

# OLYMPIA OYSTER, *OSTREA LURIDA*, RESTORATION IN SIMILK AND SKAGIT BAYS, WASHINGTON: 2019 MONITORING REPORT

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# OLYMPIA OYSTER, *OSTREA LURIDA*, RESTORATION IN SIMILK AND SKAGIT BAYS, WASHINGTON: 2019 MONITORING REPORT

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## ABSTRACT

As the only oyster native to the west coast of North America, Olympia oysters (*Ostrea lurida*) are known to contribute to the overall health of estuarine ecosystems while playing a key role in the commercial and cultural history of the Salish Sea region. Despite the obvious benefits of the species, overexploitation and degradation of water quality primarily led to collapse and the near depletion of Olympia oyster stocks in Puget Sound. In 2015, with an intended goal of creating self-sustaining Olympia oyster populations in a target region in Puget Sound, the Swinomish Indian Tribal Community began expanding their restoration work upon a previously successful pilot project. Native oysters were deployed within two different sites, Kiket (KI) and Lone Tree (LT), from 2015-2017. We collected data at each site from 2014-2019 to measure temporal change in oyster growth, recruitment, and survival. Specifically, we were interested in quantifying the biological successes or failures of our reestablished populations to determine if we should continue enhancement efforts or consider alternative restoration strategies. Significant growth was observed each year with oysters at KI experiencing faster growth rates and exhibiting larger sizes compared to LT. Despite the known presence of brooding oysters and competent larvae in the region, we found no evidence of recruitment at either site. Density, used as a proxy for survival, decreased significantly by year and site; KI oysters had lower survival rates than LT. Considering these results in tandem, we identified the lack of recruitment as our primary concern. Density and, therefore, survival would likely improve if recruitment had been measurable at these sites. We hypothesize that our restoration efforts are hindered by the lack of appropriate habitat, lower water residence time, and by the relatively small population size of our restored areas. Moving forward we plan to implement new and unique strategies to: (1) increase adult populations, (2) increase optimal settlement habitat in the lower intertidal, and (3) broaden the spatial scale of the restoration area.

**Keywords:** Olympia oyster, *Ostrea lurida*, growth, habitat, Puget Sound, recruitment, restoration, survival

## INTRODUCTION

Once dominant throughout the intertidal, oyster reefs have declined an estimated 85% on a global scale (Beck et al. 2011). A variety of reasons, not limited to overexploitation, loss of habitat, disease, and environmental degradation or mismanagement have contributed to the population decline (Kirby 2004; Ruesink et al. 2005; Grabowski and Peterson 2007; White et al. 2009b). The loss of oyster reefs is a cause for concern due to the variety of ecosystem services oysters can provide including water filtration, benthic-pelagic coupling, and sediment stabilization (Newell 2004; Coen et al. 2007; Grabowski and Peterson 2007; Allen 2009; zu Ermgassen et al. 2013). Moreover, oyster reefs structure habitat, serving other marine species in such ways as providing foraging habitat to predators or refuge for prey (Grabowski and Peterson 2007; Beck et al. 2011). In order to revitalize these ecosystem services, an increase in restoration efforts has been seen in recent years across the globe.

As the only oyster native to the west coast of North America, Olympia oysters (*Ostrea lurida*) are known to contribute to the overall health of estuarine ecosystems (Pritchard et al. 2015). Additionally, in Washington, Olympia oysters played a key role in the commercial and cultural history of the Puget Sound region. Prior to the decline of the Olympia oyster, evidence suggests the bivalve was substantially utilized by tribes throughout the Salish Sea as both a food source and for other cultural purposes such as community and spiritual gatherings (Steele 1957; White et al. 2009b; Blake and Bradbury 2012). Shell middens dating back over 4000 years, as well as the correlation of winter villages in relationship to known locations of large oyster beds, further demonstrate the importance of the species to the Indigenous peoples of the Pacific Northwest (Steele 1957; White et al. 2009b; Blake and Bradbury 2012). When tribes introduced the Olympia oyster as a food source to recent European immigrants, exploitation began on a more commercial scale (Dinnel et al. 2009). Spurred by popularization in San Francisco at the time of the gold rush, harvests reduced populations to such small quantities along the West Coast that they were considered

functionally extinct by the 1930s (Allen 2009; Dinnel 2018). Despite the clear importance of the species, overexploitation beginning in the late 1800s, followed by degradation of water quality from 1930 – 1950, led to a collapse of the commercial fishery and the near depletion of Olympia oyster stocks in the Salish Sea (Cook et al. 2000; Brumbaugh et al. 2006; Blake and Zu Ermgassen 2015).

Recognizing a need to take action to rebuild the native stocks of Olympia oysters in Washington's inland waters, the Washington Department of Fish and Wildlife (WDFW) implemented the Olympia Oyster Stock Rebuilding Plan (Blake and Bradbury 2012). The plan recommends 19 sites throughout Puget Sound as priority locations for focused restoration by 2022 with an aim to reestablish sizable, self-sustaining, source populations of this species. One of the 19 priority restoration sites, Similk Bay, is located in proximity to the Swinomish Indian Tribal Community's (SITC) reservation. For this reason, SITC Fisheries Department, partnered with WDFW and the Puget Sound Restoration Fund (PSRF), to undertake a pilot restoration project in 2012 and 2013 (Barber et al. 2015). The primary goals of the pilot project were to quantify survival and growth of the outplanted oysters and to determine if conditions in the sites would be favorable for Olympia oysters (Barber et al. 2015).

Two pocket estuaries (perched tidal lagoons) on the shores of the reservation tidelands were selected as potential habitat for outplanted oyster seed; one lagoon is located in Similk Bay and the other is located in Skagit Bay. At the completion of the pilot project, relatively high survival rates were qualitatively observed (quantitative efforts had significant issues and could not be utilized, see Barber et al. 2015). In addition, oysters in both lagoons demonstrated satisfactory growth rates and some data supported the hypothesis that spawning and recruitment may have occurred. Because SITC was encouraged by the results of the pilot restoration project, we opted to enlarge the restoration footprint beginning in 2015.

Expansion of the restoration sites in Similk and Skagit Bays began with the addition of seeded

cultch (Olympia oyster juveniles set on Pacific oyster, *Magallana gigas*, shell) from the PSRF hatchery beginning in 2015 and continuing with larger seeding events in 2016 and 2017. In their Olympia Oyster Stock Rebuilding Plan, WDFW suggests numerous metrics for measuring the progress and success of newly established restoration sites (Blake and Bradbury 2012). Following these guidelines, SITC Fisheries developed a monitoring protocol to guide our efforts toward reaching the goal of creating self-sustaining Olympia oyster populations and reestablishing the presumed ecosystem services the species provides (Greiner et al. 2015; Hunter et al. 2021). We aimed to quantify temporal change in the physical and biological parameters of areas both on and off the restoration sites. While SITC Fisheries addressed numerous scientific questions with our research (e.g., Greiner et al. 2015; Barber et al. 2016; Grossman et al. 2020; Munsch et al. 2021), for the purposes of this report we will focus on our efforts to monitor growth, recruitment, and survival of the reestablished oysters. Specifically, we were interested in quantifying the biological successes or failures of our reestablished populations in order to determine if we should continue enhancement efforts in these locations or if we needed to consider moving to other locations within the target embayments.

## METHODS

### Study area

The SITC Reservation is located on Fidalgo Island, in Washington State, on the shores of northern Puget Sound. The reservation, which is on the southeastern tip of the island, contains tidelands that extend along the western shore, where our two Olympia oyster enhancement sites are located near Lone Tree Point and Kiket Island (Figure 1). The initial restoration sites were both located in pocket estuaries, small subestuaries perched behind spits or barrier beaches (Beamer et al. 2003). These two sites, called Lone Tree Lagoon (LT) and Kiket Lagoon (KI), are surrounded by salt marsh, have tidal channels, and receive low wave and current energy (Figure 1).

Kiket Lagoon and LT, located in Similk and Skagit Bays, respectively, were selected as project sites in conjunction with WDFW and the Skagit Marine Resources Committee. Our partners selected these sites based on a variety of factors, including but not limited to, presumed nearby historical presence, water retention in the lagoons, presumed extended water residency of surrounding embayments, protection from predators, and mitigated effects from large storms. Moreover, the nature of the lagoons is such that the oysters within would not be exposed to long periods of cold air temperature during low tides as the near constant inundation would serve as a buffer to the low winter temperatures known to stress or kill the animals (Hopkins 1937). Additionally, it was hypothesized that the pocket estuary populations could eventually serve as source populations for future restoration areas on tidelands in proximity to the lagoon channel mouths.

Lone Tree Lagoon, located in Skagit Bay (SK), is approximately 1.6 hectares in size, with a barrier

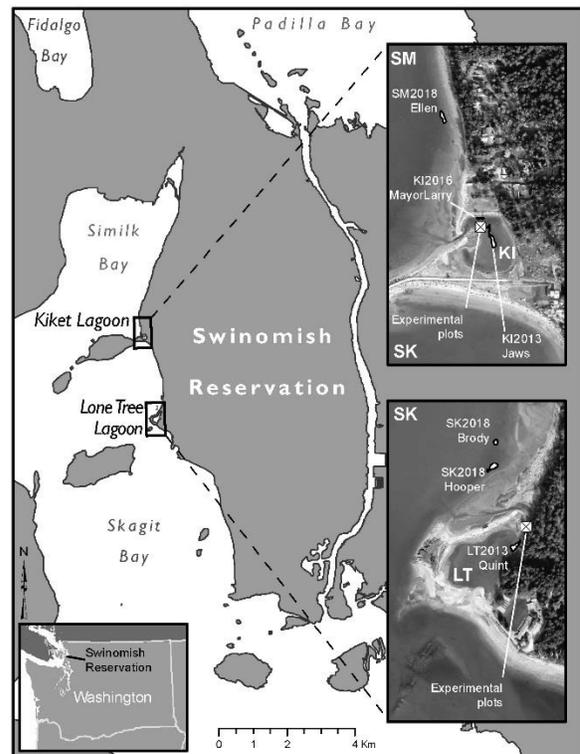


Figure 1: Location of Swinomish Olympia oyster restoration sites

beach along the western edge. Unlike KI, this lagoon receives freshwater inputs from an ephemeral stream (flowing from fall through late spring) in addition to groundwater seeps. Occasionally marine waters from SK wash into the lagoon from the barrier beach during extreme high tides or storm events [i.e., > 2.7 m above mean lower low water (MLLW)]. Marine waters enter daily during flood tides greater than 1.77 m relative to MLLW, and water exits the lagoon as the tide ebbs (Grossman et al. 2020). We placed the oysters within the lagoon at 1.91 m relative to MLLW; at the lowest tide there is at least 0.13 m of water over the oysters that are located in the channel (Barber et al. 2016).

The smaller KI, which is located adjacent to a recently restored (in 2018) tombolo connecting the mainland to Kiket Island, is approximately 0.88 hectares in size (Figure 1) (Barber et al. 2016). There is no direct freshwater input to this lagoon other than groundwater, although episodic stormwater runoff from a nearby roadway may be a concern (SITC Fisheries, personal observation). Marine waters from adjacent Similk Bay (SM) move into the channel during floods at 2.64 m relative to MLLW and exit the lagoon during ebbs, although the oysters always remain inundated. The oysters in the perched lagoon are located at an elevation of 2.21 m relative to MLLW; at the lowest tides the oysters are inundated in 0.43 m of water (Barber et al. 2016).

As our restoration efforts broadened and goals evolved, we began to expand habitat (i.e., adding unseeded Pacific oyster shell to a beach) outside both lagoons. For this habitat expansion effort,

we targeted intertidal beaches adjacent to the lagoon channel mouths with firm substrate at 0 m relative to MLLW (Figure 1). Our target substrate consisted mostly of sand, shell debris, and small rocks (i.e., cobbles, pebbles, granules). The SK shoreline immediately north of the LT channel mouth provided ideal substrate conditions at the correct elevation. However, the tidelands adjacent to the mouth of the KI channel in SM were too soft and silty for habitat expansion. Thus, we opted to select a site ~215 m north of the KI channel mouth as our other site for oyster population expansion. Both of these intertidal sites are subjected to daily tidal flux (as opposed to the pocket estuaries where the oysters remain inundated at all times) and are slightly more exposed to wave energy from surrounding SK and SM Bays (SITC Fisheries, personal observation). The site at LT is located within an area that may entrain late-stage *Olympia* oyster larvae, leading to higher potential for settlement (Grossman et al. 2020). A similar study has not yet been conducted in SM.

#### Experimental plots and restoration beds

Our work involved the creation of two different types of oyster beds, each with separate goals in mind: (1) experimental plots and (2) restoration beds. The experimental plots were established for experimental purposes (as described in Greiner et al. 2015), while the restoration beds were developed to increase the population size of the *Olympia* oyster. Lone Tree and KI lagoons each hosted both experimental plots and one or more larger restoration beds.

Table 1: Swinomish *Olympia* oyster restoration site information including: bed names, year developed, site, area, seeded vs. unseeded cultch, and years seed was added to bed

Restoration bed names	Year developed	Site	Area (year of first measurement)	Seeded or unseeded cultch	Years seed added
KI2013Jaws	2013	Kiket Lagoon	171 m <sup>2</sup> (2017)	Seeded	2013, 2015, 2016, 2017
LT2013Quint	2013	Lone Tree Lagoon	154 m <sup>2</sup> (2017)	Seeded	2013, 2015, 2016, 2017
KI2016MayorLarry	2016	Kiket Lagoon	31 m <sup>2</sup> (2017)	Seeded	2016, 2017
SK2018Brody	2018	Skagit Bay Tidelands	75 m <sup>2</sup> (2018)	Unseeded	n/a
SK2018Hooper	2018	Skagit Bay Tidelands	166 m <sup>2</sup> (2018)	Unseeded	n/a
SM2018Ellen	2018	Similk Bay Tidelands	118 m <sup>2</sup> (2018)	Unseeded	n/a

Table 2: Annual Olympia oyster (OO), *Ostrea lurida*, enhancement statistics. Number of Pacific oyster (*Magallana gigas*) shell in bags, number OO seed on shells, and number of single OO are approximate. Note that this does not include estimates of mortality and therefore, is not an estimate of the number of living oysters at the end of 2017.

Year	Bags deployed	# Pacific oyster shell / bag	# OO / Pacific shell	Total # OO seed in bags	# Single OO	Total # OO outplanted
2012	21.5	250	17.68	95,030	0	95,030
2013	50	250	4.5	56,250	0	56,250
2015	0	0	0	0	10,152	10,152
2016	173	250	3.9	168,675	5,600	174,275
2017	100	250	7.2	180,000	0	180,000
Total number of seed outplanted						515,707

### Experimental plots

Experimental plots in LT and KI consisted of three of 4.6 m<sup>2</sup> plots: (1) bare substrate (no shell added), (2) seeded cultch, or (3) unseeded cultch (Pacific oyster shell with no Olympia oysters) (Greiner et al. 2015). The seeded and unseeded plots were located in the channel near the lagoon proper at LT and in the lagoon but near the channel outlet at KI. Each experimental plot, save for the bare plots, received 16 bags of shell (bags consist of 200-250 *M. gigas* shells) across 4.6 m<sup>2</sup>.

### Restoration beds

Restoration beds were centrally located in the lagoons (Figure 1, Table 1). As of 2019, these restoration beds consist of oysters from a variety of seed years (i.e., 2013, 2015, 2016, 2017) as well as areas with unseeded cultch (placed for habitat expansion and recruitment). Bed area was not measured until after the addition of shell and seeded cultch in 2017.

In the spring of 2018, three restoration beds were established at our intertidal sites in SK and SM with the goal of expanding habitat for increased recruitment. Two beds of unseeded cultch were established north of the mouth of the LT channel and one bed was constructed north of the KI channel.

### Oyster reestablishment

Following the success of the pilot efforts in 2012 and 2013 (Barber et al. 2015), SITC received ~10,150 individual Olympia oysters from PSRF in spring of 2015. In 2012 and 2013, all of the Olympia oyster seed was delivered as seeded cultch (Table 2). In 2015, we received Olympia oysters as “singles” (larger seed not settled on any

substrate) divided into three size classes. The largest single oysters were split between the two lagoons and dispersed on and around the restoration beds initially created in 2013. The medium and small oysters were kept in grow bags over the summer in LT to protect against predation, desiccation, and weather. Once they reached appropriate size for dispersal in November 2015, the medium and small size classes were split between the two lagoons and spread onto the existing restored oyster beds.

In spring of 2016, PSRF delivered 173 bags of seeded cultch with ~168,675 Olympia oysters and 5,600 Olympia oyster singles, adding a total of ~174,275 oysters to our restoration sites (Table 2). The seeded cultch Olympia oysters were ~1 mm or smaller with a mean of 3.9 Olympia oysters per Pacific oyster shell (PSRF, unpublished data). Due to the small size of the seed, the seeded cultch was kept in bags and split between the two lagoons (88 bags in LT and 85 bags in KI) until the fall of 2016 when we built the experimental plots and expanded the original footprint of the lagoon restoration beds. The seeded cultch from PSRF was used for our seeded experimental plots, while unseeded Pacific oyster cultch was used for our unseeded experimental plots. The remaining bags of seeded cultch, 72 bags at LT and 69 bags at KI, were spread on and around the restoration beds. For the 5,600 single oysters, we split the sum total of oysters in half, and each half was then added to the existing restoration beds at LT and KI. Finally, to expand habitat and provide more opportunity for recruitment, we added 35 bags of unseeded cultch to the restoration beds in LT and KI.

In 2017, 100 bags of seeded cultch were delivered to LT and KI. Given the known mean of 7.2 *Olympia* oysters per shell from 2017 (PSRF, unpublished data), we estimated that the total 2017 enhancement included ~180,000 oysters (Table 2). The oysters, measuring ~1 mm or less, were split evenly between the two lagoons, kept in bags, and spread on the restoration beds in the fall of 2018. For all plots and beds, we qualitatively noted temporal change in bed perimeter, siltation, and shell movement and loss.

### **Monitoring**

Our project aimed to quantify growth, recruitment, and survival of the reestablished oysters at both the experimental plots and restoration beds. As noted in Barber et al. (2015), there were significant issues with the survival data collected from 2012-2014. Thus, some of these data could not be included in aspects of this current analysis (e.g., survival) but other data could be included (e.g., oyster length). Thus, data from the pilot study years are only included when appropriate. Most of the data utilized in this analysis extend from 2015 through 2019.

Each May, we used 1/16 m<sup>2</sup> quadrats to collect data on emergent substrate volume (i.e., shell) and *Olympia* oyster density and length (mm). The main impetus for data collection of emergent substrate volume was to assist us in determining potential factors that may be driving the settlement and survival of *Olympia* oyster recruits. While we collected the same quadrat data on the experimental plots versus the restoration beds, the survey design differed by reestablishment type.

### *Experimental plots*

From 2016 through present, data collection on the experimental plots consisted of five randomly placed quadrats per seeded and unseeded cultch plots. The five sample locations were generated prior to data collection in ESRI's ArcGIS Desktop 10.6. All emergent substrate and oysters (if present) were collected from each quadrat and the volume of the sample was measured. All oysters were then counted, measured, and all contents of the sample were returned to the original sample area. When sampling the seeded cultch experimental plots, the seed year of 2016

was known and consistent, thus allowing us to track survival of a single cohort. When sampling the unseeded cultch experimental plots, any recorded oysters would likely be recruits to the unseeded cultch.

### *Restoration beds*

Data collection using quadrats on the restoration beds occurred from 2015 through present. In 2015, 2016, and 2017, quadrats were deployed haphazardly within the beds. In 2018 and 2019, we modified our methods to utilize transects laid out along the longest axis of each bed's perimeter. We utilized systematic random sampling to collect quadrat samples along the transects. We started data collection on a random number between 0 - 3 m down the line (using 0.25 m increments) and then sampled quadrats every 3 m from that point until the end of the line.

In addition to the annual monitoring at LT and KI, starting in 2019, we sampled the intertidal restoration beds that were created in 2018 on the SM and SK tidelands. We utilized the same systematic random quadrat sampling along transects to collect data. For these particular quadrats we only collected emergent substrate volume and checked the shell habitat for recruitment.

Detailed descriptions of all methods are found in Hunter et al. (2021).

### **Analysis**

We used shell length to ascertain growth, shell length frequency to look for signs of recruitment and track cohort growth, and oyster density to assess survival.

### *Growth*

Since *Olympia* oysters within the experimental plots originated from the same 2016 cohort, the length data were used to discern mean oyster growth. While we were unable to tag and track growth measurements of individuals, we believe that we can generally describe growth trends by tracking the mean length of a known cohort through time. We used a two-way ANOVA and follow-up Tukey tests to determine if oyster length varied by year and site (SYSTAT 13, Sokal and Rohlf 2012). Despite log transforming

length data, we did not meet the assumptions of normality or homogeneity. ANOVA are considered robust even when the data deviate from a normal distribution (Norman 2010), however, due to the inability to meet the assumption of homogeneity, we opted to adjust the alpha to 0.01 for this analysis to protect against Type 1 error (Keppel 1991).

Restoration bed oyster length data was not used in this growth analysis as a result of being continually reseeded in 2015, 2016, and 2017 (and unable to track individual cohorts).

#### *Recruitment*

We initially intended to use data from the unseeded cultch experimental plots to track oyster recruitment, however, no recruits were recorded during the time period of this publication. While this result is noteworthy, we opted to further explore the possibility of recruitment using length frequency histograms of all measured oysters. Thus, we plotted shell length frequency by site and year to qualitatively investigate for signs of recruitment. Sampling data from both experimental plots and restoration beds were combined in these histograms.

#### *Survival*

We used density data (# oysters / m<sup>2</sup>) from the experimental plots to track temporal change in survival of the 2016 cohort by site. We tested for the effects of site, year, and their interaction on oyster density using a two-way ANOVA and follow-up Tukey tests (SYSTAT 13, Sokal and Rohlf 2012). The assumptions of normality and homogeneity were met for this analysis with untransformed data. Survival rates for each year were calculated by quantifying the change in mean Olympia oyster density per m<sup>2</sup> from the prior year and converting it to a percentage representing the number of surviving oysters from the prior year.

The addition of shell and seed to restoration beds in 2015, 2016, and 2017 meant that density metrics could not be used to accurately assess survival back to 2012 for this oyster bed type. For this reason, only data from 2018-2019 could be utilized for the restoration bed survival metric. Restoration bed density data were analyzed with

square-root transformed oyster counts using a two-way ANOVA (SYSTAT 13, Sokal and Rohlf 2012). Although we met the assumption of homogeneity for the bed density data, we did not meet the assumption of normality. Nevertheless, ANOVA are considered robust when the data deviate from a normal distribution (Norman 2010), so we did not adjust our alpha value for this analysis. Survival rates were calculated following the methods described under the experimental plots survival section.

## **RESULTS**

### **Oyster reestablishment**

Since the initiation of our restoration effort in 2013, the spatial footprint of our native oyster reestablishment project has grown to four experimental plots (bare plots not counted) and six restoration beds by 2019.

#### *Experimental plots*

Each of the four experimental plots (1 seeded cultch and 1 unseeded cultch each per lagoon) began with a shelled area of 4.6 m<sup>2</sup> per plot in 2016. Over the three years since their establishment, the plot area has shifted and spread slightly (LT) or shells have become buried under fine silt (KI). Any shift and spread of shell or shell burial was captured by the measurement of shell volume during our annual sampling. Additionally, the integrity of the true plot boundaries was restored annually.

#### *Restoration beds*

We assessed restoration bed acreage following the last addition of shell to lagoon beds in 2017. Kiket beds KI2016MayorLarry and KI2013Jaws measured 30.9 m<sup>2</sup> and 171.3 m<sup>2</sup>, respectively, while LT bed LT2013Quint was 154.1 m<sup>2</sup> (Table 1). In the two years since any addition of shell, each bed in the lagoons underwent physical changes. In LT, LT2013Quint saw the boundary extend southward as it also experienced a reduced thickness in bed depth. KI2013Jaws saw much of the shell along the eastern edge of the bed become buried. KI2016MayorLarry did not see substantial change between 2017 and 2018, but disappeared completely (no shell was found) between 2018 and 2019.

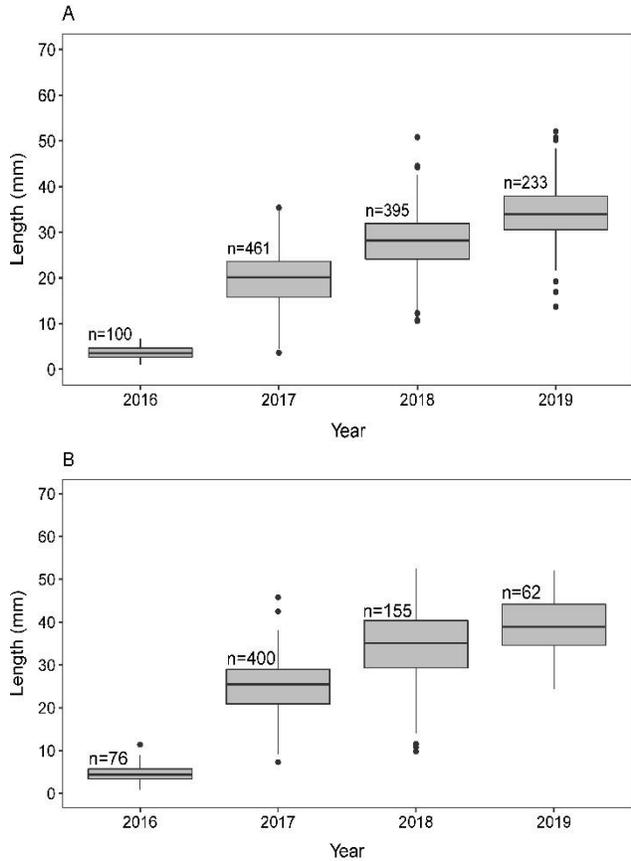


Figure 2: Distribution of experimental plot Olympia oyster shell length (mm) from 2016-2019. A = Lone Tree Lagoon and B = Kiket Lagoon.

Upon creation in 2018, the area of three intertidal restoration beds was the following: Similk Bay, SM2018Ellen (118.4 m<sup>2</sup>); Skagit Bay, SK2018Brody (75.0 m<sup>2</sup>); and Skagit Bay, SK2018Hooper (166.2 m<sup>2</sup>) (Figure 1, Table 1). In the year following the establishment of the intertidal restoration beds, bed morphology qualitatively changed consistently regardless of site. Specifically, all beds experienced a slight increase in area coinciding with a decrease in bed depth.

### Growth

#### Experimental plots

Oyster length differed significantly by site where the LT oysters remained smaller than the KI oysters following the initial seeding year of 2016 (Table 3A, Figure 2). Regardless of site, oyster length increased significantly each year (Table 3, Figure 2, all Tukey HSD pairwise comparisons by year =  $p < 0.01$ ). There was no interaction between site and year (Table 3).

Although mean growth differed each year, the oysters clearly experienced greater growth in their first full year (post-2016 outplanting) than any subsequent years (Figure 2). LT plot oysters grew from a mean length of  $3.66 \text{ mm} \pm 0.13 \text{ SE}$

Table 3: Two-way ANOVA test statistics for the effects of site and year on oyster length (i.e., growth) and density (i.e., survival).

	Type III SS	df	Mean squares	F-Ratio	p - value
<b>A: Oyster length in experimental plots by site and year (2016-2019)</b>					
Site	2.04	1.00	2.04	123.58	0.00
Year	116.80	3.00	38.93	2359.50	0.00
Site * Year	0.12	3.00	0.04	2.47	0.06
Error	30.79	1866.00	0.02		
<b>B: Oyster density in experimental plots by site and year (2017-2019)</b>					
Site	15936.15	1.00	15936.15	41.64	0.00
Year	8254.63	2.00	4127.32	10.78	0.00
Site * Year	604.87	2.00	302.44	0.79	0.46
Error	11099.20	29.00	382.73		
<b>C: Oyster density in restoration beds by site and year (2018-2019)</b>					
Site	69.77	1.00	69.77	6.02	0.02
Year	16.59	1.00	16.59	1.43	0.24
Site * Year	3.24	1.00	3.24	0.28	0.60
Error	509.64	44.00	11.58		

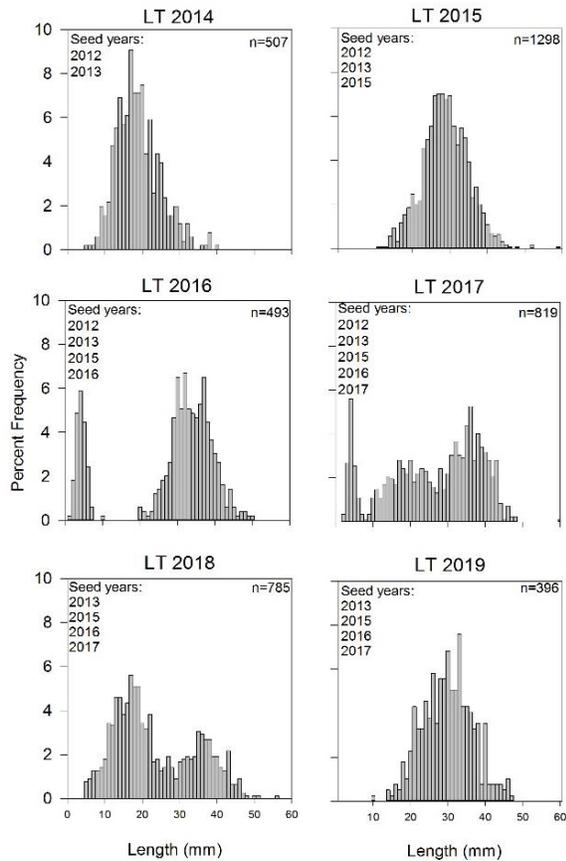


Figure 3: Lone Tree Lagoon (LT) percent frequency and distribution of Olympia oyster length (mm) by year.

( $n = 100$ ) in 2016 to  $19.81 \text{ mm} \pm 0.27 \text{ SE}$  ( $n = 461$ ) in 2017. From 2017 to 2018 the oysters grew an average of  $8.21 \text{ mm}$  to reach a mean length of  $28.02 \text{ mm} \pm 0.31 \text{ SE}$  ( $n = 395$ ) in 2018. The following year the oysters grew an average of  $6.28 \text{ mm}$  from the 2018 mean length to the 2019 mean length of  $34.30 \text{ mm} \pm 0.40 \text{ SE}$  ( $n = 223$ ) in 2019 (Figure 2A).

Similar to LT, the KI oysters exhibited the greatest growth during the initial year, followed by a decrease in the growth rate to 2019 (Figure 2B). Kiket oysters started at a mean length of  $4.6 \pm 0.21 \text{ SE}$  ( $n = 78$ ) in 2016 and grew to a mean length of  $24.92 \text{ mm} \pm 0.31 \text{ SE}$  ( $n = 400$ ) in 2017. From 2017 to 2018, the KI oysters grew an average of  $9.13 \text{ mm}$  to  $34.06 \text{ mm} \pm 0.71 \text{ SE}$  ( $n = 155$ ). Kiket oyster growth then slowed to an average annual rate of  $5.09 \text{ mm}$ , reaching a mean length of  $39.15 \text{ mm} \pm 0.83$  ( $n = 62$ ) in 2019

(Figure 2B). There was no significant interaction term between site and year (Table 3A).

### Recruitment

#### *Experimental plots and restoration beds combined*

We found no evidence of recruitment on the unseeded cultch experimental plots at KI and LT from 2016 to 2019. While single oyster seed were added in 2015, we cannot clearly distinguish this particular cohort (Figures 3 & 4). However, three cohorts (2013, 2016, 2017) are clearly visible in the LT and KI sites in 2017 (Figures 3 & 4). Qualitatively, we see the progression of growth of these three outplanted cohorts (2013, 2016, and 2017) in both lagoons with no clear evidence of new recruitment in the years following seeding (2018-2019, Figures 3 & 4).

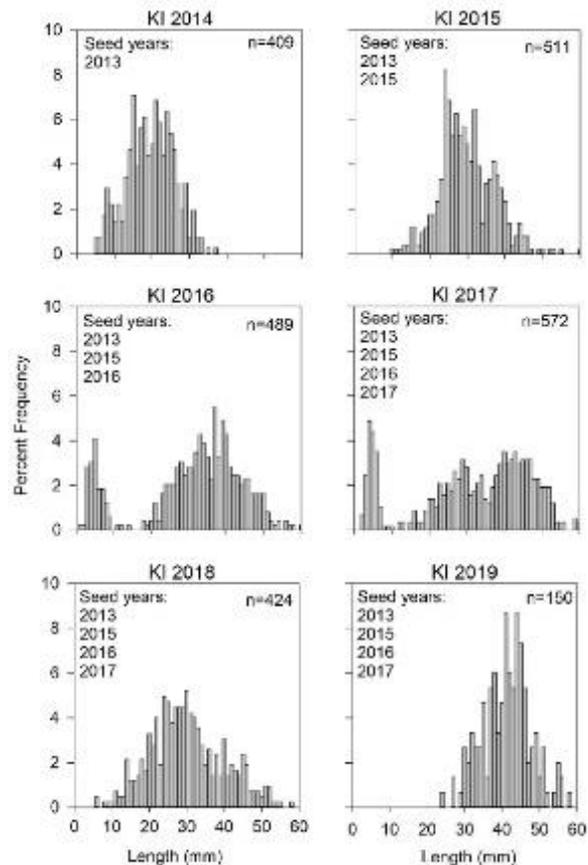


Figure 4: Kiket Lagoon (KI) percent frequency and distribution of Olympia oyster length (mm) by year.

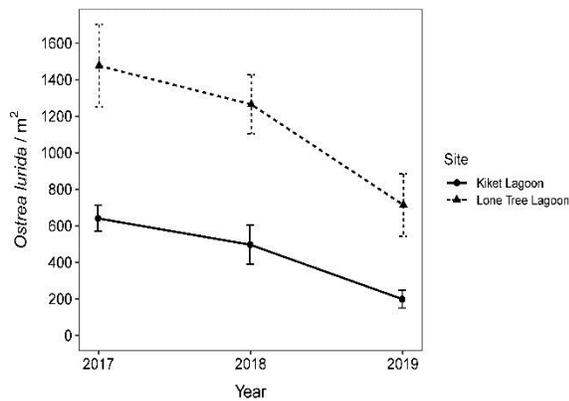


Figure 5: Mean (+/- SE) density of Olympia oysters / m<sup>2</sup> from the experimental plots at Kiket and Lone Tree from 2017-2019.

### Survival

#### Experimental plots

Oyster density decreased significantly by site and year with no significant interaction term (Table 3B, Figure 5,  $p < 0.05$ ). Oyster density, and by proxy survival, was significantly lower in KI than in LT (Figure 5). For both sites combined, oyster density differed from 2017 to 2019 (Tukey HSD,  $p < 0.01$ ) but did not differ from 2017 to 2018 or from 2018 to 2019 (Tukey HSD,  $p > 0.05$ ). Lone Tree oysters exhibited survival rates of 85.7% from 2017 to 2018 and 56.5% from 2018 to 2019. Kiket oysters exhibited a survival rate of 77.5% from 2017 to 2018, followed by a lower survival rate of 40% from 2018 to 2019 (Figure 5).

#### Restoration beds

Oyster density decreased significantly by site but not by year (Table 3C, Figure 6,  $p < 0.05$ ). There

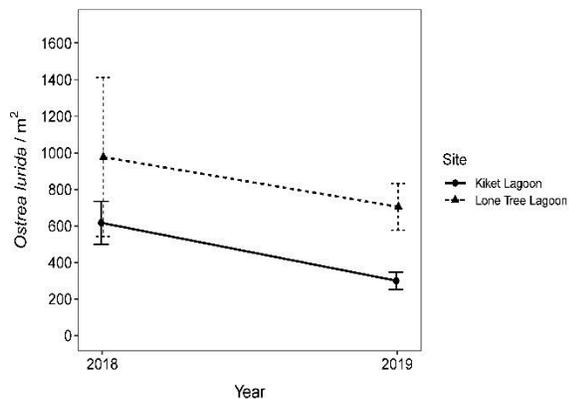


Figure 6: Restoration bed Olympia oyster mean density at Kiket and Lone Tree from 2018-2019.

was also no significant interaction between site and year (Table 3C, Figure 6,  $p > 0.05$ ). Kiket oyster density in 2018 and 2019 was significantly lower than LT oyster density in 2018 and 2019 (Figure 6). While the years were similar to each other, the data exhibit a trend in the decrease in density (and thus, survival) of oysters where KI's survival rate was 40.9% from 2018 to 2019 and LT was 64.9% from 2018 to 2019 (Figure 6).

## DISCUSSION

Our results demonstrate that the newly reestablished populations of Olympia oysters in SK and SM are capable of growing, but natural recruitment and subsequent survival are poor. Importantly, we have demonstrated in previous studies that oysters in these locations are capable of successfully reproducing and that late-stage larvae are present near the restoration sites (Barber et al. 2016; Grossman et al. 2020). Because we know these oysters can grow and reproduce, we hypothesize that our restoration efforts are hindered by the lack of appropriate habitat, lower water residence time, and by the relatively small population size.

### Oyster reestablishment

#### Experimental plots

The two experimental plots in each lagoon changed in shape over the three years since their creation. In LT, the plot shell spread so that the plots became more elongated and narrower in width, likely due to their placement in the lagoon channel. Shell spread to both the north and south, suggesting that the plots were susceptible to strong current flow on both the flood and the ebb tides. Kiket plots, located further into the lagoon from the channel, did not appear to be as susceptible to the current. Instead, these plots appear to have increased levels of sedimentation, as silt and other fines from the channel or lagoon settled out in the same locations as the plots. As a result of the increased sedimentation, some of the deeper (northern) portions of the plots were buried at KI. In the future, it would be advisable to create larger plots, so they might have a better chance of maintaining their integrity. Moreover, larger and thicker plot design may have lessened the sedimentation effects in that the bottom shell layer would have supported a subsequent layer

and offered some protection from deposition of sediment.

#### *Restoration beds*

The observed change of acreage on the various restoration beds was likely due to shell transport (current and/or wave action) and sedimentation (sedimentation is common challenge facing native oyster restoration, Ridlon et al. 2021a). The expansion of LT2013Quint to the south was likely due to tidal currents pushing shell south during the flood. This left LT2013Quint with a lesser height yet slightly larger bed perimeter. Conversely, KI2013Jaws decreased in acreage most likely from burial due to sedimentation on the eastern edge of the bed. Similar to what we suspect occurred with the KI plots, the beds were also located in an area where fine sediments suspended in the water column settled out as the velocity of flow slowed upon entry to the lagoon. Given the disappearance of KI2016MayorLarry, we surmise the northern area of the lagoon is susceptible to strong currents that could have carried the oysters to deeper inaccessible areas of the KI lagoon. Based on these observations, we suggest that future reestablishment projects, if they are situated in perched lagoons, should avoid the channels and adjacent areas. Furthermore, we would ideally determine areas with lower sedimentation rates in the lagoons before outplanting new cohorts of seeded cultch.

The three more recently created intertidal beds (SK2018Brody, SK2018Hooper, SM2018Ellen) increased in perimeter but decreased in thickness. We hypothesize that wave energy on the intertidal beaches was the likely cause of shell dispersal. We also noted a directional spread to the north for both of the SK intertidal beds, indicating these beds were susceptible to a northern longshore drift. This spread of shell may actually be beneficial in that the shell could eventually resemble a more natural bed rather than a manually constructed one with a thickness not typically seen in established beds.

### **Growth**

#### *Experimental plots*

Similar to what Barber et al. (2015) reported in their pilot study report, across all sites and all years, our reestablished *Olympia* oysters grew

each year. While mean oyster length increased significantly each year, the oysters appeared to grow the most during their first year post-outplanting and then continued growing, but at slower rates in subsequent years. Also consistent with what was reported in the pilot study, the KI oysters grew larger than their counterparts at LT (Barber et al. 2015). While the KI oysters appeared to grow faster than LT oysters, there was no interaction term indicating if one year resulted in higher oyster growth by site than another year. Significant oyster growth, regardless of year or site, demonstrates the likely role of environmental factors specific to both sites having an impact on oyster growth. These site differences could be a result of LT experiencing more freshwater input from the nearby Skagit River as well as the ephemeral stream that feeds into the lagoon (Beamer et al. 2006). Overall, our growth results reflect the fact that both sites likely support favorable growing conditions such as available food supply and sufficient salinity and dissolved oxygen (Wasson et al. 2015).

Temporal change in the frequency of oyster length was expected given the numerous years of seeding events. These data clearly demonstrate our ability to discern cohorts when introduced to the system. For example, in 2017, the length frequency histograms at LT show three peaks around 5 mm, 20 mm, and 36 mm presumably representing the cohorts from 2017, 2016, and 2013, respectively (Figure 3). By 2018, it appears the 2016 LT cohort grew to the point of becoming indistinguishable from the 2013 cohort. While we did seed oysters in 2015, this seeding used small single oysters (~20 mm), not seed on large Pacific oyster shell. Sedimentation has been shown to be responsible for higher mortality rates (Wasson 2010), thus we hypothesize that the single shells suffered higher mortality as they were more likely to be buried than their seeded cultch counterparts.

Like LT, KI oysters also had three length frequency peaks in 2017, but at different sizes of 5 mm, 28 mm, and 43 mm representing the 2017, 2016, and 2013 cohorts, respectively. In contrast to LT, the three KI cohorts became largely

indistinguishable by 2018, likely a reflection of the faster initial growth rates observed at KI.

## **Recruitment**

### *Experimental plots and restoration beds*

The apparent lack of recruitment to any of our restoration areas is disappointing and a threat to the long-term success of our restoration project. Although Barber et al. (2015) suggested that our project had potentially recorded signs of recruitment, five additional years of monitoring data have led us to surmise that our previous conclusions about recruitment were incorrect. Recruitment failure is not uncommon in Olympia oysters (Wasson et al. 2016). Indeed, Ridlon et al. (2021a) determined lack of successful recruitment as one of the top three challenges facing native oyster restoration projects along the West Coast. We know that Olympia oysters once historically occupied the SM area (Blake and Bradbury 2012; Kornbluth et al. 2022), which is one of the reasons our region was targeted for restoration. However, reestablishing native oysters in a region where they once existed does not always guarantee success. Possible explanations for lack of recruitment in our region include, but are not limited to: insufficient adult populations and consequently low larval density, low residence time in nearby waters, lack of appropriate habitat, and/or high mortality in recent settlers.

Although we know reproduction is occurring at LT and KI (Barber et al. 2016; Grossman et al. 2020) our adult populations are relatively small and may not be capable of producing high enough densities of larvae for productive recruitment (e.g., Wasson et al. 2015 notes that high adult densities can lead to significant recruitment). In comparison with other successful restoration projects, such as the population in nearby Fidalgo Bay (Dinnel et al. 2009; Dinnel 2018; Becker et al. 2020), the number of adult oysters in SK and SM that are capable of brooding is small. As of 2017, we estimate that we have outplanted a total of ~515,700 oysters between our two sites (Table 2). In comparison, scientists estimate that Fidalgo Bay had just under 3 million Olympia oysters in 2018 (Dinnel 2018). Because there is some evidence that the presence of conspecifics may play a role in successful settlement of larvae

(Becker et al. 2020), the adult populations in SK and SM may not be large enough to produce settlement cues. These rough outplanting estimates also indicate that the LT and KI oyster populations may not be capable of producing large numbers of larvae. Restoration areas with populations exceeding 1 million have been known to experience regular recruitment (PSRF 2017; Dinnel 2018). In contrast, Wasson et al. (2020) demonstrated an absence of recruitment in California's Elkhorn Slough with much smaller populations (~1000 oysters as of 2020). This suggests that we may need to bolster our adult oyster population in order to produce more larvae to improve chances of successful recruitment.

It is also possible that circulation, particularly low residence time, played a role in exporting larvae away from the restoration areas to regions with unsuitable habitat, causing local recruitment failure (Peteiro and Shanks 2015; Pritchard et al. 2016; Wasson et al. 2016). Grossman et al. (2020) found that larvae originating in the LT lagoon were more likely to be retained offshore or at intertidal locations, rather than in the lagoon from which they originated. Larvae were also more likely to be exported from LT during an ebb tide, which could have resulted in dispersal to the west and south into areas of low residence time. However, another possible trajectory would have moved larvae into a small alongshore northward gyre with more a favorable retention time (Grossman et al. 2020). Unfortunately, prior to 2018, the nearshore tidelands just west of LT lagoon lacked hard substrate (i.e., appropriate habitat for settling larvae), limiting chances for successful recruitment if larvae were entrained in this gyre during this current study period.

The lack of extensive suitable habitat in our surrounding embayments is concerning because the presence of appropriate habitat is an important factor in successful recruitment (Hopkins 1937; White et al. 2009a). Research in Fidalgo Bay demonstrated that the majority of new recruits were found in close proximity to the adult population (Dinnel 2018; Becker et al. 2020). Oyster larvae of other species have also been shown to cue into habitat-associated sounds of oyster reefs when attempting to locate suitable habitat for settlement (Lillis et al. 2014). Finally,

Olympia oysters are known to recruit to various types of hard surfaces and not just oyster shell (Groth and Rumrill 2009), but many of the coarse sand and gravel beaches near our restoration sites lack hard surfaces entirely. Thus, our project's presumed limitations in larval density were likely compounded by a lack of suitable habitat. In more recent years (2018 through current), we have transitioned our priority sites for habitat enhancement away from the lagoons to areas in the nearshore which are known to be in close proximity to competent larvae in the water column (Grossman et al. 2020). A clear need for future population expansion in our region is to increase suitable hard bottom habitat, ideally in concert with increasing adult populations.

Finally, the possibility remains that larvae did manage to recruit and then subsequently experienced high mortality due to reasons such as thermal stress, low salinity, or predation (Baker 1995). Hopkins (1937) demonstrated that Olympia oyster larvae tend to settle higher in the intertidal zone where larvae are likely more susceptible to temperature extremes. Additionally, SK and SB are known to have higher surface temperature and lower salinity than other Puget Sound sub-basins (Babson et al. 2006). These environmental characteristics are due in part to the proximity to the mouth of the Skagit River, the largest freshwater source in Puget Sound. The river is also responsible for discharging large amounts of sediment, which can be detrimental to Olympia oyster survival, particularly in the juvenile stages (Wasson 2010). Finally, predators such as whelks and crabs could have reduced populations of early settlers.

We hypothesize, however, that mortality of recently settled recruits is unlikely to be the main issue facing our restoration project. It is likely that at least a few recruits would have survived in the areas of our experimental plots (always inundated) or restoration beds (lower in elevation, less subject to temperature extremes). Predator populations are certainly present, but not in particularly high numbers and we rarely see signs of predation on adults (Swinomish Fisheries, unpublished data). Thus, we conclude that small adult populations, subsequent low larval density, and the absence of habitat are the

major factors limiting success of our restoration project.

### **Survival**

It is not surprising that oyster density, used as a proxy of survival, declined when we found no evidence of recruitment to replenish the population. Results will be discussed as a singular issue of what may be causing the decline in density regardless of site or oyster bed type.

Olympia oysters do not have a known maximum lifespan, but it has been suggested they may live over 10 years (Couch and Hassler 1989; Baker 1995). The majority of our oysters, however, are on a trajectory for much shorter lifespans. The mortality of newly outplanted oysters seen in our experimental plots in our first year of data collection was consistent with previously reported mortality rates in nearby areas (85.7% survival at LT and 77.5% at KI compared to 77.3% in the early years of Fidalgo Bay restoration) (Dinnel et al. 2005). Our data were also in agreement with mortality rates recorded in year one oysters (9.6% - 28.2%) elsewhere in the region (Gibson 1974). However, our mortality rates were higher than what the literature reported for >2 year old oysters (2018 – 2019 data) in both the experimental plots and the restoration beds. We believe environmental conditions including dynamic hydrology, salinity, temperature, sedimentation, and/or lack of habitat could be responsible for the increased mortality rates in adults. As discussed in the recruitment section, predation does not seem to significantly impact survival rates of adults at our sites.

We initially suspected that shell dispersal, due to hydrological forces, was reducing oyster density, not poor survival. Indeed, strong currents in the plot and bed locations likely moved seeded cultch and resulted in decreased shell volume on the beds (Swinomish Fisheries, unpublished data). However, although shell volume decreased each year, regardless of site, we do not think this loss in volume played a significant role in survival rates because the shell loss rate did not correlate with the decrease in oyster density (Swinomish Fisheries, unpublished data).

One possible explanation for our lower survival rates could be the relatively low salinities found at KI (9.3 - 25.6) and LT (15.3 - 26.9) (Barber et al. 2016). While the lower salinity ranges at these sites could be tolerated by Olympia oysters, salinities below 25 have been shown to negatively affect oyster size and recruitment (reviewed in Wasson et al. 2015). Prolonged exposure time to even the fringes of these salinity conditions can also act as a stressor (Wasson 2010). This indicates that the KI site, which experiences prolonged instances of salinities under 20 (Barber et al. 2016), could be a more stressful salinity environment for these oysters.

Water temperature could also be another contributing factor to the lower survival. While KI and LT populations are always inundated regardless of tide height, some oysters at the fringes of the beds are located in shallower water and at low tide have less of a buffer to protect them from extreme summer and winter temperatures. Temperatures below 10 °C and above 38 °C are known to have negative effects on Olympia oysters (Hopkins 1937; Davis 1955; Wasson 2015). Our temperature range of 9.2 - 29.3 °C (combined for LT and KI) is within the margin to stress the animal.

While temperature and salinity are likely to have an impact, we suspect it may be minimal. Instead we believe sedimentation is the predominate driver of high mortality rates. Sedimentation has been shown to be a significant cause for decreased survival at other sites and is one of the top three challenges facing restoration projects of this species (as reviewed in Ridlon et al. 2021a). Our sites, particularly KI where survival was lower, demonstrate characteristics that suggest sedimentation is taking place. The KI lagoon contains a high percentage of fine sediments and experiences low current flow (SITC Fisheries, unpublished data). Specifically, we observed KI oysters both sinking into the substrate over time and being buried into an anoxic sediment layer by the deposition of fine sediments over the top of the plots and beds.

### **Synthesis**

We have demonstrated that Olympia oysters sourced from hatchery seed are able to grow and

reproduce in the waters of northern Whidbey Basin. However, the concerning lack of natural recruitment has forced us to reevaluate our long-term restoration plans and consider alternative locations and restoration methods. Perhaps the water's residence time at our selected restoration sites is too low and we should focus on areas with higher residence time. For example, we assume that the terminal end of elongate embayments like Turners Bay or northern SM would have these longer residence times. Even if we refocus efforts on new project areas, there is still a clear need to increase local source populations and expand habitat. Indeed, recent work by Ridlon et al. (2021b) highlights our region in Whidbey Basin as one of the top 10 sites that may benefit more from the rewards of conservation aquaculture over the risks. With conservation aquaculture in mind as a potential back-up tool, we first propose attempting to minimize the risks associated with conservation aquaculture (as reviewed in Ridlon et al. 2021b) by adopting unique strategies to (1) increase adult populations, (2) increase optimal settlement habitat in the lower intertidal, and (3) broaden the spatial scale of the restoration area. As we continue towards our goal of restoring self-sustaining populations of Olympia oysters in northern Whidbey Basin, we will continue to monitor the status of our restored beds and employ novel restoration techniques.

### **Next Steps**

Beginning in 2020, we identified specific methods to address our three primary strategies to booster restoration success. To (1) increase adult populations, we have implemented a plan to transfer Olympia oyster recruits from a nearby successful restoration area (Fidalgo Bay) to SK and SM. In the spring of 2019, bags of unseeded cultch were placed in the Fidalgo Bay intertidal at known areas of high recruitment. These bags were collected post-larval settlement, washed and inspected to avoid spreading potentially harmful species [e.g., green crab (*Carcinus maenas*)], and placed strategically in SM and SK (per Grossman et al. 2020). Our 2020 pilot year resulted in the addition of ~57,000 oysters to restoration beds in SM and SK. Unseeded cultch was also placed at these areas to (2) increase optimal habitat in the lower intertidal. Lastly, Swinomish Fisheries is undergoing the process of evaluating

environmental parameters of nearby tidelands suitable for new beds to (3) broaden the spatial scale of our restoration area. We expect that it will take several years before we will know if these strategies will be effective at increasing local populations. If these expansion strategies prove ineffective, we will switch efforts to conservation aquaculture with our partners at PSRF, and continue to prioritize this important region for native oyster restoration.

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