Quality Assurance Project Plan
Olympia Oyster Restoration in Sequim Bay
2018

Prepared for:
The Northwest Straits Commission

Prepared by:
Helle Andersen
Clallam County Marine Resources Committee

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1.0 Title Page, Table of Contents, and Distribution List

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2018
Revision 1

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2.0 Abstract

The goal of this project is to expand the Olympia oyster populations in Sequim Bay contributing to the larger effort of restoring 100 acres of Olympia oyster habitat in the Puget Sound area by 2020.

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 investigated. Site assessments were conducted at Dungeness Farm and Washington Harbor and test plots were established at Pitship Point and Cline Spit. The test plots at both locations revealed that these sites were not suitable for Olympia oyster restoration because of exposure to wave and wind action. In addition, the Dungeness Farm site was deemed too exposed and sites in Washington Harbor were either too close to eelgrass beds or the landowners were not interested in allowing Clallam MRC access to their tidelands. The newly restored Dawley Road property was another potential site and 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; Clallam MRC has not yet received an answer to the request.

In May 2018 the JST proposed using a parcel of tidelands that they lease from WA Department of Natural Resources (DNR) at the head of Sequim Bay. The site is approximately 700 ft. east of the current restoration site. At the May 2018 meeting the Clallam MRC agreed to pursue this site as the new restoration site. In addition to expanding the restoration effort the study will continue to monitor the restoration effort at the Blyn site by conducting annual population surveys.

To keep the QAPP current the document will be revised as appropriate to capture any changes in the protocols or any addition of tasks.

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 candidate species by the Washington Department of Fish and Wildlife (WDFW) since 1997[1]. 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. The Clallam MRC, JST, and PSRF are working together to restore Olympia oysters in Sequim Bay. In 2012 1.5 acres of JST tidelands were established for Olympia oysters and because Olympia oysters are thriving at this site Clallam MRC and JST are working to expand the restoration effort to other parts of Sequim Bay. The project is part of a larger effort underway to restore 100 acres of Olympia oyster habitat in the Puget Sound area by 2020.

4.0 Project Goals and Objectives

The overall goal is to expand Olympia oyster populations in habitats in Clallam County, and for this project in Sequim Bay, where Olympia oysters historically thrived. Identifying new restoration sites and restoring Olympia oysters at these sites will help meet this goal. The following objectives need to be accomplished to restore Olympia oyster populations at a new site:

1. Perform an initial survey of the tidelands to identify the most suitable areas for oyster restoration.
2. Complete permit application for the restoration effort.
3. Establish test plots at the new site by spreading shells seeded with Olympia oysters from JST tidelands in Sequim Bay or from PSRF. The number and size of the plots will depend on the site and the amount of seeded clutch available.
4. Determine if the site is a viable expansion site for Olympia oyster restoration in Sequim Bay by monitoring survival of transplanted oysters through a winter season.
5. If test plots are successful based on the criteria in this QAPP, spread additional bags of seeded cultch on the restoration site. The number of bags depends of the size of the new restoration site. The seeded shells should be overwintered before spreading them on the tidelands.

[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.

6. Monitor population growth and survival for five consecutive years after applying the seeded shells; collect data to estimate population size, oyster density, and oyster size distribution. Spread additional seeded shells and/or clean shells for additional settling substrate as deemed necessary.
7. Characterize annual recruitment rate by monitoring settlement and juvenile recruitment on shell.
8. After five years assess if the Olympia oyster population at the site is self-sustainable.

4.1 New Project Site

In 2016 Clallam MRC and their partners began searching for another restoration site in either Sequim or Dungeness Bay. Site assessments were conducted at Dungeness Farm and Washington Harbor and test plots were established at Pitship Point and Cline Spit. The test plots at both locations revealed that these sites were not suitable for Olympia oyster restoration because of exposure to wave and wind action. In addition, the Dungeness Farm site was deemed too exposed and sites in Washington Harbor were either too close to eelgrass beds or the landowners were not interested in allowing Clallam MRC access to their tidelands. The newly restored Dawley Road property was another potential site and Clallam MRC is currently waiting to receive approval of a Special Use Permit that Clallam MRC submitted in September 2017 to the US Fish and Wildlife Service asking for permission to establish two test plots at their Dawley Road property in Sequim Bay.

In May 2018 Clallam MRC and JKT decided to use a parcel of tidelands that JKT is leasing from DNR at the head of Sequim Bay. The parcel is approximately ½ - ¾ acre and is located approximately 700 ft. east of the current restoration site (Figure 1), with similar habitat conditions.

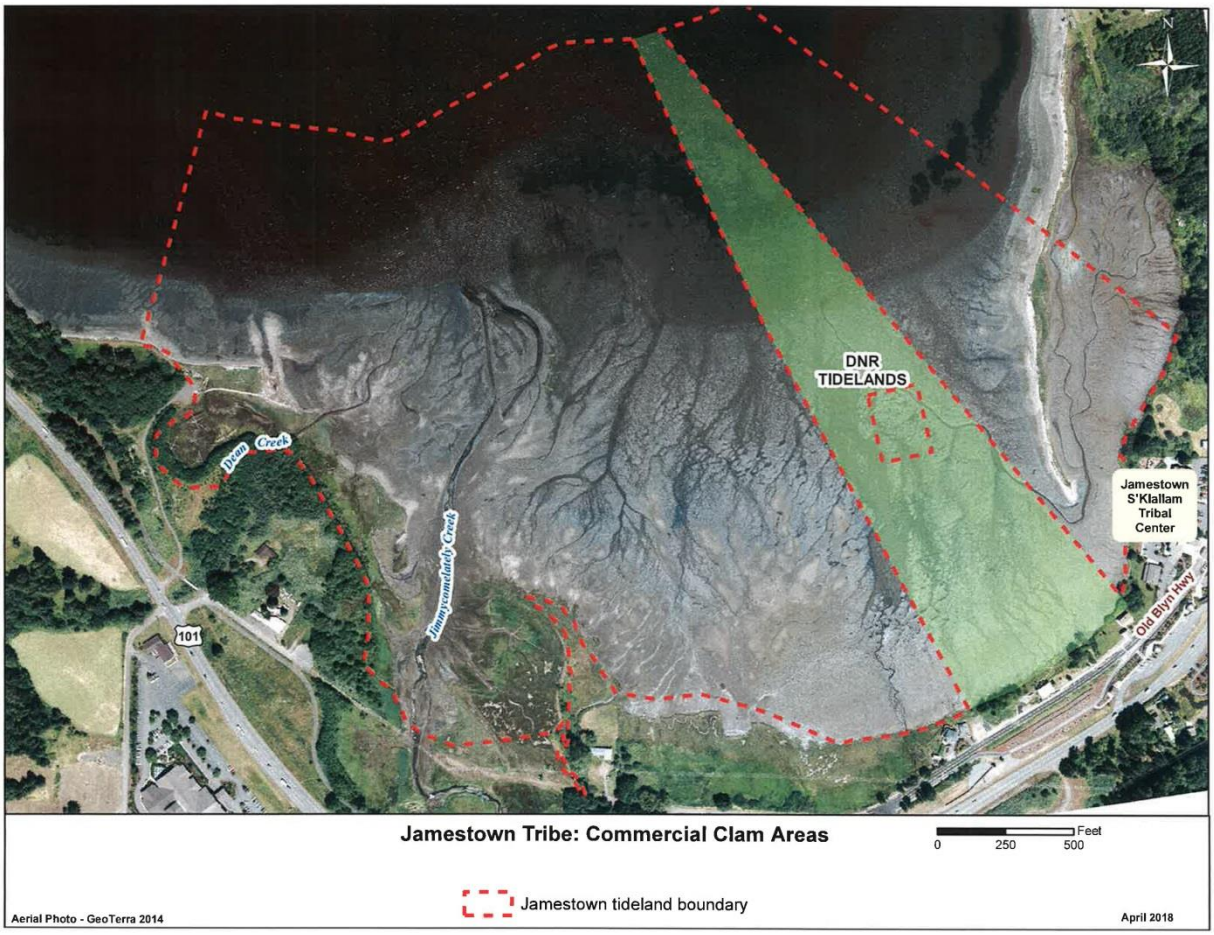

Figure 1. Map of the new restoration site at the head of Sequim Bay. The dotted red square in the green area identified as DNR tidelands outlines the potential Olympia oyster restoration area.

5.0 Organization and Schedule

5.1 Project Team

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 maintaining the official, approved QAPP. 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.

Shellfish Biologist

Elizabeth Tobin, shellfish biologist with Jamestown S'Klallam Tribe, will help execute specific tasks and make recommendations to the project manager.

Fisheries Technician

Chris Burns, fisheries technician with Jamestown S'Klallam Tribe, will help execute specific tasks and make recommendations to the project manager.

Project Quality Assurance Officer

Sasha Horst, NWSC, will provide review of this QAPP and ensure that all proposed actions meet the Washington Department of Ecology requirements.

GIS Specialist

TBD. The specialist will assist the project manager with storage and analysis of spatial data collected during the project.

5.2 Project schedule

This section describes the schedule for the restoration efforts at the new site. Because the new site is located approximately 700 ft. from the old restoration site and within 600 ft. of where seeded bags have been overwintering, several of the steps described in Section 6 will be omitted in the restoration effort based on the assumption that the site is suitable for Olympia oysters. These steps include the initial site visit, establishment of test plots, and assessment of survival and growth in the test plots.

The 100 seeded bags which were purchased in 2016 and 2017 to be used at the Pitship Point site will be spread at the new ½ - ¾ acre site in July 2018. During the following five years, shell strings will be deployed at the site over the summer months (May – August) to assess juvenile recruitment. Once a year a population survey will be conducted in late summer to assess population area and oyster density. Figure 2 summarizes the overall restoration schedule for the new site at the head of Sequim Bay.

Tasks	2018				2019-23				
	June	July	August	Sept.	May	June	July	August	Sept.
Spreading of seeded clutch									
Collect settlement and juvenile recruitment*									
Population survey of the ½ - ¾ acre site									
Data analysis									
Submittal of annual report									

* After a couple of years shell strings may be placed in May and collected in August.

Field work Data analysis Annual report

Figure 2. Overall site restoration schedule for 2018-2023.

6.0 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.

6.1 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 ft – 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 GPS coordinates will be taken. The size of the restoration site will be determined based on suitable habitat and permission by the landowner(s).

6.2 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 clutch available.

At each plot one bag will be opened and 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 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 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.

6.3 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.

6.3.1 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 using digital or dial calipers. The height is the distance from the umbo to the distal margin of the shell (Figure 4) 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.

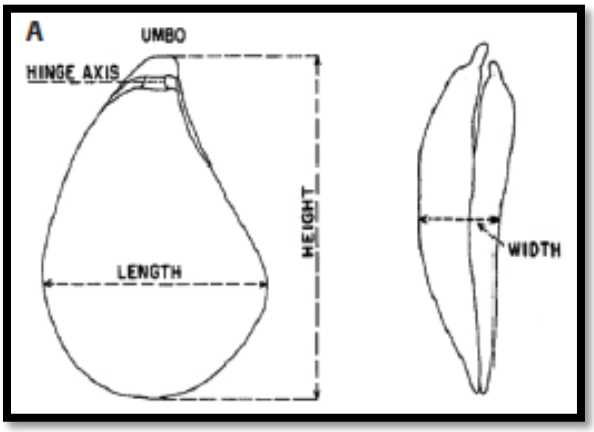

Figure 4. Height measurement[3]

6.3.2 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.

6.3.2.1 Sample Area and Number of Samples

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 (most often 0.05), 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, often 30% of the sample mean. 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

[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.

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

n = (1.96 x 18.66)/ 11.27

n = 10.53 (sample size would be 11)

6.3.2.2 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 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 6.3.2.1.

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 is required, than 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.

[3] 2016 MRC protocol written by Cheryl Lowe.

- 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_____
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 (perpendicular to) of 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 6.3.2.3.

6.3.2.3 Field Sample Analysis

On each data sheet record the transect number and quadrat number. 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 sizes 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 will be measured unless the field crew is under time constraints caused by incoming tide. In this case oysters to be measured per quadrat will be selected in an unbiased manner that correctly characterizes the size distribution of the population from the samples. One method to sub-sample oysters for measurement is to break any clumps of oysters apart and spread out all oysters collected in the sample, dividing them in half, and then dividing the selected half again if necessary. Another simpler method is to measure all oysters in every other (or every third) sample plot. 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. Height will be measured from the hinge to the longest edge of shell (see Figure 4). If a cultch shell is partly within the quadrant, only Olympia oysters set lying within the quadrant will be counted. Time and tide permitting as a quality assurance step every quadrant with the number 3 or divisible by 3 the oysters present will be re-counted and re-measured.

[4] 2016 Jefferson MRC protocol written by Cheryl Lowe.

To estimate overall coverage, place all the counted shells with edges touching so you can easily estimate the total % cover for that quadrat. Record the % cover estimate on the data sheet 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.

6.3.2.4 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[3]. 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 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.

6.4 Recruitment Monitoring

After a couple of years, Olympia oyster recruitment may be monitored at the new restoration site. 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 three shell-string collectors will be constructed, using 4-5 inch Pacific oyster shells[4]. 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. The shell strings will be installed by pressing the dowel into the substrate so that the shells are nacre-side down and the tag label is at the top.

[3] 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.

[4] 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 3. 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. 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.

7.0 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.

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.”

8.0 Data Management

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

8.1 Sample Documentation

Field data will be recorded in waterproof notebooks. The data books will be kept by the Project Manager and entered into an excel spreadsheet after the population survey. Data sheets are archived by year and maintained by the Project Manager.

8.2 Quality Control Requirements

The use of calipers will result in for accurate and consistent oyster measurements.

The standardized 0.25 m^2 size of the quadrate 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.

8.3 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 cross checked 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.

8.4 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.

8.5 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 6.3.2.1). 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 6.3.2.4). 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

8.5 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.

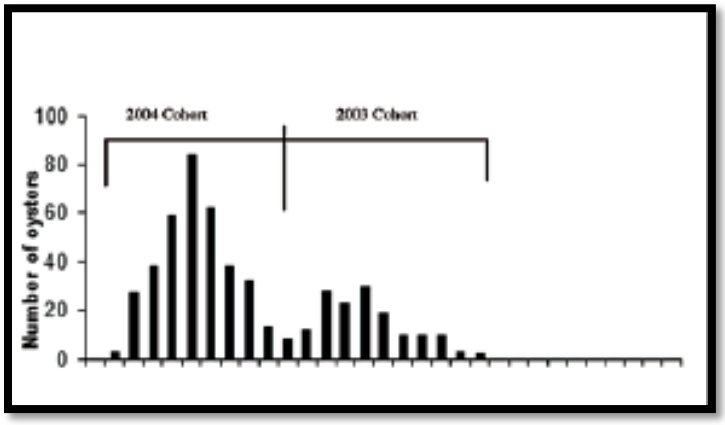

Figure 5. Example of a size-frequency distribution[3]

[3] 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.

9.0 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.
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.
3. Department of Health 2016. Shellfish Safety Map https://fortress.wa.gov/doh/eh/maps/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.

10.0 Appendices

Appendix A. Glossary, Acronyms, and Abbreviations

Glossary – General Terms

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

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

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

Nacra: mother-of-pearl

Overwintered seeded clutch: The survival rate of newly settled oysters in a clutch bag if often greater than if the shells were spread right away on the tidelands. For this reason the clutch 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 clutch: Shells, most of Pacific oyster shells, with newly settled oysters.

Seeded shell: same as seeded clutch. 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. Datasheets