Final Report to: The Nature Conservancy/NOAA Restoration Center

Olympia oyster Restoration and Eelgrass Effects in Hood Canal, WA (Subaward #GMT-PSRF-052413)

September 2015





PUGET SOUND RESTORATION FUND

382 Wyatt Way NE Bainbridge Island, WA 98110

Project Title:	Olympia oyster restoration and eelgrass effects in Hood Canal, Washington
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Final Report Narrative:

I. Abstract

The goal of this project was to successfully restore 4000 m² (~ 1 acre) of native oyster habitat in a manner compatible with a native eelgrass bed present at the same location. Based on historical evidence, native oysters (*Ostrea lurida*) reached some of their highest abundances in the Hood Canal region near the modern town of Belfair, in what is now a native eelgrass (*Zostera marina*) meadow. Few oysters are currently present, and interest in oyster restoration at this site spans state, tribal, and non-government entities. This project set out to compare two oyster restoration techniques (juvenile seeding via spat on shell, and via single oysters) for their success in generating stable or increasing oyster populations. The project also examined reciprocal interactions between native oysters and eelgrass and measured the response of native eelgrass in terms of density, size, growth, and tissue chemistry.

II. Objectives and Results

Objective 1: Restore 4000 m² of *O. lurida* at a historically important site in the Hood Canal hook where native oysters are now rare or absent. In the map below, the gray polygon indicates the location of the project site relative to the head of Hood Canal near the town of Belfair.



In 2013, PSRF produced 100 bags of seeded cultch and approximately 500 large singles for outplant at the Belfair restoration site. The seed was produced using approved conservation genetic protocols. 50 bags of seeded cultch, representing half the seeded cultch produced for this project, were outplanted August 20, 2013 within a 4,000 m² plot delineated by Jennifer Ruesink.

<u>Restoration methods</u>. Seeded cultch was spread according to an experimental design developed by Jennifer Ruesink that included ten 10x10 meter plots placed randomly along three parallel transects at -0.3 m, -0.6 m, and -1 m below mean lower low water. Five bags of seeded cultch were distributed in each Restoration plot resulting in 7% cover at a density of 13 oysters/m⁻².

15 1	3	5	High transect -0.3 m (-1') MLLW
2	8 26	20 18	Mid transect -0.6 m (-1.8') MLLW
45			Low transect -1 m (-3') MLLW

The graphic above simply shows the random placement of the 10 plots along 3 elevation transects, with numbered boxes representing plot numbers.

The 500 single oysters, considered large enough to outplant at this time, were also outplanted on the middle transect, at the north corner of the treatment plot. The remaining 50 bags of seeded cultch were stored in Thorndyke Bay throughout 2014 in order to grow additional single oysters to a size that would better survive the rigors of the experimental outplant and that would enable a simultaneous outplant of both seeded cultch and singles.

In 2014, sediment impacts at the project site necessitated a change of plan for the remaining outplants. The cultch outplanted in 2013 maintained its original coverage for a year and then disappeared under the sediments. Although fine sediment was observed at the site in 2007, the re-routing of a nearby creek in 2009 may have contributed to increased sediment accumulation, perhaps by shifting more of the sediment transport toward the project site. As a result of sediment dynamics at the site, the project team recommended in 2014 that there be no further addition of spat-on-shell directly on the sediment. The recommended plan moving forward was to 1) outplant larger seed on cultch; and 2) anchor cultch in order to avoid future burial.

To anchor the cultch, the team constructed "rosettes" of shells on wooden stakes for outplanting at the site. The oyster "rosettes" were constructed by drilling a hole in cultch and attaching it to wooden posts with wire or string, a technique used effectively to anchor cultch in eelgrass in research plots in Willapa Bay. Using this design, approximately ten cultch shells could be attached to each wooden post with two posts per square meter in order to keep overall cover at the desired 10%. The anchored cultch would prevent loss of the shell and enable better measurements of oyster growth and mortality.

In 2015, PSRF and UW transferred the remaining 50 cultch bags from the storage site to the Manchester hatchery and hand-selected large seed and singles for the purpose of constructing oyster rosettes. The new experimental treatment (pictured below) was deployed July 30, 2015. Approximately 2,000 oysters were deployed on rosettes, including 1,000 F1 single juveniles from 2013 production and 1,000 large seed on cultch from shell bags stored in Thorndyke Bay.



The construction and ouplant of oyster rosettes represents an adaptive management strategy, since neither singles nor seeded cultch outplanted in 2013 survived sedimentation at this site. A picture of Olympia oyster seed on shell used in the construction of oyster rosettes in 2015 is included below. Red circles are drawn around Olympia oysters set on Pacific oyster cultch.



The broodstock used to produce seeded cultch and singles for this project were collected in winter 2013 at various Hood Canal sites, including Dosewallips (Taylor Shellfish Farms), Triton Cove (State-owned tidelands), Jackson Cove (Hama Hama Oyster Co), and Tarboo Bay (Rock Point Oyster Co.).

An estimated 20,000 additional singles were produced in 2015 from Hood Canal broodstock. These animals are currently in the PSRF seed nursery at NOAA Manchester in Clam Bay. The 2015 Hood Canal singles will be available for outplantin in 2016.

In summary, although we were not able to achieve the intended restoration at the project site, we did achieve the objective of applying restoration techniques experimentally in a new place in order to measure response in eelgrass and see whether the benefits of both oysters and eelgrass could be realized within the same space. Ultimately, the Belfair site proved to be unsuitable to Olympia oyster out plants due to sedimentation accumulation, possibly associated with a nearby restoration project at Big Mission Creek that shifted the mouth of the creek and increased the delivery of sediments to the project site. We adaptively managed the research objective of the project by designing a different oyster treatment in 2015 (the oyster "rosettes") that would help us avoid burial and better track oysters within the eelgrass meadow. We also developed alternative strategies for restoring Olympia oysters in the southern reach of Hood Canal. Dr. Ruesink has developed a proposal for testing a mosaic strategy, involving both eelgrass and Olympia oyster out plants. Additionally, Brady Blake at Washington Department of Fish & Wildlife has identified a possible restoration site east of Belfair State Park.

A summary of Objective 1 results, prepared by Jennifer Ruesink at University of Washington, is included in Section VIII. Monitoring and Maintenance Activities.

Objective 2: Involve citizens in monitoring *O. lurida* recruitment at multiple distances away from the restoration site.

To recruit citizen spat collectors, PSRF developed a flyer and a laminated field guide to train citizen scientists in Olympia oyster identification.

You're Invited to: An Olympia oyster Training Workshop



When: May 29, 2013, 3:00-5:00 pm Where: 7281 E. Hwy 106, Union, WA (map on back)

Puget Sound Restoration Fund (PSRF) is hosting an Olympia oyster workshop to train volunteers in how to identify and monitor native oyster set in the lower reach of Hood Canal. Once trained, shoreline property owners/volunteers will deploy settlement substrate (shell) every two weeks throughout the settlement season (June – September 2013 and June – September 2014) to collect data on native oyster recruitment in the Hood Canal hook. The settlement substrate will be examined for oyster set by undergraduate students working with lenging Russing's lab at University to undergraduate students working with Jennifer Ruesink's lab at University of Washington.

The work is simple, fun and a huge help to the restoration effort. Specifically, volunteers will put out 20 shells in pre-constructed monitoring devices (spat collectors) at a low tide elevation every two weeks and pick the shells up two weeks later.

The workshop is part of a larger project to restore one acre of native oysters on Mason County tidelands near Belfair and to study the interactions between native oysters and edgrass. The Belfari site is at the end of Hood Canal in an area that historically hosted 100 million native oysters (*Ostrea lurida*) spanning 500 acres. While an estimated 10 million native oysters occur sparsely throughout all of Hood Canal today, there is low to no oyster presence near the head of Hood Canal.

Belfair represents a priority area for restoration, since oysters restored at Belfair represents a priority area for restoration, since oysters restored at this location could serve as a source population for nearby suitable habitats. In Washington Department of Fish & Wildlife's (WDFW) updated Olympia oyster Stock Rebuilding Plan, Belfair is identified as the most important site in Hood Canal for strategic, large-scale Olympia oyster restoration. The proposed project laso represents the first step in implementing a Hood Canal Olympia oyster enhancement plan developed in partnership with the Skokomish Tribe and WDFW.

stablishing a self-sustaining native oyster population in the lower reach of Hood Canal will help 1) provide complex habitat attractive to fishes, invertebrates and other marine organisms, 2) restore an intertidal habitat feature that covered 500 acres historically, and 3) explore whether eelgrass and native oysters, both habitat-formers, could be restored synergistically.

- Partners: WA Department of Fish & Wildlife Mason County University of Washington Citizen shoreline spat monitors

Please RSVP to Betsy Peabody Phone: 206.780.6947 • Email: betsy@restorationfund.org

Many Thanks to NOAA & The Nature Conservancy for funding



The flyer was distributed through known contacts in the area, including the Skokomish Tribe, nearby shellfish growers, and neighbors of Alderbrook Lodge, a popular resort along the southern shore of Hood Canal. The laminated Olympia oyster Field Guide was distributed to volunteer spat collectors and made available on PSRF's web site at the following link: http://www.restorationfund.org/sites/default/files/OlympiaOysterFieldGuide.pdf

In 2013, 11 volunteers assisted with spat collection and participated in other Olympia oyster recruitment monitoring activities. PSRF and UW conducted a field workshop May 29, 2013 to train citizen monitors in Olympia oyster identification and recruitment monitoring and to distribute the first batch of recruitment substrates, which consisted of both shellsticks and shell-carpets. Both substrates could be affixed to the substrate with stakes and zip ties. Beginning in June, citizens installed and collected both types of recruitment substrates every two weeks. When the substrates were collected, the volunteer labeled them by site, name and date of collection and stored them dry in a bucket until someone from the UW crew picked them up.

In 2014, 6 volunteers (new and returning) assisted with spat collection in the southern reach of Hood Canal. Additionally, 3 local tribes and commercial shellfish farmers assisted with broodstock collection and the deployment and collection of settlement substrate at multiple other sites in Hood Canal.

In 2015, 2 additional volunteers were recruited to assist with spat collection, and 10 volunteers were recruited to assist with rosette construction and conduct oyster population assessments at Twanoh State Park (July 2, 2015) and Belfair State Park (July 31, 2015).

All told, a total of 32 volunteers (contributing 399 volunteer hours) assisted with spat collection, population surveys, rosette construction, broodstock collection and other project activities during the project period.

A summary of Objective 2 oyster recruitment results from the 2013, 2014, and 2015 summer monitoring seasons, prepared by Jennifer Ruesink at University of Washington, is included in Section VIII. Monitoring and Maintenance Activities.

Objective 3: Test the ecological consequences of two restoration techniques for O. lurida in native eelgrass

Prior to, during, and after the 2013 native oyster outplant, Jennifer Ruesink and a graduate student conducted eelgrass measurements (shoot density and size) within each of the plots that received seeded cultch in order to test the ecological consequences of low density (10% cover) native oyster outplants.

Oyster drill populations were also monitored at this location, since drills are present within the 4,000 m² plot and were observed in breeding aggregations in spring 2013. The results of Objective 3 monitoring activities were prepared by Jennifer Ruesink at University of Washington and are summarized in Section VIII. Monitoring and Maintenance Activities.

III. Restoration Targets and Progress

Habitat Type:	Acres/miles/metric	Acres/miles/metric	Acres/miles/metric
	tons accomplished	tons accomplished to-	tons projected to be
	in this reporting	date (cumulative):	completed at end of
	period:		award:
Eelgrass/Oyster	32 m^2 Rosette area	$1000 \text{ m}^2 (2013) +$	32 m^2 area
		$32 \text{ m}^2 \text{ Rosette } (2015)$	(Note: 1000 m^2
			restored in 2013 was
			buried by sediment)

Geographic Coordinates (in decimal degrees): (WGS84)

Longitude (X-coord): -122.875006 W long	Are there mu	ltiple project	t sites fo	or
Latitude (Y-coord): <u>47.423874 N lat</u>	this award?*	Yes	X	No
(Mason County Tax parcel: 22201-00-62200)				
River Basin: Lynch Cove-Union River-Hood Canal-Nea	arshore			
Community Identifiers (community Description), Description				

Geographic Identifier (e.g. Chesapeake Bay): Puget Sound

Project Start Date: January 1, 2013 Project End Date: September 30, 2015 (amended)

IV. Permit Status

All necessary permits were obtained for this project, including:

Shoreline Exemption Permit (SHX2013-00027) ((Mason County)
Hydraulic Project Approval (131619-1) (WA Dept of Fish & Wildlife;)
State 401 Water Quality Certification (WA Department of Ecology)
Federal ESA Consultations ((NOAA, USFWS)
Nationwide Permit 18 (NWS-2013-474)	U.S. Army Corps of Engineers)

V. Species Benefitting

Anticipated benefits to species and habitat(s), including threatened and endangered species: Oysters as filter-feeding species contribute to phytoplankton removal that could otherwise decompose at depth and contribute to low-oxygen conditions in Hood Canal. Several commercial and listed species in Hood Canal may benefit from additional structured nearshore habitat for foraging and protection, including threatened Hood Canal summer chum salmon, Puget Sound Chinook and Puget Sound winter steelhead trout.

VI. Project Partners

(Project Manager; seed production; enhancement)
(Lead, enhancement design & scale),
(Pre- and post-restoration monitoring/data analysis),
(Enhancement & monitoring assistance),
(Monitoring assistance)
(Natural resource manager user group)
(Monitoring)
(Monitoring)
(broodstock collection)
(broodstock collection)

VII. Project Timeline

Final permit applications	January 2013
Broodstock collection	Winter 2013, 2014, 2015
Restoration-grade seed production	January – April 2013; January – April 2015
Pre-restoration monitoring	April - June, 2013
Implementation	June-August 2013; June-August 2015
Oyster settlement monitoring	June - Sept. 2013; June - Sept. 2014; June - Sept. 2015
Post-restoration monitoring	Jul, Oct 2013, Jan, Apr, Jul, Oct 2014/2015; ongoing
Final Report	September 15, 2015
Project End Date	September 30, 2015

Oyster recruitment monitoring and post-restoration monitoring will continue on a less frequent basis in 2016 to capture results over time, since a third outplant technique was developed in order to adapt to conditions at the site.

VIII. Monitoring and Maintenance Activities

This project involved experimental restoration of Olympia oysters within an eelgrass meadow to see whether the benefits of both could be realized within the same space. Restoration activities occurred at Belfair in lower Hood Canal, Washington, where a native eelgrass meadow currently occupies space below mean lower low water that was historically an Olympia oyster bed. Reefs of introduced Pacific oysters occur above mean lower low water, associated with old oyster dykes that were put in place to hold pools of water and culture Olympia oysters. Eelgrass at the site was not allowed to decline by more than 20%, which constrained oyster outplants to low percent cover. The site proved unsuitable for Olympia oysters to persist within the soft sediment at the site. We adaptively managed the project in 2015 by building "rosettes" that anchored cultch to avoid burial and better track survival of oysters. In collaboration with Tribes and citizen

monitors, we learned a substantial amount about recruitment of Olympia oysters in Hood Canal over three summers. Olympia oysters showed less spatial and temporal variation in recruitment than did Pacific oysters. For instance, in lower Hood Canal in 2013-2015, cumulative summer recruitment for Olympia oysters was 2, 12, 22 per shellface, and for Pacific oysters was 0.3, 370, 6 per shellface. Ovster recruitment was greater for Olympia than Pacific ovsters in 2015 in lower Hood Canal, but not in other parts of Hood Canal. These levels of Olympia oyster recruitment in lower Hood Canal would be considered a "commercial" level with regards to wild set of cultch (>3 per shell), but Olympia oysters do not persist at these levels post-recruitment given low densities of adults even where present (e.g. Twanoh oyster survey). As a result of this project, new plans and proposals have been developed for Olympia oyster restoration in lower Hood Canal that take into consideration the constraints of restoring oysters in soft-sediment eelgrass meadows. New proposed restoration sites in lower Hood Canal include shores near the Skokomish River (Washington Department of Natural Resources, NOAA Coastal Ecosystem Resiliency proposal) and east of Belfair State Park in the former delta cone associated with Big Mission Creek (Washington Department of Fish and Wildlife).

Objective 1: Successfully restore 4000 m² of *O. lurida* at a historically-important site in the Hood Canal hook where native oysters are now rare or absent.

Restoration with seeded cultch. This objective matches Structural Objective #1: Oysters in the Monitoring Plan. Oysters were monitored in Restoration plots in terms of % cover of cultch and number of O. lurida. Ten 10x10 m plots were established as Restoration plots for Olympia ovsters within native eelgrass on 20 August 2013. These ten plots were placed randomly along three parallel transects at -0.3 m, -0.6 m, and -1 m below mean lower low water. In each Restoration plot, five bags of seeded cultch were distributed. Initial cover of cultch was 7%, somewhat less than the target of 10% cover. About 13 pieces of cultch were present in each square meter, with an initial estimate of one ovster per seeded cultch, therefore initial ovster density was also lower than the target level of 50 m⁻². Adjacent plots were used for Reference plots without oyster addition. Monitoring was carried out seasonally over two years in up to seven plots. However, some statistical analyses used data from a shorter period of time when shell continued to be present on the Restoration plots. The lowest three plots (-1 m MLLW) were not accessible for sampling even on extreme low tides. Based on occasional observation, their trajectory did not appear different from that of higher plots within the eelgrass bed (-0.3 and -0.6 m MLLW). Each 10x10 m plot was subsampled with five 0.25 m² quadrats, and statistics are reported using plots as replicates (subplots within plots were combined). The timing of activities related to restoration and monitoring is detailed in Table 1.

Table 1. Timeline of monitoring and maintenance activities at the Belfair restoration site		
April 2013	Pre-restoration monitoring of eelgrass begins	
	Hatchery-produced oyster larvae settled on seeded cultch in 100 bags	
29 May to 4 Sep	Recruitment substrates replaced every two weeks for a total of 7 sampling	
2013	times at lower Hood Canal sites and Hamma Hamma	
20 August 2013	First restoration outplants: Ten 10x10 m Restoration plots established with 50	
	bags of seeded cultch	
Nov 2013	Post-restoration monitoring of oysters and eelgrass begins at seasonal	
	intervals (Nov 2013, Jan, May, July, Nov 2014, Feb, Apr, Jun 2015)	

.. ...

Mar 2014	Broodstock collected for additional hatchery production of seed, which was insufficient in quantity for restoration outplants
31 May to 9 Sep 2014	Recruitment substrates replaced every two weeks for a total of 7 sampling times at lower Hood Canal sites Hamma Hamma Dabob Bay and Port
	Gamble Bay
Nov 2014	Dramatic drop in cover of cultch in Restoration plots since July seasonal sample
Dec 2014	Broodstock collected for additional hatchery production of seed
June/July 2015	50 bags of seeded cultch from 2013 sorted to build "rosettes"
	Surveys for native and Pacific oysters across tidal elevations at Belfair, Twanoh, and Nahcotta
17 May to 30 Aug 2015	Recruitment substrates replaced every two weeks for a total of 6 sampling times at lower Hood Canal sites, Hamma Hamma, Dabob Bay, and Port Gamble Bay
30 July 2015	Second restoration outplants: Four 2x2 m plots established with rosettes and four with single juvenile oysters

The objective of restoring 4000 m² of *O. lurida* was not achieved. Oysters were added to 1000 m² (ten 10x10 m plots) as outplants of seeded cultch in August 2013, and this shell cover persisted for a year. Thereafter, a steady decline resulted in <1% cover after two years (Fig. 1). Cultch was not observed outside the Restoration plot borders, and the timing of the drop in cultch was not consistent with typical seasonal storms. We generally had to find cultch by feeling for hard surfaces, rather than seeing it. Counts of oysters dropped prior to cover of cultch (Fig. 1), indicating that oysters were dying even when cultch was still possible to find. Although we do not have counts of live oysters at the time of outplant, cultch densities were about 13 m⁻² with one oyster per cultch, still many more than 1.4 m⁻² in November, three months after outplant. Mortality continued so that only 0.4 m⁻² were present after seven months, even when cultch was still found at initial cover. These lines of evidence all point to sinking or smothering as the major source of mortality, rather than movement out of the plots to another location. Japanese oyster drills are present at the site, and some dead oysters were observed with drill holes, but most probably died from fine sediments.



Figure 1. Outplanted Olympia oysters at Belfair. A) Cover of shell (cultch); B) Density of live Olympia oysters. Outplants occurred 20 August 2013 and remained in place until summer 2014. Disappearance thereafter was probably from smothering by fine sediments. Oyster mortality occurred prior to decline in cover of cultch. Error bars represent standard errors from up to 7 Restoration plots.

<u>Adaptive management</u>. In response to the outcome of the first restoration outplant, we revised our restoration activities in the following ways: 1) Outplant remaining oysters as single juveniles or as anchored seeded cultch, totaling 32 m² area, in July 2015. 2) Collect information on potential mortality sources, including sediment that could smother oysters and non-native oyster drills that could consume them. 3) Survey native oysters across tidal elevations at Belfair and at two additional sites with some similarities to Belfair, to compare population characteristics by locale, sediment type, substratum availability, and recruitment. We report on each of these adaptive management activities in the sections below.

Restoration with single juveniles and "rosettes" of anchored seeded cultch. In the second restoration outplant, we were limited in numbers of Olympia ovsters available and reduced both the plot size and number of restoration plots. The plots were established within the eelgrass bed at elevations that will be accessible for monitoring in future. Although funding for such monitoring has expired, we will attempt to continue seasonal samples of these plots for at least a year. The plots were 2x2 m separated by 3 m. Single juveniles were added to four plots, anchored seeded cultch were added to four plots, and four plots were marked as Reference plots without oysters. Both oyster-addition treatments achieved the goal of <10% cover. Each plot for single juveniles received 260 to 290 individuals of mean shell length 26.5 mm. In the anchored treatment, we drilled a hole in each cultch and threaded ten cultch on galvanized wire, attached to a wooden garden stake to make a "rosette". We placed 14 of these "rosettes" in each 2x2 m plot, with usually one Olympia oyster per cultch. The cultch derived from hatchery efforts in 2013, which had been stored in bags in Thorndyke Bay. The average size was smaller than expected for oysters older than two years, so the oysters may have included some that settled naturally in Thorndyke Bay (Table 2). Plots were evenly divided between two tidal elevations in the eelgrass, approximately -0.3 m and -0.6 m below mean lower low water.

Table 2. Measurements of shell length of Ostrea lurida following hatchery settlement in Mar-
Apr 2013. Seeded cultch was produced in 2013 and then outplanted (50 bags) or stored in
Thorndyke Bay and used in 2015 to build rosettes (50 bags). Single juveniles were also produced
in 2013 and outplanted in 2015.

Date	Months post-	Shell length (SD)	Number	Location
	set		measured	
Aug-13	4	12.9 (2.1)	93	Hatchery singles
3-Nov-13	7	13.9 (3.0)	12	Belfair seeded cultch
19-Jan-14	9	14.0 (1.0)	3	Belfair seeded cultch
18-May-14	13	18.7 (7.1)	3	Belfair seeded cultch
11-Jul-14	15	15	1	Belfair seeded cultch
23-Nov-14	19	32	1	Belfair seeded cultch
21-Apr-15	24	20	1	Belfair seeded cultch
28-Jul-15	27	21.1 (6.0)	45	Thorndyke Bay –
				received some natural set
29-Jul-15	15	26.5 (4.4)	20	Singles moved from
				Manchester to Belfair

Sediment conditions and populations of oyster drills. Surface sediment was collected at the Belfair restoration site on 14 June 2015. The samples contained 2.87% organic content (SE=0.12%, N=3). As far as we are aware, no benchmarks exist of "suitable" sediment properties for Olympia oysters on seeded cultch. However, the conditions at Belfair clearly represent soft sediments. For instance, among 17 sites with *Z. marina* in Puget Sound sampled in 2007, only four (including Belfair = Lynch Cove) exceeded 2% organic content (Yang et al. 2013). In Willapa Bay, sites more than half-way up the estuary typically exceed 2% organic content (Ruesink et al. 2015).

Oyster drills (*Ocenebra inornata*) arrived in Washington state from Japan in the 1920s (Wonham and Carlton 2005), but their transport to Belfair is of uncertain timing. Drills consume oysters as well as barnacles, mussels, and clams. Their presence was notable during pre-restoration surveys, when drills were quantified in large belt transects and reached their highest density in April 2013 at 0.26 m⁻² near the upper edge of eelgrass. Drills were less obvious at the site in May and at lower elevations. This spatial and temporal pattern was generally repeated, that is, drills were denser in higher-elevation plots and in April 2015. In the Restoration plots with oyster outplants, we regularly detected 1-3 drills m⁻² (Fig. 2). In contrast, of 234 0.25 m² quadrats placed in reference plots from Nov 2013 to Jun 2015, only one quadrat contained oyster drills for an average density in eelgrass of 0.01 m⁻². This low density is consistent with those measured in the eelgrass bed prior to restoration, except in Apr 2013 at the upper edge of eelgrass. Because drill densities generally increased with elevation, the source of drills is likely Pacific oyster reefs above the eelgrass bed, or other factors that reduce excursions to lower elevations. Because drill densities were higher on Restoration than reference plots, as well as after outplants of oysters, the cultch appeared to attract oyster drills or serve as a surface for attachment and feeding.



Figure 2. Oyster drills (*Ocenebra inornata*) in Restoration plots at Belfair. Error bars show standard errors of 3 plots at -0.3 m and 4 plots at -0.6 m relative to mean lower low water. At Belfair, drill densities tended to increase with elevation and tracked the cover of shell on plots. Additionally, drills were more apparent during aggregations to breed each April.

Monitoring of native oyster populations at comparable sites. In July 2015, we surveyed both Olympia and Pacific oysters at Belfair and at two other sites with some similarities in habitat attributes but that differed in the presence of Olympia oysters. Twanoh, also in lower Hood Canal, shares a common water body but differs in substratum: pebbles with some fringing eelgrass patches. Nahcotta, in Willapa Bay, is similar to Belfair in substratum, with Pacific oyster reefs above mean lower low water and native eelgrass growing in fine sediment below. Nahcotta lies in a different water body from Belfair and likely has more reliable annual recruitment of Olympia oysters (Trimble et al. 2009). Surveys were conducted in quadrats along 100-m transects at five tidal elevations: $+2^{2}$, $+1^{2}$, 0^{2} , -1^{2} , and -2^{2} relative to mean lower low water. We were constrained at Nahcotta to four tidal elevations (-2^{2} MLLW was rarely accessible due to less extreme tidal amplitude) and 50-m transects across oyster reefs. Quadrat size was chosen at each tidal elevation to contain an average of at least 10 oysters per quadrat, therefore quadrat size was larger in sparser oysters, but generally ranged from 0.0625 to 1 m², with 10 quadrats per elevation.

The Belfair site was confirmed to have few Olympia oysters, as none were observed outside of Restoration plots or found during the survey (Fig. 3A). At Twanoh, Olympia oysters occurred below the elevation of Pacific oysters on the west-facing side of the site but within the elevation of Pacific oysters on the east-facing side (Fig. 3C). The highest densities of Olympia oysters among the three sites were at Nahcotta, especially within the oyster reef above mean lower low water. Olympia oysters outnumbered Pacific oysters below mean lower low water at Nahcotta within native eelgrass (Fig. 3E). Thus, Nahcotta provides evidence that Olympia oysters can successfully exist within native eelgrass at low density and opens up questions about the characteristics of soft-sediment sites compatible with Olympia oysters. The sites also differed in Pacific oysters: Nahcotta had highest densities and smallest size, especially above mean lower low water (Fig. 3B, D, F). Belfair quantitatively had sparsest Pacific oysters, because these oysters occurred in an area above mean lower low water occupied by reefs of accumulated dead shell interspersed with soft-sediment mudflat.



Figure 3. A) Density and B) size of two oyster species at Belfair. C) Density and D) size at Twanoh in lower Hood Canal. E) Density and F) size at Nahcotta in Willapa Bay. Surveys at Twanoh were primarily on the west side, but square symbols (solid = *C. gigas,* open = *O. lurida*) show east side samples near the pier, and the inset in panel C) is provided for better view of low-elevation surveys. Error bars show standard errors from 10 quadrats.

Objective 2: Involve citizens in monitoring *O. lurida* recruitment at multiple distances away from the restoration site.

This objective matches Functional Objective #1: Recruitment in the Monitoring Plan. Additionally, this recruitment monitoring was a major pathway for community involvement and outreach activities, as detailed in sections IX and X. Additionally, community members participated in the Olympia oyster surveys in 2015. Over the three summers of the project, this citizen-based recruitment monitoring expanded to collaboration with two Tribes (Skokomish, Port Gamble Sk'lallam) and to include a site with historic data (1940s-1990s) near the Taylor Shellfish hatchery on Dabob Bay. The spatial scope of recruitment monitoring spanned the length of Hood Canal, not just lower Hood Canal, which was the area of restoration activity (Fig. 4). In the historic data set, no Olympia oysters were ever reported as settling. Several explanations exist for this lack of data on Olympia oyster recruitment in the past: it may have been observed but not reported; it may have occurred earlier than shells were placed because of targeting Pacific oyster recruitment; or it may have genuinely been less than at present.



Figure 4. Hood Canal, Washington, showing the Olympia oyster restoration site at Belfair. Other sites for surveys and recruitment monitoring are denoted by circles, filled if recruitment was measured in all three years of the study, and open if measured for less than three years.

We measured oyster recruitment on the downward-facing smooth surface of 10 Pacific oyster shells. These shells had holes drilled through the center allowing them to be stacked on a wooden dowel or tied in a monolayer to plastic mesh. In 2013, we detected no difference in recruitment in these two arrangements and thereafter used only the stacked shell method (shellsticks). Shellsticks were placed at mean lower low water before June in 2013, 2014, and 2015, and were

replaced at approximately two-week intervals during low tides through September (Table 1). This duration spanned recruitment for Olympia oysters, which can occur once water temperatures exceed 13° C, and for Pacific oysters, which can occur above 17° C (Baker 1995, Rico-Villa et al. 2009, Ben Kheder et al., 2010).

Olympia oyster recruitment was similar in magnitude to Pacific oyster recruitment in 2013, but both species accumulated only a few recruits per shellface that summer (Fig. 5). In 2014, Olympia oysters accumulated about 10 recruits per shellface through the summer at all sites except Point Julia, and Pacific oyster recruitment was much greater, to several hundred on a shellface in lower Hood Canal and Hamma Hamma. Pacific oyster recruitment in 2014 was later and slightly lower (50 vs. 100s per shellface) at the northern Hood Canal sites of Dabob and Point Julia than at the more southern sites (Fig. 6). Yet a different pattern emerged in 2015, which showed greatest settlement of Olympia oysters among the three years of monitoring, but spatially patchy recruitment of Pacific oysters (Fig. 7). Shellfaces in lower Hood Canal and Hamma Hamma accumulated 30 Olympia oysters during the summer. Only Hamma Hamma and Point Julia had substantial Pacific oyster recruitment, which at Hamma Hamma accumulated to 175 per shellface through the summer. In summary, lower Hood Canal sites showed some interannual variation in recruitment of Olympia oysters, but generally better recruitment than at the shellsticks placed in northern Hood Canal bays. Based on the timing and magnitude of recruitment in lower Hood Canal, recruitment dynamics appear distinct there and not tightly coupled to the rest of Hood Canal. For instance, even in comparison to the closest site, Pacific ovster recruitment exceeded 100 per shellface at Hamma Hamma in 2015 but was only 6 per shellface in lower Hood Canal.





Figure 6. Oyster recruitment measured at two-week intervals in Hood Canal in 2014. Figure conventions follow Figure 5, except note different y-axis.



Figure 7. Oyster recruitment measured at two-week intervals in Hood Canal in 2015. Figure conventions follow Figure 5, except note different y-axis.

Objective 3: Test the ecological consequences of two restoration techniques for *O. lurida* in native eelgrass

<u>Monitoring eelgrass in Restoration and reference plots</u>. This objective focuses on response variables in native eelgrass, *Zostera marina*, and matches Structural Objective #2: Eelgrass, and Functional Objective #2: Eelgrass productivity. The objective is included as a way of testing whether Olympia oyster restoration may be compatible with no net loss of eelgrass. Because shell was present on the Restoration plots from August 2013 to July 2014, four seasonal samples

during this period were used in evaluating eelgrass response. Because single juvenile oysters were not outplanted until 2015, we were only able to evaluate response of *Z. marina* to a single restoration technique – seeded cultch at low cover. Plots were sampled at each time in five 0.25 m^2 sub-samples, in which shoots were counted and, for five representative shoots, the sheath lengths measured. At the same time, three shoots were marked with two pin-holes at the top of the leaf sheath. We returned 2-3 days later and measured the length of extension of the fastest-growing leaf (usually the second-youngest), as well as sheath length and width. We collected these density, size and growth metrics in up to seven Restoration plots and adjacent reference plots without oyster outplants. Sampling date is considered a random effect, plot pairs are a random effect, and treatment is a fixed effect in linear mixed-effect analyses.

None of the metrics of eelgrass density, size, or growth responded to the addition of seeded cultch at low density (Fig. 8, Table 3). Thus, we successfully achieved the objective of experimental Olympia oyster restoration without damaging native eelgrass.

Table 3. Results of linear mixed-effects analyses examining effects of Olympia oyster outplants on biometrics of *Z. marina*. Date and paired plots were random effects, and treatment was a fixed effect. For each response variable, results are presented for treatment effects, where P>0.05 indicates no significant difference. Number of samples was 45 for density and size, and 51 for growth (including August 2014 data).

Eelgrass response variable	Calculated t value	P value
Shoot density	0.71	0.49
Shoot size (sheath length)	1.11	0.28
Growth (maximum leaf	0.37	0.72
extension)		
Size-standardized growth,	1.06	0.30
relative to sheath length		



B Sheath length (cm)



Figure 8. Biometrics of *Zostera marina* in Restoration plots with outplanted oysters and in adjacent Reference plots from Nov 2013 to August 2014. Box plots show median and distribution of 50% (box) and remainder (whiskers) of data. Biometrics include A) density, B) size, and C) growth.



Through the two years of the study, we observed substantial temporal variation in biometrics of native eelgrass at Belfair, as well as differences by tidal elevation. Both shoot size and growth varied seasonally and were greatest in summer, with size lagging growth (Fig. 9B, C). The fastest-growing leaf extended up to 40 mm in a single day in summer, but only 10 mm per day in winter. Shoot size increased at lower tidal elevations, which is a typical pattern in *Z. marina* (Fig. 9B; Ruesink et al. 2012). Shoots were also denser at lower elevations and away from the upper

edge (Fig. 9A). Shoot density showed less seasonal variation than did other biometrics but tended to decline during the study (Fig. 9A). The design of our monitoring allowed us to test for response to oyster restoration despite spatiotemporal variability, by comparing paired Restoration and reference plots.





IX. Discussion regarding Results

- In 2014, the project team recommended that no further addition of spat-on-shell directly on the sediment occur due to sediment conditions at the project site. The cultch outplanted in 2013 maintained its original coverage for a year and then disappeared under the sediments.
- 2) The team further recommended that cultch outplanted at this site should be anchored to prevent burial. This was accomplished in 2015 by constructing oyster "rosettes" that were experimentally outplanted at the project site in order to assess both performance and impacts.

- 3) Based on analysis of site characteristics and the restoration opportunities in the southern reach of Hood Canal, the project team recommends searching for another location to conduct native oyster restoration in this area. The conditions that supported native oysters at the Belfair site historically have changed and it makes sense to try to capture and/or restore the benefits of both eelgrass and native oysters in lower Hood Canal. For instance, rather than trying to outplant Olympia oysters within the same space currently occupied by eelgrass, there may be alternative sites where an oyster band could be established above the eelgrass zone (which has been done successfully in other locations), or where a nearby site with suitable conditions could be found. Two possible alternatives are described below.
- 4) Brady Blake, the Olympia oyster lead at Washington Department of Fish & Wildlife, has recommended an alternative site for future restoration east of Belfair State Park near the current outlet of Big Mission Creek on a big sand and mud flat in state ownership. The delta associated with Big Mission Creek has shifted to the west in the wake of a dikebreaching project completed several years ago. The area now associated with the old delta cone, which is outlined in yellow in Figure 10, appears to have suitable conditions for Olympia oysters: 1) Siltation does not appear to be a problem at this site; 2) Eelgrass is not present; and 3) the area is located within the area that supported 500 acres of dense beds historically. The tidelands southward and eastward may also have potential.



Figure 10: An alternative Olympia oyster restoration site east of Belfair State Park recommended by Brady Blake at Washington Department of Fish & Wildlife

- 5) Jennifer Ruesink at University of Washington has also developed a strategy for outplanting both eelgrass and Olympia oysters using a mosaic pattern to try to achieve the benefits of both habitats within a restoration site without directly overlapping the two. Jennifer is pursuing funding to test out this particular strategy.
- 6) The vision of recreating an oyster-eelgrass nexus is worth continued exploration, in spite of the challenges presented at this particular site. Restoring each of these species within a waterbody will help create habitat complexity, which is known to confer multiple benefits for multiple species. As mentioned in our proposal, there is ample historical evidence that *O. lurida* and *Z. marina* coexisted prior to commercial oyster exploitation in some sites, although the specific case of Belfair is unknown. According to Stafford (1915), "The oysters are either covered with shallow water at low tide, or exposed for only short intervals, while the eel grass acts as a strainer in keeping the water back and preventing complete drainage, or falls over and protects the oysters from direct heat of the sun". There are also numerous sites currently where the eelgrass zone occurs below the oyster band. Moving forward, we will test out other methods for re-introducing these two habitat-formers synergistically, either in a mosaic pattern or with the oyster band re-introduced above the eelgrass band.
- 7) The Belfair project provided an opportunity to experimentally deploy Olympia oysters within an eelgrass meadow and monitor the effects on eelgrass. The experimental oyster outplant in 2013 resulted in no impacts to eelgrass. Although the site ultimately proved to be unsuitable for Olympia oysters, the project provided many valuable lessons about site selection and helped clarify parameters that are critical for site selection, including natural recruitment, sediment conditions and hydrodynamics. Insights gained during this project will help us better target project sites within the State's 19 priority areas. Parameters of interest for sediment profiling in the future include firmness, sedimentation rate, slope/grade, grain size, and organic content. Other factors such as water residence time, flow rate, and circulation patterns all play a role in which areas are most suitable for restoration. For instance, sites with protracted water residence likely enable larval retention. It is no coincidence then that many historic source populations were located near the heads of inlets and bays. Favorable retention and circulation patterns, however, can also be found elsewhere within the inlet. Gathering this information ahead of time for each of the 19 priority locations in Puget Sound will create the scaffolding on which successful restoration efforts can be built as funding becomes available.
- 8) Further benefits of the project include improved understanding of Olympia oyster populations and recruitment in the lower reach of Hood Canal and an improved strategy for restoring Olympia oysters in an area that supported a large source population historically. Recruitment data collected at multiple sites throughout Hood Canal, resulting from significant citizen, tribal, and shellfish grower engagement, helps provide a larger context for understanding recruitment results in the vicinity of the project site.

X. Community Involvement

	This reporting	To-date (cumulative):	Projected for completed award:
Volunteer Numbers:	portou.	32	10
Volunteer Hours:		399	250

2013 volunteer summary: 8 volunteers involved in a 2-hour workshop and seven 2-hour field activities throughout the summer (deployments/collections/pick-ups) contributing 112 hours; and 3 volunteers attending a two-hour spring meeting and assisting with recruitment (contributing 16 hours) for a total of **11 volunteers and 144 volunteer hours in 2013**.

2014 volunteer summary: 6 volunteers involved in seven 2-hour field visits (deployments/collections/pick-ups) for a total of 6 volunteers and 84 volunteer hours in 2014.

2015 volunteer summary: 2 volunteer spat collectors contributing 15 hours each in summer 2015 (2 x 15 hrs = 30 hours); 3 growers/tribes collecting spat (3 x 15 = 45 hours); 4 volunteers contributing 8 hours each for July 2^{nd} oyster survey at Twanoh State Park (4 x 8 hrs = 32 hours); 4 student volunteers contributing 10 hours each for rosette construction, July 30th outplant, and July 31st oyster survey at Belfair State Park (4 x 10 hours = 40 hours); and 2 interns contributing a total of 24 hours in July 2015 for a total of **15 volunteers and 171 volunteer hours in 2015**.

XI. Outreach Activities

PSRF recruited, trained and/or collaborated with 32 volunteers throughout the project period. Students, community volunteers, shoreline property owners, local tribes, and commercial shellfish farmers were involved in gathering data about native oyster settlement in both the southern reach of Hood Canal and other locations throughout the Canal, including Annas Bay, Hama Hama, and Port Gamble Bay. We exceeded our original goal, which was to train a minimum of 10 volunteers in how to identify and monitor native oyster set. Trained volunteers deployed settlement substrate every two weeks throughout the settlement season from June through August 2013, 2014, and 2015. The settlement substrate was collected and monitored by undergraduate students working with Jennifer Ruesink's lab. Other outreach activities included oyster population surveys, broodstock collection, and rosette construction. The involvement of local residents helped assess the spatial pattern of native oyster recruitment in the Hood Canal hook. Outreach paired with specific sampling activities was designed to awaken interest in native oysters and achieve 1) tangible community involvement; 2) on-the-ground restoration; and 3) a strong research component. As such, the project provided a strong nexus of community involvement and data collection that would otherwise have been impossible in scale.

XII. Supporting Materials

To help train volunteers in Olympia oyster identification, PSRF developed an Olympia oyster field Guide in August 2013. The guide can be viewed on PSRF's website at the following link: http://www.restorationfund.org/sites/default/files/OlympiaOysterFieldGuide.pdf Through pictures and habitat descriptions, the guide enables citizens to refine their "search image" in order to identify remnant populations in suitable habitats. Instructions are provided for reporting these findings to Washington Department of Fish & Wildlife and PSRF in order to expand knowledge of current distribution. The Nature Conservancy and NOAA are both acknowledged as contributors to this field guide. The guide serves multiple purposes and audiences and will be distributed as part of numerous projects and outreach activities.

	Budget Categories	Total Budgeted Grant Fund	Total Budgeted Match	Grant Funds Expended this Report	Grant Funds Expended Cumulative	Match Funds Expended this Report	Match Funds Expended Cumulative	Match Fund Source
а	Personnel	13,415	7,034	7,733.45	13,089.73	13,474	19,233.78	
b	Fringe							
c	Travel	718		285.91	967.13			
d	Equipmen t							
e	Supplies	15,394	4,480	5,000	15,426.56		11,786.49	Match: Safeway Fdn, WADNR, WAECY, PSRF
f	Contractu al	40,698	6,240	21,166.28	39,888.21		6,630	Match: Baywater (J. Davis)
g	Other	500	54,630	75	1,365.00	28,000	32,992	Match: UW (Ruesink); WDFW; Skokomish Tribe; Baywater; Volunteers & Interns
h	Total Direct Costs	70,725	72,384	34,260.64	70,736.63	41,474	70,642.27	
i	Indirect Costs	5,314	2,073	2,338.84	5,302.37	868.32	4,026.65	
j	Totals	76,039	74,457	36,599.48	76,039	42,342.32	74,668.92	

XIII. Project Budget and Expenditures (from Financial Report)

Please explain any deviations from the approved budget (note that most budget changes require prior approval):

The only notable deviations from budget resulted from the *source* of match expenditures (not the amount). PSRF provided more match expenditures (through state contracts) than budgeted (\$19,233.78 actual versus \$7,034 budgeted). Conversely, other entities provided less match than budgeted (\$32,992 actual versus \$54,630 budgeted).

Note: The table above and the budget summary below represent the accurate allocation of grant and match funds.

Briefly describe the total project cost, including the source and value of other contributing funds (leverage that is not counting towards your official match):

Grant Funds Expended:

- \$13,089.73 Personnel Costs associated with storing, transporting and outplanting seed; recruiting and training volunteers; surveying oyster populations; selecting seed for oyster rosette construction; overseeing project; and preparing reports: \$4,096.03 Brian Allen (90.6 hours @ \$45.21); \$8,264.82 Betsy Peabody (138 hours @ \$59.89); \$578.88 Derek King (24 hours @ \$24.12); \$150 intern (10 hours @ \$15)
- \$39,888.21 Contractual: \$33,648.21 to University of Washington for Jennifer Ruesink and her students to conduct monitoring activities; \$6,240 for Joth Davis to oversee hatchery operations (96 hours @ \$65)
- \$15,426.56 Supplies (\$14,939.72 for Seed; \$486.84 for 100 cultch bags)
- \$ 1,365.00 Permit and broodstock collection fees
- \$ 967.13 Travel costs: \$270.89 boat rental; 688.5 miles @ .575; 348 miles @ .555; and 195 miles @ .55
- \$ <u>5,302.37</u> Indirect (18% of \$29,483.42 for personnel, travel, and supplies, which was reduced from \$5,307.02 by \$4.65 to fit budget)
- **\$76,039.00** Total Grant Funds Expended

Match Funds Expended:

\$19,233.78 PSRF Personnel Match (covered by WA Dept. of Natural Resources agreement):
\$1,063.55, Hannah Davis: 44.5 hours @ \$23.90 developing Oly Guide
\$4,696.23, Caitlin Campbell: 231 hours @ \$20.33 preparing permit applications
\$4,824, Derek King: 200 hours @ \$24.12 maintaining seed in FLUPSY
\$8,650 PSRF hatchery staff: 346 hours @ \$25 for algae culture to support seed
production (covered by Washington Department of Ecology contract)

\$ 6,630.00	Contractor Match: \$6,630 Baywater (Joth Davis, 102 hours @ \$65 for seed production oversight				
\$11,786.49	Supplies/Seed (covered by WA Department of Natural Resources):\$9,203.84Ryan Crim: 292 hours @ \$31.52 producing seed in 2013\$2,582.65Alex Karpoff: 143.8 hours @ \$17.96 culturing algae for seed				
\$32,992.00	Other Match \$15,686 \$6,384 \$6,090 \$4,332 \$500	: University of Washington (Jennifer Ruesink) Volunteers and Interns (399 hours @ \$16/hour) WDFW (150 hours @ \$40.60/hour) Skokomish Tribe (100 hours @ \$43.32/hour) Tideflat access to store seed at Baywater (\$250/year x 2 years)			
<u>\$ 4,026.65</u>	Indirect (18% of \$22,370.27 for personnel, supplies, and travel)				

\$74,668.92 Total Match Funds Expended

The undersigned verifies that the descriptions of activities and expenditures in this progress report are accurate to the best of my knowledge; and that the activities were conducted in agreement with the grant contract. Matching fund levels established in the grant contract were met.

Bleabody

Grantee Signature:

Grantee Name: <u>Betsy Peabody</u>

References:

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