NOAA Restoration Center

National Oceanic and Atmospheric Administration
www.nmfs.noaa.gov/habitat/restoration
2008

West Coast

Native Oyster Restoration

Workshop Proceedings

August 13-15, 2007
Shelton, WA

West Coast Native Oyster Restoration, Series #2: 2007 Workshop Proceedings

NOAA Restoration Center
Office of Habitat Conservation
National Marine Fisheries Service

June 2008

U.S. Department of Commerce
Carlos M. Gutiérrez, Secretary

National Oceanic and Atmospheric Administration
Vice Admiral Conrad C. Lautenbacher, Jr., USN (Ret.)
Under Secretary for Oceans and Atmosphere

National Marine Fisheries Service
James W. Balsiger, Ph.D. Assistant Administrator for Fisheries (Acting)

Suggested Citation:

NOAA Restoration Center. 2008. West Coast native oyster restoration: 2007 workshop proceedings. U.S. Department of Commerce, NOAA Restoration Center. 86 pp.

Limited copies of this document may be obtained from:

NOAA Restoration Center
Office of Habitat Conservation
NMFS, NOAA
1315 East-West Highway, F/HC3
Silver Spring, MD 20910
(301) 713-0174

Or online at:

http://www.nmfs.noaa.gov/habitat/restoration/publications/tech_glines.html

West Coast Native Oyster Restoration: 2007 Workshop Proceedings

August 13-15, 2007
Shelton, Washington

Publication Date: June 2008

These proceedings were funded by the National Oceanic and Atmospheric Administration (NOAA) Restoration Center and California Sea Grant. All materials were compiled and edited by the workshop committee members listed below. Abstracts and presentations are included in lieu of full papers, as requested by the workshop organizers, and abstracts vary in length according to the preference of the authors. Some presentations have been modified from their original version under direction of the authors to avoid the inclusion of material that may have use limitations. Presentations have not undergone peer review, and the content is the sole responsibility of the author(s). Sponsors make no warranty with respect to the use of any information included. Views expressed are those of the authors, and do not necessarily reflect the views of the sponsors. This document does not constitute a formal publication, and is being distributed with the intent of facilitating information sharing only. Reproduction in these proceedings does not preclude supplementation and further publication of work in reviewed journals or similar publications.

Workshop Sponsored By:
NOAA Restoration Center
University of California, Davis –
Agricultural and Natural Resources Cooperative Extension
Puget Sound Restoration Fund
Taylor Shellfish Farms

Workshop committee:
NOAA Restoration Center
Kerry Griffin, Laurel Jennings, Megan Callahan-Grant
I.M. Systems Group Inc./ NOAA Restoration Center
Polly Hicks, Natalie Badrei
University of California – Davis
Agricultural and Natural Resources Cooperative Extension
Dr. Edwin Grosholz
Puget Sound Restoration Fund
Betsy Peabody

TABLE OF CONTENTS

Foreword

The Olympia oyster is the only oyster species native to the U.S. West Coast. As a filter feeding bivalve, it is an important contributor to the functioning of estuarine ecosystems, from both a water quality standpoint and because it provides three-dimensional habitat for a variety of estuarine organisms. During the 1800s and 1900s, the overall population was significantly reduced due to over-harvest, poor water quality, siltation, and the introduction of non-native predators and competitors. However, there are remnant populations, varying from a few scattered individuals to significant, self-sustaining beds comprising millions of individuals. A pre-conference field trip took participants to a Puget Sound native oyster bed that is estimated to hold up to 50 million individuals. Even with such isolated strongholds, it is clear that the overall population is greatly diminished, as compared with populations prior to European settlement. There are more examples of depleted local populations than abundant ones.

The species name of the Olympia oyster is the subject of debate. Although recent genetic research may result in a nomenclature change, we will use the commonly accepted species name *Ostrea conchaphila* in this publication.

In response to the need to restore estuarine ecosystems, many native oyster restoration projects in Washington, Oregon, and California have taken place during the past decade. These efforts continue to expand and receive funding from a variety of sources, including the National Oceanic and Atmospheric Administration's (NOAA) Community-based Restoration Program (CRP), the Puget Sound Restoration Fund (PSRF), The Nature Conservancy, and commercial shellfish growers, among others. Data gathered from these projects have demonstrated good successes, and are helping to guide practices for future restoration efforts.

Still, scientific information about native oyster restoration and the basic biology of this species is limited. Before these restoration efforts can become large-scale, we need to better understand this important organism and develop improved guidance for restoring oyster populations.

The first Native Oyster Restoration Workshop was held in 2006, in San Rafael, California. This workshop served as a catalyst for bringing together scientists, managers, and restoration practitioners from several different states. At the end, it was clear that there was still a clear need for future collaboration and opportunities to exchange information.

To continue the exchange of information, and to provide an opportunity for recent research to be presented, a second Native Oyster Restoration Workshop was held at the Squaxin Island Tribe's Little Creek Casino and Conference Center near Shelton, Washington, August 13-15 of 2007. Sponsors included the NOAA Restoration Center, PSRF, Taylor Shellfish, and the University of California at Davis. Experts from academia, industry, government, and non-profits presented and discussed the many aspects of native oyster restoration.

Field Trip

The day prior to the workshop, a dozen attendees took part in a field trip to two sites in Puget Sound. The first site, at the northern end of Case Inlet, is home to a large, self-sustaining population of native oysters. Brady Blake, with the Washington Department of Fish and Wildlife, led the site visit and provided a thorough background on the ecology, management, and policy issues of the multi-acre site. For many attendees, seeing such a dense population of native oysters was an eye-opening experience, and it served as an inspiring vision for what restoration practitioners should aim.

The second site visit was to a restoration project on Eld Inlet, managed by PSRF. This site is one of the first native oyster restoration projects in Washington State, and is an example of partnerships with willing land owners to promote and implement restoration.

Tribal Blessing

Workshop participants were honored with opening remarks and a traditional blessing by Rick Peters, a member of the Squaxin Island Tribe. Mr. Peters started working on oyster beds when he was nine years old. His grandfather had the last Olympia oyster beds in the inlet, which went out of production in 1972. Mr. Peters has a long personal and professional background related to shellfish, customary harvest, and management. He spoke about the Tribe's desire to once again see native oysters abundant in Puget Sound.

Keynote Address

Dr. Jeff Koenings, Director of the Washington Department of Fish and Wildlife (WDFW), delivered the keynote address. He described the numerous problems facing Puget Sound, including poor water quality, low populations of native shellfish, and development pressures that have placed the Sound at an ecological tipping point. He noted that the Olympia oyster is a candidate for the State of Washington's endangered species list, and discussed some of the major efforts to restore Puget Sound. He stated that major initiatives such as the Puget Sound Partnership, seek to restore the ecological functioning of the Sound, and to collaborate with smaller initiatives such as shellfish restoration.

Proceedings Structure

The presentations and discussions of the 2007 workshop are summarized here, organized by session. A summary precedes each set of session abstracts. In an effort to convey the overarching messages that came from the meeting, we have compiled a list of main conclusions, research priorities, and recommended actions on the following page. The proceedings also include a summary of the demonstrations for making concrete settling substrates.

We extend our sincere appreciation to the participants and sponsors of this year's workshop, including the Squaxin Island Tribe, the Puget Sound Restoration Fund, Taylor

Shellfish, the University of California at Davis, and the many people who helped to support the workshop.

The 2007 West Coast Native Oyster Restoration Committee:

Puget Sound Restoration Fund:
Betsy Peabody

University of California at Davis:
Dr. Edwin Grosholz

NOAA Restoration Center:
Kerry Griffin
Laurel Jennings
Megan Callahan Grant

I.M. Systems Group Inc./NOAA Restoration Center:
Polly Hicks
Natalie Badrei

Session: Populations and Perspectives

Populations and Perspectives-Session Summary

Panel Members:
Ted Grosholz, University of California at Davis
Sarikka Attoe, University of California at Davis
Susan Burke, Northern Economics, Inc.
Duane Fagergren, Calm Cove Oyster Company

During this session, speakers discussed the current and historical status of native oyster populations in California's San Francisco Bay and Washington's Puget Sound. Current studies are focusing on size class distribution of native populations and potential limiting factors to population expansion. In addition to studies examining current conditions, a wealth of historical information on oyster population and trends can be found in historical records and the in memories of oyster farmers. Also, there is an initiative to assess the economic and environmental costs and benefits of restoring native oyster populations along the West Coast.

California

Ted Grosholz and Sarikka Attoe presented a study to determine the current status of native oyster populations in San Francisco Bay and to examine potential limiting factors to oyster recovery and recruitment. In the winter of 2005/2006 there was a large rain event that resulted in a high level of freshwater input to the bay causing massive die-off of the native oyster populations. Although a number of areas in the bay appeared to be good habitat for oysters, distribution of native oysters was very limited. Predation and competition did not seem to be limiting populations, but the periodic freshwater increases may have had a greater influence. Disease was not wide spread and only had an impact on specific populations. Recruitment appears to be a main limiting factor to expansion and restoration of native oyster populations. The group is starting to look for sub-tidal populations that may be seeding the intertidal areas. Methodologies for locating these sub-tidal populations in the murky waters of San Francisco Bay are being developed.

Washington

Duane Fagergren was born into an oystering family and has many connections with other oyster growers in the southern Puget Sound. These connections have helped to provide valuable insights into historical patterns of oyster populations and methodologies for restoring these populations. Anecdotal information from these growers mirrors ideal conditions for oyster restoration habitats, such as needing gently slopping surfaces and maintaining silt free grounds with good seepage. In Washington heavy sulfur pollution from a pulp mill led to a strong die-off of native oysters. The waters had been getting better since the 1960s. In 1995 there was a prolific set of native oysters, well beyond usual recruitment levels. It is speculated that weather patterns such as the 1992 El Niño and the milder weather in 1995 may have contributed to the oyster boom. Fagergren also noted that, in addition to gathering historic information, collaboration with industry is a vital component for successful restoration. The shellfish industry has a wealth of information and resources for restoration practitioners.

Economic and Environmental Cost Assessment
Northern Economics, Inc. has developed a proposal to assess the environmental and economic costs and benefits of shellfish restoration and production in Washington State. This assessment is structured to complement the Millennium Ecosystem Assessment, by examining such benefit categories as supportive, provisioning, regulating, and cultural and by involving stakeholders in the definitions of specific benefits and costs. There is already existing support and funding for this project; however, more support and funding are needed for full implementation.

Panel Discussion
The group agreed on the need to create a central source of historic information and gray literature that is difficult to find. It was also suggested that such a clearing house of information could also hold new protocol descriptions, such as methods for identifying non-native and native larvae.

Recruitment may naturally be episodic along the entire West Coast. In addition there may be a size threshold that needs to be achieved before populations can become self-recruiting. Recruitment patterns and limitations should be better studied to understand their dynamics and impacts for restoration.

The group discussed the possible need for a document, such as a white paper, that states the direction and promise of West Coast native oyster restoration as well as guidance for answering the key questions about restoration. Most thought that this document would be useful, while some raised concerns that we do not have enough information in order to complete large-scale restoration of oysters as there is still so much information that is lacking.

Presentation (PDF)

San Francisco Bay Native Oysters: How are they doing?

Sarikka Attoe*[1], Chela J. Zabin[1,2] and Edwin D. Groholz[1]
[1]University of California, Davis, 1 Shields Avenue, Davis, CA 95616 and [2]Smithsonian Environmental Research Center at the Romberg Center for Environmental Studies, 3152 Paradise Drive, Tiburon CA 94920

Before 1900, native oysters (*Ostrea conchaphila)* were among the most abundant and ecologically important species in West Coast estuaries as well as an important fishery. Changes in hydrology, water quality and uncontrolled harvest led to the rapid decline of native oysters in California by the 1860s. Native oysters have not been harvested in San Francisco Bay for more than a century, but populations have not rebounded. Despite increasing interest in restoring oysters to the bay, a comprehensive survey of current populations had not been done and relatively little is known about the factors limiting their return.

We have surveyed the intertidal and shallow subtidal for oysters. We monitored nine sites for oyster growth and survivorship. At 13 sites, we measured the recruitment and quantified potential predators and space competitors. Oysters have also been collected for pathology analysis.

Surveys in 2006 revealed a recent die-off of oysters in the intertidal, likely due to unusually heavy spring rains. At most sites, oysters belong to a single size class, suggesting sporadic and non-recent recruitment. In 2006, recruitment was late and low. In 2007, recruitment was earlier, though still very low. Introduced drills were mainly found in the South Bay, but were not correlated with oyster densities. Total cover of potential space competitors rarely exceeds 75 percent and does not appear to limit recruitment. The incidence of parasitism and disease is low. Subtidal surveys were conducted, but only one live oyster was found

Based on this initial assessment, it does not appear oysters are limited by any of the measured biotic factors. Data suggests that small oysters are more likely to perish which suggests abiotic stresses like salinity, heat, or toxins as opposed to predation and competition. Late 2007 recruitment should provide more data on oyster survivorship. Subtidal surveys have been inconclusive due to sample size. Ecological field experiments that will address predation and competition more directly will begin in summer 2008.

Presentation 1 (PDF)

Presentation 2 (PDF)

Session: Policy, Permitting, and Regulation

Policy, Permitting, and Regulation - Session Summary

Panel Members:
Dr. Alan Trimble, University of Washington
Blain Reeves and Helen Berry, Washington Department of Natural Resources
Steve Landino, NOAA Fisheries

This session was designed to address the policy and management approaches to shellfish restoration and research in Washington, Oregon, and California. However, the California presenter was unable to attend. Dr. Alan Trimble filled the time slot, and gave a historical perspective of native oyster monitoring, recruitment, and restoration in Washington State. We have included a summary of his talk (without an abstract, ashis talk was a last-minute addition).

Dr. Trimble emphasized that the study of the native oyster requires much time and effort. A year or two of monitoring cannot substitute for decades of observations and research. Many replicates and sites are necessary to obtain an accurate picture of oyster ecology.

Because oyster set is episodic nature of oyster set, Dr. Trimble noted that it is imperative sample in the field every day, lest the set occur during a non field day. The past two years have seen significant die-off of larva during their free-floating stages. Many mollusk species succumbed in 2007, while the Pacific oysters appeared to have been hardest hit in 2007.

Finally, he noted that although the current populations are clearly depressed compared with historical numbers, we should collectively be methodical about restoration efforts. Dr. Trimble cautioned the group that because there is no imminent threat to the survival of the Olympias, it is best to avoid making mistakes in restoration efforts.

Blain Reeves and Helen Berry reviewed some of the policy and ecology of the Olympia oyster in Washington State. First, Mr. Reeves provided an overview of the Department of Natural Resources' (DNR) stewardship responsibilities on its 2.4 million acres of tidelands and bedlands. He clarified that DNR is a land management agency—not a regulatory agency—and is tasked with managing lands for multiple uses that provide a variety of benefits to the public. Conservation leasing is a new tool being used by DNR to allow, among other things, oyster restoration on DNR lands.

Helen Berry then discussed some of the review and approval criteria for conservation leasing, including management considerations, goals of the proposed lease, and how the lease would fit in with broader program goals. DNR also reviews monitoring/maintenance plans, adaptive management plans, scientific method, and the likelihood of improving habitat conditions. In summary, she reiterated that conservation leasing is a potential tool for restoring native oysters on DNR land; native oyster restoration is a recognized regional restoration priority; and scientific evaluation teams are excited about tracking developments in restoration methods.

Steve Landino provided a NOAA perspective on shellfish policy and science. Much of his office's work is in the regulatory arena, addressing both commercial shellfish and restoration activities. Under both the Endangered Species Act (ESA) and the Magnuson-Stevens Fishery Conservation and Management Act (MSA), NOAA consults with other federal agencies on any activities that may affect NOAA trust resources, including all marine and estuarine habitats. NOAA Fisheries also works proactively with groups such as the Puget Sound Partnership, environmental NGOs, and the commercial shellfish industry to address common issues. NOAA's Northwest Fisheries Science Center conducts research into propagation of fish and shellfish, and NOAA's Aquaculture Policy supports sustainable shellfish aquaculture.

In summary, Mr. Landino noted that shellfish aquaculture and restoration are important activities to the United States and to our region. The Washington State NOAA Fisheries office plays a role in permitting and environmental review of certain activities, and the NOAA Restoration Center facilitates the funding and oversight of shellfish restoration projects.

Panel Discussion
One topic raised during the panel discussion addressed the use of *C. gigas* shell as habitat enhancement, the placement of which substantially changes the existing habitat. DNR requests robust monitoring to determine the fate of the shell, infaunal population changes, and other parameters. Some audience members cautioned against using *gigas* shell for restoration purposes.

The panelists and audience discussed the potential for a white paper that would serve to document the current state of oyster restoration and science, and future direction. The paper would identify major data gaps and document the value of restoration, and could potentially influence policy.

Finally, there was discussion of the ultimate goal of restoration efforts. Although there is no great demand from the recreational community to harvest native oysters, one audience member stated her desire to see allowable recreational harvest in her lifetime. Another audience member, however, cautioned against the regulatory challenges that recreational harvest would require.

Presentation (PDF)

Policy and Ecological Considerations for Native Oyster Restoration: The Washington Department of Natural Resources Perspective

Blain Reeves and Helen Berry
Washington State Department of Natural Resources

The Washington State Department of Natural Resources (WDNR) is the trustee and steward of approximately 2.4 million acres of state owned aquatic lands. The aquatic lands managed by WDNR include approximately 68,000 acres of tidelands and 3,400 square miles of marine bedlands. Bivalve shellfish restoration projects are becoming increasingly common in Washington and it is likely that WDNR could play a significant role in the future. This presentation provides an overview of the relevant WDNR policy and ecological considerations for shellfish restoration on state owned aquatic lands.

Managing State Owned Aquatic Lands

The Washington State Legislature directed WDNR to provide a balance of public benefits for all citizens of the state (RCW 79.105.030). Public benefits for aquatic lands are varied and include encouraging direct public use and access, fostering water-dependent uses, ensuring environmental protection, utilizing renewable resources, and generating revenue for management and restoration.

Generally, WDNR relies on other federal, state and local entities with regulatory authority to review and permit activities on state owned lands before issuing a use authorization (lease). This provides some assurance that proposed activities will contain measures to mitigate for impacts to the natural resources. Because the majority of the proposed activity on state owned aquatic lands have impacts to natural resources even with mitigation measures, WDNR recently implemented a new program to better ensure environmental protection.

Conservation Leasing

Conservation leasing provides a new tool for using state owned aquatic land for conservation purposes. The WDNR established the conservation leasing program to build partnerships with other public agencies and private parties to preserve, restore, enhance and create aquatic habitat. Ultimately, this program will better assure environmental protection of critical aquatic habitats and allow for a broader perspective in managing state owned aquatic lands.

Conservation activities are defined by WDNR as preservation, restoration, enhancement, and creation actions that are completed separate from any regulatory requirement and are not used for park purposes or to provide opportunities for public access. Leasing for preservation is preferred at sites that have natural resources that should be maintained in their present state. Some examples of good candidates for preservation sites include areas with high resource production values, high biodiversity, or unique species. Restoration is done to return an aquatic area to a previously existing natural condition from a disturbed or totally altered state. It is not necessary to have complete knowledge of the pre-existing conditions. Rather, it is enough to know a certain type of habitat was there and have a goal of restoring that same habitat type. Enhancement projects improve specific functions or habitats or increase their levels of production. Generally, enhancement projects are evaluated in terms of their overall impact. Some enhancements, such as dredging to create a pond for waterfowl in a wetland that currently does not have open water, are fairly high impact. High impact activities usually require permits and result in enhancing one function at the expense of others. Creation activities occur when habitat is built in an area where it did not previously exist. Reasons for creation are not necessarily great from an ecosystem perspective. Rather, creation activities are often a response to management needs rather than habitat value or objectives. As a rule, created aquatic habitats, or wetlands, do not function as well as restored wetlands.

The WDNR has a preference for which conservation activities are implemented. Projects that demonstrate a connection to conservation activities on adjacent lands are preferred. In areas that have degraded or lost habitat, WDNR will encourage restoration and enhancement of historical aquatic habitats and functions. Projects that restore or enhance processes are preferred over those that restore or enhance features. Preservation of naturally functioning habitat is encouraged. However, at preservation sites maintenance, monitoring, reporting and outreach are required. Creation is the least desirable of the options. Creation should be considered only when it replaces critical habitat lost elsewhere or occurs in degraded areas and does not impact naturally functioning habitats.

There are three types of conservation contracts used by WDNR. Conservation leases are issued for conservation activities that occur continuously on a site (i.e. 15 or more days per month), encumber the site for greater than one year, and are exclusive in nature (i.e. the lessee has the expectation that the habitat improvements made to the site will not be disturbed by other WDNR use authorizations). Responsibility for site management lies exclusively with the lessee under conservation leases. Conservation easements are issued when a proponent requires less exclusivity (i.e. within a limited area where the conservation activity took place only). Consequently, easement proponents assume less site management responsibility than lease proponents. Conservation licenses are the lesser form of conservation contract and are issued under two scenarios; when a conservation activity is sporadic in nature (i.e. occurs on the site less than 15 days per month) and does not exceed five years in duration, or when an activity is continuous in nature (i.e. 15 or more days per month) but does not exceed one year in duration. Conservation licenses are appropriate when a project proponent has no expectation that the habitat improvements made to the site will be protected from disturbance by other WDNR use authorizations (non-exclusivity). Licenses mandate limited site management responsibility and can not be used for preservation projects.

The use of state owned aquatic lands for conservation is valued as water dependent and subjected to fees according to existing statues and rules. Conservation activities conducted under a conservation license are charged at a prorated fee based on the length of time the site is being used. The WDNR has some authority to negotiate these rates based on the level of exclusivity needed by the proponent. A formal application process is required regardless of the type of contract being pursued by a proponent. The requirements for surveys and regulatory approvals are the same with any application for use of state owned aquatic lands. With all conservation contracts, a site manager must be designated and that person will have a combination of education and experience that demonstrates capacity for aquatic natural resource management. Conservation project proponents must demonstrate the capability to fund a project through the duration of the contract and may be required to provide a performance bond, letter of credit, escrow account, or other written financial guarantee.

A conservation lease, easement and license all require a conservation plan with specific elements. The first required element is a Project Goal. This should be clearly stated and reflect an ecological endpoint. All conservation projects must have a clear Definition of Success. These should be defined as a range of acceptable outcomes to account for ecological variation. A clear Project Description must describe what is going to be done and when/where, including maps, drawings, and timelines. Conservation plans should also contain Monitoring Plans (proponents are responsible for creating the plan, conducting the monitoring and reporting to WDNR) and a Maintenance Strategy (schedule and description of ongoing maintenance activities). Conservation activities may fail and it is critical that a proponent has a defined set of actions they will undertake to ensure the projects success. This required element is called the Adaptive Management Strategy. Finally, proponents are required to describe through a regular Reporting Schedule, a synthesis of monitoring findings and their relationship to identified measures of successes.

During project development, WDNR scientific staff review draft conservation plans. The overall purpose of the review is to ensure that the project 1) supports conservation leasing program's mandate; 2) has reasonable and achievable goals; and 3) includes a well integrated monitoring plan to measure project success. As part of the review, the proposal is evaluated to ensure that it utilizes robust scientific methods and that the proposed activities are appropriate at the identified site.

Applications of Conservation Leasing to Native Oyster Restoration

WDNR's first two conservation activities have been related to native oyster restoration. The scientific review found that Olympic oyster restoration activities are potentially highly compatible with WDNR's conservation leasing goals and objectives. The biggest concern is the potential impact of adding non-native oyster shell to beaches in order to increase the amount of available hard substrate. Often habitat modification can have unintended consequences, and as land stewards it is important for WDNR to take a precautionary approach to conservation activities.

WDNR scientists identified the following priorities for native oyster restoration:

- Further experimentation with restoration methods, along with monitoring of
    - Olympia oyster recruitment and survival,

    - Effects of techniques on physical habitat characteristics, biotic community assemblage, and habitat usage;
- Standardized monitoring protocols in order to maximize comparability among sites and methods;
- Research into historical distribution of Olympia oysters;
- Research into factors limiting oyster recruitment and survival;
- Improvement of site evaluation techniques to predict areas with high probability of restoration success.

Presentation (PDF)

Session: Genetics and Nomenclature

Genetics and Nomenclature-Session Summary

Panel Members:
David Stick, Oregon State University
Nathan Wright, University of Washington
Russel Barsh and Madrona Murphy, Kwiaht, Center for Historical Ecology and the Salish Sea
William Hewson, California State Fullerton

Presentations from this session focused on genetic variation within and among remaining and historical native oyster populations, genetic techniques for studying larval dispersal, and impacts of genetic and morphological findings on the scientific nomenclature of Olympia oyster. Genetic variation of remaining oyster populations is an important piece of information for the restoration community to understand when making decisions about restoration techniques, especially seeding oysters. Populations may be genetically distinct due to existing and historic environmental conditions or they may be suffering from a bottleneck because previous influxes of new genes have been eliminated by the loss of adjacent populations.

Understanding the genetics and dispersal patterns of Olympia oysters has the added complication of historical transport of live oysters from Puget Sound into California during the Gold Rush. Populations from Vancouver Island, BC are thought to be relatively pristine because they have not been as heavily impacted from harvesting, pollution, and land use activities. Populations from Puget Sound south to Humboldt Bay, California, are potentially bottlenecked, while those further south in California are likely heavily mixed from transported oysters.

Genetic Variation

David Stick presented his study of Olympia oyster genetics from populations located from Vancouver Island, BC, to San Francisco, CA. Significant genetic variation (85.6% of the observed variation) was found among five major geographical regions including Vancouver Island, Puget Sound, Willapa Bay, Coos Bay, and Tomales Bay, indicating that these geographical areas are genetically separated. Willapa and Coos Bay were not genetically distinct from each other. Within the Puget Sound region significant genetic variation (38% of the observed variation) was found among populations from the North Sound, Central Basins, South Sound, and Hood Canal. Hatchery–propagated restoration efforts could alter the genetic structure of these populations if practitioners go outside of basins to select broodstock for seeding efforts. However, although there are observed distinctions among the large and small geographic regions, it is still unknown whether the genetic differences result from adaptive variation.

Russel Barsh and Madrona Murphy presented their on-going study to reconstruct native oyster distribution and population structures in northern Puget Sound. To help with this effort, they are testing whether genetic information from historic populations can be extracted from Olympia oyster shells. Historic genetic analyses have been conducted for other species, and preliminary tests for methodologies indicate that viable DNA remains in buried shells.

Dispersal
As noted during last year's conference, there is currently a limited understanding of Olympia oyster larval dispersal patterns, which may greatly affect restoration projects and the techniques used to successfully restore populations. Nathan Wight is developing a method for identifying and quantifying oyster larvae from samples that may contain more than one species' larva. Previous methods were time intensive, required very specific training on larvae identification, and were not 100% accurate. Nathan is examining the potential to use quantitative realtime PCR (qPCR) to both identify and quantify oyster larvae from water column samples. Because this method uses DNA, the identification is 100% accurate. The methodology has been fully developed and is now undergoing field testing.

Nomenclature
Prior to 1985, populations of Olympia oyster from its northern extent down to the Baja Peninsula were described as *O. lurida* and populations along mainland Mexico as *O. conchapila*. After 1985 these were combined into one species, *O. conchapila*. Maria Polson, Bill Hewson and their colleagues are examining morphological and genetic data to determine whether this was an appropriate nomenclature change. Using mitochondria DNA from two Mazatlan populations and multiple populations from Vancouver Island down to Baja, preliminary phylogenetic trees indicate that there should be two species. However, more genetic work—including examining samples from locations where these two potential species geographically overlap—must be completed before any nomenclature change is proposed.

Panel Discussion
The group discussed the implications of this and future genetic work on restoration actions. In Washington State there is a concern about preserving the genetic structure of Olympia oyster populations. It was noted that in addition to current genetic studies, we also need to look at historical genetic distribution as well as the implications of genetic variance for broodstocking. If populations are naturally more isolated and are not bottlenecked one would want to preserve their genetic structure. However, if populations are genetically "sick" because of reduced population size and input of new alleles, then it could be beneficial to introduce new genes. A better understanding of current and historic genetic structure and the adaptive nature of genetic differences is critical to restoration efforts.

There was support for changing the name of Olympia oyster to *O. lurida*; however, it was noted that more work is needed to make an accurate scientific nomenclature change.

Preliminary analyses of genetic structure within and among remnant populations of the Olympia oyster, *Ostrea conchaphila.*

David A. Stick*[1], Chris Langdon[1], Michael A. Banks[1] and Mark D. Camara[2]
* Corresponding author [1] Oregon State University, COMES, HMSC, Newport, OR [2] USDA-ARS, HMSC, Newport OR

The Olympia oyster, *Ostrea conchaphila*, is the only oyster species native to the Pacific Northwest. Historically, this species ranged from Southeastern Alaska southward through Baja, California in densities capable of supporting both Tribal subsistence fisheries and large commercial harvest operations. Over-exploitation, habitat degradation, and competition and predation from non-native species have drastically depleted these densities and extirpated many local populations. Ecological benefits provided by oyster reef habitats and the species' historical significance has fueled numerous restoration/supplementation efforts of the Olympia oyster. However, these efforts are proceeding without a clear understanding of existing genetic structure among populations, which could be substantial as a consequence of limited dispersal and/or anthropogenic impacts resulting in localized genetic bottlenecks or population admixture due to transplantation.

We have recently developed a number of microsatellite DNA markers in *O.conchaphila* and have used five of these to conduct preliminary analyses of genetic diversity within and differentiation among five major geographical regions: Vancouver Island (BC), Puget Sound (WA), Willapa Bay (WA), Coos Bay (OR) and Tomales Bay (CA). A hierarchal Analysis of Molecular Variance using the GDA program, found that differentiation among the five geographical regions accounted for 85.6% of the total observed genetic variation (Fst=0.0615, $p<0.05$), while 14.4% of the variation was observed among subpopulations within these regions (Fst=0.0103, $p<0.05$). Additionally, a sub-analysis of the genetic structure among extant populations within the Puget Sound region found that differentiation among four major geographical areas within the sound (North Puget Sound, Hood Canal, Central Basin and South Puget Sound) accounted for 38.2% of the total observed genetic variation (Fst=0.0079, $p<0.05$), while 61.8% of the variation was observed among the subpopulations within those areas (Fst=0.0097, $p<0.05$). Neighbor-joining trees based on Nei's genetic distances support these findings.

Some ongoing restoration efforts are utilizing hatchery-propagated oysters to supplement extant populations. We demonstrate that these efforts have the potential to inadvertently alter the genetic composition of recipient populations in either of two ways. First, we estimated pair-wise Fst and the genetic distances between the source population used as broodstock for hatchery production and two recipient populations supplemented using the resultant juveniles and found significant levels of genetic differentiation (Fst=0.0394, p=0.00002; Fst=0.0009, p=0.00357). Second, we examined how hatchery management practices and random sampling effects may generate significant founder effects by estimating pair-wise Fst and genetic distances between an extant population and hatchery-produced seed from parents collected from the same population. Again, we found significant differentiation (Fst = 0.0036 , p = 0.00015).

This is the first reported evidence of genetic structure among extant populations of the Olympia oyster, indicating limited gene flow not only between major geographical regions, but also within those regions as demonstrated by the significant genetic differentiation within the complex Puget Sound system. Our findings will help restoration efforts not only in the selection of appropriate broodstock but also in monitoring of the success of the effort itself. It is important to note, however, that our findings are based on presumably selective neutral microsatellite markers and thus should not be interpreted as evidence of local adaptation.

Figure 1. The variance among the 5 cited geographical regions of Willapa Bay, Coos Bay, Tomales Bay, Puget Sound and Vancouver Island accounted for 85.6% of the total observed genetic variation. The relationship among the sampled populations is clearly demonstrated in this neighbor-joining tree based on Nei's definition of Genetic Distance. Genetic distance is an estimate of the accumulated number of allelic differences per locus between populations. If we assume that rate of gene substitution per year is constant, then the genetic distance is linearly related to the evolutionary divergence time between populations. It may also be linearly related to the geographical distance between populations. What we see is a tight clustering of subpopulations within the defined geographical regions of Tomales Bay, Vancouver Island and the Puget Sound, but a high degree of similarity between the Willapa and Coos Bay populations.

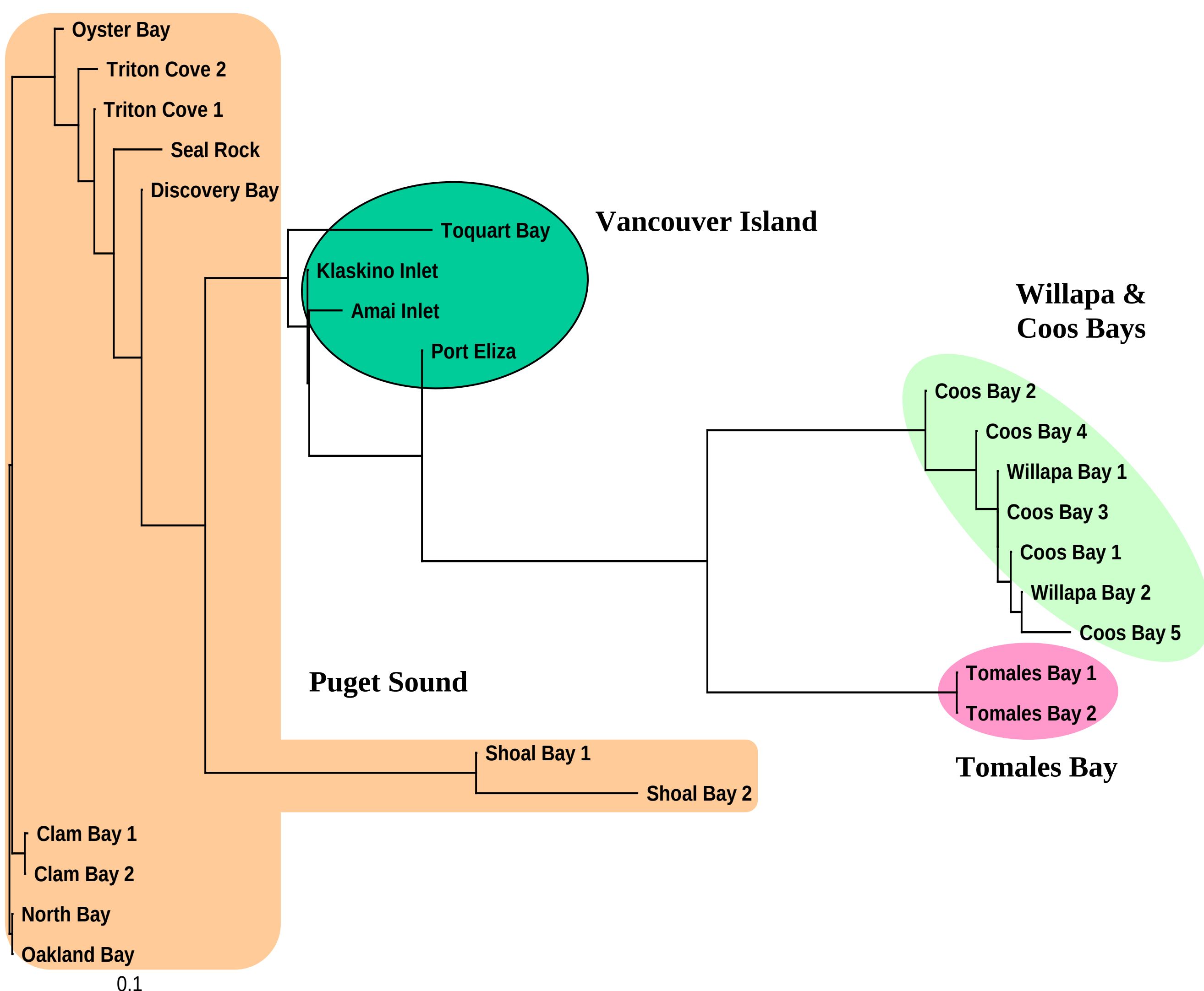
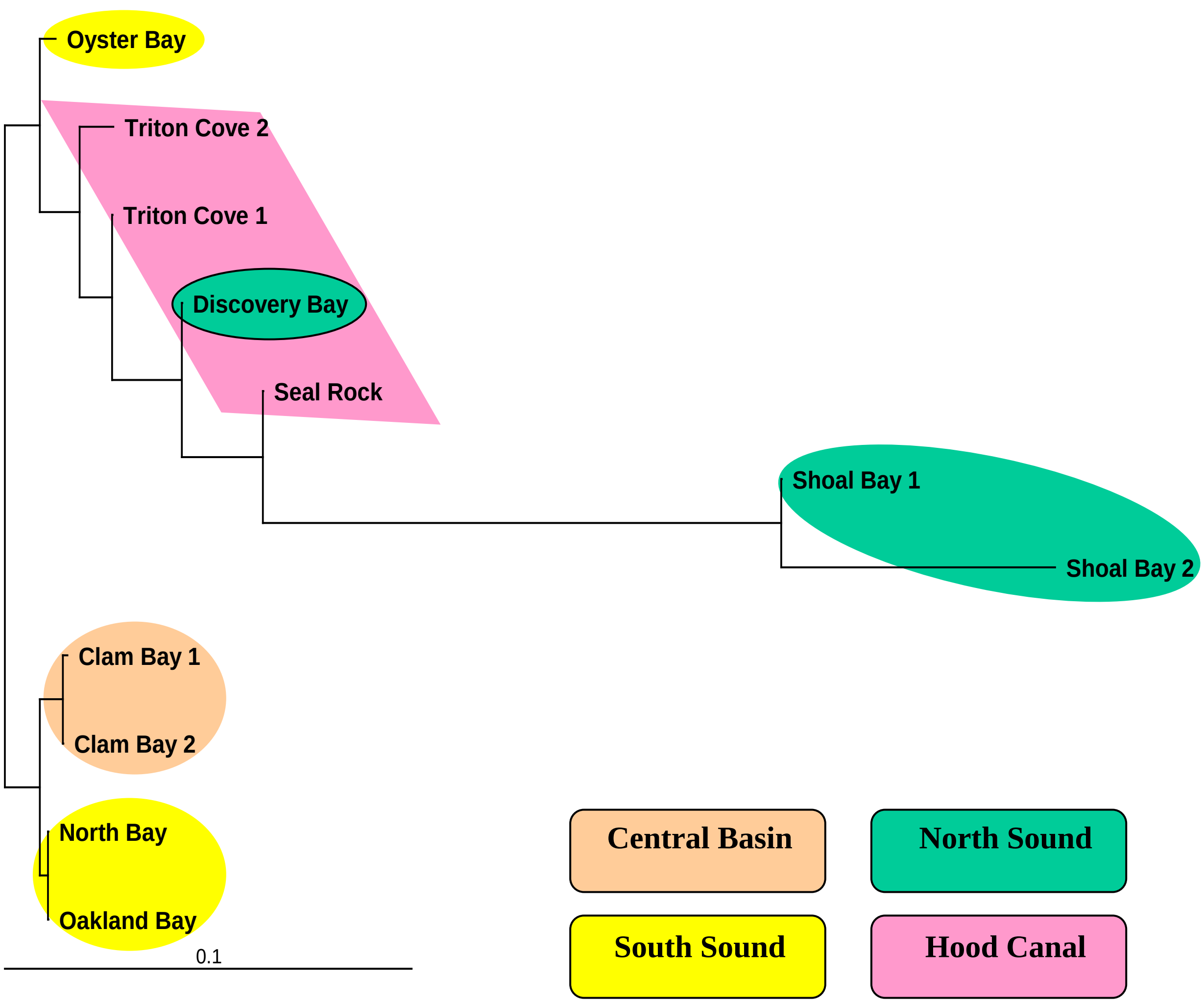


Figure 2. The variance among the 4 geographical sub-regions of Puget Sound (North Sound, Hood Canal, Central Basin and South Sound) accounted for 38.2% of the total observed genetic variation. However, as clearly demonstrated by this neighbor-joining tree, simply defining Discovery Bay as a 'Hood Canal' population instead of a 'North Sound' would have greatly increased this proportion. Still, what we see is a tight clustering of subpopulations within the defined geographical regions of Hood Canal, Central Basin, South Sound and the North Sound (Shoal Bay sites).



Presentation (PDF)

SPECIES SPECIFIC QUANTIFICATION OF OSTREA CONCHAPHILA LARVAE IN SEAWATER SAMPLES USING A DNA BASED (QPCR) ASSAY

Nathan A. WIGHT, B.VADOPALAS, J. SUZUKI, C. FRIEDMAN.
Department of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105

The west coast native Olympia oyster (*Ostrea conchaphila*) is a prime candidate for the development of a rapid, high throughput, species-specific larval identification and quantification assay. We developed *O. conchaphila* specific DNA primers and a fluorescently labeled probe that amplify a mitochondrial DNA region cytochrome oxidase 1 subunit (COI) to use in quantitative PCR (qPCR). We also developed qPCRs for the specific detection of *Neotrypaea californiensis* and *Upogebia pugettensis*, two burrowing shrimp species that have been shown to have a negative effect on oyster bed habitat. The primer and probes amplified only the target organisms. Using standard curves constructed from known quantities of larvae, we are able to rapidly and accurately identify and estimate unknown quantities of larvae. In blind tests, direct counts of *O. conchaphila* larvae did not significantly differ from qPCR estimates. DNA was fully liberated from up to 80 *O. conchaphila* larvae, and 10 *N. californiensis* larvae; PCRs were not inhibited as demonstrated by an internal positive control multiplexed into the qPCRs. Genetic based assays are an extremely useful method for sorting complex plankton samples that can be more time and cost effective than traditional microscopy techniques. Our qPCR assay may prove to be a valuable tool to monitor *O. conchaphila* restoration site productivity as well as increase our understanding of this critical life history stage.

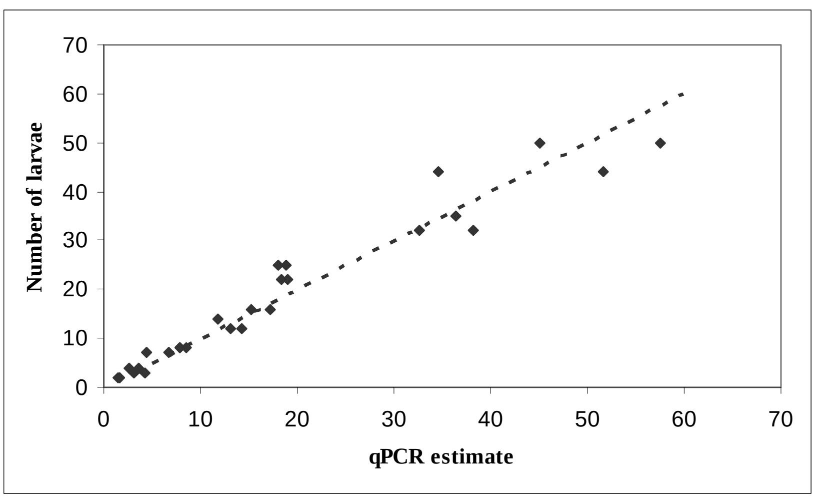


Figure 1. Relationship between the actual number of *Ostrea conchaphila* larvae and qPCR estimates. Dashed line is 1:1 correspondence (slope=1).

Presentation (PDF)

Opportunities for reconstruction of pre-Contact native oyster distribution and population structure in north Puget Sound

Russel Barsh and Madrona Murphy
KWIAHT (Center for the Historical Ecology of the Salish Sea)
P.O. Box 415, Lopez WA 98261

Managing for a successful recovery of native oysters (*Ostrea lurida*) requires an understanding of their "natural" diversity and population meta-structure. Establishing a baseline population model is confounded by the intensity of human exploitation of oyster populations prior to European contact and settlement, and commercial manipulation and ultimate destruction of oyster populations within a few decades following settlement and industrialization. Native oysters were cultivated throughout the Salish Sea, and growers not only transplanted brood stock, but also built networks of dikes to expand the oysters' shallow sub-tidal habitat. Relic populations today have presumably also been influenced by widespread restoration out-plantings of hatchery seed, and possible hybridization with the repeatedly introduced European flat oyster.

There is no scarcity of fossil shell from archaeological sites and entombed oyster reefs in the San Juan Archipelago from 100-500 years before present. Ethnographic accounts and Coast Salish oral history identify additional sites that could be tested for fossil reefs. The question has been whether useable, uncontaminated DNA could be recovered from oyster shells from archaeological deposits and sediments.

Recent research on the process by which oysters build and repair their shells has shown it likely that cells remain trapped within shell layers during the life of the animal, and may persist for some time in sediments or archaeological deposits after the animal's death.

Methods

DNA extraction was attempted on four paired specimens of *O. lurida* shells: specimens 1 and 2 were collected from an entombed reef beneath Port Stanley Lagoon (Lopez Island, WA) estimated from historical sources to be 125 years old; specimens 3 and 4 were collected from a living population in 2006, a native oyster restoration site in Fidalgo Bay (Anacortes, WA). Fragments approximately 1 cm^2 from each specimen were subjected to different decontamination treatments, prior to extraction in accordance with the Qiagen Dneasy animal tissue protocol:

(1) Sonicated for 10 minutes in a tube with 20-30 mL de-ionized water; then rinsed twice in de-ionized water and once in 100% ethanol and crushed (*OCA*).

(2) Rinsed for 10 minutes in de-ionized water, rinsed for 1 minute in 1 M NaOH, and rinsed again in de-ionized water and crushed (*OCB*).

(3) Rinsed for 10 minutes in de-ionized water, rinsed for 1 minute in 1-2% NaClO, rinsed for 1 minute in 1 M HCl and neutralized for 1 minute in 1 M NaOH, rinsed in de-ionized water again and then exposed to UV for approximately 20 minutes, then crushed (*OCC*).

(4) Rinsed for 10 minutes in de-ionized water, then gently shaken overnight at 55° C in a decalcifying solution of 0.5 M EDTA (pH 8.0), 0.5% SDS, and 100 μM proteinase K (Yang et al. 2004); then crushed and shaken an for additional 4 hours at 55° C. We then added 400 μl Qiagen DNeasy buffer AL and 400 μl 100% ethanol to the crushed shell, centrifuged the mixture at 2000 g for 5 minutes, removed the supernatant and centrifuged it at 16000 g for 5 minutes (*OCD*).

Aliquots (1 μl) of extracted DNA were run on 1% agarose gels containing 2 μl ethidium bromide per 100 ml. No bands were observed. Extracted DNA was then amplified using three sets of primers for related species in the absence of publicly available sequences for *Ostrea lurida*.

(1) Primers for 5S rDNA that amplify in Ostreidae and other bivalves including *Mytilus galloprovincialis*, *Ostrea edulis, Ostrea stentina, Crassostrea angulata,* and *Crassostrea. gigas* (Cross and Redbordinos 2006): MT1 (5'-CGTCCGATCACCGAAGTTAA), MT2 (5'-ACCGGTGTTTTCAACGTGAT).

(2) Primers designed to amplify a portion of the non-transcribed spacer in the 5S rDNA region of one closely related species, *Ostrea edulis* (Cross and Redbordinos 2006): ED1 (5'-GACTTGCCATTTTAGAGGGTCT); ED2 (5'-TGTTTAATTGGTGATAACGATGA).

(3) Forward and reserve primers for two highly polymorphic *Ostrea edulis* microsatellites (Launey et al. 2002): Oedu H15-F (5'-TTTTGACTCTGTGATATCGAC); Oedu H15-R (5'-TAATGATTTCGTTCGTTGAC); Oedu J12-F (5'-GCTGTATTTCCATCAATTCGAG); Oedu J12-R (5'- TCGTCACCTCCCTCTCAGAG).

PCR reactions were initiated with 9 μl Invitrogen Platnum PCR Super Mix, 1 μl template DNA, 0.05 μl 10 mM forward primer and 0.05 μl 10 mM reverse primer. PCR reactions with MT and ED primers were run on a Biometra thermolcycler and cycled at 94° C for 5 minutes, followed by 56 cycles at 94° C for 45 seconds, 59° C for 45 seconds, and 72° C for 1 minute, with a final elongation step at 72° C for 10 minutes. PCR reactions with microsatellite primers were run on a Biometra thermolcycler and cycled at 94° C for 2 minutes, followed by 56 cycles of 94° C for 1 minute, 50° C for 1 minute, 72° C for 75 seconds, with a final elongation step of 72° C for 5 minutes. All PCR products were run on 1% agarose gels containing 2 μl ethidium bromide per 100 ml in 0.5X TBE at 100V, and visualized using a Biorad ChemiDoc XRS and Quantity One software. OCA, OCB and OCC were run for 30 minutes (Figures 3a and 4a); OCD were run for 15 minutes (Figures 3b and 4b).

Results

DNA that can be amplified using the MT and ED primers was extracted from both recent and 100 year-old shell. Microsatellite primers developed for *Ostrea edulis* did not consistently produce bands from the extracts, however. Bands amplified with MT and ED primers were smaller than reported in *Ostrea edulis.* Decontamination methods appear to have affected the amplification; sonication alone and NaOH alone produced the most amplification, but may have allowed non-target DNA to be extracted. It is possible that *Ostrea* shell is too translucent to expose to UV without penetrating the inner shell lamellae and destroying target DNA. Decalicification prior to extraction did not improve amplification.

A balance must be maintained between removing contamination and preserving target DNA. Method OCC without UV may represent a reasonable compromise. Future work will require more specific primers (such as those developed by Stick et al and Wright et al. in these proceedings). Our results show that the recovery of DNA from *Ostrea* shell is feasible, but that further work to optimize extraction and recover useful genetic data is necessary.

Presentation (PDF)

YOU SAY *CONCHAPHILA,* I SAY *LURIDA*

Maria P. Polson*, William E. Hewson*, Douglas J. Eernisse, Patrick K. Baker§ and Danielle C. Zacherl*.
*California State University, Fullerton and §University of Florida.

Despite its relevance to recent restoration efforts, the taxonomic controversy regarding the recent reclassification of the Olympia oyster, *Ostrea conchaphila* (Carpenter 1857), has remained unresolved. Historically, the Olympia oyster was classified as *O. lurida* (Carpenter 1964) with a geographic range extending from Sitka, AK, to Cabo San Lucas, Baja California. *O. conchaphila*, which was not classified as the sister taxon, had a distribution from Mazatlan, Mexico to Panama. A study published in 1985 reclassified the two species as one with *conchaphila* as the senior synonym, although *lurida* was prevalent in the literature. Here we use a molecular approach to test the single or two species hypotheses with samples from Willapa Bay, WA, the type locality of *O. lurida* and samples from Sinaloa, Mexico, near the type locality of *O. conchaphila* (Mazatlan, Mexico). Based on our analyses of two mitochondrial DNA (mtDNA) markers, whose products are 16S ribosomal RNA (16S) and cytochrome oxidase III (CO3), samples of *O. conchaphila* from Willapa Bay and samples from Sinaloa, Mexico comprise two well-supported distinct taxa.

Maximum parsimony (MP) and neighbor joining (NJ) trees, for the 16S dataset, resulted in similar topologies. All samples from Las Tijeras (LT) and Las Ratas (LR) grouped into a well-supported sister group (hereafter referred to as the "Southern group") to samples from Willapa Bay (WB) and localities in southern California and northern Baja (hereafter referred to as the "Northern group"). Bootstrap support for the relationship between the two sister taxa was 71% and 92% for MP and NJ respectively. For the combined 16S and CO3 datasets, the MP and maximum likelihood (ML) trees resulted in similar topologies. ML support for the Northern group was 95% and 82% for Southern group. In this total evidence tree however, there was a lack of support for the two clades as sister groups. The MP tree indicated 95% and 98% support for the Northern and Southern groups respectively and 69% support for the two groups as sister to each other. Corrected pairwise sequence comparisons for 16S, indicate these two groups last shared a common ancestor 1.5 to 3.9 mya (2.06% sequence divergence).

Post-hoc morphological comparisons uncovered no significant support for morphological distinction between the two taxa, as results on the characters examined overlap between the two groups. This underscores the difficulty associated with the use of a morphological species concept. We also did not detect the zone of overlap between the two species. Samples from the seven localities in southern California and northern Baja, California all grouped with *O. lurida* across both mtDNA markers and molecular models. In the future, sampling from other locations around the Baja California Peninsula is warranted to determine the existence, if any, of a zone of overlap. The only mention of such a zone was in a 1959 study by Hertlein, which also described three separate morphotypes found in southern California. Considering the high degree of phenotypic plasticity in oyster shell morphology, it is possible that a zone of overlap was never present in southern

California and northern Baja California. Instead, the 1959 study might have documented evidence of the high degree of phenotypic plasticity exhibited by this group of oysters. That our morphological analyses were inconclusive should not come as a complete surprise given that *O. lurida* fits the description of a cryptic species. Based on our novel 16S and CO3 sequence data we have provided evidence which supports the original classification by Carpenter (1857, 1864) that identified *Ostrea conchaphila* and *Ostrea lurida* as separate and unique oyster species. We therefore believe that *O. lurida* should be reinstated as the Olympia oyster.

Presentation (PDF)

Session: Monitoring and Invasives

Monitoring and Invasives – Session Summary

Panel Members:
Sumudu Welaratna, San Jose State University
Kate Henson, Department of Biology, University of Washington
Eric Buhle, Department of Biology, University of Washington

During this session the speakers discussed a specific Olympia oyster predator and its complicating behaviors with respect to reestablishing native populations. In addition, the discussion centered on native oyster recruitment success in California and possible next steps for restoration professionals.

Washington

Kate Henson opened the session with her research on Japanese oyster drills. Japanese oyster drills were discovered in Puget Sound in the 1920s and their current range is from California to British Columbia. Drills can consume three small oysters per week and have been found to impact restoration sites and have to cause commercial beds to be abandoned. Past research has shown that mortality due to drills is variable in oysters. Highest predation occurs where Olympia oyster population density is low, while the presence of other species such as barnacles can alleviate predation pressure on oysters.

Methods for controlling Japanese oyster drills include the use of chemicals, hand removal, and restricted transfer. Kate Henson's research compared two oyster control methods at two locations in Liberty Bay, Washington. Her preliminary results show that hand removal does not have a significant impact on drill density and is very time consuming. Hand removal did not reduce drill density at the scale tested. Ms. Henson also completed a small-scale drill dispersal experiment where she removed all drills from plots and then reintroduced the drills from a central point. The drills had high levels of movement within the two-week period after reintroduction. The greatest impact for restoration efforts can be made when predator density is decreased and at the same time the prey density is increased.

California

Sumudu Welaratna presented on recruitment studies carried out by Save the Bay from 2001 to 2006. At five sites around the San Francisco Bay, they counted and measured oysters, monitored other colonizers, and recorded water quality information and tide levels. A second study was launched in 2007 to examine recruitment patterns within the Bay. This study is currently examining recruitment rates over time, to determine whether recruitment rates are correlated with different substrates, water quality, or presence of other settling organisms. Ms. Welaratna has deployed three substrates for monitoring oyster settlement (shell string, PVC plate, and shell bags) at six sites to represent the Central and South San Francisco Bay.

Panel Discussion

The panel explored several issues and questions about monitoring and invasives. Questions were raised about the length of time needed to determine whether recruitment was happening and the need for continued experimentation with substrates in order to offer suitable recruitment substrates for oysters. Ms. Welaratna recommends the development of a San Francisco Bay Native Oyster Intertidal Survey Protocol to create a more comprehensive picture of the state of the bay. The topic of invasive Japanese drills proved to be thought-provoking. Several questions that were discussed involved costs and benefits of hand removal of drills, predation rates on other species such as barnacles, and behavioral characteristics observed in the drills.

Presentation (PDF)

Control Strategies for Japanese Oyster Drill and Implications for Restoration and Management of Olympia oyster (*Ostrea conchaphila*) in Liberty Bay, Washington

Kate Henson and Eric Buhle
Department of Biology, University of Washington, Seattle, WA

The Olympia oyster (*Ostrea conchaphila*) is vulnerable to predation by the invasive Japanese oyster drill (*Ocinebrina inornata*). In Liberty Bay, the Puget Sound Restoration Fund and The Nature Conservancy are working to facilitate the recruitment of Olympia oysters by adding Pacific oyster (Crassostrea gigas) cultch in 2005, 2006 and 2007. Previous research suggests the presence of Japanese oyster drills in Liberty Bay may pose a significant threat to the restoration of the Olympia oyster population. We are conducting research to test the feasibility of reducing drill densities and to quantify the benefits of drill control for Olympia oysters in the context of this restoration project. We are using a controlled, replicated experiment to compare the cost-effectiveness of two control methods: (1) visual search and removal of drills from the substrate, and (2) the use of bait (barnacle-encrusted stakes) to concentrate drills for removal. The experiment is replicated in a pre-existing habitat patch and a recent shell addition area, allowing us to ask whether the costs and benefits of drill control differ for established and colonizing oyster populations. Our results will help to inform the allocation of resources for predator control in native oyster rebuilding efforts.

Our preliminary results indicate that there is no significant difference in mean drill density between the control, removal from substrate and removal from bait plots. We predicted that this is due to the small relative scale of the removal and the scale and intensity of the removal would need to be increased to have a significant impact on drill density. We tested these predictions by conducting a drill dispersal experiment to help understand drill movement in the experimental areas in Liberty Bay. Drills were collected, marked and released from a central point. We returned two weeks later to record movement and found that drills traveled meters from the release point. This supports the idea that drill movement is significant and explains why small scale removal may be ineffective. Preliminary conclusions indicate that in addition to increasing prey population (O. Conchaphila), predator density (*O. inornata*) should be reduced although this proves to be challenging.

Presentation (PDF)

Session: Restoration Projects Round 1

<u>Restoration Projects Round 1 – Session Summary</u>

Panel Members:
David Couch, City of Arcata
Rob Brumbaugh, The Nature Conservancy
Joth Davis, Baywater Inc.

This session focused on the result from two restoration projects: one in Humboldt Bay, California and the other in Liberty Bay, Washington. Both bays have limited substrate available for oyster colonization.

Humboldt Bay, Calilfornia

South Humboldt Bay is a designated oyster preserve; however, no restoration actions had been started until recently. David Couch has been conducting restoration efforts in the Bay for the City of Arcata. Because there was natural oyster spat set at his sites, he placed rock and cultch shell over a total of 3 to 4 acres. When he has returned to the bay, no live oysters were found to be remaining. Mr. Couch hypothesizes that sediment changes during the winter (changes that do not occur in the summer) resulted in unfavorable conditions. In addition, oyster drills may have had an impact, but more research needs to be done on this topic. In other areas of the Bay, Mr. Couch has seen oysters move past a dike and a tide gate to colonize suitable habitat. In conclusion, he noted that past efforts to establish higher diked beds have failed. Instead, he recommends using only native species in a restoration effort and to place shell in a low tide area for better restoration results.

Liberty Bay, Washington

Joth Davis has been conducting research on substrate restoration projects in Liberty Bay since 2005. At this site, 2 acres of habitat in the lower intertidal zone were enhanced to promote native oyster larval recruitment, and thereby increasing water filtration and providing complex habitat for invertebrates and fish substrate was distributed over a 3-year time. Dr. Davis is monitoring native oyster settlement as well as associated invertebrates and fish recruitment. From this project, his research team has already found that exotic species have caused some predation to the native oysters and that there is the high potential for a large barnacle sets to out-compete the oysters for space.

For example, in 2005, he used 1/4 meter square quadrat samples, and calculated the mean oyster shell cultch density for sampled plots to be 60.08 (±21.35) shells per $1/4M^2$. He then found the Olympia spat abundance at 22.25 (±11.71) per $1/4M^2$, or 90 oysters per square meter. They were counted when they were about 9 months old, after they had survived a winter. The calculations were repeated in 2006 and the results were; replicate (N=36) 1/4 meter square quadrat samples taken in May, 2007 and the Olympia spat abundance = 10.2 (±0.48) per M^2. In the future, the development of standardized data gathering protocols for Olympia oyster enhanced sites will in the future include additional information on invertebrate colonization and use by mobile invertebrates and fishes.

Mr. Brumbaugh explained that The Nature Conservancy is relatively new to shellfish restoration, but they have some insight into the processes and complexities. He gave the group some characteristics of restoration as well as some collected thoughts. He reminded the group that shellfish are ecosystem engineers—i.e., they are a fundamental building component for a healthy marine ecosystem. Although TNC is new to restoration, they do have some experience gained from their current shellfish restoration projects in progress in 12 states, and dozens of other projects, which include at least one NOAA partnership. The TNC recently embarked on a global assessment of shellfish (mostly reef-forming bivalves, mussels and oysters), which is similar to the current global coral reef assessment. They ranked the bivalve habitats as high/med/low condition and risk, in order to prioritize protection and restoration efforts. Overall, they focused on ecosystem services in the context of shellfish restoration, in addition to value of harvest, recreation, etc. Finally, he commented on his observation that U.S. systems are "frayed around the edges," but things are not as bad as in other parts of the world. Restoration professionals should be patient—there is no urgency out here. He noted that the "semi-educated hand-waving" up to this point is not helpful, and that a more educated effort is called for in order to see real progress.

Panel Discussion

During the panel discussion a number of questions were raised and the resulting discussion is summarized below.

- Researchers had found that the substrate additions to the sites had undergone some sinking, but the rate of sinking had tapered off after the initial placement.
- In Liberty Bay, Dr. Davis noted that he had not seen any settlement of the commercially grown Pacific oysters, but he has noticed an immediate change in the benthic communities after the substrate enhancement.
- Kate Henson, who presented in another session, pointed out that adults oyster can travel great distances and have been known to cross freshwater and mud flats.
- Research has shown that larvae preferentially recruit to live oyster shell rather than other substitutes.

Presentation 1 (PDF)

Presentation 2 (PDF)

Session: Restoration Projects Round II

Restoration Projects Round II - Session Summary

Panel Members:
Robert R. Abbott Ph.D., MACTEC Engineering and Consulting Inc., Petaluma CA
Brian Mulvey, Kleinfelder Inc., Santa Rosa, CA
Pamela Archer, Jessica Miller, and Chris Langdon, Oregon State University
Dick Vander Schaaf, The Nature Conservancy

The second session focusing on results from restorations covered two experimental restoration projects, one in San Francisco Bay, California near Marin and the other in Netarts Bay, Oregon. The Marin project, led by Bud Abbott, has experimented with oyster restoration in a unique partnership with the Marin Rod & Gun Club on habitat previously resembling a "featureless mudscape." The emphasis of this project is on testing different methods of substrate enhancement and spat monitoring using strings, stakes with rosettes of shell, pallets, oyster reef rows (pyramid-shaped and Lincoln Log style mounds), and "coral heads" built out of 30 bags piled up in four staggered rows. As predicted, there has been some shrinkage and subsidence into the mud. However, there is also quantitative data indicating that multiple species are benefiting from the substrate placement. Herring are spawning on the reefs; crabs and shrimp are present; Goby are laying eggs using the cup shape of the shell; eelgrass is showing up between reef structures; and there appears to be more bird activity. Also, according to informal, qualitative data gathering during annual fishing derbies, angling appears to be about three times better near reefs than on the other side of the pier. Overall, in a level of effort comparison, Bud and his co-authors offer this assessment: stakes require the highest level of effort to install, and mounds require the lowest level of effort. In a spat set rate comparison: mounds get the highest set rate and strings the lowest. Preliminary cost/benefit assessment therefore indicates reef mounds are the best method.

Pamela Archer, a graduate student at Oregon State University, presented her study on an oyster project in Netarts Bay, the sixth largest estuary in Oregon. Her project examined optimal outplant densities that would re-create a self-sustaining oyster bed without negatively affecting the surrounding eelgrass beds. The site was aptly chosen, since "Netarts" means "oyster" and the area is known to have contained oysters in the past. The project also focused on whether the addition of oysters and substrate would impact eelgrass. While there is historic evidence of oyster/eelgrass coexistence as essential fish habitat, the project needed to be implemented in such a way that it resulted in no net loss of eelgrass. During the course of her project, Ms. Archer did not observe any oyster mortality related to shrimp. Shrimp appeared to be moving eelgrass around, but there many shoots still were emerging. For sediment changes, she observed some pockets of anoxia, but the impact of this change is unknown. Future efforts on this project include spreading blank shell to monitor for natural recruitment and modeling the physical structure of Netarts Bay. To measure the self-sustainability of oyster populations in Netarts Bay, Ms. Archer recommends focusing on natural recruitment, the presence of multiple age classes, and larvae presence.

Panel Discussion

As part of the question and answer session, there was a lengthy discussion about water quality and the presence of exotic species in San Francisco Bay. Water quality in San Francisco Bay is not good enough to legally harvest oysters for human consumption and, while water quality is improving, the bay is still a long way from being able to support aquaculture. As such, the focus of San Francisco Bay oyster projects is on ecosystem restoration. Water clarity is about 1 foot and is therefore not clear enough to observe fish movement around reefs using underwater video. The root of the turbidity is a combination of gold mining, which releases sediment from upper systems; a deep water shipping channel that is constantly dredged; and the omnipresence of mudflats surrounded by steep, riprapped shorelines.

San Francisco Bay also has more invasive species than most places. Hydrozoans are the main biomass weighing down structures, along with mussels and some other exotics. In reefs, exotic clams recruit into the shell bags. In South Bay, exotic sponges become a problem. Bryozoans will eventually cover oysters. There are oyster drills at the South Bay sites, but not at more northern sites. Though green crabs are prevalent in the bay, they have not yet been seen in association with oyster projects.

In regard to fish observations off the pier in the San Francisco study and the connection between oyster restoration and fishing, Jack smelt, striped bass, cod, leopard sharks, bat rays, anchovies, and California halibut have all been caught off the pier. The reefs seem to be acting as fish aggregation devices and the scent of the fishing bait carries from pier to reefs. It's unclear yet whether people are making a connection between fishing and oyster work, but Club members often select sites near reefs. Remnant ostrea populations are being found as deep as -3, -4. These lower elevation refugia seem to be part of the species survival strategy.

Ms. Archer included a photograph in her presentation of an *O. crassostrea* (Pacific oyster) amongst natives and posed the question of whether this was good or bad. While the Pacifics overgrow the natives, they also provide substrate. There seem to be plusses and minuses, depending on the goals of the project. In San Francisco Bay, a few *crassostrea* showed up and there was a highly publicized effort to remove invasive exotics, but the presence of *crassostrea* need not rule out the presence of natives, according to Bud Abbott.

Presentation (PDF)

Olympia Oyster Habitat Construction Methods and Results: 2005-2007

Robert R. Abbott[1], Ph.D. Rena Obernolte[1] and Brian Mulvey[2]
[1]MACTEC Engineering and Consulting Inc., Petaluma, CA and [2]Kleinfelder Inc., Santa Rosa, CA

A variety of applications of Pacific oyster (*Crassostrea gigas*) shell have been used to create habitat for Olympia oyster (*Ostrea conchaphila*) spat settlement in San Francisco Bay over the last three years. The methods include shell strings, shell bags on pallets, shells on stakes, shell bags stacked in different configurations and loose shell placed directly on the mud bottom. The results from these methods are summarized and then compared to evaluate which one is most efficient, considering labor to deploy and monitor, as well as recruitment success.

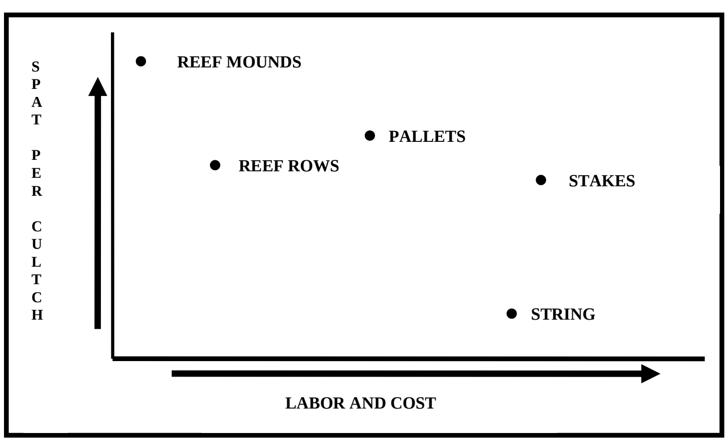
Thirty strings with 24-cultch shells per string, with 1" PVC spacers were suspended from the Marin Rod and Gun Club (MRGC) recreational angling pier in June, 2005. The strings became heavily encrusted with mussels, bryozoans, tunicates and hydrozoans (~20-kg) and most had to be removed in December 2005 to protect the railing on the pier. The average set rate on the strings was only 1.27-oyster spat/cultch shell. Most spat settled on the lower cultch shells since the higher shells were often out of the water due to strong tidal currents.

Also in the summer of 2005, artificial reefs constructed using wood loading pallets as platforms for keeping a pyramid of 10-bags of cultch shells off the bottom. Eight pallets were placed on subtidal mudflats offshore of the MRGC and four pallets were set up near the Port of Redwood City. The wood pallets are still in fairly good shape at the MRGC but the pallets near Redwood City, in the South Bay, were completely disintegrated by the fall of 2006, largely due to wood boring worms. The average spat set rate on the cultch bags piled on the pallets was 3.75 spat/cultch shell. Wood and PVC stakes with rosettes of oyster cultch were also placed in the subtidal mudflats offshore of the MRGC. The rosettes of cultch were approximately one foot off the bottom. The stakes became encrusted with fouling organisms, and were found to be expensive and time consuming to construct, set in place and monitor, relative to other methods of providing hard surfaces for natural spat sets. All the stakes were removed in November 2006. The set rate on the stakes averaged 3.1 spat/cultch shell.

A second method of building artificial reefs was tested at the MRGC in June, 2006. Three 75- ft. long reef rows were set up running parallel to the shore at three depths (-0.1-ft., -2-ft., and -4-ft. MLLW). One-third of each reef row consisted of oyster shells scattered in an elongated mound. The other sections were comprised of cultch bags stacked either pyramid-style or Lincoln log-style. A 6-ft. wide "surge" channel separated reef row sections. The stacked bags were pinned in place with 5-ft. long 3/8-in rebar hooks pressed into the mud. At the end of a year of observation it became apparent that the cultch scattered on the bottom was being rapidly covered with sediment and dispersed by currents. At the end of a year the cultch bags in the reef rows were stable and supported the growth of numerous oysters. The average set rate on the reef rows was approximately 3.4 spat/cultch shell.

In June 2007, a third method was tested that involved building 26-mounds, the functional equivalent of a reef coral head or reef ball, were set up at -2 ft MLLW on the mud flats at the MRGC. The mounds consisted of about 30-35 bags of cultch stacked and held firmly in place by four, 3/8" rebar bent in an “L”. The 26-mounds utilized approximately 720-cultch bags with approximately 25-50 cultch shells per bag. The mound construction method was relatively simple and took approximately 80-person-hours of field construction work. It took a half a day for two people to layout the mound field with PVC pipe markers, and approximately one person day to cut twenty-six, 20-foot long rebar into 5-foot long sections, transport and bend them into “L hooks”. With two skiffs ferrying cultch to five volunteers in the water, it was possible to place the 720-bags or approximately 27-cubic yards of bagged cultch around the marked locations in approximately 4-hours. Each skiff had a crew of 2-4 volunteers to operate the skiff, anchor and keep it in positions and hand bags to the volunteers in the water. It took 8-volunteers working 4-12 hours (~40 volunteer hours) on shore to move the cultch from the loading pallets to the skiffs, provide miscellaneous support and clean up. This project activity was filmed for a documentary titled “Saving San Francisco Bay”, for a PBS presentation scheduled for April 2008.

Monitoring samples of cultch from the bags forming the mounds in August, just two months after the construction of the mounds showed an average of 5.6-spat per cultch suggesting a population of approximately 100,000 spat on the 26 mounds. A review of the level of effort and costs for the different methods, and then comparing the recruitment results, indicates the mound construction method provides the best results for the least cost.



Cost-Benefit Assessment

* By December 2007, monitoring indicated an average of 13.8 spat per cultch shell on the mounds, suggesting a native oyster population on the new mounds of approximately 250,000-spat.

Presentation (PDF)

Session: The Human Dimension

Human Dimension - Session Summary

Panel Members:
Marilyn Latta, Save the Bay
Betsy Lyons, The Nature Conservancy
Brian Allen, Puget Sound Restoration Fund

What is the role of the public, community members, landowners, and volunteers in supporting native shellfish restoration efforts?

Marilyn Latta discussed the work of her organization, Save the Bay, which has focused on connecting the public with San Francisco Bay. Marilyn noted that working with the communities and existing groups around the Bay has helped create public support for native oyster restoration and achieve faster project results. Marilyn's organization has engaged volunteers in data collection, public education and outreach, and hands-on restoration. Marilyn noted that volunteers benefit from feeling connected to specific locations that they can return to over time to observe changes. And being involved in oyster restoration helps educate the public about oysters, their ecological importance and how individuals can contribute to the health of the Bay. Future Save the Bay projects include developing a website that is shared among agencies and organizations working on native oysters and organizing a Bay-wide community native oyster survey, similar to the Audubon Society's Christmas Bird Count. Marilyn stressed that although challenges are associated with involving the public as volunteers in native oyster restoration, the benefits are well worth the effort.

Betsy Lyons and Brian Allen discussed their joint work on native oyster restoration sites in Liberty Bay, Woodard Bay, and Frye Cove, Washington. The goals of their project were to test restoration methods and to restore substrate for natural recruitment in areas with historic populations. Project management challenges have included: getting access to restoration sites; securing permits (due in part to lack of familiarity with native oyster restoration on the part of agency permitting staff); timing (established "fish windows" for in-water work often do not coincide with ideal windows for native oysters); the need to balance on-the-ground restoration with monitoring, research, funding and reporting; and keeping the community involved and supportive.

Brian and Betsy shared insights from past projects on site selection, monitoring, habitat improvement, and defining success. Brian noted that "true" success at a site would include several well-represented year classes and periodic recruitment. These measures cannot be reached within the timeframe of a single grant or award, which presents challenges when evaluating and reporting on the results of projects to funding entities.

Presentation 1 (PDF)

Presentation 2 (PDF)

Session: Outcomes, Conclusions, and Next Steps

Outcomes, Conclusions, and Next Steps

Outcomes

The 2nd Annual West Coast Native Oyster Restoration Workshop provided a good venue for information exchange on a number of restoration fronts and an ideal spring board for field trips and shellfish consumption. A number of growers served fresh oysters for slurping (thank you Chelsea Farms, Calm Cove Oyster Co., and Taylor Shellfish) and Xinh's Clam and Oyster House hosted a dinner attended by more than half of the workshop participants (thank you Taylor Shellfish). While there was some discussion about the need for more substantive working sessions and fewer presentations at future workshops, there was general consensus that the West Coast workshop is the only place where people actively working on native oyster restoration can gather to learn about what everyone else is doing first-hand. The venue is important because much of this information is new and unpublished and because there is no other forum where people can interact with each other and with the experts. The larger national conferences lack the critical mass or time to foster in-depth discussions.

There was also much interest in a hands-on demonstration of how to construct concrete egg cartons that can serve as effective spat collectors (thanks to David Couch and Sarrika Attoe).

Long-awaited news on genetics was met with considerable interest by all, since it has a direct bearing on where and how restoration should be undertaken. The Hatfield work clearly showed the appearance of genetic differences among the five major sites (Tomales, Coos, Willapa, Puget Sound and Vancouver) and the appearance of genetic differences among and within the four Puget Sound basins (Hood Canal, North Sound, Central Basin, and South Sound). What cannot be answered yet are: 1) whether or not these populations are locally adapted; and 2) how the information should be interpreted and used to guide restoration projects. Future workshops will need to explore fundamental goals that drive current restoration. If our primary goal is to preserve genetic diversity, then we should not produce seed. If our primary goal is to provide ecological benefit, then we should pursue restoration strategies that enable re-colonization of habitat by larvae naturally present in the water *or* practice very careful hatchery production using as many brood animals as possible, if seeding seems warranted.

Conclusions

Federal and state partners concur that efforts should be expanded to improve shellfish restoration. Olympia oysters are known to be ecologically important and their populations, although still present, are a fraction of historic levels. In Washington State, shellfish restoration projects "are welcomed as part of the larger Puget Sound Partnership effort to restore the health of estuary by 2020," according to Steve Landino with NOAA Fisheries. Similarly, The Nature Conservancy is placing significant emphasis on the role of shellfish restoration within larger estuarine restoration efforts. The Nature Conservancy's commitment to native shellfish restoration at the national and international level is based on their value in providing ecosystem services, according to

Rob Brumbaugh. TNC-sponsored shellfish restoration projects are currently being pursued in 12 states with dozens of projects in some of those states. These projects fit within the larger context of TNC's global assessment, which characterizes shellfish beds and reefs as among the most impacted of all ecosystems. Although populations in the United States are better off than in many other parts of the world, shellfish restoration is likely to continue to be a main focus area of TNC's marine initiatives. The West Coast workshop (and the experts in attendance) can and should play a role in 1) elevating the need for native shellfish restoration; and 2) improving materials and methodologies. However, there is also a shared understanding that we should continue to take an experimental approach. Olympia oyster restoration is still in a developmental phase, and the projects we do undertake should further the science rather than just accomplishing restoration. Important questions remain about how and where to restore Olympia oyster populations, what broodstock to use and how to determine the scale of individual restoration efforts. While current projects demonstrate that Pacific oyster cultch works, for instance, questions remain about using non-native cultch to change a naturally occurring, soft-substrate environment. The West Coast workshop can and should be used as an effective forum for improving both the state of our knowledge and the practice of restoration.

Next Steps

There were discussions about whether or not the West Coast workshop should be held annually. Although there was interest in a 2008 workshop, there seemed to be agreement on two things: 1) the workshop should take place later in the fall and not during the prime field season; and 2) the workshop should have more working sessions with the express purpose of developing specific products (e.g. white papers, monitoring procedures) in order to advance collective needs and priorities and generally take better advantage of the expertise represented at the workshop.

Specific features of a future workshop might include:

- A working session to train participants in larval identification
- A working session to discuss/standardize monitoring techniques (Note: Yet to be determined is whether or not draft products should be developed in advance of the workshop or whether the working sessions are intended to be used for developing a draft.)
- A presentation session for sharing new information – for instance, successful techniques/projects or the results of the Vancouver Island trip to assess the large remnant oyster population,
- Guest speaker on the history of native oysters in the area – tribal member/farmer
- Research highlighting historic distribution/ factors limiting recruitment and survival
- A working session to develop an "Olympia Oyster White Paper" for the purpose of informing managers at higher levels and elevating native oyster restoration as a funding priority. Among other things, the white paper would succinctly elucidate the importance of Olympia oysters and the range and nature of current Olympia oyster restoration efforts, and provide a compendium of research priorities

relevant to restoration efforts along the West Coast. The white paper might also be used to inform regulators about preferred methodologies and best practices, since it is unlikely at present that a permit could be obtained for large-scale deployment of concrete spat collectors.

Session: Demonstrations

Demonstrations – making concrete settling tiles

Sarrika Attoe, UC Davis
David Couch, City of Arcata

This year's workshop included demonstrations on making concrete settling tiles. Tiles are typically used for monitoring timing and abundance of natural spatfall, for collecting natural spat for use in restoration, or for settling substrate in hatcheries. These manufactured tiles are useful when clean oyster shell is not available, or when there is a need for a more precise and known surface area of settling substrate.

The demonstrations were useful and directly relevant to the work being done by many of the workshop attendees, and the sunny warm day provided a perfect opportunity to head outside for the demonstrations.

Sarrika Attoe demonstrated the "Seameant" recipe used by the San Francisco Bay Native Oyster Working Group. Although the homemade tiles can be utilitarian, commercially-available cement pavers may provide a better settling substrate in some cases. Oyster larvae are fickle, and practitioners must experiment to determine the best settling substrate in each locale.

> *Ingredients:*
> *Burlap*
> *2 parts oyster shell powder (we use Jerico brand Pearl Powder)*
> *1 part Portland cement (plain not quick set or anything)*
> *approximately 1 part water*
> *wax paper or hard plastic*
>
> *Cut Burlap into desired size and shape. Mix shell powder, Portland cement, and water to make a milkshake consistency. Dip burlap into Seameant mixture. Shape into desired shape (i.e. flat, ridged, or on mold). Let dry for 24 hours. Repeat mixing, dipping, and drying twice more. Deploy and you have a handy-dandy recruitment collector with a known surface area!*

David Couch demonstrated his method for egg carton spat collectors, which essentially consists of egg cartons and wet cement. The convoluted shape of the egg cartons provides small niches, eddies, and good microhabitat for larval oysters. However, the surface area is more challenging to calculate than with flat tiles.

After the concrete-dipped egg cartons are allowed to dry, they can be placed in mesh bags lengthwise, with knots tied in the mesh bag in between egg cartons. With a float tied to one end, the concreted egg cartons hang down vertically in the water column. This method allows for spat collection at a variety of depths.

Session: Posters

We were pleased to have several posters at our Monday evening session. The posters covered a range of topics, and all were a meaningful addition to the workshop.

Temporal and Spatial Variation in Settlement of *Ostrea conchaphila* in Newport Bay, CA

Lily A. Sam and Danielle Zacherl.
Southern California Ecosystems Research Program,Department of Biological Science, California State University, Fullerton.

Ostrea conchaphila is the only oyster native to the west coast of the United States. Over-harvesting in the early 1900s paired with pollution from pulp mills led to massive population crashes. There are still remnant populations throughout its range and some restoration efforts are currently occurring in areas between northern California and Washington. Before planning to restore populations of *O. conchaphila*, it is important to learn about constraints on current population growth. For example, knowledge about temporal and spatial variation of *O. conchaphila* larval settlement could potentially help maximize the collection of spat in order to enhance settlement within an existing population. Settlement of *O. conchaphila* was assayed at six sites throughout Newport Bay, CA using settlement tiles that were collected every spring tide. We hypothesized that settlement would vary both spatially and temporally among populations located within Newport Bay, California. Preliminary findings show that settlement varied significantly among sites, with highest settlement typically occurring at sites in the upper bay. We also found that settlement varied temporally with maximal settlement occurring in mid-August at most sites. Future studies will attempt to pinpoint variation in growth rates and survivorship among settlers from sites throughout Newport Bay in an effort to understand what factors limit population density.

Restoration of Native Oysters, *Ostrea conchaphila*, in Fidalgo Bay, Washington

Paul Dinnel[1], Betsy Peabody[2] and Tristan Peter-Contesse[2]
[1]Western Washington University and [2]Puget Sound Restoration Fund

Abstract

The Olympia or native oyster, *Ostrea conchaphila*, is native to the Pacific Coast of North America and was common in Puget Sound prior to the arrival of European settlers. Over harvest in the late 1800s, combined with severe pollution in the first half of the 20th century from pulp and paper mills, drove many Puget Sound beds to near extinction. Skagit County Marine Resources Committee (Skagit MRC), working in cooperation with shellfish industry, tribal, and community partners, initiated a project to establish several native oyster beds in Fidalgo Bay near Anacortes, Washington. The project goal is creation of one or more self-sustaining native oyster beds. Thus, oysters on these beds must survive, grow, spawn and produce larvae that recruit to the beds and surrounding areas. Native oyster seed on Pacific oyster cultch were planted in Fidalgo Bay during 2002, 2003, 2004 and 2006. Survival and growth of planted seed has been excellent at one site (Trestle Site), but poor in a second site. With the addition of seed on cultch during four years and augmentation of the Trestle Site with five cubic yards of Pacific oyster shell in 2006, a structured oyster bed is gradually emerging. Deployment of temperature sensors in 2006 showed that water temperatures easily reached the minimum temperature for gameteogenesis and spawning. Examples of larval spawning (veligers in the mantle cavity) and natural post-larval recruitment to the Trestle Site were documented in 2006. Several new sites within and around Fidalgo Bay are being evaluated for future restoration efforts.

How do upstream watershed land use patterns affect bacterial loading in coastal intertidal habitat of shellfish?

Amanda Hillman
Portland State University

Abstract

Restoration of an oyster native to the West Coast, *Ostrea conchaphila*, has been the focus of many restoration efforts. Many studies have examined the marine environment to determine where restoration efforts will be most effective. However, few if any studies have begun to examine the terrestrial influence on these habitats. The study proposed here will determine if there is a correlation between ocean and terrestrial systems regarding bacterial loading. The main product of this project will be a predictive model that uses watershed land use patterns to predict water quality and ecosystem health of downstream nearshore marine habitats. This predictive model will be useful for focusing habitat restoration efforts and placement of shellfish aquaculture operations. For these reasons, this model will contribute to the knowledge necessary for decreasing human health risk associated with eating shellfish by identifying areas to avoid with respect to fecal pollution. Being able to predict where bacterial loading will be the lowest, this model will support a shellfish industry that will have fewer closures resulting in a more economically successful industry.

Project Summary and Background

Restoration of native shellfish populations has become increasingly common along the West Coast in recent years, with support from the National Oceanic and Atmospheric Administration (NOAA), NGOs, and other partners. The West Coast native oyster, *Ostrea conchaphila*, has been the target of most of these restoration efforts. The overall population of this oyster remains much lower than historic levels, and is mostly off-limits to recreational harvest. However, with the ultimate goal of restoring robust, self-sustaining populations, it is reasonable to expect that recreational harvest may be allowed sometime in the future. In light of this probability, it is important to assess human health considerations associated with consumption of shellfish. The same considerations should be applied to proposed commercial shellfish operations.

The goal of this project is to generate a correlation between estuarine, ocean, and terrestrial systems with regards to the shellfish consumption and human health. It is aimed at monitoring the compositional aspect of coastal marine ecosystem, specifically bacterial loading from fecal pollution. The main objective is to determine if watershed land use will affect bacterial loading, including fecal coliform and *E.coli*, in shellfish tissue, water and sediments of the corresponding intertidal zone.

For all project components, a combination of field and laboratory research and GIS database analysis will be used to gain detailed information about current land use practices of five coastal watersheds. The study sites include: Cascade Head at the mouth of the Salmon River Watershed, Yachats Bench at the mouth of the Yachats River, Coquille Point at the mouth of the Coquille River watershed, Cape Blanco at the mouth of the Sixes River watershed and the Chetco Point at the mouth of the Chetco River watershed.

Hypothesis

Different land use patterns will affect bacterial loading in downstream coastal environments.

Land Use Pattern	Expected Effect on Bacterial Loading in downstream Loading in downstream Coastal Environment
~Agriculture	**Increase** loading by using manure as a fertilizer and by increasing overland flow due to monocropping.
~Logged	**Increase** loading by removal of vegetation that allows infiltration of overland water.
~Human Population Density	**Increase** loading by increasing bacterial source.

Methodology

Objective: Determine Effect of Land Use on Fecal Pollution in Nearshore Environments

Task 1.

Monitor fecal coliform and *E.coli* in water column in coastal areas adjacent to river drainages of watersheds with different types of land use.

Every month water samples will be collected by lowering a 120 ml sterile bottle into ankle deep water and allowing the bottle to fill while avoiding areas with bird droppings (Ferguson et. al., 2005). The samples will be stored at 5-10°C and processed within 6 hours of sampling time (Ferguson et. al., 2005).

The Colisure procedure from IDEXX, a method approved by the EPA and used by Casteel et. al. (2005), will be used to enumerate the fecal coliforms. The samples will be diluted to no dilution, 1:10 and 1:100 to ensure coliform counts are quantified within the 6hr time period. Then 100ml of each dilution will be filtered and put into Shrink Banded Disposable Vessels (IDEXX #WV120SB-20) along with the Colisure reagent (IDEXX #WCLS20). The entire 100ml sample with reagent is then put into a Quanti-Tray 2000 (IDEXX #WQT-2K) and sealed. The sample, now in the tray, will be incubated at 37 °C for 24-48 hours and quantified by colorimetric and/or fluorescent detection. Yellow is indicative of no coliform, magenta is total coliform and magenta+fluorescent is *E. coli*. Software from IDEXX is then used to calculate the most probable number of fecal coliforms and *E.coli*.

Task 2:

Monitor accumulation of fecal coliform and *E.coli* in intertidal sediments and mussels. Sediment is an important aspect of water quality as some bacteria can live in sediments and can later be resuspended into the water column (Ferguson et.al, 2005). Mussels are also important to monitor because they allow bacteria to get incorporated into the nearshore food chain which may have ecological impacts on nearshore ecosystems.

Samples will be taken at the same time as the seawater samples and will be prepared by adding 100mL sterile water to a preweighted amount of sediiment. Then sediment is will then be mixed with the water by shaking for 30 seconds. Then the sediment is allowed to settle for 15 seconds. Next 50ml of the water added to the sediment is mixed with 50mL of sterile water and processed using the Colisure procedure explained above.

Mussels will be sampled once every other month. They will be transported back to the lab in a cooler containing ice and stored at 4-8 degree C for up to 4 days before processed.

Once in the lab the mussels will be cleansed to remove detritus and fouling agents (Power et. al., 1990) using 70% ethanol. They will then be immersed in a solution containing 7% $MgCl_2$ and seawater (1:1, vol/vol) for 30 minutes to anesthetize them (Power et. al., 1990). This is to prevent expulsion of bacteria from gut which may be caused by stress. After being fully anesthetized the entire mussels is dissected out if it shell and chopped finely with a sterile scissors in sterile water. The solutions are then homogenized using a tissue miser for 15 seconds at 20,000rpm.

The homogenate will be diluted (1:10, 1:100 and 1:1000) and inoculated into 5 tubes of each dilution containing lauryl sulfate tryptose lactose broth (Difico) and incubated at 37° C for 24 hours. The samples will be measured for gas production using a durham tube. Samples with gas production, which is a presumptive indication of coliforms present, will be transfer into a new tube containing Brilliant Green lactose bile broth (Difico), and incubated for 24 hours at 37° C to confirm coliform growth. Samples that are positive will be examined by MPN method to determine the approximate number of fecal coliform per gram of tissue. Samples that are posiblve in the Brilliant green media will be inocculated into a tube containing EC media and incubated at 45 degrees C for 24 hours to test of E.coli. The samples posibtive for E.coli will also be quantified using MPN.

<u>Products of Study</u>

~Continual monitoring of bacterial levels in water, sediment and mussel tissue of coastal areas corresponding to 5 Oregon watersheds from July 2007 thru June 2008.

~A predictive model that is useful in designating areas that will be least influenced by land for oyster restoration.

Preliminary Results

Sample	Water (Ave CFU/100mL)	Sediment (Ave CFU/100g)
Salmon	1364.0	9.8
Yachats	677.3	7.9
Coquille	0.7*	0.5
Sixes	2.8*	43.5
Chetco	2.3*	3.0

Discrepancy between methods- Samples with * were not diluted when processed.

Site Locations

Site	GPS Coordinates
Salmon	N 45°02'50.3" W 124°00'38.7"
Yachats	
Coquille	N 43°07'05.1" W 124°25'57.9"
Sixes	N 42°51'18.3" W 124°32'47.4"
Chetco	N 42°02'44.2" W 124°16'19.1"

Acknowledgements

A special thank you to the NOAA Restoration Center for funding this project. I would also like to thank Kerry Griffin for providing advice and getting this project proposal into the right hands. Last but not least I would like to thank Elise Granek who will be providing advice as well as lodging and transportation dollars for many of the field trips.

Survival and growth of native and Pacific oysters in Willapa Bay, Washington

Arwen A. Norman and Jennifer L. Ruesink
Department of Biology, University of Washington, Box 351800, Seattle, WA 98195, USA.

Native oysters (*Ostrea conchaphila*) were replaced commercially in Washington in part because the introduced Pacific oyster (*Crassostrea gigas*) grew faster to a larger market size. However, the two species have not been compared for growth under common garden conditions. In 2006, native and Pacific oysters recruited naturally to ceramic tiles placed intertidally in August. We set up an experiment to examine the effects of Pacifics on native oysters, carried out by removing Pacifics from half of the tiles when they were moved to their permanent location at MLLW. We followed growth and survival of both species from September to the following July. Native oysters exceeded Pacifics in recruitment, but had much lower survival and growth. Native oysters grew 1.5 mm/mo from September to March, and 3.4 mm/mo from March to July. Over the same time period, Pacific growth was 3.4 mm/mo and 6.2 mm/mo. Thus, both species grew twice as fast in spring/summer than in fall/winter, and Pacifics grew twice as fast as natives. At the end of 10 months, only 8% of natives still survived, as compared to 40% of Pacifics. Recruitment and survival were relatively poor for both species, and no space competition occurred. As there was no evidence of predation, we infer that mortality was due to physical factors, possibly both cold and heat stress given that natives survived poorly in both seasons.

Appendices

Workshop Agenda

MONDAY

Presenter	Time	Title
Betsy Peabody *Puget Sound Restoration Fund* Kerry Griffin *NOAA Fisheries*	1:00 – 1:20	Opening remarks, workshop introduction
Rick Peters *Squaxin Island Tribe*	1:20 – 1:40	Tribal Blessing
TBD	1:40 – 2:00	Keynote address

Populations and Perspectives

Ted Grosholz *UC Davis*	2:00 – 2:15	Overview of oyster populations in Central California
Sarikka Attoe *UC Davis*	2:15 – 2:30	San Francisco Bay native oysters: How are they doing?
Susan Burke *Northern Economics, Inc.*	2:30 – 2:45	A proposal to assess the benefits and costs of Washington State mollusk production and restoration
Duane Fagergren *Calm Cove Oyster Company*	2:45 – 3:00	The perspective and role of industry in native oyster restoration
	3:00 – 3:15	**Break**
	3:15 – 4:30	**Panel Discussion – Mimicking the native seascape**
	5:00 – 6:30	**Reception and Poster Session – Grand Ballroom**
	6:30 – 9:00	**Dinner (on site)**

TUESDAY

	7:00 – 8:15	**Breakfast**

Presentations – Policy, Permitting, and Regulation

Natalie Cosentino-Manning *NOAA Fisheries*	8:30 – 8:45	Update on San Francisco Bay oyster restoration working group
Blain Reeves and Helen Berry *WA Dept of Natural Resources*	8:45 – 9:00	WA state DNR perspective - Policy and science in the regulatory arena
Steve Landino *NOAA Fisheries*	9:00 – 9:15	NOAA's perspective on shellfish policy and science
	9:15 – 9:35	**Panel Discussion – Policy, Regulation, and Permitting**
	9:35 – 9:45	**Coffee break**

Genetics and Nomenclature

David Stick *Oregon State University*	9:45 – 10:00	Preliminary analyses of genetic structure within and among extant populations of the Olympia oyster, *Ostrea conchaphila*
Nathan Wight *University of Washington*	10:00 – 10:15	Species specific quantification of *Ostrea conchaphila* larvae in seawater samples using a DNA based (QPCR) assay
Maria Polson (substitute)	10:15 – 10:30	You say *conchaphila*, I say *lurida*
	10:30 – 11:00	**Panel Discussion – One species or two?**
	11:00 – 12:30	**Lunch**

Monitoring and Invasives

Kate Henson *University of Washington*	12:30 – 12:45	Control strategies for the Japanese oyster drill and implications for restoration and management of the Olympia oyster in Liberty Bay, WA
Sumudu Welaratna *San Jose State University*	12:45 – 1:00	San Francisco Bay native oyster recruitment study of 2006-07 and the development of shared protocol for monitoring efforts
	1:00 – 1:20	**Questions and Discussion**
Sarikka Attoe and David Couch	1:20 – 1:45	**Demonstration – Larval settling substrate**
	1:45 – 2:00	**Break**
	2:00 – 3:00	**Topic Discussion** (monitoring or substrate enhancement)

Restoration projects Round I

David Couch	3:00 – 3:15	Arcata's native oyster project in Humboldt Bay, 2007 update
Stan van de Wetering *Confederated Tribes of the Siletz*	3:15 – 3:30	Yaquina Bay, Oregon native oyster restoration
Joth Davis *Baywater, Inc.*	3:30 – 3:45	Liberty Bay, WA restoration projects
	3:45 –4:30	**Questions and Discussion**
	6:00 – 9:00	Optional dinner at Xinh's Restaurant, Shelton

WEDNESDAY

	7:00 – 8:15	**Breakfast**

Restoration projects round II

Bud Abbott *MACTEC, Inc.*	8:30 – 8:45	Olympia oyster habitat construction methods and results: 2005-2007
Jessica Miller *Oregon State University*	8:45 – 9:00	Re-establishment of the native oyster, *Ostrea conchaphila*, in Netarts Bay, Oregon
Russel Barsh *Center for the Historical Ecology of the Salish Sea*	9:00 – 9:15	Opportunities for reconstruction of pre-Contact native oyster distribution and population structure in north Puget Sound
	9:15 – 9:45	**Questions and Discussion**
	9:45 – 10:00	**Break**
		The human dimension
Marilyn Latta *Save the Bay*	10:00 – 10:15	Volunteers on the half shell! Native oyster Restoration in San Francisco Bay using community volunteers
Betsy Lyons and Brian Allen *Puget Sound Restoration Fund*	10:15 – 10:30	Native oyster restoration in South Puget Sound
	10:30 – 11:30	**Panel – The human dimension** Funding opportunities New direction/the next big thing (new shellfish species, multi-species experiments, sustainable aquaculture, etc) Industry's role in restoration Small group discussions by state Review workshop, next steps "Bin" items
	11:30 – 12:00	Wrap up, next steps

Adjourn

2007 West Coast Native Oyster Restoration Workshop Attendees

Last Name	First Name	Affiliation	State	Email
Abbott	Bud	Mactec	CA	rrabbott@mactec.com
Allen	Brian	Puget Sound Restoration Fund	WA	brianleeallen@hotmail.com
Adams	John	Taylor Shellfish	WA	JohnA@taylorshellfish.com
Archer	Pam	OR State University	OR	parcher@coas.oregonstate.edu
Attoe	Sarikka	UC Davis	CA	saattoe@hotmail.com
Badrei	Natalie	NOAA	CA	natalie.badrei@noaa.gov
Berry	Helen	WA DNR	WA	helen.berry@dnr.wa.gov
Blake	Brady	WDFW	WA	Blakebcb@dfw.wa.gov
Brumbaugh	Rob	TNC	RI	rbrumbaugh@tnc.org
Buhle	Eric	UW	WA	buhle@u.washington.edu
Burke	Susan	Northern Economics Inc	WA	susan.burke@norecon.com
Callahan-Grant	Megan	NOAA	OR	megan.callahan-grant@noaa.gov
Camara	Mark	OSU	OR	Mark.Camara@oregonstate.edu
Chesney	Bryant	NMFS SWR	CA	bryant.chesney@noaa.gov
Couch	Dave	City of Arcata	CA	bg_ranch@sbcglobal.net
C-Manning	Natalie	NOAA	CA	natalie.c-manning@noaa.gov
Davis	Joth	Baywater, Inc.	WA	jdavis@bainbridge.net
Dinnel	Paul	Shannon Point Marine Lab	WA	padinnel@aol.com
Erickson	Aleta	Jamestown S'kkallam Tribe	WA	aerickson@jamestowntribe.org
Fagergren	Duane	Puget Sound Action Team	WA	DFagergren@PSAT.WA.GOV
Fredell	Andy William	CSU Fullerton	CA	fredell@csu.fullerton.edu
Friedman	Carolyn	UW	WA	carolynf@u.washington.edu
Green	Rhoda	PSRF	WA	rhodagreen@yahoo.com
Griffin	Kerry	NOAA	WA	kerry.griffin@noaa.gov
Guy	Dan	NOAA	WA	dan.guy@noaa.gov
Hamilton	Laura	NMFS	WA	laura.hamilton@noaa.gov
Henson	Kate	U. Washington	WA	hensok@u.washington.edu
Hewson	William	CSU Fullerton	CA	whewson@fullerton.edu
Hicks	Polly	NOAA	WA	polly.hicks@noaa.gov
Hillman	Amanda	Portland State University	OR	hillmana@ohsu.edu
Hunter	Matt	ODFW	OR	Matthew.V.Hunter@state.or.us

Jennings	Laurel	NOAA	WA	laurel.jennings@noaa.gov
Landino	Steven	NOAA	WA	steven.landino@noaa.gov
Langdon	Christopher	Hatfield Marine Science Center	OR	Chris.Langdon@oregonstate.edu
Latta	Marilyn	Save the Bay	CA	mlatta@savesfbay.org
Leavens	Liz	Padilla Bay NERR	WA	eleavens@padillabay.gov
Lehto	Jason	NOAA	WA	jason.a.lehto@noaa.gov
Hamilton	Laura	NMFS	WA	laura.hamilton@noaa.gov
Henson	Kate	U. Washington	WA	hensok@u.washington.edu
Hewson	William	CSU Fullerton	CA	whewson@fullerton.edu
Hicks	Polly	NOAA	WA	polly.hicks@noaa.gov
Hillman	Amanda	Portland State University	OR	hillmana@ohsu.edu
Hunter	Matt	ODFW	OR	Matthew.V.Hunter@state.or.us
Jennings	Laurel	NOAA	WA	laurel.jennings@noaa.gov
Landino	Steven	NOAA	WA	steven.landino@noaa.gov
Langdon	Christopher	Hatfield Marine Science Center	OR	Chris.Langdon@oregonstate.edu
Latta	Marilyn	Save the Bay	CA	mlatta@savesfbay.org
Leavens	Liz	Padilla Bay NERR	WA	eleavens@padillabay.gov
Lehto	Jason	NOAA	WA	jason.a.lehto@noaa.gov
Lyons	Betsy	TNC	WA	blyons@TNC.ORG
Madeira	Josh	UCS B	CA	jmadeira@bren.ucsb.edu
McGraw	Kay	NOAA Restoration Center	CA	kay.mcgraw@noaa.gov
Michaels	James	USFWS	WA	james_michaels@fws.gov
Norman	Arwen	UW	WA	aan2@u.washington.edu
Peabody	Betsy	Puget Sound Restoration Fund	WA	betsy@restorationfund.org
Peters	Rick	Squaxin Island Tribe	WA	spikenpam@hotmail.com
Reeves	Blain	WDNR	WA	blain.reeves@dnr.wa.gov
Rohrbach	Morgan	Puget Sound Restoration Fund	WA	morgan@restorationfund.org
Ruesink	Jennifer	UW	WA	ruesink@u.washington.edu
Schirato	Margie	WDFW	WA	schirmms@dfw.wa.gov
Sparkman	Eric	Squaxin Island Tribe	WA	esparkman@squaxin.nsn.us
Stick	David	OSU	OR	david.stick@oregonstate.edu
Toy	Kelly	Jameston S'Klallam Tribe	WA	ktoy@jamestowntribe.org
Trimble	Alan	UW	WA	trimblea@u.washington.edu

Vadopalas	Brent	UW	WA	brentv@u.washington.edu
van de Wetering	Stan	Siletz Tribes	OR	stanv@ctsi.nsn.us
Vander Schaaf	Dick	TNC	OR	dvanderschaaf@tnc.org
Welaratna	Sumudu	San Jose State University	CA	welaratna@yahoo.com
Wight	Nathan	UW	WA	naw4@u.washington.edu
Zacherl	Danielle	California State Fullerton	CA	dzacherl@exchange.fullerton.edu