OLYMPIA OYSTER, *OSTREA LURIDA*, PILOT PROJECT IN NORTHERN PUGET SOUND, WASHINGTON: 2014 MONITORING REPORT

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SWINOMISH INDIAN TRIBAL COMMUNITY

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ABSTRACT

Historically Olympia oysters, *Ostrea lurida*, played an important economic, ecological, and cultural role as Washington's only native oyster. Yet due to overexploitation, loss of habitat, and other human-related factors, only ~5 % of the once-known beds remain in Puget Sound. In 2012 and 2013, the Swinomish Indian Tribal Community began a small-scale Olympia oyster restoration effort in two pocket estuaries (lagoons). Our intent was to eventually establish self-sustaining populations that could act as larval sources for additional sites in northern Puget Sound. The primary goals during this pilot project phase were to quantify survival and growth of the outplanted seed by site and seeding year in order to determine if one, or both, of the lagoons could serve as an optimal location for further restoration work. Relatively high survival rates in both lagoons were qualitatively observed, although survival appeared to decline slightly with an increase in barnacle recruitment. Oysters in both lagoons grew faster than oysters in a different restoration site in northern Puget Sound and the oysters in one lagoon grew faster in the spring while oysters in the other lagoon grew faster in the summer. Finally, our length frequency data indicated that spawning and recruitment may have occurred in the lagoons during the summer of 2013. Our data suggest that both pocket estuaries are viable sites for Olympia oyster restoration. As a result, the tribe will expand research and restoration endeavors within the two lagoons; these efforts will include the development of baseline physical and biological parameter datasets that will allow us to determine the status of the restoration project and assess the need for adaptive change through time.

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

INTRODUCTION

Oyster reefs have declined worldwide as a result of overexploitation, loss of habitat, disease, and environmental degradation or mismanagement (Kirby 2004, Brumbaugh et al. 2006, Grabowski & Peterson 2007). Specifically, an estimated 85% of oyster reefs have been lost on a global scale despite the importance of these organisms as ecosystem engineers that filter water, provide structured habitat for the associated ecological community, and stabilize shorelines (Jackson et al. 2001, Brumbaugh et al. 2006, Beck et al. 2011). Because fishing practices for oysters typically involves the removal of their habitat (shell), overfished reefs and their related communities have struggled to recover even after cessation of fishing pressure (Jackson et al. 2001, Trimble et al. 2009). Over the past several decades oyster restoration efforts have grown in popularity as mostly developed nations attempt to reestablish their fisheries as well as the ecosystem services these bivalves once provided estuarine communities (Jones et al. 1994, Brumbaugh et al. 2006, Coen et al. 2007).

Prior to European settlement along the west coast of North America, the only oyster found in the area was the native or Olympia oyster, *Ostrea lurida*. Despite the small size of this species (mean shell length is 35 – 45 mm), it played a large role in the economic, ecological, and cultural history of the North American west coast (Steele 1957, White et al. 2009). In the Puget Sound region of Washington State, shell middens dating back over 4,000 years contained large numbers of Olympia oysters, indicating that this species was utilized by coastal tribes as an important food source and possibly for commerce (Steele 1957, Hurst 2003, Blake & Bradbury 2012). Exploitation of native oyster reefs by European colonizers began in San Francisco in the early 1800s and harvests expanded northward to Oregon and Washington (Kirby 2004). By the early 1900s the fishery nearly extirpated oyster beds in Puget Sound while the remaining beds were further stressed by severe water pollution (Dinnel et al. 2009, Blake & Bradbury 2012). Demand for oysters, however, did not deteriorate with the decline of Olympia oysters and attention turned to cultivating non-native species in the region. Currently, cultivated *Crassostrea gigas* (the non-native Pacific oyster) is the most common oyster found in Washington while only ~5 % of native oyster beds (circa 1850) remain in Puget Sound and Olympia oyster habitat in many areas along the west coast is considered functionally extinct (Blake & Bradbury 2012, zu Ermgassen et al. 2012).

In response to a growing concern about native oyster populations in Puget Sound, the Washington Department of Fish and Wildlife (WDFW) developed the Olympia Oyster Stock Rebuilding Plan (Cook et al. 1998, Blake & Bradbury 2012). This plan identified 19 sites throughout Puget Sound as target restoration locations for rebuilding oyster reefs with the goal of creating large, self-sustaining source populations (Blake & Bradbury 2012). Most restoration efforts to date have taken place in south and central Puget Sound by the non-profit organization Puget Sound Restoration Fund (PSRF). Until 2012, however, northern Puget Sound only had one active restoration site in Fidalgo Bay. The success of the Fidalgo Bay efforts (e.g., Dinnel et al. 2009) encouraged PSRF and WDFW to attempt expansion of restoration work into other target sites in the northern Sound.

The Swinomish Indian Tribal Community (SITC) is located in northern Puget Sound, an area that traditionally supported extremely large (e.g., ≥ 2,000 acres in Samish Bay) beds of *O. lurida* (Blake & Bradbury 2012). The tidelands of the Swinomish Reservation extend into Similk Bay, one of the priority restoration sites identified by WDFW due to the high probability that this historic native oyster population functioned as a source population for other beds in the area. In 2012 and 2013, PSRF provided SITC with the seeded cultch necessary to initiate a pilot restoration project at two pocket estuaries on the reservation in Skagit and Similk Bays. These seeded cultch were placed in flowing channels that remain inundated throughout all tidal cycles within both estuaries. This would have provided the seed with an ideal habitat since Olympia oysters are sensitive to freezing temperatures and siltation. Our primary goals during this pilot project were to quantify survival and growth of the outplanted seed by site and seeding year in order to determine if one, or both, of the lagoons could serve as an optimal location for further restoration work. Once outplanted, we also

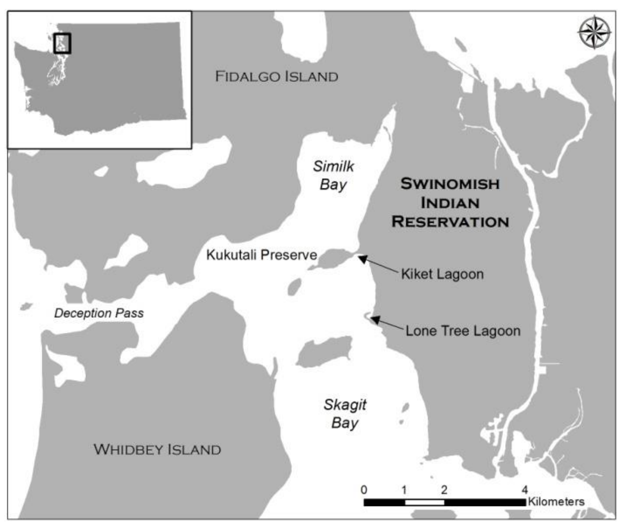

Figure 1: Location of Lone Tree and Kiket lagoons in Skagit and Similk Bays.

examined the cultch for evidence of oyster recruitment.

METHODS

In 2012 we divided and placed 21.5 bags of seeded cultch, containing approximately 91,660 individual Olympia oysters in the Lone Tree (LT) and Kiket (KI) lagoons (Figures 1 and 2). In 2013, 24 and 26 bags with a combined total of ~56,000 individual oysters were placed in the LT and KI lagoons, respectively. All seed was provided to SITC by PSRF. The seeded cultch was outplanted in August 2012 or in June 2013. For both years the seeded cultch was kept in bags over the winter to provide some protection against desiccation, weather, and predation. Cultch at each lagoon was spread into single 2*2 m plots the following spring such that at the end of the 2013 summer each lagoon had approximately 8 m^2 of seeded cultch (4 m^2 of 2012 seed and 4 m^2 of 2013 seed).

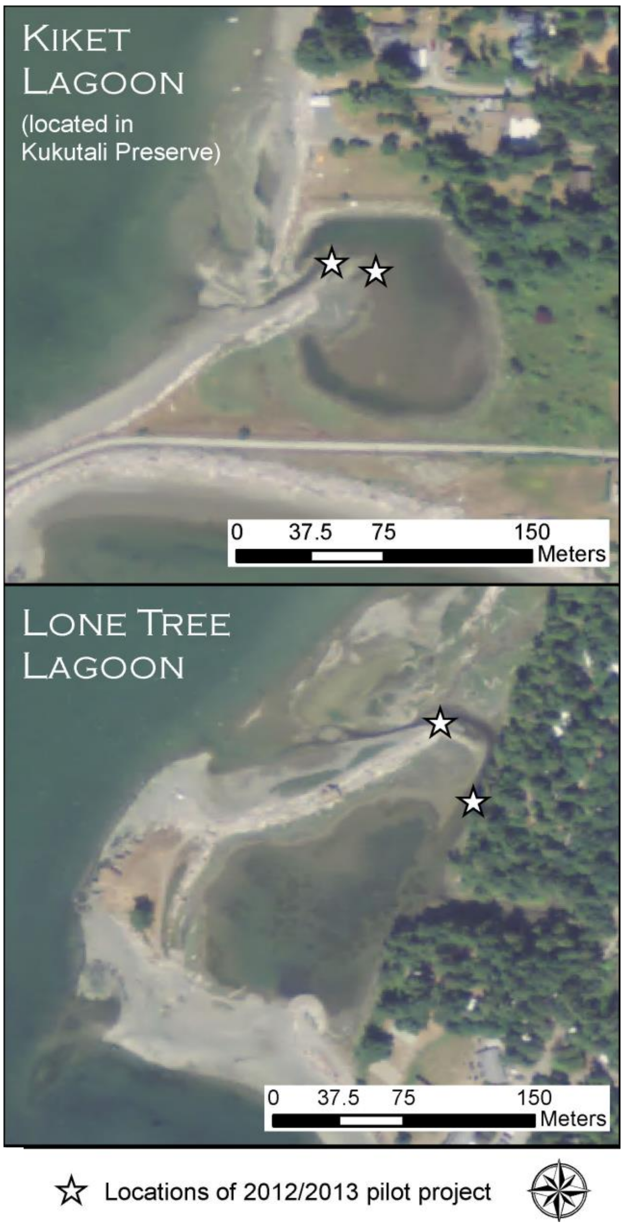

Figure 2: Specific locations of the Olympia oyster pilot project within the pocket estuaries.

We used data from the PSRF hatchery to determine the mean number of Olympia oyster seed per shell for the 2012 seed prior to outplanting the seed. Because no hatchery data were provided for the 2013 seed, we estimated the mean number of seed per shell approximately two weeks following the dispersal of the seeded cultch in the lagoons. A "shell" was defined as a single *C. gigas* valve or several *C. gigas* valves fused together. We did not record the length of the *C. gigas* valves.

In order to assess survival and growth of the 2012 seed, we gathered data from haphazardly selected bags at each site in May 2013. Ten bags were sampled and within each bag we haphazardly gathered 10 shells; thus, 100 samples were gathered at each site (LT and KI). For each shell the length of all living oysters was recorded. During the winter following the 2012 seeding, the KI cultch was presumably moved by strong storms to inaccessible depths in the lagoon. Thus, 2013 sampling of the 2012 seed was only completed at the 4 m^2 LT2012 plot. Sample size was reduced to 10-15 haphazardly selected shells in subsequent sampling periods.

The 2013 seed was outplanted in late June; seed size and abundance were recorded in early July 2013. Because the seeded cultch needed to remain in the bags for the upcoming winter, we collected data from three shells per bag at each site. In April 2014, we returned and sampled three shells per bag at each lagoon (not all bags were initially recovered at KI). Data were collected on the same parameters described for the 2012 seed. 2013 cultch was removed from the bags and spread into one 4 m^2 plot in April or July for LT and KI, respectively.

Analysis

For each sampling period, we used the estimated number of shells added to the lagoons (~250 shells per bag from PSRF's hatchery) and the mean number of oysters per shell to calculate the total number of living oysters by seed year. Survival could not be estimated for the 2012 KI seed because these oysters had been most likely moved by a storm to unreachable locations in the lagoon. Once the data were plotted, it became clear that our method for estimating survival was flawed (survival appeared to increase for two out of the three measured populations). No statistical analysis was conducted; rather, revised methods for sampling in the future will be discussed.

Table 1: Two-factor ANOVA results on Olympia oyster length by site and sampling month.

	SS	df	MS	F	*p*
Site	1,134.52	1	1,134.52	22.29	< 0.00
Sample month	71,191.37	3	23,730.46	466.245	< 0.00
Site * Sample month	1,342.52	3	447.506	8.792	< 0.00
Error	75,989.24	1,493	50.897		

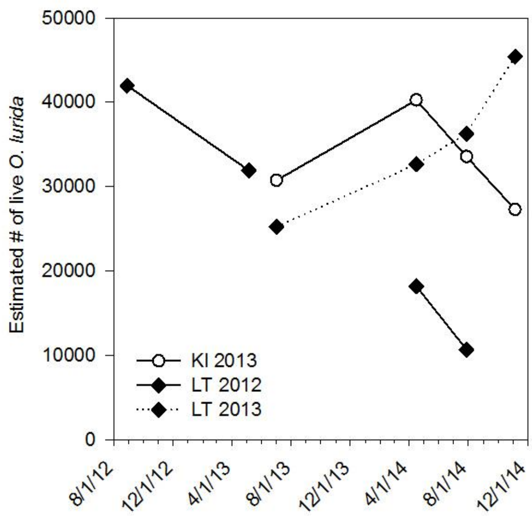

Figure 3: Olympia oyster survival from 2012-2014. KI = Kiket Island and LT = Lone Tree. *Please note the discussion section's description of some problems associated with these data.

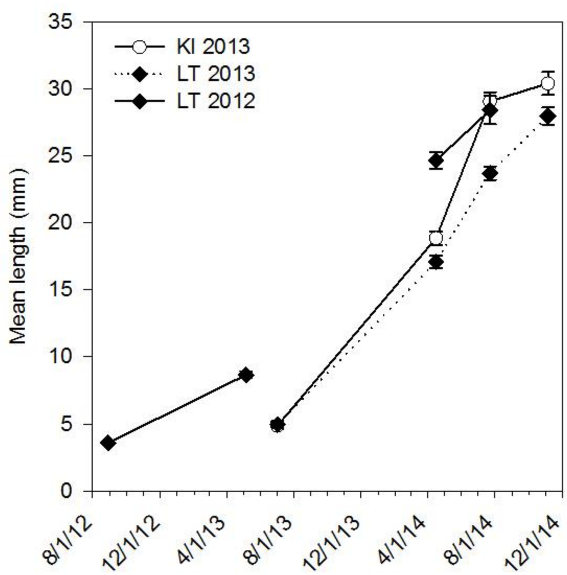

Figure 4: Mean (+/- SE) Olympia oyster growth from 2012-2014. KI = Kiket Island and LT = Lone Tree.

We used a two-factor ANOVA with follow-up Tukey tests to look for possible differences in mean oyster length by site and time for the 2013 seed. A one-way ANOVA with follow-up Tukey tests was used to examine growth data from the LT2012 seed. Data from August 2012 were not used in this analysis because we only had an estimate of mean length from the PSRF hatchery and no raw data; thus, this analysis only included data from the months of May 2013, April 2014, and July 2014. We separated the analyses by year seeded because the LT2012 seed were located in a different area of the lagoon and were never in contact with the LT2013 seed. An adjusted alpha of 0.01 was used for both analyses because, even with transformations, we could not meet the assumptions of an ANOVA (Keppel & Wickens 2004).

Finally, we examined differences in the length frequency distributions using the non-parametric Kolmogorov-Smirnov (KS) test for three different sampling periods (April 2014, July 2014, and November 2014) for both seed years. We used a Bonferroni-adjusted alpha value if multiple pairwise comparisons (KS test) were conducted on the frequency data (Sokal & Rohlf 1995). Lone Tree 2012 seed were not measured in November 2014. These length frequency data were also plotted in histograms to allow for a qualitative assessment of the possibility of recruitment in 2013. We determined that spat <10 mm in size on the 2012 and 2013 seeded cultch would be unlikely one year following the outplanting. Thus, if we recorded the presence of seed <10 mm in July 2014, the seed was potentially the result of natural recruitment.

SYSTAT 13 was used for all data analyses.

RESULTS

The survival of the KI2013 oysters increased initially and then declined, while LT2013 survival increased with each sampling period (Figure 3). The LT2012 results show an overall decline in survival over time (Figure 3). Plausible reasons for these results will be discussed.

Despite potential problems with our quantitative survival data, we qualitatively noted that the LT2012 seed survival appeared to have declined somewhat over the years. This decline may have occurred concurrently with

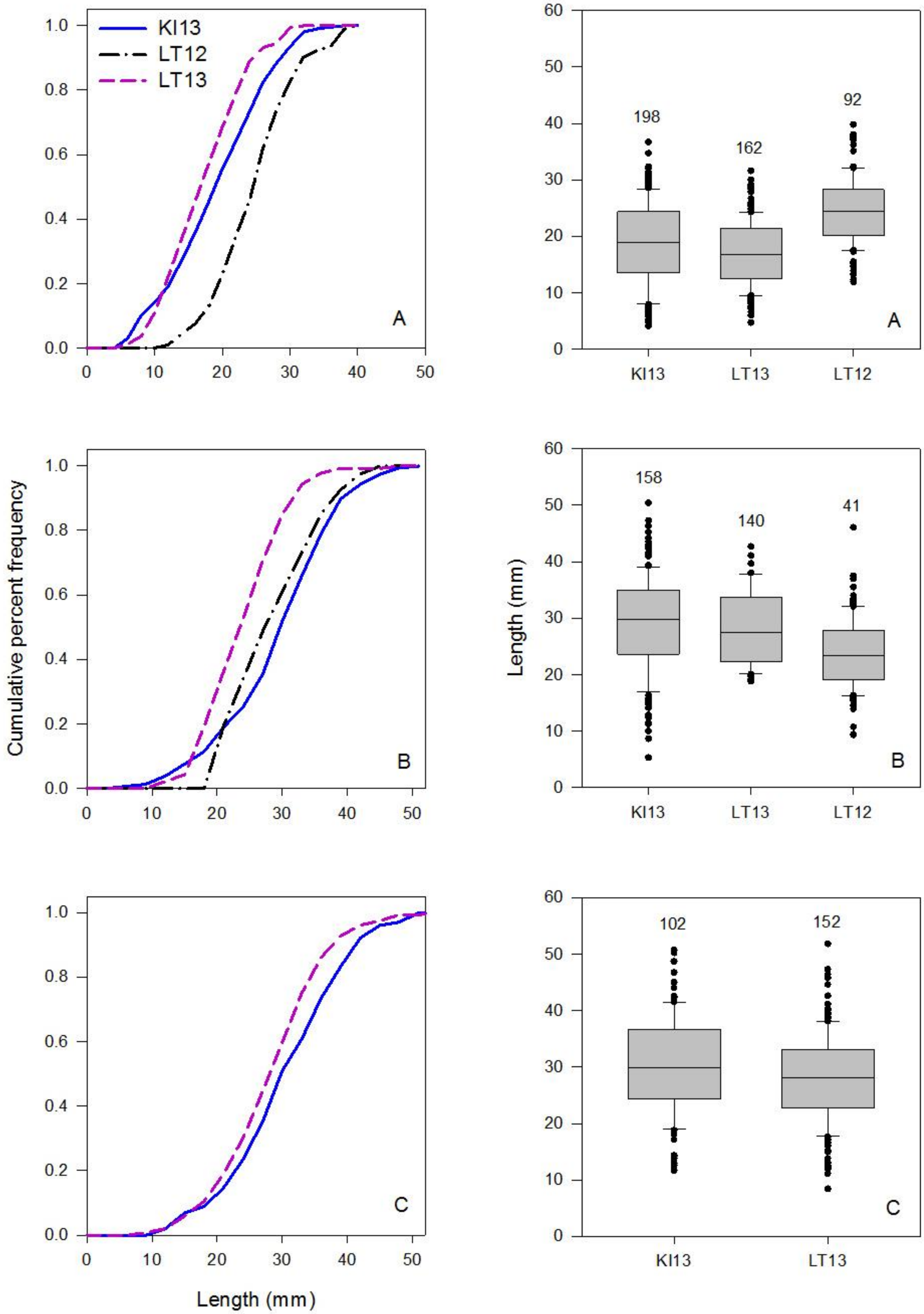

Figure 5: Cumulative percent frequency (left) and distribution of Olympia oyster length by site and sampling month. A = April 2014, B = July 2014, and C = November 2014. Median = solid line in box plots. KI = Kiket Island and LT = Lone Tree. Numbers in box plots represent sample size.

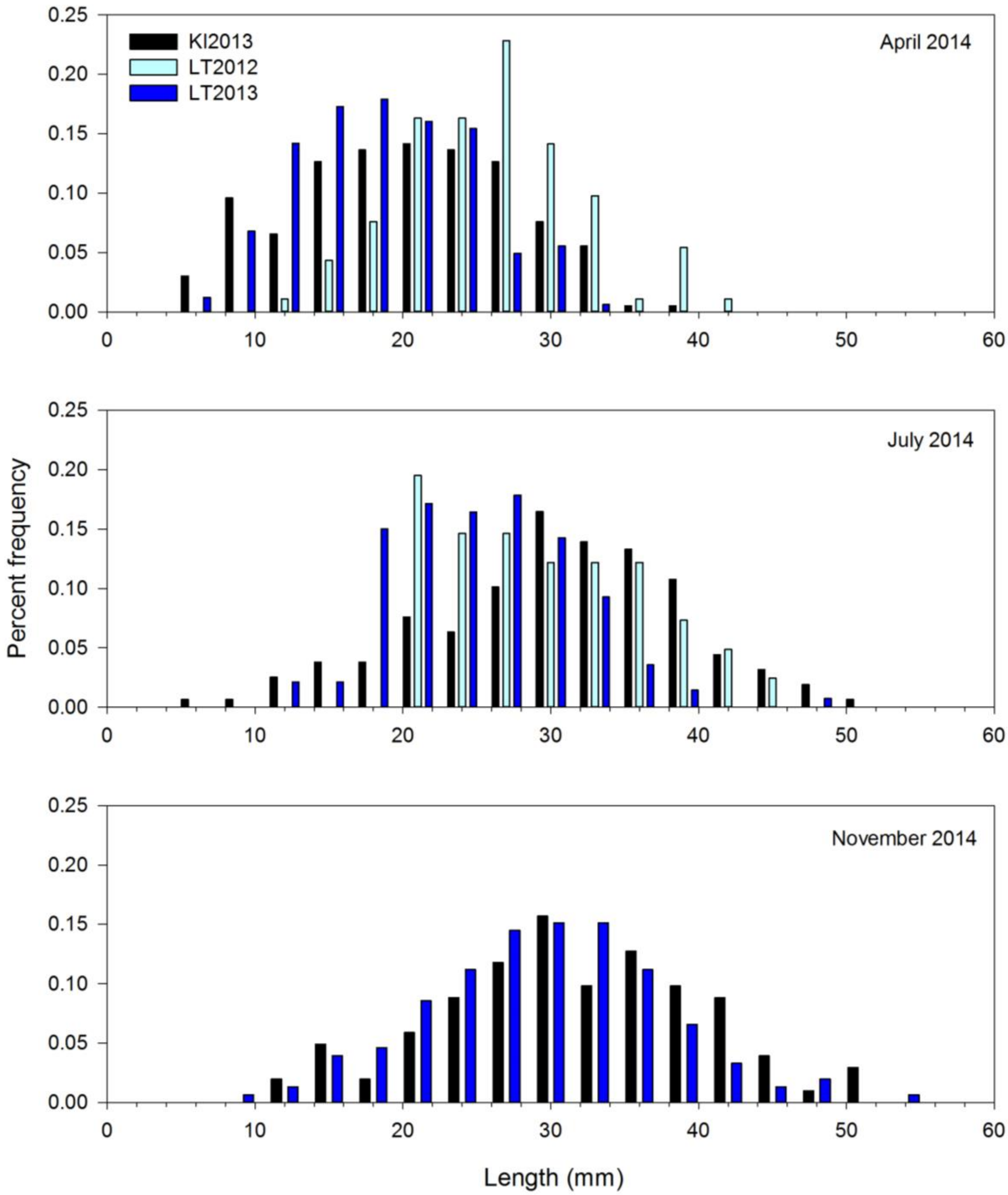

Figure 6: Percent frequency of Olympia oyster length by site and sampling month. KI = Kiket Island. LT = Lone Tree.

high barnacle recruitment and survival on the cultch in 2013. The 2013 seed survival from both locations appeared to be high and no significant barnacle recruitment was noted on the cultch in 2013 or 2014.

All 2013 seed grew from July 2013 to November 2014 and the KI seed grew faster than the LT seed (Table 1, Figure 4). Significant interactions (Tukey test, $p < 0.000$, for all) were found for all 28 possible follow-up comparisons except July 2013 KI vs. LT, April 2014 KI

Table 2: Kolmogorov-Smirnov results comparing length frequency distributions of oysters by site and seeding year.

	April		July		November	
Bonferroni *p*-value	0.016		0.016		0.05 (not adjusted)	
	D	*p*	D	*p*	D	*p*
KI2013 vs. LT2012	0.357	< 0.000	0.146	0.502		
KI2013 vs. LT2013	0.160	0.021	0.366	< 0.000	0.162	0.082
LT2012 vs. LT2013	0.485	< 0.000	0.278	0.017		

Blank spaces indicate that no analysis was completed for a particular comparison

vs. LT, July 2014 KI vs. November 2014 KI, July 2014 KI vs. November 2014 LT, and November 2014 KI vs. LT. The 2012 seed from LT grew significantly in the three tested months ($F_{2, 369} = 533.2$, p = < 0.000). May 2013 oysters grew from a mean length of 8.7 ± 0.26 SE mm to a mean length of 24.7 ± 0.62 mm in April 2014 (Tukey test, p < 0.000, Figure 4). From April 2014 to July 2014 the oysters grew to 28.4 ± 1.4 mm (Tukey test, p < 0.000, Figure 4). LT2012 seed measured in May 2013 were smaller than the LT2012 seed measured in July 2014 (Tukey test, p < 0.000, Figure 4).

In 14 months the LT2012 seed grew an average of 19.7 mm to a mean length of 28.41 mm ± 1 SE (n = 41) for a two-year old oyster. The LT2013 seed grew an average of 22.9 mm in 15 months to a mean length of 23.7 mm ± 0.5 SE (n = 140) and the KI2013 seed grew an average of 25.5 mm to a mean length of 30.4 mm ± 0.86 SE (n = 102) for ~18 month old oysters. Of particular interest, KI2013 seed grew an average of 10.2 mm from April 2014 to July 2014, while the LT2013 seed grew an average of 6.6 mm during the same time period.

In April 2014, the LT2012 seed were significantly larger than the 2013 seed from LT or KI (Table 2, Figures 5 and 6). The 2013 seed from LT and KI were similar to one another in terms of length frequency distributions in April 2014. By July 2014, the KI2013 length frequency distribution was the same as LT2012 seed, whereas the LT2013 seed were still significantly smaller in length from the KI2013 seed (Table 2, Figures 5 and 6). November 2014 revealed no difference between KI2013 and LT2013 seed (Table 2, Figures 5 and 6).

DISCUSSION

Our qualitative and quantitative results suggest that Lone Tree and Kiket lagoons are viable sites for Olympia oyster restoration. In addition to encouraging survival and growth observations, these lagoons offer firm substrate and high potential for the expansion of restoration projects within and around the lagoons.

Allen et al. (2015) assessed Olympia oyster survival by measuring changes in mean oyster density while Dinnel et al. (2009) successfully calculated survival by counting live and dead oysters on cultch. Because we received our oysters from PSRF while they were very small (< 5 mm mean length), we opted to retain the oysters in the shell bags in order to increase survival during the oysters' first winter. Thus, we did not obtain initial density estimates since the seed were not spread in the lagoons. We did, however, calculate the mean number of Olympia oyster seed per shell based on data provided by the PSRF hatchery or on data that we collected when the bags were placed in the lagoons. Our definition of a "shell" likely caused many of the obvious problems seen with our results (i.e., increases in survival are unlikely when measuring the same cohort through time, Figure 3). Our definition of a "shell" was not standardized because: (1) a "single" shell could also be several valves fused together, and (2) Pacific oyster valves vary greatly in length. This lack of standardization likely invalidates our survival estimates.

It is possible, of course, that our definition of a "shell" was not the problem with these data. The increase in survival of the 2013 seed could have been due to an initial poor estimate of the number of living oysters, while the data following the outplanting were more accurate. Another explanation for the recorded increase could be that we misidentified and counted newly settled false jingles (*Pododesmus macroschisma*) as Olympia oysters. Yet, this is not likely as we were field-trained by an expert (P. Dinnel, personal communication) in identifying species that look similar to newly settled Olympia oysters. Finally, new oyster recruitment could easily explain at least some of the increase in "survival" because we may have counted new recruits as part of the older cohort. In 2014 we did note the presence of <10 mm oysters on the 2012 and 2013 cultch. If the older 2012 seed spawned in 2013 or even early in 2014, the <10 mm individuals on the 2013 cultch may have been recruits from that spawning event. While it is plausible that these "recruits" may partially explain our results, based on our observations it is unlikely that the recruits would fully account for the survival increase. It is more likely that our data represent a combination of possible new recruitment and the lack of standardization in *C. gigas* valve size.

In the future, we will use mean oyster density and the area of the oyster bed to estimate the total number of live *O. lurida* in an area. If future seed needs to remain in growout bags, we will record initial oyster length for growth data but density will not be estimated until the seeded cultch is spread. Future methods will incorporate the use of 1/16 m^2 haphazardly-placed quadrats. All cultch will be collected within the quadrat and Olympia oysters will be counted and measured. The volume of emergent habitat (i.e., oyster shell) will also be recorded.

Although our quantitative data were clearly problematic, our qualitative observations were sufficient for the purposes of this pilot project. The minor decline in LT2012 seed survival may have been due to the significant barnacle settlement that occurred on the cultch and the increased competition for space (Trimble et al. 2009). The 2013 seeded cultch from both sites did not experience high barnacle settlement, perhaps explaining why we did not observe any obvious declines in oyster seed survival. Based on the size of the barnacles on the 2012 cultch, the barnacle set occurred in 2013 but prior to the placement of the 2013 seeded cultch. Overall, our observations demonstrate that native oyster survival is likely to be very high in these locations, especially during years where barnacle settlement is low.

It is not surprising that the oysters grew significantly from the time of outplanting to November 2014. It is interesting, however, to note that the KI2013 seed grew larger more quickly than the LT2013 seed, although by November 2014 the LT seed were similar in size to the KI seed. One plausible explanation for the slower initial growth in the LT seed is the fact that this particular lagoon receives more freshwater input (from an ephemeral stream and the Skagit River) than the Kiket lagoon (Beamer et al. 2006, S. Grossman, personal communication). Indeed, Wasson et al. (2014) found reduced growth rates in Olympia oysters that were exposed to lower salinities. Freshwater input is likely to be higher in the spring at this lagoon than at KI; perhaps oyster growth rates only increase at LT when the salinity increases toward mid-summer (as a result of decreased flow from Lone Tree Creek and the Skagit River). We aim to quantify differences in lagoon water properties during the next few years.

Dinnel et al. (2009) observed that Olympia oysters in Fidalgo Bay reached 35-45 mm in three years of growth. He found that one particular cohort of oysters grew approximately 15.6 mm in 15 months, or approximately 1.04 mm per month. We have found slightly faster growth rates in Lone Tree and Kiket lagoons of ~1.5 mm per month. This makes sense because the oyster beds in the lagoons are consistently inundated with water in the channels, whereas the beds in Fidalgo Bay are exposed to low tides on a regular basis. Thus, the LT and KI oysters have the ability to feed at all times and are exposed to fewer stressors such as temperature change. Using our growth rate calculation, we estimate that the Olympia oysters at LT and KI could reach ~55 mm within a three year growth period.

We expected to find differences in the length frequency distributions of the oysters depending on their seed year, and we did find this difference during the spring 2014 sampling where the LT2012 seed were larger compared to the 2013 seed from both sites (Figure 5). While not statistically significant, the KI2013 seed had broader length distributions in all 2014 sampling periods (Figure 5), possibly explained by the potential new recruitment and the faster spring growth of the Kiket oysters. As mentioned previously, we speculate that increased competition for resources from barnacles on the LT2012 cultch may have contributed to the fact that the KI2013 and LT2013 seed were similar in length frequency distribution to the LT2012 seed by the summer of 2014. Differences in water properties by lagoon may have also contributed to the recorded lag in spring growth in the LT2013 seed. Regardless of the LT2013 growth lag, both lagoons have shown great potential to provide prime habitat for favorable growth in Olympia oysters; this result is encouraging for the expansion of restoration efforts in both areas.

Based on our growth and length frequency data, we determined that none of the oysters in the lagoons should have been <18 mm in size by the spring of 2014. Recruitment is one of several possible reasons for the presence of small (<10 mm) seed found on the 2012 and 2013 cultch (Figure 6). Olympia oysters are known to mature within 5-6 months (Baker 1995) and the 2012 cultch would have been capable of reproducing in the summer of 2013. The 2013 seed, however, were approximately three months old in July of the same year and should not have been capable of reproduction during the 2013 summer. Importantly, the 2013 seeded cultch was outplanted in the lagoons just before the start of the known peak settlement period in July for northern Puget Sound oysters (note that these data are from Fidalgo Bay and may not be representative of peak settlement timing in Skagit and Similk Bays; Allen et al. 2015). Thus, both the 2012 and 2013 cultch could have provided habitat for settling larvae in the summer of 2013 and, interestingly, some of the smallest individuals were located on top of older Olympia oysters. It is important to note that we only found minimal evidence of small recruits in

November 2014, when one would expect to find recruits if the oysters had reproduced in the summer of 2014 (Figure 6). Other plausible explanations for smaller oyster size classes would be that the smaller individuals were located on sub-optimal positions on the *C. gigas* valves, were partially buried in silt, or were on *C. gigas* valves with abnormally high densities of native oysters and competition for space limited their ability to grow larger.

Blake and Bradbury (2012) suggest that restoration efforts should meet their plan metrics in three out of 10 years before success can be determined. As we near three years of monitoring, we believe the data from this pilot project demonstrate that both sites have high potential for success. We recently received additional funding from a US Fish and Wildlife Tribal Wildlife Grant (Grant Award F14AP00495) to expand our research and restoration efforts within the two lagoons. Before the restoration work grows larger in effort and physical size, we must develop a baseline of physical and biological parameters in the lagoons to determine the status of the restoration effort and assess the need for adaptive change through time. In the state's restoration plan, WDFW clearly establishes measureable benchmarks for describing the original conditions of the restoration site and determining the status of the restoration effort (Blake & Bradbury 2012). Our long-term Swinomish Olympia Oyster Monitoring Plan (Greiner et al. 2015) incorporates Blake and Bradbury's (2012) suggestions and will include annual resampling efforts of the oyster beds (survival, recruitment, growth, settlement, etc.) as well as the quantification of baseline biological and physical parameters. The pilot project oysters described in this report will be used in our recruitment benchmark study in order to determine what time of year they begin brooding and when larval settlement may occur in or near the lagoons. Additional seeded cultch will be outplanted in 2015 and/or 2016 and all other measurable benchmarks will be recorded from this younger cohort. The results from the long-term monitoring study will also provide essential data to areas currently lacking research, especially in regard to the ecosystem services provided by Olympia oysters and how those services might influence other species and ecosystems at larger scales.

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