Quality Assurance Project Plan
Olympia Oyster Restoration in Sequim Bay
2020

Prepared for:
The Northwest Straits Commission

Prepared by:
Helle Andersen
Clallam County Marine Resources Committee

CLALLAM COUNTY
MARINE RESOURCES COMMITTEE

Publication Information

This study has been funded in part by the United States Environmental Protection Agency's (EPA) National Estuary Program through the Puget Sound Partnership, via Interagency Agreement Number 2020-19 with the Department of Ecology/Northwest Straits Initiative, of which the Marine Resources Committees are a member. The Northwest Straits Commission is comprised of seven Marine Resources Committees in Northwest Washington. The Marine Resources Committees were established by county governments in 1999, as an advisory body to county government, and are part of the regional coordinating organization, the Northwest Straits Initiative.

Each study conducted for the EPA using National Estuary Program funds must have an approved Quality Assurance Project Plan (QAPP) describing the objectives of the study and the procedures to be followed to achieve those objectives. The plan and final reports for this project will be available on the Clallam MRC's website at http://www.clallamcountymrc.org/ and in the annual reports. Neither document necessarily reflects the views and policies of the EPA, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. This QAPP is valid through July 2025.

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http://www.clallamcountymrc.org/

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1.0 Title Page, Table of Contents, and Distribution List

Quality Assurance Project Plan
Olympia Oyster Restoration in Sequim Bay
July 2020

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2.0 Abstract

The Olympia oyster (*Ostrea lurida*), Washington's only native oyster, forms three-dimensional biogenic habitat extensively used by fish, invertebrates and other marine organisms.[1] However, extensive overharvesting led to large declines in Olympia oyster populations throughout Puget Sound. The Washington Department of Fish and Wildlife identified Sequim Bay as a potential Olympia oyster restoration site and in 2012 Clallam County Marine Resources Committee, in partnership with Jamestown S'Klallam Tribe and Puget Sound Restoration Fund, began an Olympia oyster restoration project. This QAPP will serve as a guide to collect a baseline of biological data and will put forward a set of guidelines for current and future MRC members or staff on steps to quantify ecological parameters, evaluate restoration success, and facilitate adaptive management practices. Components of the QAPP include pilot project duration, monitoring methodology, and monitoring frequency. Implementation of this plan will inform restoration potential in Sequim Bay, as well as planning, research, project development, and implementation of future Olympia Oyster Restoration projects.

3.0 Background

The Olympia oyster (*Ostrea lurida)* is the only native oyster of the North American Pacific Coast and once thrived in coves, inlets and other protected tidelands in Puget Sound. Olympia oysters have been listed as a State Candidate species since 1997[1]. State Candidate species are those that Washington Department of Fish and Wildlife (WDFW) may review for possible future listing as State Endangered, Threatened, or Sensitive species. The Olympia oyster is also included in WDFW's Priority Habitats and Species (PHS) List, a catalog of habitats and species considered a priority for conservation and management; all State Candidate, Endangered, Threatened and Sensitive species are automatically included in the PHS List. Although Olympia oysters occur throughout their historic range, their relative abundance has been drastically reduced to an estimated 4% of historic core populations due to a combination of over harvesting, pollution and non-native oyster cultivation[1].

In 2012, Jamestown S'Klallam Tribe (JST) partnered with Clallam Marine Resources Committee (Clallam MRC) and Puget Sound Restoration Fund (PSRF) to restore Olympia oysters on 1.5 acres of their tidelands in Sequim Bay. The restoration effort was successful which prompted Clallam MRC and their partners in 2016 to search for another potential restoration site in Sequim Bay and Dungeness Bay. Several potential sites in Sequim and Dungeness Bay were

[1] Blake, B., & Bradbury, A. Washington Department of Fish and Wildlife Plan for Rebuilding Olympia Oyster (*Ostrea lurida*) Populations in Puget Sound with a Historical and Contemporary Overview. Washington Department of Fish and Wildlife.

investigated but none of these sites was suitable for Olympia oyster restoration because of exposure to wave and wind action.

In May 2018, the JST proposed using a 0.3 acre parcel of tidelands that they lease from Washington Department of Natural Resources (DNR) at the head of Sequim Bay. The site is approximately 700 ft. east of the current restoration site. In July 2018, more than 100 bags of Olympia oyster seed were moved from the area they have overwintered to the new restoration site and in August 2018 a crew of volunteers and tribal biologists spread the shells with the oyster seed.

In September 2017, Clallam MRC submitted a Special Use Permit to the US Fish and Wildlife Service asking for permission to establish two test plots at their Dawley Road property in Sequim Bay. In September 2018, the permit was granted and the two test plots were established in early October 2018. In fall 2019 the two test plots were assessed for survival and growth and it was decided not to go forward with restoration at this location. No further exploration for additional restoration sites was conducted in 2019 or is planned for 2020.

This project is part of a larger effort underway to restore 100 acres of Olympia oyster habitat in the Puget Sound area by 2020.

3.0 Project Goals and Objectives

The overall goal is to expand Olympia oyster populations in habitats where Olympia oyster historically thrived in Clallam County. Thriving populations have primarily been identified in Sequim Bay and in 2012 Clallam MRC, JST and PSRF identified the goal of restoring 2 acres of Olympia oyster habitat in Sequim Bay as part of the overall 2020 goal of 100 acres for all of Puget Sound. To reach this goal the following objectives had to be achieved:

1. Identify suitable habitats within Sequim Bay for Olympia oyster restoration
2. Establish test plots within these areas
3. Quantify the survival and growth of the out-planted seed in the test plots to determine the feasibility of Olympia oyster restoration
4. Based on successful test plots establish restoration area(s)
5. Track the oyster population(s) in the restoration area(s) and ensure that sufficient settling substrate is available
6. In 2020 document if the goal of 2 acres of Olympia oyster habitat has been achieved
7. Continue the restoration effort in Sequim Bay in partnership with JST and PSRF

3.1 Old Restoration Site

Restoration at the old site commenced in 2012 and by summer 2018 the population covered an area of 1.5 acres. The annual population survey in 2018 found that a sizeable portion of the oyster bed (~0.5 acres) had expanded west of the restoration site boundary. The 2018 survey results indicated that the restoration efforts have, in general, been successful as Olympia oysters appear to be surviving, growing, reproducing and expanding their population area. However, the shellfish biologists determined that the population is limited by the lack of suitable substrate and in the winter of 2019 JST purchased additional clean shell substrate and spread it at the site in summer 2019.

The annual population survey will be conducted at the old restoration site at the end of the summer 2020 to estimate the abundance, delineate the population perimeter and identify the size range of the Olympia oysters. Future annual population surveys will continue to be conducted at the end of summer.

3.2 New Restoration Site

In May 2018, Clallam MRC and JST decided to use a parcel of tidelands that JST is leasing from DNR at the head of Sequim Bay. The parcel is approximately 0.3 acre and is located approximately 700 ft. east of the current restoration site (Figure 1), with similar habitat conditions. In July 2018 more than 100 bags of Olympia oyster seed were moved from the area they have overwintered to the new restoration site and in August a crew of volunteers and tribal biologists spread the shells with the oyster seed. Because the new site has limited suitable substrate for oyster larvae to settle on, the Clallam MRC and JST applied for funds to buy clean shells in the spring of 2019. A total of 850 bags of shells were purchased and spread at the site in summer of 2019.

The annual population survey will be conducted at the new restoration site at the end of the summer 2020 to estimate the abundance, delineate the population perimeter and identify the size range of the Olympia oysters.

Figure 1. Map of the two restoration sites at the head of Sequim Bay. The dotted red square in the green area identified as DNR tidelands outlines the new Olympia oyster restoration area. The old site is the yellow square.

4.0 Organization and Schedule

4.1 Project Team

Technical oversight and training will be provided by co-manager staff from JST and WDFW. The Clallam MRC coordinates with WDFW shellfish experts for technical advice, training for project volunteers when needed, and data review. The surveys and data management are led by shellfish biologist staff at JST. This system provides technical experts to be involved in all aspects of the project and to provide training and on site leadership for volunteers.

Clallam MRC Lead

Lyn Muench, Clallam MRC member, will be the Clallam MRC lead on the restoration project. Ms. Muench will also provide advice and guidance on the project based on more than 30 years' experience in shellfish culture. Ms. Muench will review all project deliverables submitted to Northwest Straits Commission (NWSC).

Project Manager

Helle Andersen, Clallam MRC Project Coordinator, will be responsible for project oversight and be responsible for keeping the official, approved QAPP updated and revised every year. Ms. Andersen will consult with, and receive guidance from Elizabeth Tobin and Chris Burns, both of JST, to ensure completion of project tasks, relative to the project timeframe and consistent with accomplishing project outcomes. Helle has a Masters in Marine Biology from Aarhus, Denmark.

Shellfish Biologist

Elizabeth Tobin, shellfish biologist with JST, will help execute specific tasks and make recommendations to the project manager. Elizabeth has a Ph.D. from School of Oceanography, University of Washington and has been working as a shellfish biologist since 2014.

Fisheries Technician

Chris Burns, fisheries technician with JST, will help execute specific tasks and make recommendations to the project manager. Chris has an AAS in Fisheries from Peninsula College and has worked in fisheries related work and aquaculture since 1990. In 2008, he started working full time as a Natural Resources Technician for JST.

Project Quality Assurance Officer

Arati Kaza, Department of Ecology, will review this QAPP and ensure that all proposed actions meet the quality standards appropriate to the goals and scope of the project.

GIS Specialist

TBD. Jamestown S'Klallam Tribe GIS Department. The specialist will assist the Shellfish Biologist with storage and analysis of spatial data collected during the project.

WDFW Shellfish Biologist

Brady Blake, WDFW Shellfish Biologist, will examine any species identification that comes into question while analyzing shell strings for Olympia oyster settlement. Brady has a BS from Western Washington University and has worked on shellfish enhancement, restoration and disease prevention for WDFW since 1989.

4.2 Project schedule

This section describes the schedule for the restoration efforts at the two sites (Old Site and New Site; Figure 1). An annual population survey will be conducted at the sites at the end of the

summer to estimate the abundance, delineate the population perimeter and identify the size range of the Olympia oysters.

Figure 2 summarizes the overall restoration schedule for the two restoration sites at the head of Sequim Bay in the years 2020-25.

Tasks	2020-25 July	August	Sept.
Population survey at the old restoration site*			
Population survey at the new restoration site*			
Data analysis			
Submittal of annual report			

* After a couple of years collection of settlement and juvenile recruitment using shell strings may occur by placing the strings in May and collecting them in August.

Field work Data analysis Annual report

Figure 2. Overall site restoration schedule for 2020-2025.

4.3 Project Budget and Funding

Because the two restoration sites are on tribal tidelands most of the work is done by tribal biologists and technicians. The Clallam MRC members and volunteers assist in the annual population surveys and with any substrate enhancement that may occur in any given year. Clallam MRC receives EPA National Estuary Program funds through a grant from the Northwest Straits Commission. Those funds will be used to cover the cost of the JST tribal shellfish biologist to lead annual population surveys and analyze that data.

Table 1. Estimated Project Budget for 2020-21

Cost Item	Estimated Annual Cost
Staff (JST Shellfish Biologist)	$2,500
Supplies and materials	$0
Total Cost	**$2,500**

5.0 Sample Collection Procedures

This section describes all the major field tasks, from the initial site visit until completion of the project, conducted during an Olympia oyster restoration effort in an area with none or very limited numbers of Olympia oysters. These tasks are described in this QAPP to document the procedures for all tasks conducted by Clallam MRC and its partners during the restoration efforts in Sequim and Dungeness Bay, which commenced in 2012.

5.1 Initial Site Visit

On a suitable minus tide the potential restoration site will be visited by an experienced shellfish biologist. The biologist will assess the suitability of the tidelands using the following criteria:

- Protected bay or inlet
- Suitable substrate – not too muddy or rocky
- Influx of freshwater – near a stream or seepage
- Appropriate tidal height of approximately 0 feet – Mean Low Lower Water (MLLW)
- Low slope of the intertidal area
- Low risk of being washed out by stream or by wind, wave or tidal action
- No eelgrass bed – eelgrass beds will make it difficult to obtain the needed permits

If suitable habitat is identified, the area will be delineated and Global Positioning System (GPS) coordinates will be taken. The size of the restoration site will be determined based on suitable habitat and permission by the landowner(s).

5.2 Initial Test Plot Placement and Seed Count and Size Survey

After identifying a suitable habitat, the next step is to establish test plots within the future restoration site. The number and size of the plots will depend on the site and the amount of seeded cultch available.

At each plot one bag will be opened, 10 shells will be randomly selected and all live and dead Olympia oysters will be counted on each shell to generate an estimate of the total number of Olympia oysters being out-planted. The oyster cultch will then be spread at each plot in a circle with a 1-meter radius (3.1 m^2 or 8.5 ft^2). The coordinates of center of the test plots will be recorded using a handheld GPS.

The population of Olympia oysters in the test plots will be assessed in the spring after being exposed to the habitat through the winter months following the same approach described above. If the estimated population at a test plot is stable, with respect to the initial population estimate, a restoration effort will be initiated at that site by spreading seeded cultch. Each time seeded cultch is added to the test plots or restoration site a subsample of 10 randomly selected shells will be collected to estimate the total number of Olympia oysters being out-planted and the shell height of the Olympia oysters will be measured before the seeded shells are distributed at the site.

Depending on the number of shells being examined, either all Olympia oysters will be measured or a subset of the oysters will be measured (for example the first 20 Olympia oyster spat found on the randomly selected shells). Shell heights will be measured to the nearest millimeter with

calipers. If any Pacific oyster set is discovered (distinguishable in oysters 4mm and larger) these oysters will be counted and recorded separately, but no shell heights will be collected. The ratio between Olympia and Pacific oysters will be noted.

5.3 Growth and Survival Survey

Growth and survival of the Olympia oysters will be monitored both in the test plots and in the restoration site, if the decision is made to continue the restoration effort based on the outcomes of the test plots. The following sections outline the survey methods for monitoring growth and survival in the test plots and in the restoration site.

5.3.1 Test Plots Growth and Survival Survey

The survival in the test plots at the potential restoration site will be assessed in the spring following exposure to the habitat through the winter months. Thirty shells from each plot will be randomly selected and the number of live and dead Olympia oysters will be counted. The number of live Olympia oysters will be compared to the number of live oysters collected when shells were originally spread to get an estimate of the survival rate.

The shell height will be measured on the first 100 Olympia oysters 2mm and larger using dial calipers, and precision will be recorded to the nearest millimeter. All individuals assisting with population surveys will be trained how to read the dial calipers prior to the start of the survey. The height is the distance from the umbo to the distal margin of the shell (Figure 3) and the measurement will be taken to the nearest mm. The shell height measurements will be used to derive an oyster size-frequency distribution and for future growth estimates.

In addition, qualitative observations will be made regarding loss of shell and any mortality such as obvious signs of predation by oyster drills.

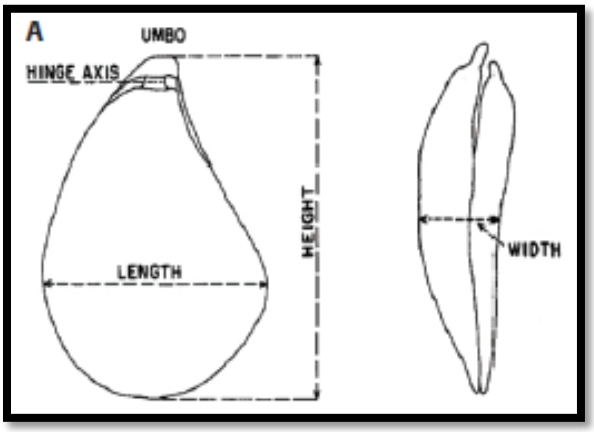

Figure 3. Height measurement[3]

5.3.2 Restoration Area Growth and Survival Survey

If the decision is made to expand the restoration effort at a site beyond the test plots, the Olympia oyster population will be surveyed annually in the summer months. The following sections outline the methodologies for sample area, sample size, sampling survey method, and delineation of the oyster reef.

5.3.2.1 Sample Area and Number of Samples

The number of samples to be collected is calculated in advance. For existing sites, field staff use data from previous surveys to calculate the sample size. If a new site were to be added, a pilot sampling effort would be conducted in advance in order to calculate the minimum sample size for the survey. The samples will be collected using a 0.25 m^2 quadrat (0.5 m x 0.5 m). The sample size or number of quadrats will be determined using the following equation[2]:

$n = z_\alpha^2 \sigma^2 / d^2$

Where n is sample size, α is the significance interval (0.05 for this study), z_α is the z-value from a standard normal distribution for the chosen α (1.96 for α=0.05), σ^2 is the variance of the population, and d is the maximum allowable absolute difference between the true population mean and the estimated population mean, 30% of the sample mean for this study. Sigma (σ) is usually unknown but may be estimated from the standard deviation (SD) of pilot samples (assuming that you are covering the range of densities with this sampling).

To estimate σ, take a minimum of five (replicate) pilot samples and determine the mean and standard deviation of the Olympia oyster density obtained from these pilot samples. Obtain the variance (σ^2) by squaring the standard deviation, and then use this calculated variance in the above equation. Based on standards that are commonly accepted in fisheries literature [confidence interval (CI) of 95%, with a maximum allowable distance (d) of 30% of the mean and α of 0.05] enough samples should be collected to ensure that the coefficient of variation (CV), which is the ratio of the standard deviation to the mean, is approximately 0.5. Example sample size calculation:

Example of calculation:

Oyster densities (oysters/m^2) from five pilot samples: 16, 26, 35, 47, 64

Mean = 37.60; σ = 18.66, CV = 0.50

z_α for α of 0.05 and a 95% confidence interval = 1.96

d = 0.30 x 37.60 = 11.27

[2] Baggett, L.P., S.P. Powers, R. Brumbaugh, L.D. Coen, B. DeAngelis, J. Greene, B. Hancock, and S. Morlock, 2014. Oyster habitat restoration monitoring and assessment handbook. The Nature Conservancy, Arlington, VA, USA.

n = (1.96 x 18.66)/ 11.27

n = 10.53 (sample size would be 11)

5.3.2.2 Sampling Survey Method

To eliminate bias a stratified random sample population survey protocol will be used[3]. Transects will be laid out 25 feet apart along the northern side (landward facing side) of the restoration site. The starting position of the first transect and the quadrat starting position for each transect will be randomized. The minimum number of samples collected using the 0.25 m^2 quadrat will be calculated as described in Section 5.3.2.1.

Depending on the calculated number of required samples the number of transects may need to be adjusted. The exact number of samples for each transect will be based on the width of the restoration site and the random sampling start. If it is determined that greater or fewer samples are required, then one transect can be omitted or added with an adjustment of the distance between transects.

Equipment:

- GPS & notebook for writing notes
- 150' tape measure
- Camera (or two) for quadrat photos
- Data sheets (on waterproof paper), pencils, clipboards
- Calipers
- Quadrats
- Stakes or pin flags to mark transect start, end and quadrat centers.
- Flagging and 20 wire flags
- Volunteer sign-in forms

Select seven random numbers less than 20 using a random number table or online generator https://www.random.org/. These numbers will be used to locate the start of the first transect placement and of the first quadrat placement for each transect. The surveyor should determine his or her pace length (pace = one step of one leg) prior to the start of the survey.

1. Identify the northwest and northeast corners of the restoration site based on the following coordinates and identify a compass bearing (direction to walk along the northern edge of the restoration site)
   NW_____
   NE_____

[3] 2016 MRC protocol written by Cheryl Lowe.

2. Beginning at the northwest corner of the plot walk [1st random number] of paces in the identified compass direction along the edge of the restoration site to the beginning point of Transect 1. Mark with a stake for beginning of Transect #1. Record the GPS coordinates for start of transect.
3. Walk the number of paces equivalent to 25 ft. (based on individual pace length) along the edge of the restoration site and mark the beginning of Transect #2. Record the GPS coordinates for start of transect.
4. Walk [25 ft. number of paces] along the northern edge and mark beginning of Transect #3. Record the GPS coordinates for start of transect.
5. Walk 10 more paces along the northern edge and mark beginning of Transect #4. Record the GPS coordinates for start of transect.
6. Walk [25 ft. number of paces] along the northern edge and mark beginning of Transect #5. Record the GPS coordinates for start of transect.
7. Walk [25 ft. number of paces] along the northern edge and mark beginning of Transect #6. Record the GPS coordinates for start of transect.
8. From each transect starting point, facing 90 degrees south of (perpendicular to) the identified compass bearing delineating the northern edge, lay out the tape until you reach the southern edge of the restoration site to delineate the transect. Record the GPS coordinates at the end point of each transect.
9. Go back to the northwest starting point of Transect #1 and walk [2nd random number] of paces. Put a wire flag at that point. You will place the quadrat frames on the ground with the stake at the center and the tape running through the center of the quadrat.
10. Walk the number of paces equivalent to 12 ft and place another wire flag/quadrat in the same orientation. Repeat to the end of the Transect #1.
11. Repeat for each transect, starting at:
    a. [3rd random number] of paces for Transect #2
    b. [4th random number] of paces for Transect #3
    c. [5th random number] of paces for Transect #4
    d. [6th random number] of paces for Transect #5
    e. [7th random number] of paces for Transect #6
12. For each quadrat placed along the transect, you will:
    a. Take a photo
    b. Count the total number of spat or adult oysters found within the quadrat
    c. Record the size of each spat found on each shell piece using calipers
    d. Estimate the percent cover of oyster shell

For further details on these tasks, see Section 5.3.2.3.

5.3.2.3 Field Sample Analysis

On the standard field datasheet record the transect number and quadrat number (datasheet attached in Appendix B). Start by taking a photo of the quadrat and time the photo was taken for future cross reference.

Start with the lower right quarter section of each quadrat[4]. Pick up each shell piece and inspect it for Olympia oyster spat or adults. Using calipers record all the spat or adult shell heights found on each shell, set it aside in a bucket or container. OR, just move all the shell out of one quarter section and then start inspecting and moving them into that first quarter section. All remaining counted shells can be placed on the ground in that first quarter section. Examine all substrate within each sample, count live and dead Olympia oysters, and measure shell heights. The shell height of all oysters from each sample plot will be measured unless the field crew is under time constraints caused by the incoming tide. In this case, shell heights will be subsampled in an unbiased manner by measuring all oysters in every third sample plot (those divisible by 3) along the survey transects. As with determining the number of sample plots, the sample size analysis can be used to determine the minimal number of individuals that need to be measured. Shell height will be measured from the hinge to the longest edge of shell (see Figure 4). If a cultch shell is partly within the quadrat, only Olympia oysters set lying within the quadrat will be counted. For quality assurance, all participants in the survey take turns measuring shell heights of all oysters within the first sample plot measured to ensure consistency. If shell height measurements are inconsistent, the lead surveyor will show survey participants how to correctly measure the shells and read the dial calipers. The survey will not commence until all survey participants are measuring shell height in a consistent manner.

To estimate overall coverage, place all the counted shells with edges touching so you can easily estimate the total percentage cover for that quadrat. Record the percentage cover estimate on the datasheet at the top of the shell count for that quadrat, then proceed to the next quadrat in that transect and repeat the tasks. When you get to the end of the transect line, make sure you have recorded the end point on the GPS. Then move to the next transect.

5.3.2.4 Determination of Oyster Reef and Surveyed Area

The last task in the restoration site growth and survival survey is to walk the perimeter of the oyster reef with a survey grade GPS unit to determine the size of the reef[5]. In the handbook by Baggett et al., the Reef Area (used to calculate density estimates) is defined as “the total area of summed patches of living and non-living oyster shell substrate within the restoration footprint.” Make continuous measurements or collect as many GPS locations as possible as the perimeter is walked; large numbers of data points may be required to accurately define the reef

[4] 2016 MRC protocol written by Cheryl Lowe.

[5] Baggett, L.P., S.P. Powers, R. Brumbaugh, L.D. Coen, B. DeAngelis, J. Greene, B. Hancock, and S. Morlock, 2014. Oyster habitat restoration monitoring and assessment handbook. The Nature Conservancy, Arlington, VA, USA.

perimeter. For determination of reef area, the edge of the reef is defined as a continuous line where the percent coverage of surficial living or non-living shell substrate (or alternate material) is equal to or greater than 25%.

Coordinates will be entered into a mapping software (e.g., ArcGIS, ESRI, Redlands, CA), and the area of the plotted oyster reef will be determined and reported in m^2.

5.4 Recruitment Monitoring

If annual population assessments indicated that restoration efforts at a site appear successful based on an increase in oyster abundance, expansion of the population area and/or if recruitment is identified from shell height measurements (shell height < 10 mm from cultch that was not seeded the same year), Olympia oyster recruitment may be monitored at the restoration site. The decision to monitor for recruitment will be based on the above indicators no earlier than 2-3 years after the new restoration site has been established.

Use of shell-string collectors is based on the Puget Sound Restoration Fund's (PSRF) method of using shell stacks to assess natural recruitment and is used at Olympia oyster restoration sites around Puget Sound by multiple organizations. Shell-string collectors will be deployed at three sampling locations within the restoration area. Each sampling location will be marked with a GPS to ensure that future sampling can occur at the same locations. The shell strings will be collected in late summer after a minimum of three months' deployment.

The shell-string collectors will be constructed using Pacific oyster shells[6] that are 4-5 inches long. A 3/8 inch drill will be used to place a hole in the middle of each shell, 11 shells will be placed nacre side down onto a 22-inch section of ¼-inch wooden dowel with a label at one end. Cloth backed, waterproof adhesive tape (duct tape) and a grease marker will be used to label location, station and replicate on each. At least two dowels (ideally 3) with 11 shells each will be deployed at each site. The shell strings will be installed ~12 inches apart by pressing the dowel into the substrate so that the shells are nacre-side down and the tag label is at the top.

[6] Puget Sound Restoration Fund 2015. Olympia oyster recruitment: Long-term Puget Sound settlement and juvenile monitoring. A protocol by PSRF, Bainbridge Island, WA. 4 pp.

Figure 4. Shell-string collector assembly.

The top 10 shells (nacre side only) from each shell string will be analyzed under a dissecting microscope at 10x magnification in a systematic fashion. The number of live Olympia oyster settlers and post settlement mortalities will be recorded from each shell. Once a settler is observed magnification is increased to 32x to confirm identification and marked with a pencil to prevent double counts. Shells will be thoroughly rinsed to remove sediment build up before counting. All live oyster juveniles and oyster mortalities will be counted. Dead shells will be identified by gaping or open shells. Shell heights of up to 10 live and dead oysters encountered on each shell will be measured using calipers to the nearest millimeter and recorded. The oyster shells will be analyzed within 48 hours from being removed from the water. Once removed, the shell sticks will be placed in a bucket and covered by a seawater-moistened towel/rag to minimize desiccation of spat. Care will be taken to ensure that the shells do not rub against each other or the side of the bucket resulting in knocking of settled spat off the shells.

6.0 Five Year Assessment of Restoration Effort

After five years, the restoration effort will be assessed to determine if the Olympia oyster population at the restoration site is self-sustainable, and to determine an appropriate monitoring frequency to assess long-term trends.

During a work session at the 2016 MRC conference in Port Townsend the topic of “when a restoration effort is completed/successful” was discussed. As no conclusion was reached this section of the QAPP will be revised based on future discussions among MRC members and agencies working on Olympia oyster restoration. No structured meeting has taken place since 2016.

A few criteria indicating a successful restoration effort were discussed at the 2016 conference. Several participants suggested a number of Olympia oysters ranging from around 30 to 100 individuals/m^2. Another suggested criterion was determining the population as "self-sustainable." No clear definition of this term was established.

Data collected from the Sequim Bay restoration sites will help contribute to a better regional understanding of Olympia oyster restoration methods, timelines, and strategies to reach the collective goal of self-sustaining populations and how those success metrics are defined. The Northwest Straits Commission and partners are planning to hold regional discussions to provide more guidance.

7.0 Data Management

This section describes the different data management tasks conducted throughout the project.

7.1 Sample Documentation

Field data will be recorded in waterproof notebooks. The data books will be kept by the Shellfish Biologist and entered into an excel spreadsheet after the population survey. Hard copies of data sheets are archived in physical folders by year as is the electronic spreadsheets on the computer. All JST files are automatically backed up by cloud-based servers on a daily basis.

7.2 Quality Control Requirements

The calipers used will be double scale plastic Vernier calipers with a measuring range of 0 - 150 mm (0 - 6 inches) and precision of 1 mm. No calibration of manual calipers is needed beyond making sure they are cleaned properly after use so they slide smoothly and the numerical values can be easily read.

The standardized 0.25 m^2 size of the quadrat will result in consistent subsample sizes during the population survey. If any species identification comes into question while analyzing shell strings for Olympia oyster settlement, shells will be saved for further examination by WDFW Shellfish biologist Brady Blake. The GPS will be calibrated according to the service manual before use.

7.3 Validation and Verification Methods

The Project Manager will ensure the data forms are completed and checked for any errors. The Project Manager will enter the data into Excel spreadsheet files. The Shellfish Biologist will use pictures of the datasheets and enter data into an Excel spreadsheet. Spreadsheets will be crosschecked for errors and all errors will be corrected. The Project Manager will summarize the data and provide project partners with a copy of the results. The Shellfish Biologist will verify the summaries and collaborate with the Project Manager to produce the final report, which will be proofed by the Project Lead.

If errors are suspected or identified on the field data sheets or in the Excel spreadsheets, the Project Manager will file a Corrective Action Statement based on the error(s) found. The Corrective Action Statement will include the following information: date the error(s) was identified and by whom; a clear description and/or file name of the datasheet and/or spreadsheet where the error(s) occurred; a detailed explanation of how the error was dealt with, and what corrective actions were taken. This Corrective Action Statement will be filed in the same physical or electronic folder where the data sheets or spreadsheets are stored, and a copy of the statement will be provided to the Project Lead. In the case where a final report is submitted before the error is identified, a correction to the final report will be made and submitted to the Project Lead.

7.4 Analytical Methods Requirements

The Project Manager will enter all formulas for calculation of parameters and basic statistics to determine the results of the population survey, which include population size estimate, density, growth, and size distribution. The Shellfish Biologist will check all the formulas. If any errors are found, the Project Manager will correct the errors and the Shellfish Biologist will verify corrections have been made. The Shellfish Biologist and Project Manager will work together to organize and write the final report.

7.5 Calculation of Population Size and Density Estimates

Two density estimates will be calculated based on the number of Olympia oysters observed in the 0.25 m^2 quadrat samples collected during a survey (for determination of number of samples see Section 5.3.2.1). The total estimated density of Olympia oysters in the restoration site will be calculated as follows:

Restoration site area: 0.5 Acres = 2023.43 m^2
Area sampled: No. of collected samples x 0.25 m^2 = area sampled m^2
Number of Olympia oysters/m^2: No. of observed Olympia oysters/area sampled = number of oysters/m^2
Estimated population in restoration site: number of oysters/m^2 x 2023.43 m^2 = total number of Olympia oysters

The area of the Olympia oyster reef will be calculated using ArcGIS or another mapping software and the GSP data collected in the field. The reef area will be determined using the definition of “a continuous line where the percent coverage of surficial living or non-living shell substrate (or alternate material) is equal to or greater than 25%” (see Section 5.3.2.4). The total estimated density of Olympia oysters in the reef area will be calculated as follows:

Reef area: calculated using ArcGIS and field data = m^2
Area sampled: No. of collected samples within the reef area x 0.25 m^2 = reef area sampled m^2

Number of Olympia oysters within the reef area/m^2: No. of observed Olympia oysters/reef area sampled = number of oysters within the reef area/m^2

Estimated population in reef area: number of oysters/m^2 x reef area m^2 = total number of Olympia oysters.

Uncertainty will be reported as 95% confidence intervals around the population estimate: $\bar{x} \pm \hat{z} * \sigma/\sqrt{n}$, where $\bar{x}$ is the sample mean, σ is the standard deviation, n is the sample size and z^*is the value from the standard normal distribution for the desired confidence level (95% CI = 1.96).

7.5 Calculation of Size-Frequency Distribution and Growth

Oyster size-frequency distribution is a measure of how the oyster population is distributed across various size classes, and provides information about oyster growth and the survivorship and mortality of cohorts. Figure 5 shows an example of a size-frequency distribution. The average annual growth can be calculated from the size-frequency distributions.

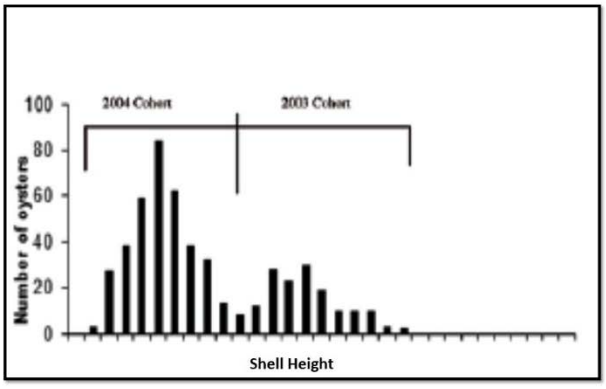

Figure 5. Example of a size-frequency distribution[7]

[7] Baggett, L.P., S.P. Powers, R. Brumbaugh, L.D. Coen, B. DeAngelis, J. Greene, B. Hancock, and S. Morlock, 2014. Oyster habitat restoration monitoring and assessment handbook. The Nature Conservancy, Arlington, VA, USA.

8.0 References

1. Blake, B., & Bradbury, A. Washington Department of Fish and Wildlife Plan for Rebuilding Olympia Oyster (Ostrea lurida) Populations in Puget Sound with a Historical and Contemporary Overview. Washington Department of Fish and Wildlife, 25 pp. https://erb7v36cnte3ws6d42lk0gge-wpengine.netdna-ssl.com/wp-content/uploads/2019/02/olympia_oyster_restoration_plan_final.pdf
2. Baggett, L.P., S.P. Powers, R. Brumbaugh, L.D. Coen, B. DeAngelis, J. Greene, B. Hancock, and S. Morlock, 2014. Oyster habitat restoration monitoring and assessment handbook. The Nature Conservancy, Arlington, VA, USA, 96 pp. http://www.oyster-restoration.org/wp-content/uploads/2014/01/Oyster-Habitat-Restoration-Monitoring-and-Assessment-Handbook.pdf
3. Department of Health 2020. Shellfish Safety Map https://fortress.wa.gov/doh/biotoxin/biotoxin.html
4. Lowe, C. 2016. Olympia Oyster Monitoring--Discovery Bay 2016. Jefferson County Marine Resources Committee. August 1, 2016. Protocols approved by WDFW Shellfish Biologist Brady Blake, August 2015.
5. Puget Sound Restoration Fund 2015. Olympia oyster recruitment: Long-term Puget Sound settlement and juvenile monitoring. A protocol by PSRF, Bainbridge Island, WA. 4 pp.

9.0 Appendices

Appendix A. Glossary, Acronyms, and Abbreviations

Glossary – General Terms

Clean shell: shells, most often Pacific oyster shells, without any live organisms attached

Cultch: same as clean shells. Shells, most often Pacific oyster shells, without any live organisms attached.

Cultch bag: A large mesh bag approximately 2-3 ft. long filled with clean shells (most often Pacific oyster shells).

Nacre: mother-of-pearl

Overwintered seeded cultch: The survival rate of newly settled oysters in a cultch bag if often greater than if the shells were spread right away on the tidelands. For this reason, the cultch bag is often overwintered or kept through the summer months to give the oysters a chance to grow and increase their likelihood for survival.

Recruitment: Recruitment includes settlement and some period of post-settlement survival, whose duration varies depending on the researcher's objective.

Reef or Reef Area: The total area of summed patches of living and non-living oyster shell substrate within the restoration footprint.

Seeded cultch: Shells, most of Pacific oyster shells, with newly settled oysters.

Seeded shell: same as seeded cultch. Shells, most of Pacific oyster shells, with newly settled oysters.

Settlement: Occurs once the larva has become permanently attached to the substrate or has metamorphosed into its final benthic form.

Shell-string collector: The collector is used to estimate the settlement of oyster larvae. It is made of 11 4-5 inch Pacific oyster shells. A hole is drilled in the middle of each shell and the shells are placed with the nacre side down on a ¼-inch wooden dowel with a label at one end.

Spat: Newly settle oyster larvae.

Acronyms and Abbreviations

Acronyms and abbreviations used in this QAPP:

ft	Feet
ft^2	square feet
GIS	Geographic Information System software
GPS	Global Positioning System
m	Meter
m^2	Square meter
MLLW	Mean low low water
MRC	Marine Resources Committee
NWSC	Northwest Straits Commission
QA	Quality assurance
QAPP	Quality Assurance Project Plan
QC	Quality control
WDFW	Washington Department of Fish and Wildlife

Appendix B: Standard Field Datasheets

Field Datasheet
JST 2019

Olympia Oyster Population Survey - Oyster Count Datasheet

Site: Survey Date:

Sampler(s): Survey Time:

Page of

Sample Plot Number	# Pacific	# Live Olympia	# Dead Olympia	**Sample Plot Number**	# Pacific	# Live Olympia	# Dead Olympia

Field Datasheet
JST 2019

Olympia Oyster Shell Height Measurements

Site: Survey Date:

Sampler(s): Survey Time:

mm	#	mm	#
< 1		43	
2		44	
3		45	
4		46	
5		47	
6		48	
7		49	
8		50	
9		51	
10		52	
11		53	
12		54	
13		55	
14		56	
15		57	
16		58	
17		59	
18		60	
19		61	
20		62	
21		63	
22		64	
23		65	
24		66	
25		67	
26		68	
27		69	
28		70	
29		71	
30		72	
31		73	
32		74	
33		75	
34		76	
35		77	
36		78	
37		79	
38		80	
39		81	
40		82	
41		83	
42		>84	

Notes: