2015 Olympia Oyster, *Ostrea lurida*, Brooding Results From Northern Puget Sound

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Introduction

Olympia oyster, *Ostrea lurida*, restoration efforts have increased recently in an attempt to re-establish this ecosystem engineer to its native habitat along the eastern Pacific coast. 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.

Understanding reproductive activity within specific *O. lurida* populations is particularly important to restoration efforts because spawning conditions appear to vary geographically (Pritchard et al. 2015). Water temperature is thought to be a primary metric in the initiation of spawning activity. Once a critical temperature threshold is reached, *O. lurida* spawn and brood their larvae for a period of 10 to 12 days as they progress from unshelled early-stage larvae (i.e. "white sic") to shelled late-stage larvae (i.e. "black sic") (Hopkins 1937). While it is relatively straightforward to quantify the onset of brooding and larval development in conjunction with the collection of environmental data, only a few published records exist (Hopkins 1937, Carson 2010) and results are likely to vary by site.

The goals of our research were to:

1. Quantify the onset of reproductive activity in northern Puget Sound and relate these data to water temperature and previous reproductive studies.
2. Determine if there was a difference in brooding by site and time.
3. Describe temporal change in larval development.

Methods

The Lone Tree (LT) and Kiket (KI) lagoons are located in Skagit and Similk Bays, respectively, in northern Puget Sound on the Swinomish Reservation (Fig. 1). The Olympia oyster plots are located in lagoon channels that are inundated with water even at extreme low tides. Olympia oysters were outplanted to both sites in 2012, 2013, and 2015.

Figure 1. Map of restoration sites within the Swinomish Reservation.

Sampling methods:

- Water temperature was logged at 15 min. intervals at each site from April to October 2015 using HOBO UA-002-64 and daily minimum water temperature (DMWT) was determined.
- Oysters were collected from three to five 1/16th m^2 quadrats per site, totaling roughly 500 individuals per lagoon per week from 6 May through 25 August 2015 (n=17 sampling dates) (Fig. 2A).
- Individuals were desiccated for a minimum of 45 minutes and transferred into a treatment of 75g/L Epsom salt mixed with a 50/50 seawater/freshwater solution for 45 minutes (Fig. 2B) (Heare et al. in prep).
- Oysters were removed from the solution and open individuals were inspected for brooding (Fig. 2C & 2D) using a black zip tie to visually check for brooded larvae (Fig. 2E).
- Length of brooding oyster and the stage of larval development were recorded (Fig. 2F).

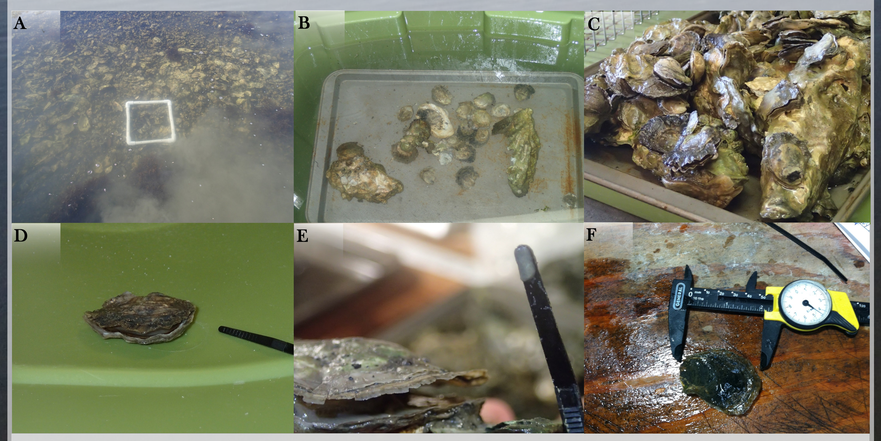

Figure 2. Depiction of sampling methods from collection to documentation of brooding oysters.

Results

- Early-stage larvae were recorded at LT on 6 May when the DMWT was 10.6°C. Late-stage larvae were first documented at LT on 21 May with a DMWT of 10.8°C (Figs. 3 & 4). Brooding activity was detected at LT for almost a full month before the previously reported critical DMWT threshold of 12.5°C for southern Puget Sound (Hopkins 1937) was recorded.
- Brooding, including the presence of late-stage larvae, in KI oysters was first recorded on 11 May 2015 at a DMWT of 12.6°C (Fig. 4).
- We found no significant difference in mean percent brooding by site (two-way ANOVA on arc-sin transformed data, $F_{1,104} = 0.005$, $p = 0.944$) and no significant interaction between site and date ($F_{16,104} = 1.129$, $p = 0.339$).
- The number of oysters brooding changed temporally ($F_{16,104} = 14.13$, $p < 0.000$). Follow-up Tukey tests showed significantly more oysters brooding in late May to mid-June (Fig. 3).
- No obvious temporal pattern was noted in the proportion of early-stage larvae versus late-stage larvae by site and time (Fig. 4).

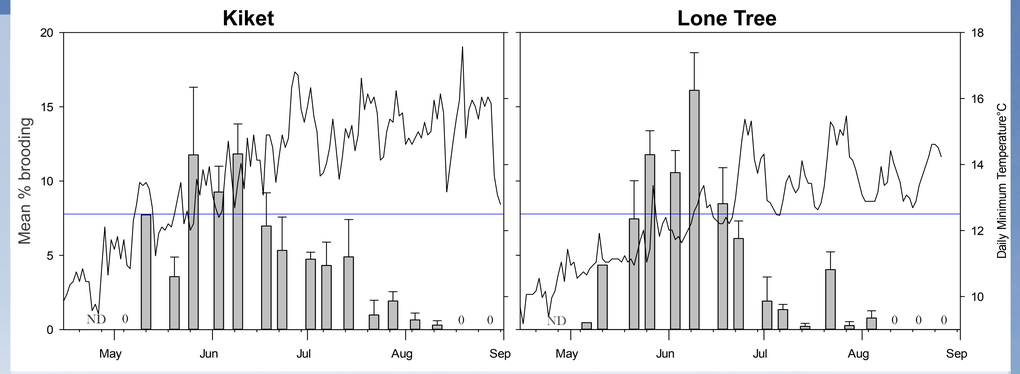

Figure 3. Mean percent brooding for *Ostrea lurida* at Kiket Lagoon and Lone Tree Lagoon from 6 May to 26 August, 2015 and associated daily minimum water temperature (°C). Horizontal blue line represents the previously supported critical temperature threshold (12.5°C) needed to initiate spawning in Puget Sound (Hopkins 1937). ND = no data and 0 = zero brooders.

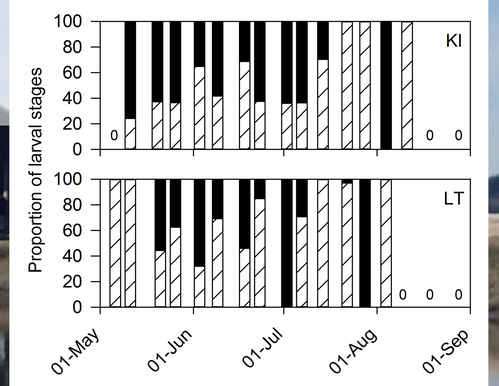

Figure 4. Larval stage proportions in brooding female *Ostrea lurida* at Kiket Lagoon (KI) and Lone Tree Lagoon (LT) from 6 May to 26 August 2015. 0 = zero brooders.

Discussion

- *O. lurida* are capable of brooding at temperatures colder than what has been previously reported in the literature [12.5 °C in Puget Sound, 14.5 to 16.0 °C in Oregon, 16.0 °C in California (Hopkins 1937 & Pritchard et al. 2015)].
- The presence of late-stage larvae in the brooding oysters indicates that larvae had successfully reached viable development several weeks before 12.5 °C was recorded at LT or KI.
- *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.
- Because KI minimum water temperatures were higher than LT, one would expect to record brooding first in KI oysters. Yet, no significant difference in mean percent brooding by site was found; this most likely means that water temperature represents just one factor of many that initiates brooding.
- Results from this study indicate that northern Puget Sound brooding peaks in late May and early June (Fig. 3). Thus, peak recruitment could be expected to occur in northern Puget Sound by mid to late June.
- An unseasonably warm winter may have been partially responsible for the surprisingly early start to brooding. Studies in a similar northern latitude species show it is possible to initiate incomplete reproduction in the fall. These gonads can then overwinter until more favorable conditions are attained in spring (Joyce et al. 2013).
- This study demonstrates the importance of collecting site-specific data due to the possibility of inter-annual and/or site variation.

Suggestions

Future high latitude brooding studies should start quantifying reproductive activity in mid-April and consider sampling twice a week to ensure documentation of early brooders. Furthermore, we suggest monitoring water temperature and other environmental conditions (e.g. salinity, pH, chlorophyll α) in order to examine which environmental metrics are influencing reproductive timing at study sites. These results may improve the ability of restoration managers to capitalize on spat recruitment and settlement and promote restoration success.

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