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Alleviating Regulatory Impediments to Native Shellfish Aquaculture - PI Roberts

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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.

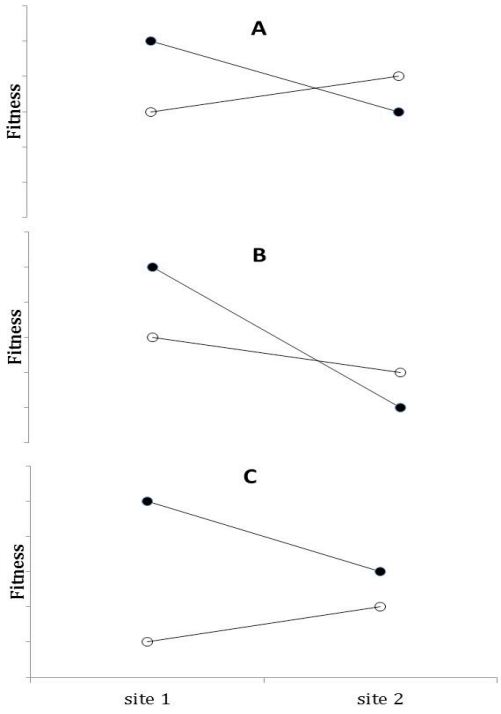


Figure 1. Evidence for local versus foreign adaptation for site 1 population (solid circles) over site 2 population (open circles) is illustrated in panels A (best overall performance at local sites) and B (better relative performance at local sites). Panel C illustrates overall better performance for 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.

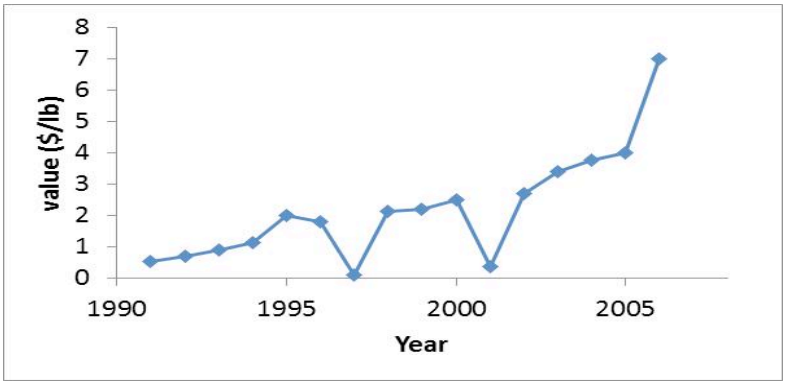


Figure 2. Olympia oyster value, 1991-2006 (FAO.org accessed 04-10-2012)

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).

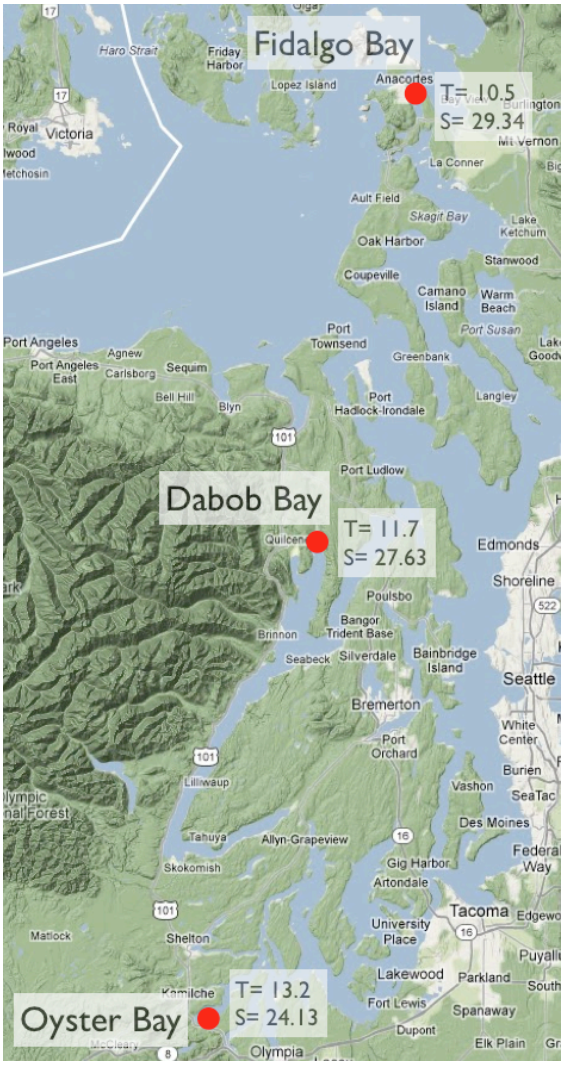


Figure 3. Map indicating three sites where Olympia oysters will be characterized for both phenotypic and molecular traits following a reciprocal transplant experiment. Mean annual temperature - ˚C (T) and mean salinty - *PSU* (S) are shown.

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.

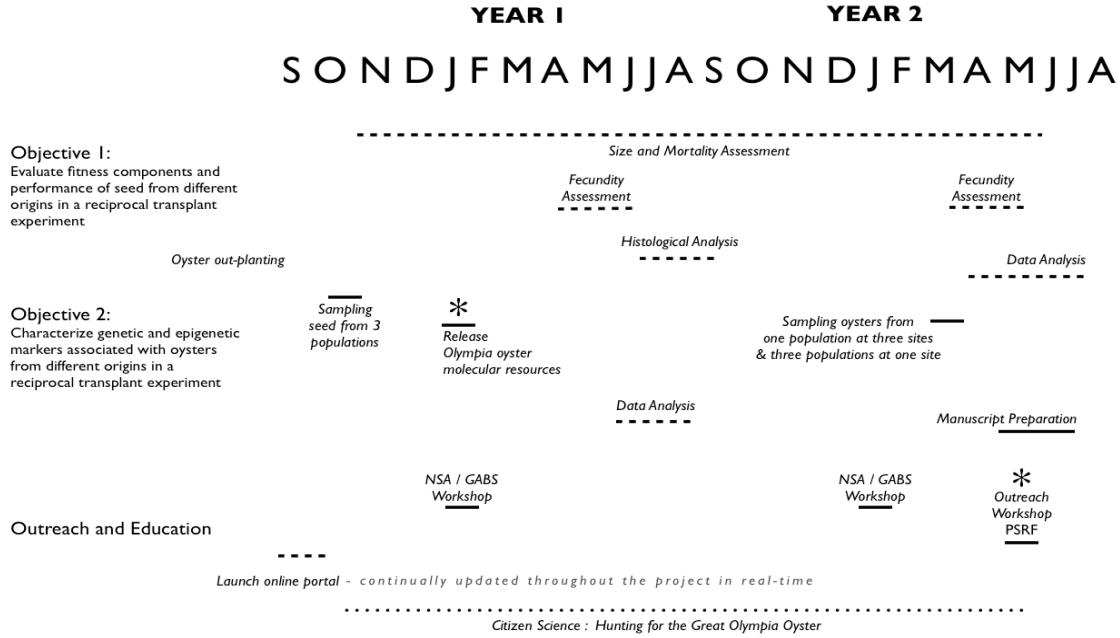
PERFORMANCE MEASURES

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

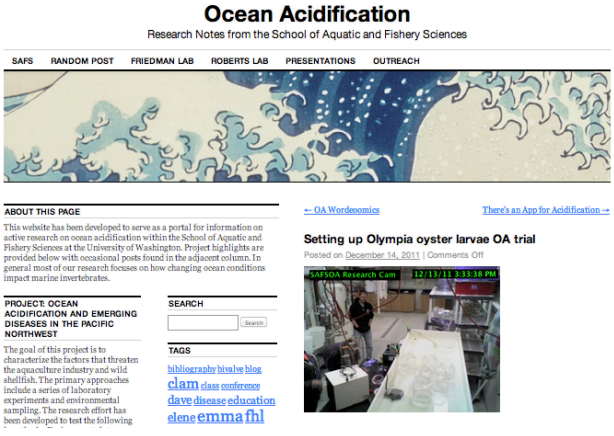


Figure 4. Example of an online portal. We will develop a similar portal for the current project. The website shown here is used to disseminate research results related to projects focused on ocean acidification.
Website address: safsoa.wordpress.com

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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Washington Sea Grant
Subaward Budget Worksheet

Parent Project PI: Roberts
Parent Project Title: ALLEVIATING REGULATORY IMPEDIMENTS TO NATIVE SHELLFISH AQUACULTURE
Subaward Institution or Organization Name: Puget Sound Restoration Fund
Budget Period (9/1/12 - 8/31/13):

A. SALARIES AND WAGES	mo. salary	benefits % (decimal: 0.XX)	sea grant mos. effort	grantee mos. effort	**SG FUNDS**	**GRANTEE SHARE**
1. SENIOR PERSONNEL						
Joth Davis	10075	*Included in salary*	1		$10,075	$0
Name					$0	$0
Add more lines as required					$0	$0
2. OTHER PERSONNEL: **Identify as:** Professional, Research Associate, Res. Asst/Grad. Student, Prof. School Student, Pre-Bachelor Student, Secretarial-Clerical, Technician, Other.						
Name					$0	$0
Name					$0	$0
Add more lines as required					$0	$0
Total Salaries and Wages					$10,075	$0
B. FRINGE BENEFITS					$0	$0
C. PERMANENT EQUIPMENT - *No Indirect Costs*						
e.g.						
Describe						
Add more lines as required						
Total Permanent Equipment					$0	$0
D. EXPENDABLE SUPPLIES AND EQUIPMENT						
E. TRAVEL						
1. Domestic - US and its Possessions (inc. Puerto Rico)						
2. International						
Destination						
Add more lines as required						
Total Travel					$0	$0
F. PUBLICATIONS AND DOCUMENTATION COSTS						
G. OTHER COSTS						
Other Cost not subject to indirect						
Describe						
Add more lines as required						
Total other Cost not subject to indirect					$0	$0
Other Cost subject to indirect						
Describe						
Add more lines as required						
Total other Cost subject to indirect					$0	$0
Tuition						
TOTAL DIRECT COSTS					$10,075	$0
H. INDIRECT COSTS	**Rate** (decimal: 0.XX)					
Sub-award organization/institution IDC rate. Enter '0' for no IDC. NOTE: IDC is calculated on all costs except PERMANENT EQUIPMENT, OTHER COST - NOT SUBJECT TO IDC and TUITION. For other IDC agreement configurations contact WSG at wsgrfp@uw.edu.					0	0
TOTAL COSTS					10,075	0

Washington Sea Grant
Subaward Budget Worksheet

Parent Project PI: Roberts
Parent Project Title: ALLEVIATING REGULATORY IMPEDIMENTS TO NATIVE SHELLFISH AQUACULTURE
Subaward Institution or Organization Name: Puget Sound Restoration Fund
Budget Period (9/1/13 - 8/31/14):

A. SALARIES AND WAGES	mo. salary	benefits % (decimal: 0.XX)	sea grant mos. effort	grantee mos. effort	**SG FUNDS**	**GRANTEE SHARE**
1. SENIOR PERSONNEL						
Joth Davis	10075	*Included in salary*	1		$10,075	$0
Betsy Peabody	10075	*Included in salary*	0.49		$4,940	$0
Add more lines as required					$0	$0
2. OTHER PERSONNEL: **Identify as:** Professional, Research Associate, Res. Asst/Grad. Student, Prof. School Student, Pre-Bachelor Student, Secretarial-Clerical, Technician, Other.						
Name					$0	$0
Name					$0	$0
Add more lines as required					$0	$0
Total Salaries and Wages					$15,015	$0
B. FRINGE BENEFITS					$0	$0
C. PERMANENT EQUIPMENT - *No Indirect Costs*						
e.g.						
Describe						
Add more lines as required						
Total Permanent Equipment					$0	$0
D. EXPENDABLE SUPPLIES AND EQUIPMENT						
E. TRAVEL						
1. Domestic - US and its Possessions (inc. Puerto Rico)						
2. International						
Destination						
Add more lines as required						
Total Travel					$0	$0
F. PUBLICATIONS AND DOCUMENTATION COSTS						
G. OTHER COSTS						
Other Cost not subject to indirect						
Describe						
Add more lines as required						
Total other Cost not subject to indirect					$0	$0
Other Cost subject to indirect						
Describe						
Add more lines as required						
Total other Cost subject to indirect					$0	$0
Tuition						
TOTAL DIRECT COSTS					$15,015	$0
H. INDIRECT COSTS	**Rate** (decimal: 0.XX)					
Sub-award organization/institution IDC rate. Enter '0' for no IDC. NOTE: IDC is calculated on all costs except PERMANENT EQUIPMENT, OTHER COST - NOT SUBJECT TO IDC and TUITION. For other IDC agreement configurations contact WSG at wsgrfp@uw.edu.					0	0
TOTAL COSTS					15,015	0

Puget Sound
RESTORATION FUND

590 Madison Ave North
Bainbridge Island, WA 98110
www.restorationfund.org
(206) 780 – 6947

April 18, 2012

Dr. Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington
Seattle WA 98105

Dear Steven,

As a subcontractor identified in the University of Washington's proposal entitled ALLEVIATING REGULATORY IMPEDIMENTS TO NATIVE SHELLFISH AQUACULTURE, Puget Sound Restoration Fund agrees to perform the following services:

Year 1:
Joth Davis will oversee field sites and sampling and conduct fecundity assessments and growth analyses at a cost of $10,075 (155 hours @ $65/hour).

Year 2:
Betsy Peabody will plan and run a one-day workshop to discuss genetic considerations of native shellfish production among a broad group of scientists, regulators, resource managers and stakeholders at a cost of $4,940 (72 hours @ $65/hour); and

Joth Davis will oversee field sites and sampling and conduct fecundity assessments and growth analyses at a cost of $10,075 (155 hours @ $65/hour).

Thank you for including Puget Sound Restoration Fund in this research.

Sincerely,

B Peabody

Betsy Peabody
Executive Director

Support and match letters

National & Washington State Resource Managers

Goetz (NOAA), Childers (WDFW)

Washington State Tribes

Sparkman (Squaxin Tribe), Toy (Jamestown S'Klallam Tribe), Barber (Swinomish Tribe)

Washington State Commercial Shellfish Growers

Taylor (Taylor Shellfish Co.), Steele (Rock Point Oyster Co.)

Washington State Restoration Practitioners

Peabody (Puget Sound Restoration Fund), Dinnel (Skagit County Marine Resources)

Nationwide Support

Hetrick (Alutiiq Pride Hatchery, Alaska), Geiger (Florida Fish and Wildlife Commission),

Rumrill (Oregon Fish and Wildlife), Tettelbach (Long Island University, New York)

Additional Letters

King (WA Sea Grant), Blake (WDFW)

National & Washington State Resource Managers

Goetz (NOAA), Childers (WDFW)

UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE
Northwest Fisheries Science Center
Manchester Research Station
Po Box 130
Manchester, WA 98353

12 April 2012

Dear Steven,

I am writing this letter to confirm both my enthusiastic support and willingness to serve as a Co-PI on the proposal to be submitted to the NOAA Sea Grant Aquaculture Research Program entitled, "Alleviating Regulatory Impediments to Native Shellfish Aquaculture". This research effort provides a scientifically sound approach to increase sustainable aquaculture production of the native Olympia oyster. The integrated approach taken in this project, along with the research outcomes, will not only benefit the Puget Sound region, but will be applicable to restoration and commercial aquaculture across the United States where natural populations exist.

As you are aware, NOAA has embarked on a National Shellfish Initiative, with the Washington Shellfish Initiative being the first implementation of NOAA's effort to increase commercial production and restore native shellfish. In conjunction with this initiative, our facility in Manchester, WA was awarded $155,000 this year to construct a new shellfish hatchery for restoration activity. This hatchery is expected to be in operation this fall and we are looking forward to working together with your group to produce larvae from oysters used in this project. These seed will not be directly used in the transplant experiment described in the proposal, but will allow us to continue this research direction beyond the extent of the award. In addition, I am happy to offer my advice and expertise on the genetic and epigenetic components of the project. We have significant experience using high-throughput sequencing to characterize commercially important fish in both wild and cultured conditions. I understand as a federal scientist, I will be an uncompensated partner on this project.

I am looking forward to working with you and the other investigators on this exciting project.

Sincerely,

Rick Goetz
Supervisory Research Scientist
NOAA Northwest Fisheries Science Center
Manchester Research Station
rick.goetz@noaa.gov

State of Washington
DEPARTMENT OF FISH AND WILDLIFE

Mailing Address: 600 Capitol Way N · Olympia, WA 98501-1091 · (360) 902-2200, TTY (800) 833-6388
Main Office Location: Natural Resources Building · 1111 Washington Street SE · Olympia, WA

April 16, 2012

National Oceanic and Atmospheric Administration (NOAA),
Sea Grant Aquaculture Research Program

Dear Sir/Madam

On behalf of the Washington Department of Fish and Wildlife (WDFW), please accept this letter of support for the grant proposal titled "Alleviating Regulatory Impediments to Native Shellfish aquaculture" submitted by Dr. Steven Roberts, School of Aquatic and Fishery Sciences, University of Washington.

Rebuilding the wild Olympia oyster population in Puget Sound will require hatchery origin seed to achieve the Department's long term native oyster restoration objectives. Due to genetic conservation concerns WDFW currently requires strict genetic conservation protocols to be followed in the production and use of native oyster hatchery seed for restoration on public tidelands. Furthermore, WDFW discourages the use of generic hatchery seed for restoration purposes on non-public tidelands and only supports use of seeding where extant natural reproductive is absent or determined to be insufficient.

The Department's shellfish management team believes the proposed research will provide critical answers regarding genetic fitness and compatibility of hatchery derived Olympia oysters with remnant wild stocks. The results of this research will provide State and Tribal resource managers information necessary for updating and developing protocols and guidelines for use of hatchery seed for native oyster restoration.

In closing, WDFW strongly encourages the NOAA Sea Grant Aquaculture Research Program to support this proposed project and believe Dr. Robert's team at the University will implement the project as proposed in a professional and timely manner.

Sincerely,

Rich Childers

Rich Childers
Puget Sound Shellfish Manager
Washington State Department of Fish and Wildlife
1000 Point Whitney Road
Brinnon WA 98320

Washington State Tribes

Sparkman (Squaxin Tribe), Toy (Jamestown S'Klallam Tribe), Barber (Swinomish Tribe)

SQUAXIN ISLAND TRIBE

April 12th, 2012

Dr. Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington
Seattle, WA 98105

Dear Dr. Roberts,

Native shellfish resources have always been important to the Squaxin Island Tribe both culturally and commercially. While historically the Tribe relied on wild stocks of shellfish, today wild stocks are still of primary importance but aquaculture practices are commonly used to enhance populations for restoration and to increase tribal harvest opportunity. The maintenance of genetically adapted native stocks is an important component of sustainable treaty fisheries. Likewise, the ability to improve aquaculture and restoration production of native species is also of importance to the Tribe as these techniques are increasingly used to reestablish populations and enhance existing populations.

On behalf of the Squaxin Island Tribe, I am writing in support of your research project " ALLEVIATING REGULATORY IMPEDIMENTS TO NATIVE SHELLFISH AQUACULTURE." This research would contribute to a better understanding of interactions between wild and hatchery produced stocks which would inform regulatory entities in their decision making with regard to hatchery reared native shellfish. In addition the evaluation of fitness and performance of seed in different areas will increase the efficiency of future hatchery production. These two out comes provide for a win-win situation for wild stock managers and aquaculturists alike.

Sincerely,

Eric A Sparkman

Eric Sparkman
Shellfish Biologist
Squaxin Island Tribe

Natural Resources Department / 2952 S.E. Old Olympic Hwy. / Shelton, WA 98584
Fax (360) 426-3971 / Phone (360) 426-9781

JAMESTOWN S'KLALLAM TRIBE

1033 Old Blyn Highway, Sequim, WA 98382 360/683-1109 FAX 360/681-4643

April 5, 2012

Dr. Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington
Seattle, WA 98105

Dear Dr. Steven Roberts,

On behalf of the Jamestown S'Klallam Tribe, I am writing in support of the proposed research project, "Alleviating regulatory impediments to native shellfish aquaculture" Shellfish resources are important both commercially and culturally to all of the Puget Sound Tribes. The Jamestown Tribe has found the shellfish aquaculture permitting process to be very cumbersome and costly. There are many regulatory agencies involved and coordination between the agencies appears to be minimal. There has been a serious decline in native shellfish species such as native littleneck clams and Olympia oysters. This research will hopefully identify where the redundancies exist and find solutions to stream lining the permitting process, which will also help accelerate the restoration of native species in Puget Sound. Please contact me @ 360-681-4641 if you have any questions.

Sincerely,

Kelly Toy
Shellfish Manager
Jamestown S'Klallam Tribe

Phone (360) 466-3163
Fax (360) 466-5309

Swinomish Tribal Community
A Federally Recognized Indian Tribe Organized Pursuant to 25 U.S.C. § 476
P.O. Box 817 • 11404 Moorage Way
LaConner, Washington 98257

4/3/2012

Dr. Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington
Seattle WA 98105

Dear Dr. Roberts:

The Swinomish Indian Tribal Community is pleased to submit this letter in support of your grant application, entitled "Alleviating Regulatory Impediments to Native Shellfish Aquaculture", to the NOAA Sea Grant Aquaculture Research Program 2012.

The Swinomish Fisheries Department is particularly interested in high-quality research programs that promote the maintenance and/or restoration of the Puget Sound ecosystem while encouraging the sustainable use of resources within the Sound. Native oysters, *Ostrea lurida*, could play a significant role in the restoration of the Sound by providing valuable ecosystem services. Furthermore, these oysters have historically played an important cultural role in local tribal communities; restoring this species could also re-establish aspects of tribal culture and history.

The research proposed by your team is especially interesting to the Swinomish Fisheries Department for several reasons: (1) understanding the genetic diversity and local adaptations of native oysters could help the Tribe develop better restoration and management practices for future outplantings, (2) results could encourage the Tribe and other stakeholders to prudently expand existing aquaculture programs to include native oysters and (3) the Tribe values careful restoration techniques and would benefit from the return of this culturally-significant species.

We wish you success with your proposal and we look forward to the presentation of your results. Furthermore, we would be interested in participating in various aspects of the research if a collaboration was of interest to this project.

Thank you.
Sincerely,

Julie Barber
Shellfish Biologist
Swinomish Indian Tribal Community

Washington State Commercial Shellfish Growers

Taylor (Taylor Shellfish Co.), Steele (Rock Point Oyster Co.)

Taylor Shellfish Farms

April 13, 2009

Dr. Steven Roberts
School of Aquatic & Fishery Sciences
University of Washington
1122 N.E. Boat Street
Seattle, WA 98105

Dear Dr. Roberts:

I am writing to confirm our collaboration in your proposed work entitled "*Alleviating Regulatory Impediments to Native Shellfish Aquaculture*" for consideration to the 2012 NOAA Sea Grant Aquaculture Research Program. My understanding is that you will be performing an experiment where Olympia oysters from different populations from Puget Sound including Fidalgo Bay, Oyster Bay and North Dabob Bay will be grown together at each of these locations in a reciprocal transplant study to examine a suite of genetic and life history parameters that relate to estimates of fitness (e.g. adaptation) among the populations based on measuring phenotypes and relating these to observed genetic differences among and between oysters and oyster populations. I recognize that alleviating impediments to regulations impacting the culture of native species depends upon a better understanding of the genetic underpinnings of adaptation in marine invertebrates in general and that using Olympia oysters as a model organism has significant benefits as this species is the object of significant restoration/restocking efforts in Washington State and along the west coast of North America.

In terms of direct match and other support, we can provide space on our intertidal property in southern Puget Sound (Oyster Bay) for you to place experimental oysters. Our understanding is that you and your students will be performing the necessary sampling work over the course of the study. We will be pleased to enable access to the site once established and will be happy to assist as we can in the maintenance of the oyster cages over the course of the study.

I also recognize that this proposal will assist greatly in better understanding genetic adaptation in this species and will have importance to the aquaculture industry generally as the culture of native species is a common practice and an increasingly important component of the shellfish industry here in Washington State.

I wish you well and hope your proposal is funded. We depend on research conducted at the University of Washington and other research institutions to enable our industry to thrive and appreciate the opportunity to collaborate with you on this project.
Please let me know if I can be of further assistance.

Best Wishes,

William Taylor
Taylor Shellfish Farms, Inc.

April 11, 2012

Dr. Steven Roberts
School of Aquatic & Fishery Sciences
College of the Environment
University of Washington
Box 355020
Seattle, WA 98195-5020

Dear Dr. Roberts:

I am writing to express my support of your proposal being prepared for submission to the NOAA Sea Grant Aquaculture Research Program 2012 entitled "Alleviating Regulatory Impediments to Native Shellfish Aquaculture". We at Rock Point Oyster Company are specifically interested in assisting your work with Olympia oysters to better understand the genetic underpinnings of adaption in marine bivalves through the provision of space necessary to maintain and grow trays of oysters on our intertidal properties here at the north end of Dabob Bay in Tarboo Bay. I understand you will be growing Olympia oysters from three distinct populations that include oysters produced from broodstocks from North Sound, South Sound and Hood Canal as part of a reciprocal transplant study. We are excited to assist as we can in providing the space for inclusion of trays on our intertidal farm.

We in the shellfish aquaculture community recognize that alleviating impediments to regulations impacting the culture of native species depends upon a good understanding of the genetic underpinnings of adaptation in marine invertebrates in general. Using Olympia oysters as a model organism has great benefit as this species is of fundamental importance and the object of significant restoration/restocking efforts in Washington State and along the west coast of North America. We recognize that this proposal will assist greatly in better understanding genetic adaptation in this species and will have importance to the aquaculture industry generally as the culture of native species is a common practice and an increasingly important component of the shellfish industry here in Washington State.

I wish you well and hope your proposal is funded. We at Rock Point are poised to assist as needed in terms of provision of the modest space required to help establish the study site on our property.

Best wishes,

David Steele

David Steele, CFO

Rock Point Oyster Company

Washington State Restoration Practitioners

Peabody (Puget Sound Restoration Fund), Dinnel (Skagit County Marine Resources)

Puget Sound
RESTORATION FUND

590 Madison Ave North
Bainbridge Island, WA 98110
www.restorationfund.org
(206) 780 – 6947

April 5, 2012

Dr. Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington
Seattle WA 98105

Dear Steven,

Puget Sound Restoration Fund strongly supports the University of Washington's proposal entitled ALLEVIATING REGULATORY IMPEDIMENTS TO NATIVE SHELLFISH AQUACULTURE, which is being submitted for funding to the NOAA Sea Grant Aquaculture Research Program 2012.

In 2010, partners in Olympia oyster restoration embarked on a 10-year goal to enhance 100 acres of native oyster habitat within the 19 historic areas identified as restoration priorities by Washington Department of Fish & Wildlife. These efforts received a big boost in December 2011 when Governor Gregoire and Dr. Lubchenco with NOAA launched the Washington Shellfish Initiative, which includes a goal to restore native oyster populations.

Several strategies are needed to rebuild core populations in historic areas. In areas that lack remnant populations, it is necessary to produce restoration-grade seed. To ensure the genetic diversity of outplanted seed, PSRF and UW have1) co-developed genetic protocols with Washington Department of Fish & Wildlife; and 2) collected genetic samples from multiple locations to assess stock structure. To proceed with restoration at a larger scale, we need to understand local adaptation and address regulatory barriers.

The proposed project will assess two broodstock locations (Fidalgo Bay and Dabob Bay) used to support Olympia oyster seed production for 2012 enhancement sites. Genetically diverse, restoration-grade seed will be outplanted at these sites in summer 2012. Seed oysters will also be held in Little Clam Bay, adjacent to the NWFSC Manchester Research Station, where NOAA and PSRF are establishing a shellfish restoration hatchery. These seed oysters will be used for further tests of local adaptation.

To support this project, Puget Sound Restoration Fund will provide $50,000 in private matching funds. This includes $25,000 from The Russell Family Foundation, which is funding the 2012 Port Gamble Bay native oyster project, and $25,000 from the Skagit Restoration Initiative, which supports Fidalgo Bay enhancements. As a subcontractor, PSRF will also coordinate a one-day workshop to discuss genetic considerations of native shellfish production among a broad group of scientists, regulators, resource managers and stakeholders.

Thank you for your consideration.

Sincerely,

B Peabody

Betsy Peabody
Executive Director

Skagit County Marine Resources Committee

1800 Continental Place, Mount Vernon, WA 98273

ph: 360/336-9400 fax: 360/336-9478 www.skagitcounty.net/SMRC

Dr. Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington
Seattle WA 98105

4 April 2012

Dear Steven:

This is a letter of support for your proposal to NOAA titled "Alleviating regulatory impediments to native shellfish aquaculture". As the lead person for the Skagit County Marine Resources Committee (Skagit MRC) on our 10 year old native oyster restoration project, I lend my personal support to the proposed project and can verify that we will have plenty of community volunteers to assist as needed on the project in the Skagit County area. These volunteers include other Skagit MRC members, highly trained and dedicated WSU Beach Watchers, and other interested community members.

Working with many partners over the course of the last 10 years, we have established the first restoration bed of native oysters in the North Puget Sound region in Fidalgo Bay, near Anacortes, WA. Densities of native oysters in our main restoration plot are now at a density of about 130 oysters/m^2 and we have had successful natural post larval recruitment in five of the ten years since the start of the project in 2002. Because of that success, we will be expanding our restoration efforts to four new North Sound locations during the summer of 2012, and will be distributing Pacific oyster shells in various new locations in Fidalgo Bay to encourage formation of additional native oyster beds resulting from natural spawning and subsequent post larval settlement.

And, as a Marine Scientist at WWU's Shannon Point Marine Center, I can offer some level of laboratory support for the proposed native oyster work and support from undergraduate students who are part of our MIMSUP (January-May) and REU (June-August) programs. Both of these undergraduate intern programs are supported by National Science Foundation grants and the students are always very eager to take part in interesting projects.

I look forward to being a partner on the proposed project,

Paul A. Dinnel, PhD
Marine Scientist
Shannon Point Marine Center, WWU
1900 Shannon Point Road
Anacortes, WA 98221

Nationwide Support

Hetrick (Alutiiq Pride Hatchery, Alaska), Geiger (Florida Fish and Wildlife Commission), Rumrill (Oregon Fish and Wildlife), Tettelbach (Long Island University, New York)

Alutiiq Pride Shellfish Hatchery
PO Box 369
Seward, AK 99664
907 224-5181 224-5282 fax
jjh@seward.net

Dr. Gene Kim
NOAA Sea Grant
1315 East-West Highway
SSMC3, R/SG
Silver Spring, MD 20910

April 12, 2012

Dear Dr. Kim,

I am pleased to support the proposal "Alleviating Regulatory Impediments to Native Aquaculture" submitted by Dr. Steven Roberts, Associated Professor from the University of Washington School of Fisheries.

I believe Dr. Stevens has assembled a very strong team of investigators with some of the foremost researchers on the west coast. This group has an excellent chance for success.

As Director of the Alutiiq Pride Shellfish Hatchery I am often confronted with many of the issues this proposal will address. The most significant is predicting the possible impacts of out stocking for enhancement purposes or for potential consequences from aquatic farming operations. Without science based research regulators always take an extremely cautious approach or prohibit projects from moving forward.

I see the results of this research to be directly applicable to Alaska as we develop the hatchery technology to produce king crabs and sea cucumbers.

I urge you to fund this project.

Sincerely,

Jeff Hetrick, Director

Florida Fish and Wildlife Conservation Commission

Commissioners
Kathy Barco
Chairman
Jacksonville

Kenneth W. Wright
Vice Chairman
Winter Park

Ronald M. Bergeron
Fort Lauderdale

Richard A. Corbett
Tampa

Aliese P. "Liesa" Priddy
Immokalee

Charles W. Roberts III
Tallahassee

Brian S. Yablonski
Tallahassee

Executive Staff
Nick Wiley
Executive Director

Greg Holder
Assistant Executive Director

Karen Ventimiglia
Chief of Staff

Fish and Wildlife Research Institute
Gil McRae
Director

(727) 896-8626
(727) 823-0166 FAX

4-11-2012

Brent Vadopalas

Principal Research Scientist

School of Aquatic and Fishery Sciences

University of Washington

Re: Letter of support for aquaculture grant application

To Whom It May Concern:

I am writing this letter of support for Dr. Roberts' et al. proposal.

In Florida, we face similar decisions regarding the optimal genetic nature of broodstock for shellfish restoration projects, often with many parallels to the Olympia oyster attempted restoration. Florida's highly altered habitats have resulted in very fragmented marine landscape, a situation which has likely reduced gene flow between subpopulations. Currently, we are discussing two potential options: selection of broodstock from the relatively healthy, stable and diverse core or selection of broodstock from incipient recovering populations. The logic of the first choice aims to maximize genetic diversity during restoration. The logic of the second choice assumes that those individuals currently present nearest the targeted restoration are genetically predisposed to be more successful in that locale. This proposal offers yet another possibility for selecting localized broodstock in that epigenetic factors may also be present that could be beneficial.

This proposal may shed light on the issue and thereby lead to a methodology capable of guiding future decision processes in Florida's shellfish restoration strategies.

Sincerely,

Dr. Stephen P Geiger - Research Scientist

Molluscan Fisheries - Marine Fisheries Research

FFWCC-FWRI

Managing fish and wildlife resources for their long-term well-being and the benefit of people.

Fish and Wildlife Research Institute
100 Eighth Avenue SE
St. Petersburg, Florida
33701-5020
Voice: (727) 896-8626
Fax: (727) 823-0166
Hearing/speech-impaired:
(800) 955-8771 (T)
(800) 955-8770 (V)
MyFWC.com/Research

Oregon
John A. Kitzhaber, M.D., Governor

Department of Fish and Wildlife
Marine Resources Program
2040 SE Marine Science Drive
Newport, Oregon 97365
(541) 867-4741
FAX (541) 867-0311

MEMORANDUM

DATE: 11 April 2012

TO: Dr. Steven Roberts, University of Washington

FROM: Dr. Steve Rumrill (Oregon Department of Fish and Wildlife / Marine Resources Program)

RE: Letter of support for research proposal

Dr. Roberts;

I am pleased to offer my strong support and recommendation to advance your collaborative research proposal titled "*Alleviating Regulatory Impediments to Native Shellfish Aquaculture.*" It is encouraging that you have assembled such an excellent and well-credentialed team, and that your interdisciplinary group has come together at this time to focus on the problematic issue of predicting the impacts of culturing native shellfish species for the dual purpose of ecological restoration and commercial production.

As you acknowledge in your proposal, legitimate concerns have been raised about the potential impacts of cultured native shellfish on recovering natural populations. This issue is unresolved by existing technical information for populations of native Olympia oysters (*Ostrea lurida*) in Puget Sound and many other locations along the Pacific coast of North America. New characterizations of genetic and epigenetic characteristics are required for this species that can be coupled directly with empirical results from common-garden and reciprocal-transplant field experiments to obtain definitive evidence of the impacts of mariculture activities on multiple metrics of ecological performance. The information generated by your collaborative research will be of widespread and immediate utility to provide better guidance to regulatory agencies and other groups that are anxious to undertake new mariculture activities designed to further enhance the recovery of Olympia oysters throughout the Pacific northwest region.

As a practitioner in the restoration and recovery of native Olympia oysters in Oregon estuaries, I look forward to the results and practical findings generated by the research. You have taken rigorous approach to the design of your characterization of the genetic and epigenetic structure of the wild and cultivated oyster populations, and the comparative experimental evaluation of fitness and ecological performance will be invaluable. I have no doubt that the work products generated by your top-caliber team will help address the fundamental problems and hurdles that currently impede substantial progress with the broad-scale restoration and recovery of native shellfish at many locations along the coast.

Sincerely,

Dr. Steve Rumrill, Shellfish Program Leader
ODFW Marine Resources Program
2040 SE Marine Science Drive
Newport, OR 97365
541-867-0300 ext. 245
Steven.s.rumrill@state.or.us

14 April 2012

Dr. Gene Kim
NOAA Sea Grant
1315 East-West Highway
SSMC3, R/SG
Silver Spring, MD 20910

Dear Dr. Kim:

I am writing in strong support of the proposed project "Alleviating Regulatory Impediments to Native Shellfish Aquaculture" which is being submitted for funding by Dr. Steven Roberts and colleagues to the NOAA Sea Grant Aquaculture Research Program. The project is well thought out and will help answer critical questions, repeatedly raised by geneticists and managers, about the potential genetic impacts of transplanted hatchery-reared shellfish stocks on native populations. The information provided by this project will help assure the sustainability of aquaculture – for *Ostrea lurida* (the focus of this study) as well as other shellfish species cultured in the United States for restoration programs and commercial production.

As the leader of a successful program to restore bay scallop populations in eastern New York over the last 7 years, I am fully cognizant of the importance of the information that will be generated by the proposed project. Dr. Roberts collaborated with my team in the early years of the NY bay scallop restoration program and I can personally attest to the quality of his work. His collaborative team of tribal and non-tribal shellfish farmers, resource managers, and restoration practitioners are also highly qualified.

In sum, I believe that the project proposed by Dr. Roberts and his colleagues will yield results that are very important to the sustainability of shellfish aquaculture in the US. I highly recommend this project for funding.

Sincerely,

Stephen T. Tettelbach, Ph.D.,
Professor of Biology
LIU-Post
720 Northern Blvd.
Brookville, NY 11548-1300

Additional Letters

Washington Sea Grant
University of Washington
P.O. Box 488
Shelton, WA 98584

360.432.3054 • fax: 360.432.3055
wsg.washington.edu

April 12, 2012

Dr. Steven Roberts
University of Washington
Box 355020
Seattle, WA 98584

Dear Dr. Roberts,

I am looking forward to working with you on the "*Alleviating Regulatory Impediments To Native Shellfish Aquaculture*" project being submitted to the NOAA Sea Grant Aquaculture Research Program. To complement the ecological and molecular approaches you and your research partners will use to provide information to resource managers on the population structure of Olympia oysters, I will be leading a gumshoe investigation of private tideland owners. This citizen science effort referred to as the "Hunt for the Great Olympia Oyster" will result in an unprecedented mapping of resources throughout Washington State.

This effort will be incorporated into the Bivalves for Clean Water (BFCW) program. The BFCW program provides outreach to private tideland owners to implement Best Management Practices regarding non point source pollution control and shellfish culture through a variety of techniques and will be expanded to teach landowners how to search for and identify Olympia oysters. Not only will this information help Washington Department of Fish and Wildlife in regulatory decisions concerning restoration aquaculture activity, but will offer an outstanding educational opportunity for community members to be engaged in science. During the second year of the proposed project I will assist in hosting of a workshop highlighting the overall projects' research results as part of the Sound Science Seminar series that I host for Washington Sea Grant.

As part of our effort I understand that you are requesting funds to cover travel, GPS equipment, and salary support for Janis McNeal. Janis is the Program Coordinator in our South Sound office that will be the main contact for tideland owners sleuthing out Olympia Oysters.

Sincerely,

Teri King
Teri King
Aquaculture Specialist

State of Washington
DEPARTMENT OF FISH AND WILDLIFE

Mailing Address: 600 Capitol Way N · Olympia, WA 98501-1091 · (360) 902-2200, TTY (800) 833-6388
Main Office Location: Natural Resources Building · 1111 Washington Street SE · Olympia, WA

13 April 2012

Dr. Steven Roberts
School of Aquatic and Fishery Sciences
University of Washington
Seattle WA 98105

To Whom It May Concern:

In support of Dr. Roberts *et al* research proposal the Department will provide 80 hours of staff time in 2012 from a Fish & Wildlife Biologist 3 and an additional 80 hours of support from the same position in 2013. Salary and benefit/overhead rate per hour is $34.18 for $2734.40 in each year and total grant match of $5468.80. Staff support will include assisting with project design, site selection, project set up, monitoring, conferring with project partners, reviewing results and conclusions.

Sincerely,

Brady Blake
Native Oyster Lead Biologist
Washington State Department of Fish and Wildlife
Point Whitney Shellfish Lab
1000 Point Whitney Road
Brinnon WA 98320