LOW TEMPERATURE BROODING OF OLYMPIA OYSTERS (*OSTREA LURIDA*) IN NORTHERN PUGET SOUND

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ABSTRACT   Olympia oysters (*Ostrea lurida*) traditionally played important ecological, economic, and cultural roles as the only oyster native to the west coast of North America. Yet overfishing, pollution, and the cultivation of nonnative oysters have driven many historic beds toward or into extinction. Restoration efforts have increased recently in an attempt to reestablish this important ecosystem engineer. In 2012, the Swinomish Indian Tribal Community began a small-scale Olympia oyster enhancement effort in two tidal lagoons with the intention of eventually establishing self-sustaining populations. To properly time future expansion efforts with peak reproductive activity, brooding status was quantified from oyster populations within both lagoons throughout the spring and summer of 2015. Brooding data were compared with water temperature and salinity. Results from this study clearly indicate that northern Puget Sound oysters located in tidal lagoons are capable of brooding at 10.5°C, two degrees colder than has been reported in the literature. Oysters in one lagoon brooded for almost an entire month before the known critical daily minimum water temperature threshold of 12.5°C was reached. These results suggest possible local adaptation or habitat-specific reproductive maturation, and are relevant for Olympia oyster restoration and expansion efforts.

KEY WORDS: native oyster, Olympia oyster, *Ostrea lurida*, restoration, reproduction

INTRODUCTION

Oysters play an important role as estuarine ecosystem engineers that filter water, provide structured habitat, and stabilize shorelines throughout the world (Grabowski & Peterson 2007, Grabowski et al. 2012). Oyster reefs have declined as much as 85% globally as a result of overexploitation, loss of habitat, disease, and environmental degradation or mismanagement (Kirby 2004, Beck et al. 2011). In response to concern over the loss of these organisms and their associated habitat, restoration efforts have grown in an attempt to reestablish fisheries and the ecosystem services these bivalves once provided (Brumbaugh et al. 2006, Coen et al. 2007).

The Olympia oyster, *Ostrea lurida* (Carpenter, 1864), is the only oyster native to the eastern Pacific Coast. Historically, this foundation species played an important economic, ecological, and cultural role within its former range from Sitka, Alaska, to Baja California (Steele 1957, Polson & Zacherl 2009). Recently, however, *O. lurida* beds have diminished considerably due to fishing effort, pollution, logging, mining, and habitat loss (Kirby 2004, Pritchard et al. 2015); in Washington's Puget Sound, only approximately 5% of Olympia oyster beds (circa 1850) remain (Blake & Bradbury 2012). Because of this decline, the past 15 y have seen an increase in restoration and enhancement efforts throughout the current range of the oyster (Cook et al. 2000, Dinnel et al. 2009, Blake & Bradbury 2012, Wasson et al. 2015). These endeavors should attempt to expand populations through natural reproduction to lower the risk of unintended genetic impacts (Camara & Vadopalas 2009), and include long-term monitoring as part of their restoration plan (Baggett et al. 2015). To evaluate performance of restoration efforts, several authors have offered suggestions on environmental and/or biological criteria that should be quantified

(Blake & Bradbury 2012, Baggett et al. 2015, Wasson et al. 2015). It is important that these metrics are measured on a site-to-site basis because oysters can exhibit local adaptation, and each restoration site will have unique environmental parameters (Bible & Sanford 2015).

Water temperature is a key environmental metric, partially because of the role it plays in initiating *Ostrea lurida* spawning (reviewed by Baker 1995 and Pritchard et al. 2015). Once a critical temperature threshold has been attained, these viviparous, protandrous, serial bidirectional hermaphrodites spawn and begin brooding their larvae for 10–12 days before releasing D-stage veligers into the water column (Coe 1931, Hopkins 1937). During the brooding process, larvae progress from unshelled early-stage larvae (i.e., "white sic") to shelled late-stage larvae (i.e., "black sic") (Hopkins 1936, Carson 2010). Although it is relatively straightforward for researchers to quantify the onset of brooding activity and larval development in conjunction with the collection of environmental data, only a few published records exist, and results are likely to vary by site (Hopkins 1937, Carson 2010, Heare et al. in preparation).

Although northern Puget Sound traditionally supported extremely large (e.g., approximately 810 hectares in Samish Bay: Blake & Bradbury 2012) beds of *Ostrea lurida*, the majority of restoration and enhancement efforts have been focused in south and central Puget Sound (but see Dinnel et al. 2009). In 2012, the Swinomish Indian Tribal Community (SITC) began a pilot *O. lurida* enhancement project at two tidal lagoons (i.e., pocket estuaries) in northern Puget Sound (Barber et al. 2015). Because Olympia oysters are sensitive to extreme temperatures and siltation (reviewed in Wasson et al. 2015), pocket estuaries provide an ideal habitat, since the enhanced beds can be located in flowing channels that remain inundated throughout all tidal cycles. Although restoration efforts have taken place in this type of habitat before, few studies exist that describe how *O. lurida*

behave in this unique environment (but see Wasson 2010), and none of these studies, to our knowledge, have evaluated brooding activity in tidal lagoons.

The primary goal of this research was to quantify the onset of reproductive activity in northern Puget Sound tidal lagoons and to relate these data to temperature and salinity. Secondary goals were to determine if there was a difference in brooding by site and time and to describe temporal change in larval development. Importantly, these goals fit within the broader SITC restoration objectives defined in the work of Greiner et al. (2015) by specifically addressing the aims to (1) assess Olympia oyster population enhancement success and (2) measure abiotic habitat parameters.

MATERIALS AND METHODS

Study Site

This study took place on SITC reservation tidelands (48.41° N, –122.6° W) in the northern Puget Sound region of Washington State (Fig. 1). The two enhancement sites, Lone Tree (LT) and Kiket Lagoons (KI), are geomorphically considered pocket estuaries or tidal channel lagoons, small subestuaries located behind spits or barrier beach landforms in submerged valleys or small creek deltas. These pocket estuaries are typically surrounded by saltmarsh and have tidal channels, lower wave and current energy, and some freshwater input (Beamer et al. 2003).

Lone Tree Lagoon is approximately 1.6 hectares in size, with a barrier beach along the western edge (Fig. 1). Marine waters from Skagit Bay primarily enter the lagoon through the outlet channel located at the northern portion of the lagoon, but the barrier beach also overwashes from the south at the highest tides [i.e., >2.7 m above mean lower low water (MLLW)]. The enhancement plots within the lagoon are 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. This site receives freshwater inputs from groundwater seeps and Lone Tree Creek, an ephemeral stream flowing into the lagoon during fall through late spring months.

Kiket Lagoon, is located just north of the tombolo connecting the mainland to Kiket Island (Fig. 1). Unlike LT, KI does not have a direct source of freshwater input other than groundwater and occasional stormwater runoff. Kiket Lagoon is smaller than LT at 0.88 hectares with the oyster enhancement plots in the lagoon at an elevation of 2.21 m relative to MLLW; at the lowest tides, the oysters are inundated in 0.43 m of water.

Environmental Parameters

Temperature (HOBO Temperature/Light Logger UA-002–08) and conductivity (HOBO Conductivity Logger U24–002-C, both logging in 15-min intervals,) probes were deployed April 15, 2015, approximately 7 cm above the substrate directly next to the oyster enhancement plots. At LT, a separate temperature and conductivity logger was also suspended from a float
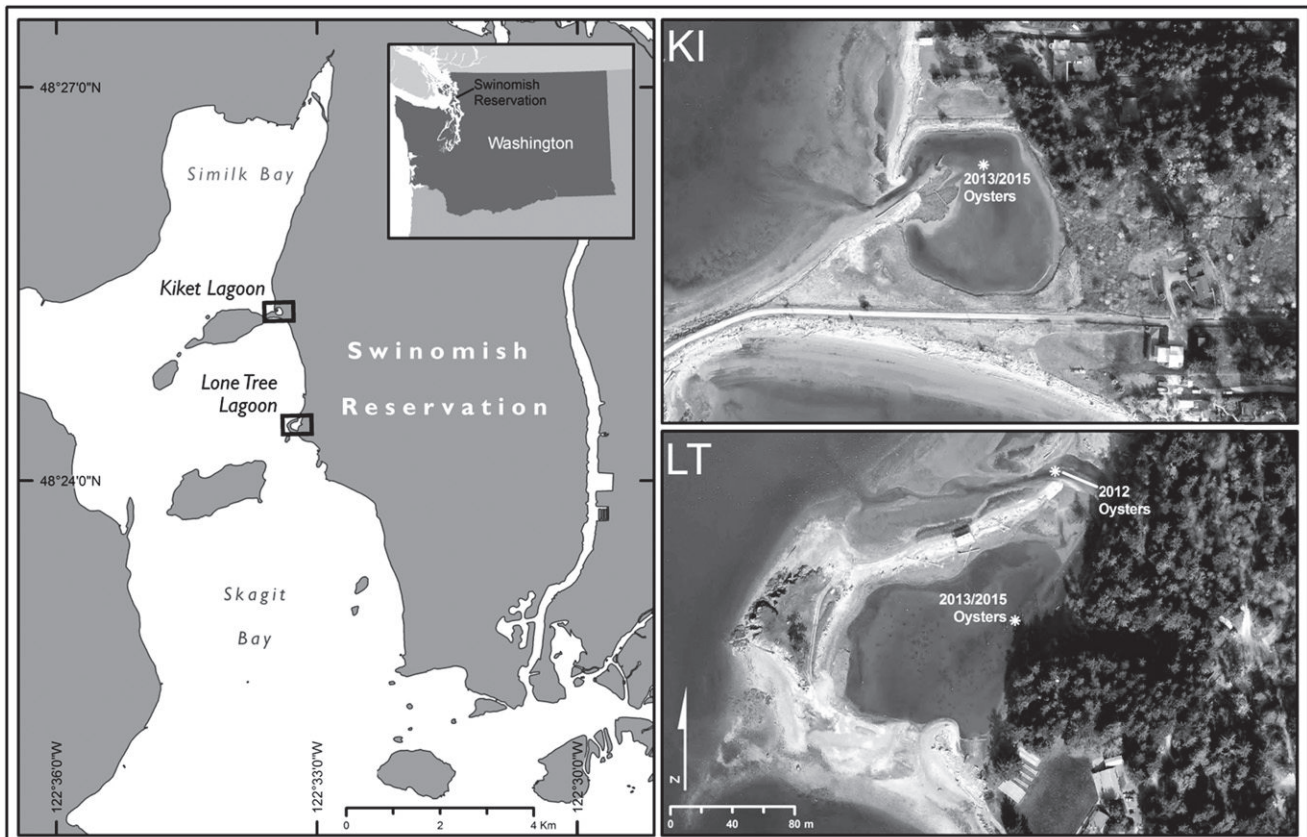

Figure 1. Map of northern Puget Sound *Ostrea lurida* enhancement sites in tidal lagoons. KI = Kiket Lagoon and LT = Lone Tree Lagoon. Stars indicate location and year of oyster seed outplanting.

roughly 30 cm below the water surface adjacent to the oyster enhancement plot. Salinity data from LT were used from the mid-column logger due to fouling issues with the logger located just off the bottom. All other data are presented from the bottom loggers located near the oysters. Conductivity loggers were periodically audited to ensure that data were within the manufacturer's published error range. Daily minimum values for temperature and daily mean values for salinity were calculated from the dataset; invalid data points were omitted from analyses.

Brooding

Between both sites, *Ostrea lurida* seed set on *Crassostrea gigas* (Thunberg, 1793) shell (cultch) were evenly outplanted during the summers of 2012 and 2013, and single individuals of *O. lurida* were outplanted in April 2015 (Barber et al. 2015). All oyster seed were bred from northern Puget Sound broodstock (i.e., Fidalgo Bay). Because *O. lurida* is capable of reproduction within 5–6 mo of age, all oyster seed would have been able to reproduce at the time of this study (Coe 1931, reviewed in Pritchard et al. 2015). A 2012 winter storm event moved the 2012 KI cultch to an inaccessible depth in the lagoon, thus, brooding data from KI are only from the 2013 cultch and the 2015 single oysters. The LT data were separated by population since the LT 2012 cultch were located in a different area of the lagoon from the combined LT 2013 cultch and 2015 single oysters.

From May 5 to August 26, 2015, all the oysters in three to five $1/16 \, m^2$ quadrats were collected per site (oysters were not collected from quadrats during the first two sampling dates). Although quadrats were haphazardly placed in different locations on the oyster bed during each sampling session, it is plausible that some individuals may have been resampled. Nonlethal methods were used to assess brooding status following methods detailed by Heare et al. (in preparation) and Hintz et al. (in preparation). Briefly, individuals were desiccated for a minimum of 45 min and transferred into a treatment of 75 g/L magnesium sulfate (final concentration) mixed with a 50/50 seawater/freshwater solution for 45 min. Individual quadrats were kept in separate tubs of solutions. Oysters were then removed from the solution, the number of open individuals was recorded, and the open individuals were inspected for brooding activity. A 5-mm-wide black zip tie was used to gently sweep the inside of the mantle to extract larvae to easily determine the development stage of the brooded larvae (white, early-stage development, or black, late-stage development) (Hopkins 1936, Carson 2010). Length of the brooding adult oyster was recorded. Oysters were placed in a 100% seawater bath for a minimum of 45 min before being returned to their respective pocket estuaries.

Brooding and temperature data were compared with the only known daily minimum temperature threshold (12.5°C) for initiating brooding in Puget Sound (Hopkins 1937); relationships between brooding and salinity were also described. A two-way analysis of variance (ANOVA; SYSTAT 13) was used on arcsine-transformed data with follow-up Tukey's tests to determine if there was a difference in mean percent brooding by site and date (Sokal & Rohlf 1995). Although the transformed data did not meet the assumption of normality, ANOVA are considered robust even when the data deviate from a normal

distribution (Norman 2010). A Kolmogorov–Smirnov test was used to determine if there were differences in the length-frequency distribution of brooding oysters by site (Sokal & Rohlf 1995).

RESULTS

Environmental Parameters

Throughout the 17-wk monitoring period, water temperature was lower whereas salinity was higher at LT versus KI (Table 1, Fig. 2). At LT, the daily minimum temperature reached 12.5°C on May 27, 2015, but this temperature was not sustained until June 9 (Fig. 2). Periods of warming and cooling at LT corresponded with the neap and spring tidal cycle, respectively. Water temperatures at KI exceeded 12.5°C from May 9 to 12 during a neap tide and dropped below 12.5°C for about 2 wk (with a 1-day temperature spike) before maintaining the theoretical minimum temperature threshold for brooding of 12.5°C for the remainder of the summer (Fig. 2). Weekly minimum temperature patterns at KI did not appear to correspond as closely with tidal cycles compared with LT.

Brooding

A mean of 475 ± 19 SE oysters were sampled per lagoon per week (*n* = 17 sampling dates). Brooding *Ostrea lurida* were recorded on the first day of sampling at LT on May 6, 2015, when the daily minimum water temperature (DMWT) was 10.6°C and the salinity was 26 (Fig. 2). The presence of early-stage larvae at LT on May 6, 2015, indicates that the oysters could have been brooding approximately 1–3 days prior to collection when the DMWT was 10.5°C and salinity 23 (Figs. 2 and 3; Hopkins 1936, 1937). No brooding oysters were recorded at KI on May 6, 2015 (Fig. 2). The following week *O. lurida* at LT were brooding at a DMWT of 11.3°C and a salinity of 25 (Fig. 2). On the same day brooding was recorded for the first time at KI with a DMWT of 12.6°C and a salinity of 20 (Fig. 2). The presence of late-stage larvae at KI on May 11, 2015, indicates that the oysters were likely brooding approximately 5–10 days prior to collection when the DMWT was 11.7°C (Figs. 2 and 3; Hopkins 1936, 1937). Late-stage larvae were first recorded at LT on May 21, 2015, with a DMWT of 10.8°C and a salinity of 26 (Figs. 2 and 3). Lone Tree oysters brooded larvae for almost an entire month before the previously reported critical DMWT threshold of 12.5°C was recorded on May 27, 2015 (Fig. 2; Hopkins 1937).

Lone Tree 2012 brooding data were combined with LT 2013/2015 data for other analyses because there was no statistical

TABLE 1.

Water property monitoring results for Lone Tree and Kiket lagoons from April 15 to August 31, 2015.

| Site | Seawater temperature (°C) | | Salinity | |
	Range	Mean ± SE	Range	Mean ± SE
Lone Tree	9.2–23.6	14.7 ± 2.5	15.3–26.9	22.9 ± 1.6
Kiket	9.5–29.3	17.5 ± 3.6	9.3–25.6	19.8 ± 2.3

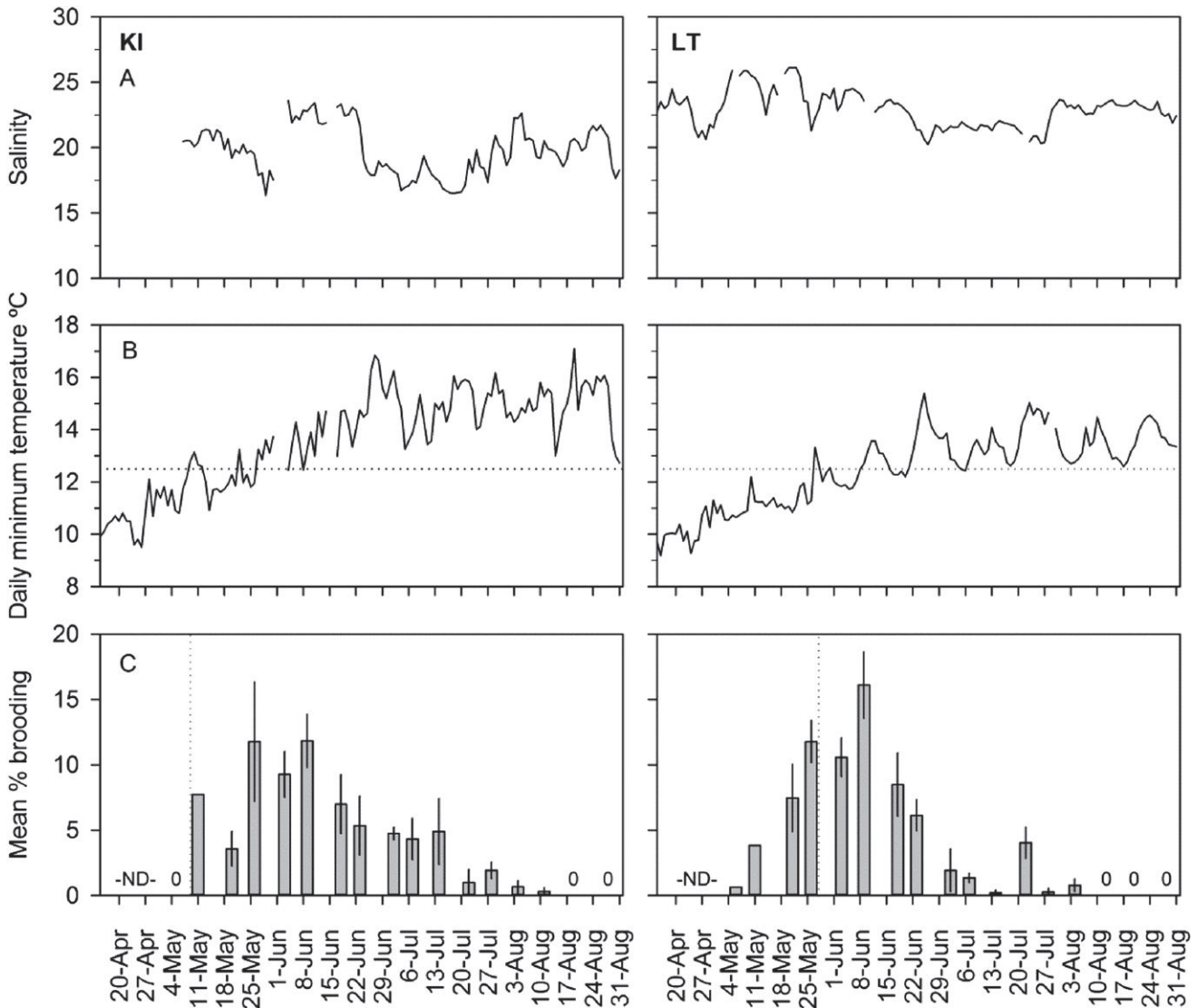No salinity data exist for Kiket before May 6, 2015.

Figure 2. The 2015 timing of *Ostrea lurida* brooding in Kiket (KI) and Lone Tree (LT) lagoons in relation to (A) salinity and (B) daily minimum water temperature. (C) Mean percent brooding is shown with SE bars. Dotted lines in graphs B and C represent the previously reported critical temperature threshold (12.5°C) needed to initiate spawning in Puget Sound *O. lurida* (Hopkins 1937). ND, no data collected.

difference in mean percent brooding between these two populations (one-way ANOVA, arcsine-transformed data, $F_{1,73} = 0.3$, $P = 0.59$; LT 2012 mean = 3.9% ± 0.88 SE and LT 2013/2015 = 4.6% ± 0.91 SE). No difference was found in mean percent brooding by site (KI mean = 4.6% ± 0.64 SE and LT mean = 4.3% ± 0.65 SE; Fig. 2, Table 2). Follow-up Tukey's tests revealed a temporal difference in mean percent brooding where significantly more oysters were brooding in late May and early-to-mid June versus July and August, and more oysters were brooding in late May versus early May (Fig. 2, Table 2). No interaction between date and site was detected (Table 2), and no obvious temporal pattern was noted in the proportion of early-stage larvae versus late-stage larvae by site and time (Fig. 3), although the proportion of oysters containing early-stage larvae at KI appears to increase slightly through time. Brooding oysters at KI had a statistically different length distribution (slightly larger) than LT oysters ($D = 0.317$, $P < 0.000$; Fig. 4).

DISCUSSION

This research clearly shows that Olympia oysters are capable of brooding at temperatures colder than what has been previously reported in the literature (12.5°C for Puget Sound; Hopkins 1936, 1937). Furthermore, the presence of late-stage larvae in the mantle of brooding oysters indicates that larvae had successfully reached late-stage development approximately 2 wk before 12.5°C was first recorded at LT.

At the southern range of Olympia oysters, Coe (1931) found that the critical temperature for spawning was 16°C (daily mean temperature), whereas common critical temperatures in the higher latitudes of Oregon and Washington range from 14°C to 16°C (Strathmann 1987, Oates 2013). No reliable study on brooding and critical temperature thresholds exists from the northernmost range of this oyster species in British Columbia (Gillespie 2009), but Pritchard et al. (2015) noted that it might
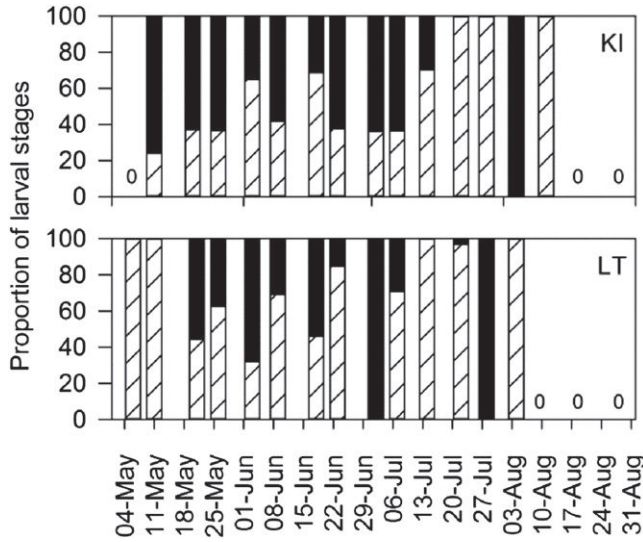
Figure 3. Larval stage proportions in brooding *Ostrea lurida* at Kiket (KI) and Lone Tree (LT) lagoons from May 6 to August 26, 2015. White hatch bars indicate early-stage larvae and black bars indicate late-stage larvae.
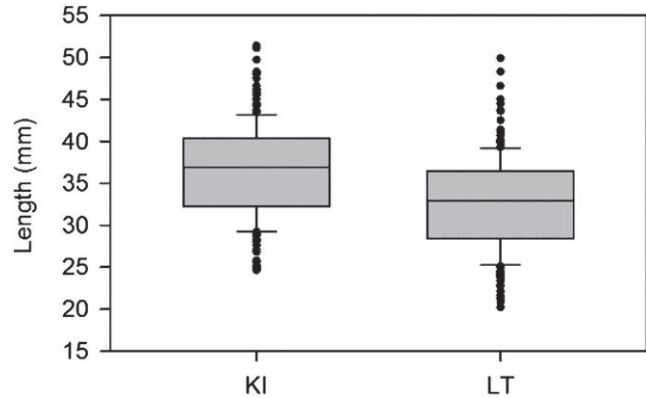

Figure 4. Length distribution of brooding *Ostrea lurida* at Kiket (KI) (*n* = 242) versus Lone Tree (LT) lagoons (*n* = 255). Solid line indicates median.

be 13°C. Hopkins' (1936, 1937) field research is the most relevant to this investigation because he determined the 12.5–13°C DMWT threshold for Olympia oysters in southern Puget Sound and noted that this was related to high tides. Recently, Heare et al. (in preparation) reported results from northern Puget Sound that agree with Hopkins' (1936, 1937) findings, although they did not check for brooders until mid-May, and the SITC results indicate that the oysters could have been brooding before this time. Importantly, Hopkins' (1936, 1937) research was conducted on oyster populations located in diked mud flats such that the oysters were constantly inundated even at low tide; to an extent this diked environment presumably mimics certain aspects of environmental conditions found in tidal lagoons. Yet, at least for LT, these data indicate that northern Puget Sound oysters are capable of brooding at colder temperatures. This analysis lends support to results showing that temperature thresholds for Olympia oyster recruitment (not brooding) can vary widely across embayments (Seale & Zacherl 2009), and that this species may exhibit an example of local adaptation (i.e., northern Puget Sound broodstock) in a marine bivalve mollusc (Bible & Sanford 2015, Heare et al. in preparation). Furthermore, this study demonstrates the importance of collecting site-specific data due to the possibility of interannual and/or site variation.

TABLE 2.

Two-way ANOVA test statistics for the effects of site (Lone Tree or Kiket lagoons), time, and their interaction on mean percent brooding.

	df	Sum of squares	Mean square	F	P
Site	1	0.00	0.000	0.005	0.944
Date	16	0.255	0.016	14.125	0.000
Site × date	16	0.020	0.001	1.129	0.339
Error	104	0.117	0.001		

Brooding studies conducted on subtidal *Ostrea lurida* populations would have been equally important for comparison, but to our knowledge, no such studies exist. Reproductive studies conducted on a different species within the Ostreidae family [*Ostrea chilensis* (Philippi, 1844)] have found that subtidal populations are capable of brooding at 12°C (Brown et al. 2010). There is also evidence from other oyster species that subtidal populations are exposed to lower physiological stress and higher suspension feeding opportunities (Brousseau 1995, Bishop & Peterson 2006). Although it has been shown that fouling organisms can create increased stress (e.g., reduced growth) on subtidal oyster populations (Bishop & Peterson 2006, Trimble et al. 2009), significant fouling (barnacles and mussels) was only noted on the LT 2012 oysters (see Barber et al. 2015), and no difference was detected in shell length (J. S. Barber, unpublished data) or mean percent brooding between this cohort and the LT 2013/2015 oysters. As long as fouling is not a serious impediment, *O. lurida* populations located subtidally or in tidal lagoons may be capable of brooding at colder temperatures because of increased feeding opportunities or lower physiological stressors. It is, however, certainly possible that factors aside from constant inundation play a role in determining the onset of brooding in this species.

Fall and winter temperatures experienced by oyster populations could also influence subsequent reproductive activity (Joyce et al. 2013). Gametocytes in a high-latitude population of *Ostrea edulis* (Linnaeus, 1758) were recorded when water temperatures were 0–1°C, possibly as a result of warmer autumn water temperatures initiating a second incomplete round of reproductive activity prior to winter dormancy (Joyce et al. 2013). The winter of 2014 to 2015 was warmer than average for the Puget Sound region (National Oceanic and Atmospheric Administration 2015), and tidal lagoons were likely subjected to variability in air temperature (depth never exceeds 1.5 m) (e.g., Hopkins 1936). Although DMWT during this study did not reach the theoretical brooding threshold of 12.5°C until late May to early June, daily average water temperatures at LT did reach 12.5°C as early as April 9, 2015 and a daily maximum water temperature of 12.5°C was recorded on March 24 (S. K. Grossman, unpublished data). It is plausible that these high-latitude oysters may have initiated a second round of reproductive activity due to warmer temperatures in the fall and/or winter, resulting in the ability of these

oysters to reproduce earlier at cooler temperatures the following spring. Of course, extended winter cold periods may be important for Olympia oyster gonad development (R. Crim, Puget Sound Restoration Fund, personal communication, February 2016), but this determination cannot be made based on temperature data from the SITC analysis. Nevertheless, these results suggest a need for researchers to reconsider using DMWT as a proxy for when to initiate reproductive studies.

Other known influences on reproduction in *Ostrea lurida* include salinity, dissolved oxygen, and/or food availability (Kimbro et al. 2009, Oates 2013, reviewed in Wasson et al. 2015). Although dissolved oxygen and chlorophyll *a* were not measured in this particular study, SITC was able to measure salinity. Although Olympia oysters have been shown to tolerate a range of salinity, exposure to levels below 25 can result in a reduction in reproduction and growth (Cheng et al. 2015, reviewed by Wasson et al. 2015). At the SITC study sites, however, brooding activity occurred at lower salinity levels. Furthermore, KI and LT mean salinity levels were 8.0 and 4.9 lower, respectively, than the mean salinity of 27.8 recorded by Hopkins (1937) in southern Puget Sound. Although KI had consistently lower salinity levels than LT (Table 1), the similar proportions of brooders between these two tidal lagoons imply that salinity levels did not affect brooding at KI. Perhaps the physiological stress of lower salinity at KI was countered by the warmer temperatures in the lagoon (Cheng et al. 2015). Similarly, the cooler temperatures at LT did not appear to affect the ability of oysters to brood; it is possible that the higher salinities in this lagoon opposed the stressors of the cooler temperature and encouraged brooding. Indeed, despite different environmental conditions, brooding peaked in both lagoons at the end of May through mid-June (Fig. 2).

It is important to note that these results are based on a single year of sampling, therefore it is plausible that these data are anomalous for northern Puget Sound tidal lagoons. Climate change and increased sea surface temperature, however, could make these potentially anomalous results more common.

In regard to larval development in the mantle cavity, the SITC results closely mirror those of Hopkins (1937) and Carson (2010) where early-stage larvae and late-stage larvae co-occurred throughout the spring and summer. Although the presence of late-stage larvae is not an indicator of recruitment success, it is a relatively reliable indicator of brooding success (Hopkins 1936, 1937), and can be used to estimate the timing of peak recruitment (Carson 2010). Results from this study indicate that northern Puget Sound brooding peaks in late May and early June, with no obvious second peak except perhaps at LT in late July (Fig. 2). Thus, peak recruitment

could be expected to occur in northern Puget Sound by mid-to-late June.

The age of the oyster primarily determines the individual's ability to initiate reproduction for the first time (reviewed in Pritchard et al. 2015), but oyster size plays a role in its fecundity (Hopkins 1937, Cole 1941). Thus, because the KI oysters were larger than the LT oysters, one could hypothesize that more larvae may be available for self-seeding enhancement efforts at this tidal lagoon. Although SITC did not obtain estimates of the number of larvae per brood, these data paired with different temperature and tidal elevations may be helpful to future restoration efforts (R. Crim, Puget Sound Restoration Fund, personal communication, February 2016).

To increase the likelihood of a successful restoration program, sites must be located in areas that offer a combination of characteristics that are essential to Olympia oyster reproduction, survival, and growth (Blake & Bradbury 2012, Pritchard et al. 2015, Wasson et al. 2015). The timing of brooding and recruitment within a particular habitat can clearly vary, and these results suggest that brooding studies at higher latitudes or subtidal locations should start quantifying reproductive activity by early-to-mid April when the DMWT is approaching 10°C. Further enhancement efforts should monitor other factors in addition to temperature and salinity (e.g., pH, overwintering conditions, chlorophyll *a*) to best determine how other parameters may influence the timing of spawning (Baggett et al. 2015). Finally, expansion efforts in northern Puget Sound should install unseeded cultch near currently existing *Ostrea lurida* beds by mid-May to ensure placement prior to the release of larvae from existing populations. Ultimately, this study demonstrates the importance of collecting data from specific restoration locations, as local site variability can clearly influence reproductive activity.

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