebook entries, progress reports, and images from the field. Our research group has significant experience in this: both the labs of Dr. Roberts and Dr. Friedman are dedicated to open science (*e.g.* Ocean Acidification: Research Notes from SAFS - safsoa.wordpress.com). An advantage of this platform is that it offers the public a means to engage and ask questions. With stakeholders around the United States poised to follow our progress, we expect their interactions with colleagues to increase our exposure. We will measure the effectiveness of our online platform via integrated website analytics. At the initiation of this research effort we will conduct a national online survey (supported by infrastructure of the University of Washington's information

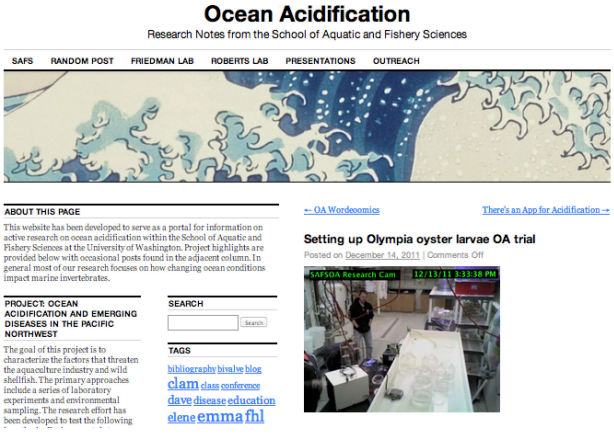


Figure 4. Example of an online portal. We will develop a similar portal for the current project. The website shown here is used to disseminate research results related to projects focused on ocean acidification.
Website address: safsoa.wordpress.com

technology resources) of at least 300 individuals about their current state of knowledge regarding local adaptation in cultured marine invertebrates. Follow-up surveys performed at the end of the project will allow us to quantify our effectiveness. We will augment the online survey with public engagement activities in cooperation with Washington Sea Grant and the Puget Sound Restoration Fund.

Citizen Science

A key component of our public outreach plan lies with our partnership with Teri King, Washington Sea Grant Marine Water Quality Specialist, who will oversee a citizen science effort to map Olympia oyster populations on private land ("*The Hunt for the Great Olympia Oyster*". This effort will not directly address the question of local adaptation, but will provide Washington Department of Fish and Game (WDFW) with critical information on locations of remnant populations in possible need of restoration. Washington Sea Grant has an impressive group of volunteers in place in the region, and for this project they will be submitting samples (shells and photos) of Olympia oysters and their surrounding habitat to Brady Blake (WDFW) along with information on date, time, location, and contact information. These data will be assessed for quality and added to the WDFW Olympia Oyster database. Paul Dinnel (Skagit County Marine Resources) has also indicated that Skagit County has a group of dedicated community volunteers (see Dinnel letter of support). This citizen science network of volunteers can educate and share the rationale and outcomes of the transplant experiment and molecular analysis. At the initiation of the mapping effort, the larger context of the project will be explained via oral presentations and information pamphlets (provided by the PI), including details on how to obtain more information through our website.

Professional Community Outreach

An important outreach goal is to effectively share our research results with the aquaculture community (e.g. growers, scientists, resource managers). The investigators on the current project are active participants in an annual international meeting for those involved in genetics and breeding of shellfish. This workshop (GABS- Genetics and Breeding of Shellfish - formerly known as WERA-99), held in conjunction with the National Shellfisheries Association annual meeting, is of seminal value for scientists, resource managers, and shellfish producers to share information and experiences. During these workshops we will share the results of our research efforts. Similarly, we will present our findings at a variety of local (Salish Sea, Pacific Coast Shellfish Growers Association, Sea Grant Shellfish Growers conferences), national and international conferences (World Aquaculture Society, National Shellfisheries Association, Association for the Sciences of Limnology and Oceanography). Our research will provide critical science that will assist managers across the United States in making informed decisions with respect to aquaculture and native species (see letters of support: Childers- Washington Department of Fish and Game; Blake- Washington Department of Fish and Game; S. Geiger- Florida Fish and Wildlife Conservation Commission; Rumrill- Oregon Department of Fish and Wildlife).

Workshop on Aquaculture of Native Shellfish

In collaboration with the Puget Sound Restoration Fund we will convene a one-day workshop focused on local adaptation in cultured native marine molluscs, which will include presentations and panel discussions by local and national experts (see letter of support: B. Peabody- Puget Sound Restoration Fund). Invited attendees will include interested NGOs, Sea Grant Marine Advisors, Tribal and State resource managers, commercial shellfish growers, and volunteer participants in our citizen science program to educate participants about local adaptation in native oysters and other aquaculture native species.

The outcome of the workshop will include: 1) a list of research priorities that can be incorporated into future work and used to guide hatchery propagation of native shellfish species; and 2) an inventory of the specific resources (financial and technical) available from different partners that can be used to create a more coordinated recovery program based on sound genetic understanding and conservation.

COORDINATION WITH OTHER PROGRAM ELEMENTS

The proposed project involves a suite of partners from industry (Taylor Shellfish, Rock Point Oyster Company), academia (University of Washington), community (Puget Sound Restoration Fund), tribes (Squaxin, Jamestown S'Klallam), and state and federal regulatory agencies (Washington Department of Fish and Wildlife, NOAA) (see corresponding letters of support). These partnerships will not only ensure appropriate methods and research results are attained, but they will also provide effective means to leverage resources and coordinate with other program elements.

As part of separate, ongoing research funded by Washington Sea Grant, our group has recently been able to generate significant transcriptomic resources for the Olympia oyster. As part of this research focused on ocean acidification, a 40,000 contig transcriptome has been produced from high-throughput sequencing. These data will allow us to potentially annotate SNPs identified from RAD-Seq. Given that DNA methylation is primarily found in the coding sequencing in oysters (Roberts and Gavery 2012), the transcriptome data will be particularly relevant in adding value to the epigenetic data.

At the core of the proposed research is the coordination of experimental partners that aligns with goals of the NOAA's National Shellfish Initiative, the Washington State Shellfish Initiative and the Puget Sound Restoration Fund. Not only are we leveraging the years of successful Olympia oyster restoration efforts spearheaded by the Puget Sound Restoration Fund, but in collaboration with the NOAA Manchester Research Station's new shellfish hatchery we will work toward conserving marine resources while providing for sustainable use.

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Current and Pending Funding - Steven Roberts

Current Funding

Title: DNA Methylation as a Mechanism to Increase Adaptive Potential in Invertebrates
Source of Support: National Science Foundation
Total Award Amount: $243,090
Total Award Period: 5/1/2012 - 4/30/2014
Time Committed: 2.0 months
Student Support: Claire Ellis
Role: PI
Relationship- Similar methods used - epigenetic analysis.

Title: Sablefish Broodstock Development and Functional Genomics
Source of Support: NOAA Contract
Total Award Amount: $349,407
Total Award Period: 9/15/2011 - 9/14/2013
Time Committed: 1.0 months
Student Support: Doug Immerman, Andrew Jasonwicz
Role: PI
Relationship- Similar methods used; genomic resource development.

Title: Effects of ocean acidification on declining Puget Sound molluscan calcifiers.
Source of Support: Washington Sea Grant
Total Award Amount: $400,300
Total Award Period: 2/1/2010 - 6/30/2013
Time Committed: 0.2 months
Student Support: Dave Metzger, Liza Ray, Emma Timmins-Schiffman
Collaborators: Carolyn Friedman
Role: Co-PI
Relationship- Research results (transcriptome) will be used in current project.

Pending Funding

Title: Alleviating Regulatory Impediments to Native Shellfish Aquaculture -
Source of Support: NOAA Aquaculture Program 2012
Total Award Amount: $427,371
Total Award Period: 9/01/2012 - 8/30/2014
Time Committed: 7.5 months (over 2 years)
Student Support: TBD
Role: PI

Brent Vadopalas Current & Pending Grants

Current

Agency: Washington Sea Grant
Title of Project: Effects of ocean acidification on declining Puget Sound calcifiers
% Time on Project: 32
Total Award: 302928
Period of Support: Feb 2010-Jan 2013
Location: Washington state: Quilcene, Friday Harbor, Seattle

Agency: NOAA Protected Resources
Title of Project: PIT tag development, tissue preservation methods, and genotyping of Pinto abalone (Haliotis kamtschatkana)
% Time on Project: 15
Total Award: 25330
Period of Support: Sept. 2011-June 2012
Location: Washington state: Quilcene, Mukilteo

Agency: Washington Sea Grant
Title of Project: Effects of early exposure of Pacific oysters to ocean acidification on subsequent performance
% Time on Project: 17
Total Award: 171442
Period of Support: Feb 2012-Jan 2014
Location: Washington state: Quilcene, Puget Sound, Seattle

Pending

Agency: NOAA
Title of Project: Alleviating regulatory impediments to native shellfish aquaculture
% Time on Project: 17
Total Award: 427371
Period of Support: Oct. 2012-Sept. 2014
Location: Washington state, Olympia, Hood Canal, and Anacortes

Agency: NOAA
Title of Project: Hatchery Production and Demonstration Growout of Purple-Hinge Rock Scallops
% Time on Project: 17
Total Award: 328714
Period of Support: Oct. 2012-Sept. 2014
Location: Washington state: Quilcene and Manchester

Agency: NOAA
Title of Project: Synthesis of environmental and human dimensions information to support aquaculture regulatory decision for Pacific geoduck clams in Puget Sound, WA
% Time on Project: 8
Total Award: 328714
Period of Support: Oct. 2012-Sept. 2014
Location: Washington state: Seattle

Current and Pending Proposals: Jonathan P. Davis

Current Projects:

Project Title: ***Effects of early exposure of Pacific oysters to ocean acidification on subsequent performance***
Washington Sea Grant Program
Amount Funded: $171,442
Funding Period: February 2012 to January 3014
Location: Washington State: Quilcene, Friday Harbor, Seattle
Person-Months Per Year Committed to the Project: 1.5 Months

Project Title: ***Effects of ocean acidification on declining Puget Sound calcifiers***
Granting Agency: Washington sea Grant Program.
Amount Funded: $478,092
Funding Period: February 2010 to January 3013.
Location: Washington state: Mukilteo, Quilcene, Friday Harbor, Seattle
Person-Months Per Year Committed to the Project: 2 Months

Project Title: ***Biosecure domestication of native geoduck clams***
Granting Agency: NOAA Saltonstall Kennedy Program
Amount Funded: $261,267
Funding Period: February 2009 to January 30 2013.
Location: Washington State: Quilcene, Washington
Person-Months Per Year Committed to the Project: 2.5 months

Project Title: ***Preparation for larger scale Olympia oyster restoration in Puget Sound***
Granting Agency: TNC NOAA Partnerships Funds
Amount Funded: $120,420
Funding period: October 1 2011 - December 31, 2013
Person-Months per Year Committed to the Project: 2.0

Project Title: ***Shellfish Initiative Project, NW Straits NFWF Funds, Port Gamble Bay Russell Family Foundation Project***
Amount Funded: 227, 040
Funding Period: October 2011 to September 2013
Person-Months per Year Committed to the Project: 2.0

Project Title: ***Abalone Restoration in the Pacific Northwest***
Granting Agency: NOAA Fisheries Protected Resources Program Office (PRPO)
Amount Funded: $3500 (Sub-contract to Baywater, Inc.)
Funding Period: September 2011 to August 31, 2014
Person-Months per Year Committed to the Project: 0.25

Project Title: ***Quartermaster Harbor bivalve mariculture nutrient mitigation analysis and outreach***
Granting Agency: Russell Family Foundation
Amount Funded: $5000 (Sub-contract to Baywater, Inc.)
Funding Period: January 2012 to December 2013
Person-Months per Year Committed to the Project: 0.25

Pending Projects:

Project Title: ***Planning for Sustainable Shellfish Aquaculture in Complex Multiple Use Environments***
Granting Agency: NOAA Sea Grant Aquaculture Research Program

Project Title: ***Hatchery Production and Demonstration Grow-out of Purple-Hinge Rock Scallops***
Granting Agency: NOAA Sea Grant Aquaculture Research Program

Project Title: ***Alleviating Regulatory Impediments to Native Shellfish Aquaculture***
Granting Agency: NOAA Sea Grant Aquaculture Research Program

Project Title: ***Restoration of Native Oysters and Ecosystem Effects in Hood Canal, Washington***
Granting Agency: TNC/NOAA Community Based Restoration Matching Grants Program

Current and Pending Funding – Frederick Goetz

Great Lakes Fishery Commission - "Morphological, physiological and genetic differentiation of lake trout morphotypes from lake superior" F. Goetz (PI) & S. Sitar. School of Freshwater Sciences, University of Wisconsin-Milwaukee, 03/01/2011-02/28/2013. $59,235. 1 man month. Not related to current proposal.

Great Lakes Fish and Wildlife Restoration Act – "Restoration Grant: Quantifying genetic, phenotypic, and reproductive differences of siscowet and lean lake trout reared in a controlled environment" F.Goetz (PI), Shawn Sitar. School of Freshwater Sciences, University of Wisconsin-Milwaukee 10/02/2010-10/01/2012. $79,800. 1 man month. Not related to current proposal

EPA - "Scaling the sublethal effects of methylmercury to population level effects in Great Lakes Perch: a multi-tiered approach using an adverse outcome pathway framework." Cheryl Murphy PI (Goetz CoPI with others). School of Freshwater Sciences, University of Wisconsin-Milwaukee, 04/01/2010-03/31/2013. $500,000. 0.5 man months. Not related to current proposal.

Great Lakes Fishery Commission - "Estimating the sublethal effects of lamprey parasitism on lipid allocation, reproduction and population dynamics of lake trout." Cheryl Murphy PI (Goetz, CoPI with others). School of Freshwater Sciences, University of Wisconsin-Milwaukee, 04/01/2010-03/31/2012. $108,800. 0.5 man months. Not related to current proposal.

NOAA National Cooperative Research Framework - "Reproductive life history analysis of sablefish populations off the Washington and Alaskan Coasts" PI F.W. Goetz and CoPI P. Swanson. NOAA Manchester Research Station, NWFSC, $300,000. 03/01/2012-03/01/2013. 1 man month. Not related to current proposal.

NOAA Internal Aquaculture Grants Program - "Genetic Analysis of Wild Sablefish in the Eastern Pacific Ocean for Broodstock Development" – PIs K. Nichols and F.W. Goetz, $99,878 . NOAA Manchester Research Station, NWFSC, 3/01/2012-3/01/2013. 1 man month. Not related to current proposal.

NOAA Internal Aquaculture Grants Program – "Technologies to preserve and extend the milt of sablefish for aquaculture operations" – PI F.W. Goetz, and CoPIs P.Swanson, A. Luckenbach. $78,717. NOAA Manchester Research Station, NWFSC, 3/01/2012-3/01/2013. 1 man month. Not related to current proposal.

NOAA Internal Aquaculture Grants Program - "Establish a shellfish aquaculture program and facility at Manchester WA: Year 1: Olympia oyster restoration in Puget Sound." W. Dickhoff PI, B. Peabody, J. Davis, T. Flagg, D. Maynard, F. Goetz (CoPIs). $155,000. 04/01/2012-4/01/2013. 0.5 man months. Not directly related to the objectives of the current grant but a direct resource for future studies on the Olympia Oyster and other shellfish and a resource for graduate students.

Pending

Great Lakes Fishery Commission - "Reproduction in lake trout morphotypes surrounding isle royale" F. Goetz PI (and others). $227,080, School of Freshwater Sciences, University of Wisconsin-Milwaukee, 03/01/2013-02/28/2015. 1.5 man months. Not related to current proposal.

Current and Pending Proposals: Carolyn Friedman

Pending Projects:

Current Proposal

Current Projects:

Project Title: Effects of early exposure of Pacific oysters to ocean acidification on subsequent performance
Granting Agency: Washington sea Grant Program. (Principal Investigator)
Amount Funded: $171,442
Funding Period: February 2012 to January 3014.
Location: Washington state: Quilcene, Puget Sound, Seattle
Person-Months Per Year Committed to the Project: Cal.: Acad. 0.5 FY 2012, 0.1 FY 2013: Sumr: 1

Project Title: Effects of ocean acidification on declining Puget Sound calcifiers
Granting Agency: Washington sea Grant Program. (Principal Investigator)
Amount Funded: $421,288
Funding Period: February 2010 to January 3013.
Location: Washington state: Mukilteo, Quilcene, Friday Harbor, Seattle
Person-Months Per Year Committed to the Project: Cal.: Acad. 2.1 FY 2010, 2.23 FY 2011, 1.45 FY 2012: Sumr: 1.2

Project Title: Understanding the roles of competing bacterial endosymbionts in abalone health, management and restoration
Granting Agency: California Sea Grant. (Principal Investigator)
Amount Funded: $325,734
Funding Period: July 2010 to January 2013.
Location: California coastal regions and Seattle, WA
Person-Months Per Year Committed to the Project: Cal.: Acad.: 1.5 Sumr: 0

Project Title: Optimizing hatchery methods for pinto abalone
Granting Agency: NOAA Protected Resources (Co-Principal Investigator)
Amount Funded: $32,496
Funding Period: September 2010-December 2012.
Location: Mukilteo, Washington
Person-Months Per Year Committed to the Project: Cal.: Acad.: Sumr: 0

Project Title: PIT tag development, tissue preservation methods, and genotyping of Pinto abalone (*Haliotis kamtschatkana*)
Granting Agency: NOAA Protected Resources (Co-Principal Investigator)
Amount Funded: $25,330
Funding Period: September 2011-June 2012.
Location: Mukilteo, Washington
Person-Months Per Year Committed to the Project: Cal.: Acad.: Sumr: 0.25