

SWINOMISH OLYMPIA OYSTER MONITORING PLAN

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Useful definitions:

Oyster reef or bed = physical structures with a layer of living oysters on top of the non-living shell deposits of prior oyster generations

Seed = very young oysters with small shell length

Cultch = the substrate to which the oysters attach, typically other oyster shell

Seeded cultch = very young oysters attached to oyster shell

Unseeded cultch = bare, UV-sterilized shell with no attached young oysters

Acronyms:

BACR = Before After Control Restoration

PSRF = Puget Sound Restoration Fund

SITC = Swinomish Indian Tribal Community

SOOMP = Swinomish Olympia Oyster Monitoring Plan

SOORP = Swinomish Olympia Oyster Restoration Plan

SRSC = Skagit River System Cooperative

WDFW = Washington Department of Fish and Wildlife

1.0 Purpose of the SOOMP

The Swinomish Olympia Oyster Monitoring Plan (SOOMP) is intended to serve as a guide to assess the long-term effectiveness of Olympia oyster restoration efforts on the Swinomish Indian Tribal Community's (SITC) tidelands. Components of the SOOMP will include our project duration, monitoring methodology, sampling timeline, and reporting protocol. Methods and rationale of the SOOMP apply to a 10-year period beginning one year prior to the addition of Olympia oysters (*Ostrea lurida*) to restoration sites on the Reservation. The overarching goal of the SOOMP is to guide current and future SITC staff on steps to quantify ecological parameters, evaluate restoration success, and facilitate adaptive management practices. Results from this plan will advise the planning, research, development, and implementation of future projects identified by the Swinomish Olympia Oyster Restoration Plan (SOORP). The 50-year SOORP will address restoration needs and recommend appropriate actions to effectively achieve the Tribe's restoration goals for *O. lurida*.

2.0 Swinomish Olympia Oyster Restoration Project Partners

The Swinomish Olympia oyster restoration project was initiated as a feasibility study by Swinomish Fisheries Department (Fisheries), Puget Sound Restoration Fund (PSRF) and Dr. Paul Dinnel in 2012 (Figure 1). Since that time, Swinomish has collaborated with numerous partners to employ a science-based approach to restore Olympia oysters on Swinomish Reservation tidelands including the Department of Environmental Protection at SITC as well as the Skagit River System Cooperative (SRSC), Puget Sound Restoration Fund (PSRF), Skagit Marine Resources Committee, Washington Department of Fish & Wildlife (WDFW), Conservation, Research and Education Opportunities International (CREOi), and the Kukutali Preserve Management Board.



Figure 1. Swinomish Fisheries and Puget Sound Restoration Fund staff deploying *Ostrea lurida* seeded cultch for 2012 pilot study in Lone Tree Lagoon.

3.0 Introduction

3.1 Restoration Project Background

Historically, Olympia oysters played an important economic, ecological, and cultural role as Washington's only native oyster. Yet, due to overexploitation, loss of habitat, pulp mill pollution, and other human-related factors, only ~5 % of the once-known beds (circa 1850) remain in Puget Sound. While individuals and small assemblages can be found scattered within its historic range, *O. lurida* habitat in many areas along the west coast is considered functionally extinct (Blake & Bradbury 2012, zu Ermgassen et al. 2012).

In response to a growing concern over native oyster populations, WDFW listed *O. lurida* as a State Candidate species and placed it on the Priority Habitats and Species List establishing it as a priority for conservation and management (Blake & Bradbury 2012). The restoration of native oyster beds was also listed as a primary goal for the National Shellfish Initiative (NOAA 2011) and the Washington State Shellfish Initiative (Washington State 2011). To guide efforts in rebuilding Olympia oyster reefs, WDFW developed the Olympia Oyster Stock Rebuilding Plan in 1998; in 2012 the plan was updated to include information gleaned from recent restoration efforts (Cook et al. 1998, Blake & Bradbury 2012). Within this plan, WDFW identified 19 target restoration sites throughout Puget Sound with the ultimate goal of rebuilding Olympia oyster reefs to create large, self-sustaining source populations (Blake & Bradbury 2012). Most restoration efforts to date have taken place in south and central Puget Sound; until 2012 northern Puget Sound only had one active restoration site in Fidalgo Bay. The success of the Fidalgo Bay efforts (e.g. Dinnel et al. 2009, Allen et al. 2015) encouraged agencies to expand work into other target northern restoration sites, including the SITC tidelands that extend into Similk Bay.

In addition to recognizing the importance of site selection, projects in Washington and California have promoted effective restoration strategies by publishing information on enhancement techniques and monitoring metrics (Peter-Contesse & Peabody 2005, Grosholz et al. 2008, Brumbaugh et al. 2006, Brumbaugh & Coen 2009, Dinnel et al. 2009, White et al. 2009, Blake & Bradbury 2012, Wasson 2010, Wasson et al. 2014, Allen et al. 2015, Pritchard et al. 2015). While these publications are extremely helpful toward restoration efforts, a literature gap still remains in our scientific understanding of the benefits and services that restored *O. lurida* beds provide to the ecosystem.

Most of our current knowledge of oyster ecosystem services is derived from scientific studies conducted on eastern oysters (*Crassostrea virginica*). This large oyster species is known to enhance denitrification rates, increase fish and invertebrate abundance, filter the water column, provide food, and protect coastlines (Brumbaugh et al. 2006, Grabowski & Peterson 2007, Beck et al. 2011). While biological and structural differences between the two species may result in varying degrees of ecosystem services provided (e.g. Ramsay 2012, zu Ermgassen et al. 2013), it is assumed that many of these same ecosystem services will likely return to restored Olympia oyster beds (Blake & Bradbury 2012). For example, an unpublished report based on data from an Olympia oyster restoration site in southern Puget Sound quantified an increase in the diversity of epibenthic species (including juvenile salmonid prey) on *O. lurida* beds compared to sandy substrate without emergent habitat (PSRF 2009). Our restoration project will strengthen this understanding by implementing a

long-term monitoring study to quantify abiotic and biotic changes that may occur due to the presence of native oyster beds.

To assess the viability of Olympia oyster restoration on the Swinomish Reservation, a pilot study was conducted in 2012 by Swinomish Fisheries, PSRF, and Dr. Paul Dinnel. PSRF provided 21.5 bags of Olympia oyster seeded cultch that were outplanted in the Lone Tree and Kiket lagoons. In 2013, an additional 24 and 26 bags were placed in the lagoons, respectively. The 2012 and 2013 cultch were each placed into 2x2 m plots to monitor survival and growth from August 2012 to May 2013. The 2012 and 2013 *O. lurida* from both lagoons showed significant growth and qualitatively exhibited high survival (Barber et al. 2015). Results from this pilot study encouraged further development of *O. lurida* restoration efforts on Reservation tidelands and led to the creation of this SOOMP. To ensure the cultch from the pilot study would not influence subsequent long-term studies in the two lagoons, all cultch was removed from the delineated study plots and relocated in October 2014, returning the original plots to bare substrate.

3.2 Restoration Project Goals

The ultimate goal of SITC's Olympia oyster restoration effort is to enhance the population of a historically-important native species as well as the assumed services the species provides to the ecosystem. Specifically, we plan on establishing new Olympia oyster populations on the Reservation and quantifying biological and physical parameters that could demonstrate the ecologically-important role these oysters presumably play in the environment. This goal is in accordance with SITC's Natural Resources Code, STC Title 18, Chapter 1 which dictates at 18-01.010 the necessity to (1) "preserve, protect, and enhance the fishing, hunting, and gathering resources and traditions of the Swinomish Indian Tribal Community for current and future generations" and (2) promote resource management "in accordance with cultural tradition and the best available scientific information" (SITC 2006). Furthermore, the Swinomish Climate Change Initiative encourages the support of projects that have potential to address specific adaptation options recommended in the Impact Assessment Technical Report (SITC 2009). Maintaining healthy shorelines and shellfish beds is a high priority for the Tribe. Olympia oyster restoration projects will assist in SITC's response to the impending impacts of climate change on shellfish and their associated habitats.

3.3 Restoration Project Description

Pocket estuaries were selected as our initial target restoration sites in Skagit and Similk Bay because Olympia oysters are sensitive to freezing temperatures and siltation; these pocket estuaries provide ideal native oyster habitat since the restored beds will be located in flowing channels that remain inundated throughout all tidal cycles. Furthermore, this unique shoreform is known to provide important habitat for out-migrating juvenile salmonids (including ESA-listed Chinook salmon), forage fish species (Simenstad & Fresh 1995, SRSC & WDFW 2005, Beamer et al. 2006), and other economically and culturally-important species such as Dungeness crab (Ramsay 2012). Within the selected restoration sites, *O. lurida* habitat will be enhanced by adding 9.2 m² of seeded and unseeded cultch in 2015. Following the installation of the cultch, we will measure oyster survival and growth in addition to investigating the new reefs for signs of reproduction or recruitment.

Additionally, abiotic parameters will be routinely monitored to help provide context to observed biotic changes within and between the restoration areas. For example, it is well understood that synergistic effects of high water temperatures and low salinities are significant stressors for Olympia oyster growth and survival (Wasson et al. 2014). The Swinomish pocket estuary sites are routinely exposed to higher water temperatures than surrounding ambient waters. This, coupled with the close proximity of the sites to the Skagit River (the largest freshwater stream in Puget Sound), ephemeral streams, and ground-water inputs depressing salinity highlight the need to monitor physical habitat characteristics. Understanding the variability around these metrics will also help to make determinations about how climate change could alter the physical habitat in the future. Thus, over a 10-year period, environmental stressors and factors known to support Olympia oyster communities will be assessed by monitoring the following parameters at each restoration: water properties, sediment characteristics, and physical setting.

SITC and our collaborators will also quantify biological parameters before and after enhancement. We hypothesize that our restoration effort will increase habitat complexity and subsequently change species diversity in the pocket estuaries on the Reservation. In addition to enhancing native oyster populations, we are particularly interested in quantifying the potential associated change in salmonid prey abundance within these pocket estuaries. To investigate this hypothesis and increase our understanding of the ecological role of native oyster reefs, we will collect epifaunal species samples while SRSC concurrently samples juvenile salmonid abundance and diet data.

To address all of these goals, we will specifically ask the following questions with this monitoring plan:

1. Does Olympia oyster population enhancement result in, or have the potential to result in, a self-sustaining population within the restoration site?
2. Does Olympia oyster population enhancement result in, or have the potential to result in, exportation of larvae from the seeded lagoons and post-larval recruitment outside of the restoration site?
3. Does monitoring abiotic parameters (i.e., water properties, substrate, and beach morphology) provide data needed to inform future restoration activities? Does adding cultch to lagoon channels create a localized sediment trap, erosion over time, or no change?
4. Does the restoration effort change epifaunal species diversity on and off oyster reefs?
5. Is there evidence that known juvenile salmonid prey utilize Olympia oyster reefs when juvenile salmonids are present in pocket estuaries?
6. Is there evidence that species found in juvenile salmonid gut samples are found living on the Olympia oyster reefs?

3.4 Restoration Project Objectives

The restoration project consists of three objectives within which all six questions are addressed:

Objective 1: Assess Olympia oyster population enhancement success

This objective will determine the success of our restoration efforts toward creating a self-sustaining Olympia oyster population by collecting data on the following criteria identified by WDFW (Blake & Bradbury 2012): survival, reproduction, recruitment, and expansion.

Objective 2: Measure abiotic habitat parameters

Habitat parameters will be monitored at all study sites to assess environmental stressors and known factors that support Olympia oyster communities (e.g., suitable substrate, water temperature, etc.) that may be influencing oyster growth and abundance. These measurements are also likely to aid in explaining biotic variability within and between the lagoons. Water-property parameters (i.e., temperature, salinity, and relative light intensity) will be measured *in situ* and at moored stations throughout the study area. Since the availability and ability of a site to sustain suitable substrate is an important factor that determines successful Olympia oyster restoration (Wasson et al. 2014), data on substrate composition will also be collected. We will also monitor the geomorphic profile in the treatment areas in order to assess if the addition of cultch (or no cultch additions as in our control site) has the potential to change elevation over time.

Objective 3: Quantify ecological change in three pocket estuaries that may be due to the presence of an Olympia oyster reef

In order to assess potential changes in invertebrate communities within the pocket estuaries, we will measure sessile, mobile, and epibenthic invertebrate abundance

throughout the 10-year monitoring plan. We will also be collaborating with SRSC to assess juvenile salmonid use of the pocket estuaries and prey diet. In conjunction with the epibenthic data, the salmonid data will provide valuable information on how Olympia oyster reefs may benefit salmon restoration.

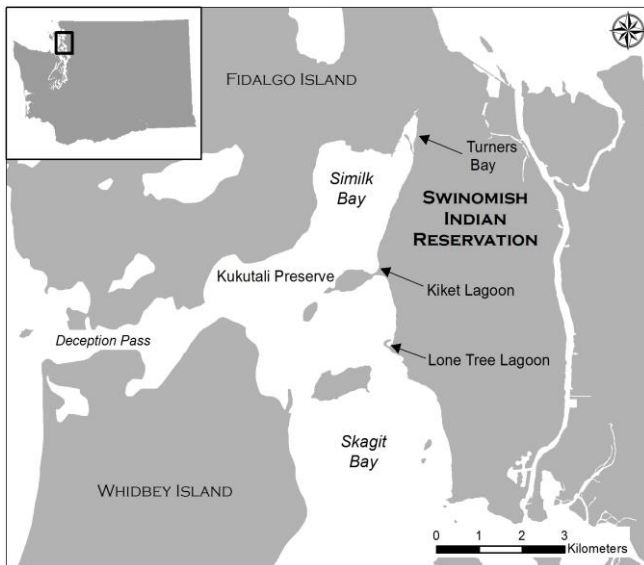


Figure 2. Location of the Swinomish Indian Reservation and the three pocket estuaries (lagoons) involved in the *Ostrea lurida* restoration project.

4.0 Study Area

The study sites for this restoration project are located in three pocket estuaries (Kiket Lagoon, Lone Tree Lagoon, and Turners Bay) on the west side of the Swinomish Reservation (Figure 2). The two treatment sites, Kiket and Lone Tree, will each receive 4.6 m² of




☆ Location of restoration sites 

Figure 3. Locations of *Ostrea lurida* restoration project treatment sites at Kiket and Lone Tree Lagoons.

seeded cultch and 4.6 m² of unseeded cultch in the summer of 2015. Turners Bay will function as the control site for the abiotic parameters and salmonid data since no cultch will be added. If deemed appropriate, Turners Bay will also serve as a control for the invertebrate sampling. Kiket and Lone Tree lagoons were selected based on their identification as priority restoration sites in the Skagit Chinook Recovery Plan, which aims to improve the quality and capacity of these pocket estuaries and Skagit Bay at large to support all six wild stocks of Skagit Chinook salmon (SRSC & WDFW 2005). Because native oyster beds have the potential to increase the abundance of juvenile salmon prey (PSRF 2009), our efforts are likely to compliment salmonid restoration efforts in these ecologically-important pocket estuaries.

4.1 Site Descriptions

All three initial study sites are geomorphically considered pocket estuaries or tidal channel lagoons; however, each lagoon has unique physical characteristics including substrate composition and freshwater input.

Kiket Lagoon, a tidally-influenced lagoon, is located just north of the tombolo connecting the mainland to Kiket Island and is within the boundaries of Kukutali Preserve (Figure 3). Unlike Lone Tree Lagoon and Turners Bay, Kiket Lagoon does not have a direct source of freshwater input other than groundwater and occasional stormwater

runoff. Kiket Lagoon is also the smallest of the three pocket estuary environments but it is potentially the least impacted by anthropogenic disturbances. The lagoon itself is roughly 0.88 hectares and surrounded by marsh and riparian habitats.

Lone Tree Lagoon is a tidally-influenced lagoon that is approximately 1.6 hectares with a barrier beach along the western edge (Beamer et al. 2006, Figure 3). Marine waters from Skagit Bay primarily enter the lagoon through the outlet located at the northern portion of the lagoon but the barrier beach also overwashes from the south at the highest tides. Lone Tree Creek, an ephemeral stream, flows into the lagoon from the east. In addition to the ephemeral stream, Lone Tree Lagoon is likely influenced by freshwater from the Skagit River. The inner lagoon floor is mainly composed of soft sediments, transitioning into mixed gravel and shell at the start of the tidal channel. There is minimal marsh habitat at this location and a high instance of human use (primarily during summer months).

Turners Bay is a tidal channel lagoon (Beamer et al. 2006) and the largest such lagoon on the western shore of the Swinomish Reservation at roughly 14 hectares (Figure 4). Freshwater inputs include seeps from surrounding bluffs, wetlands, and stormwater drainage. Historically, this site has experienced degraded water quality due to failing septic systems in the northern portions of the lagoon and from stormwater inputs which drain from Highway 20 and Reservation Road.



☆ Control site



Figure 4. Location for *Ostrea lurida* restoration project control site in Turners Bay.

5.0 Methodology

5.1 BACR Design

Our long-term monitoring survey will follow the established principles of a BACI design (Before-After-Control-Impact) although we will use the acronym “BACR” to refer to “restoration” efforts rather than an impact (Schroeter et al. 1993, Brumbaugh et al. 2006). In this case, data will be collected in 2015 *before* enhancing the Olympia oyster population and habitat in the lagoons. All subsequent sampling will occur *after* the addition of Olympia oyster seed and habitat into the two treatment lagoons. The *restoration* areas will be the sections in the lagoons where we spread our seeded and unseeded cultch. The unseeded cultch will provide settlement habitat for future Olympia oyster natural recruitment and will be assessed for spatfall. The same monitoring data, excluding the *O. lurida* benchmark metrics, will be collected at our control site, Turners Bay where no cultch will be added. Importantly, the BACR design will allow for our analysis to take natural variation within and between the lagoons into account. While it is ideal to use three survey sites with identical physical and biological properties, it is unrealistic to find such conditions in the field. By incorporating Turners Bay as a control site, our long-term monitoring will account for changes that occur on a larger scale throughout Skagit Bay and help identify changes that may have occurred directly as a result of restoration.

5.2 Sampling Methods

In the two treatment lagoons, a 13.9 m² study area comprised of three 4.6 m² plots with seeded cultch, unseeded cultch, and no cultch will be flagged and delineated with a hand-held GPS unit and measuring tape. At the control site, only two 4.6 m² plots will be flagged and delineated, neither plot will have cultch added because this is the control lagoon although for record keeping purposes one plot will be referred to as “bare” and the other as “seeded”. For sessile, mobile, and invertebrate sampling we will use ArcGIS to randomly select sample sites within the seeded cultch and bare plots. The juvenile salmonid sampling and abiotic monitoring will occur in all three study areas. All equipment will be calibrated, maintained, and operated according to the manufacturer’s specifications prior to deployment.

5.21 Objective 1

Olympia oyster survival, growth, recruitment, reproductive activity, and possible expansion will be quantified annually at the restoration lagoons during low tide events (Table 1).

I. Benchmark metrics

- a. Survival and growth will be surveyed in late March or early April in seeded cultch areas using a 1/16 m² quadrat. Five randomly selected quadrats will be sampled at each treatment lagoon. Within each quadrat, all the shell will be removed and placed into a bucket filled with seawater to measure shell volume. The number of live and dead (gaping) Olympia oysters will be counted. All live oysters will be measured to the nearest millimeter. Data will be analyzed to determine density, survival, and growth.
- b. Concurrent with the survival and growth survey, recruitment data will be collected in seeded and unseeded cultch areas using a 1/16 m² quadrat. At 10 randomly selected quadrats (five from seeded and five from unseeded), all shell will be assessed for signs of postlarval recruitment. Olympia oyster spat will be counted and measured to the nearest millimeter. This sampling will occur in the spring under the assumption that we will be looking for spatfall from the previous year.
- c. Reproductive activity will be assessed in 2015 by monitoring brooding activity once a week from the beginning of April to the end of August. Three to four 1/16 m² quadrats will be randomly placed in the seeded cultch areas. All shell within the quadrat will be collected and brought back to the lab for processing. Data will be collected by individual quadrat providing a measurement of the number of individuals brooding/m². Individuals will be removed from the seawater for 45 minutes then immersed in a 75g/l solution of heptahydrate sulfate mineral epsomite (Epsom salt) and a 50/50 mix of freshwater/seawater for 45 minutes. Each sample will be evaluated for brooding by visually inspecting open individuals for the presence of larvae, also referred to as “sic” (Heare et al. in review). Reproductive condition, sic stage (white, grey, or black), and shell length will be recorded. Post-sampling, oysters will soak in seawater for 45 minutes so individual oysters can recover before being returned to the area they were collected from.
- d. Expansion potential will be monitored by deploying shellstring spat collectors near the study areas in the treatment lagoons. Following methods by White et al. (2009), replicate shellstrings will be deployed in early May near the treatment areas just above the substrate at -0.3 m relative to mean lower low water (MLLW). Locations will be selected based on the feasibility of oyster larval transport and deployed in

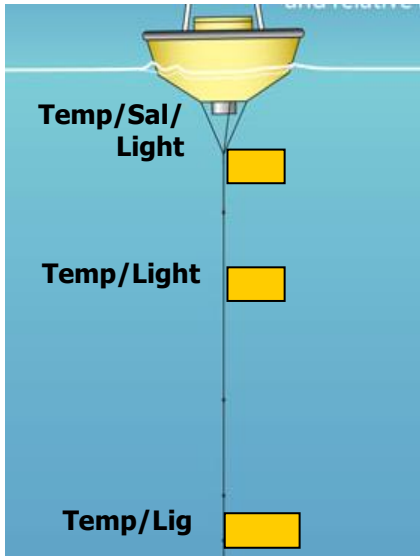


Figure 5. Moored instrument suite diagram.

sets of three. One set will be placed within each treatment lagoon, another set will be put outside of the lagoon near its outflow and the final set should be deployed a reasonable distance away from the lagoon, such as the south side of Kukutali for Lone Tree. A shellstring consists of 11 UV-treated Pacific oyster, *Crassostrea gigas*, valves drilled through the center and stacked cup-side down on a wooden dowel. Shellstrings will be recovered in late August and replaced with new shellstrings the following May. After the shells have been collected, the top ten shells will be examined under a dissecting microscope for newly recruited Olympia oysters (White et al. 2009). New recruits will be identified by size and symmetrical shape from other bivalves (Loosanoff et al. 1966, Trimble et al. 2009). Each identified recruit will be counted and measured.

5.22 Objective 2

Habitat parameters will be monitored year-round in and outside of Lone Tree and Kiket lagoons.

I. Moored suite monitoring

Relative differences in water properties will be examined among sites through the deployment of moored water property instruments. Water property measurements for temperature [degrees Celsius (°C)], salinity [practical salinity units (psu)], and relative light intensity (a proxy for turbidity) will be collected using Onset HOB0 Pendant Temperature/Light Data Loggers and HOB0 Conductivity Data Loggers. Figure 5 shows the general set-up of the moored instrument suite along with what parameters will be monitored by depth. Instruments will be deployed in fixed locations secured by weights and a buoy. Each suite will have a temperature/salinity sensor at roughly 0.5 meters below the water surface (BS), a temperature sensor at 5 m BS (or other appropriate depth adjusted height), and a temperature/light sensor fixed at the bottom. The sampling interval on all loggers should be set at 15 minutes. Scheduled maintenance and downloading of data from the instruments will occur once per month.

II. Sediment/substrate characterization

We will examine the surface and subsurface sediments/substrate at each restoration plot using a 1/16 m² quadrat (n = 5) to estimate percent cover of each substrate type including: shell (type), cobble (> 6 cm), pebble (4 mm to 6 cm), granule (2 to 4 mm), sand (“gritty” up to 2 mm), and silt/clay (smooth between your fingers).

III. Channel beach morphology

At each of the restoration plots and control site a cross-section profile of elevation will be surveyed using a Real Time Kinematic – Global Positioning System (RTK-GPS) to determine if, over time, there is any sediment scour or deposition at the study sites. These elevation profiles will be collected once per year.

- IV. When feasible, these data will also be collected at Turners Bay. However, due to the nature of the tidal exchanges at Turners Bay, the moored instrument suite cannot be deployed in this location.

5.23 Objective 3

Epifaunal, sessile, and mobile invertebrate surveys will occur at all three study lagoons. Data collected in 2015 will provide a baseline for characterizing the ecological communities prior to the introduction of *Olympia* oysters. The first “after” survey will occur in 2016 and then every four years to monitor any changes post-enhancement.

I. Sessile and mobile invertebrates

Using stratified random sampling, 10 1/16 m² quadrats will be placed on the substrate (Table 1). The sides of the quadrat will have vertical walls to ensure mobile invertebrates are retained for sampling. The first team collecting data will focus their effort on data collection while the quadrat is in the water. During this time, five out of the ten quadrats will be used for substrate data collection (see 5.22 II) and percent cover estimates of barnacles, mussels, and sponges. *Olympia* oysters and diatom film will be recorded as present or absent water (Appendix A). For all ten quadrats, the water-based team will then remove all mobile and sessile invertebrates and shell from the quadrat and place them into a container. Another team on the shore will identify invertebrates to the lowest feasible taxonomical unit and count or record presence/absence (Appendix B). Datasheets and the database note what species are counted versus recorded as present/absent (Appendices A & B). Samplers should review datasheets from the previous year of sampling to familiarize themselves with the data collection and expected species. Species not listed on the datasheets are still recorded as a count or as present/absent. The shore team only records algal species as present/absent.



Figure 6.
Epibenthic
pump

II. Epibenthic invertebrates

Epibenthic invertebrate sampling will occur biannually in late February or early March and late April or early May to correspond with the presence of juvenile salmonids in the pocket estuaries (Beamer et al. 2006). Organisms will be sampled with a battery-powered device modified to suction epifauna off of bottom substrate following suction-sampling methods used by PSRF (2009) and Toft et al. (2013) (Figure 6). Using random sampling, 24 sample sites will be selected prior to the survey using ArcGIS and uploaded into a handheld GPS unit (12 on oysters once they are present, 12 bare substrate). For each sample, the battery-operated pump will run for 10 seconds in approximately 0.6 m (depth) of water. The outflow of the pump will pass through a 106 micrometer mesh sieve and the retained material will be rinsed into a jar with filtered water from the sample site. All retained materials will be fixed in 10% buffered neutral formalin solution then switched to 70% isopropyl alcohol in a jar labeled with date, time, site name and sample number. The samples will then be sent to an invertebrate ID contractor for identification and quantification to the lowest feasible taxonomical unit.

III. Juvenile salmonid surveys

The Skagit River System Cooperative (SRSC) will conduct small net (24.4-meter length) beach seine surveys to quantify juvenile salmonid use of the three lagoons as well as juvenile salmonid diet surveys to determine what species juveniles are feeding on. Sampling dates will occur as close to the epibenthic invertebrate sampling dates as possible. Methods for both surveys follow the protocol outlined in Beamer et al. (2005).

6.0 Documentation

At each field sampling event the date, time, sample site, and sampler name will be recorded in a field notebook. Any problems or abnormalities with sampling procedures, instruments or site conditions will be entered in the notebook. Information on data gathered in the field will be recorded on field forms and stored in digital formats. Field forms will be checked in the field for missing information and prior to entry into the appropriate database. All data generated through laboratory analysis will be recorded on lab sheets and approved by the applicable project lead before entry into the database.

All field sheet information will be entered into an Access database for storage and analysis. All digital records will be kept on the SRSC internal server which is backed up weekly. The original and digital copy of the field forms will be kept indefinitely by the Swinomish Fisheries Department. Raw data files will be downloaded from instruments and kept in digital form at SITC and with the appropriate project representative. All results will be shared after QA/QC tasks have been performed by their respective organization.

7.0 Analysis

Throughout the long-term study, statistical and qualitative analyses will be conducted to assess the effectiveness of the restoration efforts and changes in ecological communities within and between lagoons before and after the introduction of Olympia oysters. For all parametric statistics, if the assumptions of homogeneity of variance and normality cannot be met, we will lower our alpha value from 0.05 to 0.01 (Keppel & Wickens 2004) or use non-parametric statistics when appropriate.

7.1 Objective 1

A two-factor ANOVA will be used to determine if site and time have any effects on mean oyster density, survival, recruitment, reproductive activity, or length. If we want to examine differences in the length frequencies of oysters by site at a particular time, we will conduct pairwise comparisons (Kolmogorov-Smirnov tests) on the cumulative percent frequency of Olympia oyster length by site. We will use a one-factor (location) ANOVA to look for differences in mean oyster recruitment (mean abundance) on the shellstrings.

7.2 Objective 2

Habitat metrics will be quantified at each study area and compared between sites and years. The moored instrument water property dataset will be analyzed concurrently with National Oceanic and Atmospheric Administration tide, current, and meteorological data and U.S. Geological Survey river discharge data from the Skagit River for additional regional context. Simple summary statistics will be used to examine substrate and channel morphology over

time. Water properties will be examined to determine timing and duration of events such as periods of low salinity (stressor) or water temperatures above 12°C (supportive factor). One-factor ANOVAs will be used to determine if metrics differ by lagoon at a particular time. When appropriate, multi-factor ANOVAs may be used for analyses.

7.3 Objective 3

The established principles of a BACR design allow for the use of a nested three-factor ANOVA (nesting factor = lagoon, factor 1 = before or after restoration, factor 2 = time, factor 3 = oyster reef, no oyster reef, or Turners Bay control). More advanced statistics may be utilized in addition to the nested ANOVA to develop a more accurate understanding of the data. Depending on the data being examined, we will use mean species densities and/or species richness or diversity index values for the analyses. SRSC will provide either their own analysis of the juvenile salmonid abundance and gut content data or they will recommend the best methods for analysis of their data to Fisheries staff.

8.0 Reporting

Results and recommended amendments to the SOOMP will be compiled into biennial technical reports that will inform the five year review of the SOORP and support adaptive management in the Tribe's restoration efforts. The reports will include essential information to areas currently lacking in scientific literature, especially in regard to the potential ecosystem services provided by native oysters and how those services might influence other species and ecosystems at larger scales. The biennial reports will also be disseminated through the Tribe's publication website (<http://www.swinomish.org/resources/publications.aspx>) to other departments within the Tribe, project partners and funders, and other interested parties to promote the implementation of the best available science in future restoration efforts throughout the Salish Sea.

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Table 1: Olympia oyster restoration project sampling methods (m = meters, mm = millimeters, μm = micrometers).

Parameter	Frequency/Timing	Sampling method	Proposed sample size	Equipment
Survival benchmark*	Once a year; Late April-early May	Collect counts of living Olympia oysters in quadrats randomly placed on seeded cultch	5 – 1/16 m ² quadrats per treatment lagoon	<ul style="list-style-type: none"> • 1/16 m² quadrat
Growth benchmark*	Once a year; Late April-early May	Measure live Olympia oysters to the nearest mm in quadrats randomly placed on seeded cultch and volume	5 – 1/16 m ² quadrats per treatment lagoon	<ul style="list-style-type: none"> • 1/16 m² quadrat • calipers • clear bucket with volume units
Recruitment benchmark*	Once a year; Late April-early May	Count and measure spat to the nearest mm in quadrats randomly placed on seeded and unseeded cultch	10 – 1/16 m ² quadrats per treatment lagoon (5 in seeded plot, 5 in unseeded cultch plot)	<ul style="list-style-type: none"> • 1/16 m² quadrat • calipers
Reproductive activity benchmark (i.e. brooding)	Every week; April through August for one or two years	Collect shell in quadrats randomly placed in seeded cultch, subject samples to anesthesia, visually inspect individuals for brooding, measure brooding individuals to the nearest mm, and return shell to site	3 to 4 – 1/16 m ² quadrats per treatment lagoon	<ul style="list-style-type: none"> • 1/16 m² quadrat • Epsom salt • calipers • buckets • totes
Expansion benchmark	Once a year; Deploy in May, collect in late August or early September.	Place 3 shellstrings at -0.3 m relative to MLLW. Examine shell under dissecting microscope, identify Olympia oyster recruits, count and measure recruits to the nearest mm. When possible, place shellstrings at additional locations.	3 – shellstrings with 11 <i>C. gigas</i> shells	<ul style="list-style-type: none"> • sterile <i>C. gigas</i> shell • wood dowels (.95cm) • drill • duct tape • dissecting microscope • calipers
In situ water-property parameters *	Every two weeks; May through August	Collect spot measurements at locations within the lagoon, at the study plots, and in adjacent bay	2 replicate samples per sampling location	<ul style="list-style-type: none"> • YSI 6920 or equivalent multi-parameter sonde • calibration solutions
Moored suite water-property parameters*	Year-long deployments March-February; 2015-17, 2020-21, and 2024-25.	Mooring suites monitor water properties at near-surface (~0.5 m), mid-water column (~2 to 5 m), and bottom locations within the water column (Figure 5). These suites will be deployed within the restoration sites and in the adjacent bays	Yearly deployment with loggers measuring water-properties at 15-minute intervals.	<ul style="list-style-type: none"> • Onset HOBO temperature-conductivity logger • Onset HOBO temperature-light pendant • buoy & chain • weights (e.g. brick)

Parameter	Frequency/Timing	Sampling method	Proposed sample size	Equipment
Channel/beach morphology*	Once in 2015, 2016, 2020 & 2024 Summer	Elevation profile transects will be measured at each of the restoration sites and the control	Minimum of 3 transects per restoration site/control.	<ul style="list-style-type: none"> • RTK-GPS
Substrate composition*	Once in 2015-2017, 2020 & 2024 Late April-early May	Collect substrate percent cover data	10 – 1/16 m ² quadrats per treatment lagoon (5 in seeded plot, 5 in bare plot)	<ul style="list-style-type: none"> • 1/16 m² quadrat
Sessile and mobile invertebrates*	Once in 2015-2017, 2020, & 2024 Late April-early May	Collect sessile and mobile invertebrates and shell in quadrats randomly placed in stratified random sites, identify invertebrates to lowest feasible taxa, return shell to site	20 – 1/16 m ² quadrats per lagoon (10 in seeded plot, 10 in bare plot)	<ul style="list-style-type: none"> • 1/16 m² quadrat
Epibenthic invertebrates	Twice in 2015, 2016, 2017, 2020, & 2024 Late February-early March and late April-early May	Collect epibenthic samples in stratified random sites, sieve samples to 106 µm and preserve first in 10% buffered formalin then transfer to 70 % isopropyl, send to contractor to identify organisms to lowest taxa	24 samples per lagoon (12 in seeded plot, 12 in bare plot)	<ul style="list-style-type: none"> • epibenthic suction pump • 106 µm sieve • 10% buffered neutral formalin solution • monosodium phosphate • sodium phosphate • isopropyl alcohol • 250 ml specimen containers • scintillation vials
Juvenile salmonid surveys	Once in 2015, 2016, 2017, 2020 & 2024 Late April-early May	Collaborators (SRSC) and/or contractors will determine methods	To be determined	<ul style="list-style-type: none"> • Collaborators will provide

* indicates sampling that can occur simultaneously

Table 2. Data quality objectives for water property measurements using a YSI 6920 V2 sonde, Onset TempV4, and HOBO Water Level Logger U20-001-02. Units include milligrams per liter (mg/L), percent (%), millisemens per centimeter (mS/cm), practical salinity units (psu), degrees Celsius (°C), pH standard units, millivolts (mV), and meters (m).

Sensor	Units	Range	Resolution	Method Accuracy	Audit Precision Limit
YSI ROX Optical Dissolved Oxygen	mg/L	0 to 50 mg/L	0.01 mg/L	0 to 20 mg/L: ± 0.1 mg/L or 1% of reading	± 0.5 mg/L
	% Saturation	0 to 500%	0.10%	0 to 200 %: ± 1 % or 1% of air saturation	N/A
YSI Conductivity 6560	mS/cm	0 to 100 mS/cm	0.001 to 0.1 mS/cm	$\pm 0.5\%$ of reading + 0.001 mS/cm	± 0.3 mS/cm
YSI Salinity	psu	0 to 70 psu	0.01 psu	$\pm 1\%$ of reading or 0.1 psu, whichever is greater	N/A
YSI Temperature 6560	°C	-5 to +50°C	0.01°C	± 0.15 °C	N/A
YSI pH 6561	pH units	0 to 14 units	0.01 units	± 0.2 units	± 0.2 units
Depth - Shallow	m	0 to 9.1 m	0.001m	± 0.02 m	N/A
Onset HOBO Temp/Light Logger	°C	-20°C to 50°C 0 to 320,000 lux	0.14°C	± 0.53 °C	N/A
Onset HOBO Water Level Logger	°C	-20°C to 50°C	0.1°C	± 0.37 °C at 20°C	N/A
	m	0 to 30.6 m	0.41 cm	1.5 cm to 3 cm in water	N/A
Onset HOBO Conductivity Logger	°C uS/cm	5 to 35°C 5,000 to 55,000 uS/cm	.01°C 2 uS/cm	± 0.02 °C 5% of readings in waters within a range of $\pm 3,000$ uS/cm	N/A

Table 3. Swinomish Olympia oyster restoration project sample naming convention.

Data Type	Example	Description				
Quadrat	LT15QB107	Site LT = Lone Tree Lagoon KI = Kiket Lagoon TB = Turners Bay	Year	Sample type QB = Quadrat Bare Plot QS = Quadrat Seeded Plot QU = Quadrat Unseeded Plot*	Number of times sampled 1	Identification number 01 - 12
Epibenthic	KI15ES204	Site LT = Lone Tree Lagoon KI = Kiket Lagoon TB = Turners Bay	Year	Sample type EB = Epibenthic Bare Plot ES = Epibenthic Seeded Plot EU = Epibenthic Unseeded Plot*	Number of times sampled 1	Identification number 01 - 14
Pilot	LT15P0305	Site LT = Lone Tree Lagoon KI = Kiket Lagoon SM = Similk	Year	Sample type P = Pilot Study	Number of times sampled 01 - 05	Identification number 01 - 10 (but could go higher if needed)
Brooding	KI15B0204	Site LT = Lone Tree Lagoon KI = Kiket Lagoon	Year	Sample type B= Brooding	Week of brooding 01 - 15	Identification number 01 - 05
Shellstring	FS15M103	Site SM = Similk Bay TB = Turners Bay FS = Flagstaff Island KN = Kiket North KI = Kiket Lagoon KS = Kiket South LN = Lone Tree North LT = Lone Tree Lagoon LS = Lone Tree South	Year	Shellstring location L = Lagoon Proper C = Channel of Lagoon M = Mouth of Lagoon S = Shoreline	Month collected 1=Collected in August 2=Collected in May	Identification number 01 - 06

*These codes exist although we are not currently sampling the unseeded cultch plots for these particular types of data collection.

Appendix A: Substrate and percent cover biota datasheet

Date(yyyymmdd):		Site:	Plot:			
Surveyor:						
		QUADRAT (1/16 m2)				
<i>Naming convention ex: LT15QB102</i>		QuadID				
		Time				
PERCENT COVER		Photo ID				
Substrate	BE drock					
	BO ulder (>25cm) head size or greater					
	CO bble (6-25cm) billiard ball to head size					
	PB = (0.4cm-6cm) pea to billiard ball size					
	GR anule (0.2-0.4cm) bb size to pea size					
	CS = Coarse Sand (beebee size)					
	FS = Fine Sand (salt/sugar)					
	SI = Silt					
	CL = Clay					
	SD =Shell Debris (broken-up shell fragment)					
	SH = Shack (w hole or half clam shells)					
WO = w ood debris						
Biota (also % cover)	<i>barnacles</i>					
	<i>mussels</i>					
	<i>sponges</i>					
	<i>O. lurida</i>					
	<i>diatom film</i>					
Notes:						

Appendix B: Mobile and sessile invertebrate datasheet

Date(yyyymmdd):	Site:	Plot:		
Species Identifier:	QUADRAT ID (1/16 m ²)			
INVERTEBRATE COUNTS				
<i>Balanus glandula</i> (presence)				
<i>Balanus crenatus</i> (presence)				
<i>Chthamalus dalli</i> (presence)				
<i>Red filamentous algae</i> (presence)				
<i>Red algae blade</i> (presence)				
<i>Encrusting red algae</i> (presence)				
<i>Ulva lactuca</i> (presence)				
<i>Mastocarpus sp.</i> (presence)				
<i>Prionitis sp.</i> (presence)				
<i>Pagurus hirsutiusculus</i>				
<i>Mytilus trossulus</i>				
<i>Tectura persona</i>				
<i>Tectura scutum</i>				
<i>Nucella lamellosa</i>				
<i>Hemigrapsus oregonensis</i>				
Notes:				