Olympia oyster (*Ostrea lurida*) spat settlement in the Gorge Waterway

2019

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WORLD FISHERIES TRUST

Contents

Figures

1.0 Introduction

Oysters are important foundation species (*e.g.* Kimbro & Grosholz, 2006) that provide ecosystem services (Pritchard et al. 2015) and nourishment for a variety of organisms, including people. *Ostrea lurida* – commonly known as the Olympia oyster – is the only oyster species native to the Pacific coast of North America. It once occupied many bays and lagoons along its range, but few healthy populations of *O. lurida* remain today. Past and present disturbances, such as historical overfishing, habitat degradation via urbanization, pollution, invasion from introduced species, and climate change (Kirby, 2004; Pritchard et al. 2015), have drastically reduced their numbers. In 2003, *O. lurida* was listed as a species of Special Concern under the Species at Risk Act (SARA). While a series of restoration and recovery projects have been launched in Puget Sound; so far, the official Canadian recovery plan is focused on monitoring to better understand the stability of remnant populations and factors affecting their abundance (Gillespie, 2009).

Understanding *O. lurida* reproduction and recruitment is a key element for successful recovery initiatives. Spat collectors are a well-established methodology for monitoring recruitment of oysters as a proxy of annual reproductive success in a population. World Fisheries Trust (WFT) has monitored the settlement and post-settlement success of Olympia oysters in the Gorge Waterway (Victoria, BC) since 2014. Despite environmental stressors and the presence of non-native, fouling organisms, populations throughout this waterway are extraordinarily robust (Fisheries and Oceans Canada, 2009). In this report, we look at the recruitment rates of Olympia oysters at two sites on the Gorge Waterway during the 2019 season and compare them to the five previous survey years (2014-2018). These results provide good insight into the unique recruitment dynamics of *O. lurida* in the Gorge and they will help to guide future restoration efforts in this location and elsewhere.

2.0 Methods for 2019

The methods used in this report are identical to those described in reports from previous years. Details of the general methods are outlined below.

2.1 Preparation of Settlement Stakes

Oyster spat settlement stakes consist of five Pacific oyster shells with a central hole stacked with their interior sides down on 60 cm long PVC pipes of ½” diameters (Figure 1A). These shells were sourced from the upper edge of the Timothy Oyster Company’s shell piles (above tideline), in Lantzville, BC. These are estimated to have been out of the water for at least 2 years. Relatively flat, single shells of average size were selected and scrubbed with a coarse brush to remove dirt. They were soaked for 24 hours in [8%] chlorinated water to kill any potentially invasive species, dried in the sun before storage, and used as needed. After drilling central holes with a 7/8” cement drill bit, these shells were placed on the stakes, spaced one inch apart with spacers made from clear PVC hose with a 1” inside diameter. The top shell was placed 1” from the top of the stake. Both the bottom and top shells were further secured to the PVC pipe by lacing Zap-straps through 5mm holes in both ends of the oyster stake; the top Zap-strap also secured a 2 ½” x 1 ¾” identifier label made of Coroplast® corrugated plastic and labeled with a waterproof ink felt pen (Figure 1A). We assigned specific labels for each stake placed in the Gorge and for each shell on the oyster stakes, numbered 1 to 5 from top to bottom (Figure 1B).

Figure 1. A) Weathered intertidal bi-weekly settling stake. B) Shell labeled for preservation and later analysis.

2.2 Stake deployment

Like in 2018, only the Craigflower site was monitored for spat settlement in 2019. This location has a muddy substrate with rocks and debris of various sizes, including shells of dead bivalves (Figure 2). Like many other areas of the Gorge, the scattered, hard debris on the benthos and small debris in the mud provide suitable habitat for *O. lurida* provided they can stay above the sediment. However, based on the results of all previous survey years, we believe that the Craigflower Bridge site supports the densest breeding population of *O. lurida* in the Gorge. The fast current in these narrows keeps the bottom relatively clear of mud, and the pilings of the bridge provide substantial solid vertical surface that remains submerged throughout the tidal cycle. The vertical orientation protects the oysters from sedimentation. These bridge pilings were also constructed with horizontal ridges specifically to facilitate oyster settlement and survival.

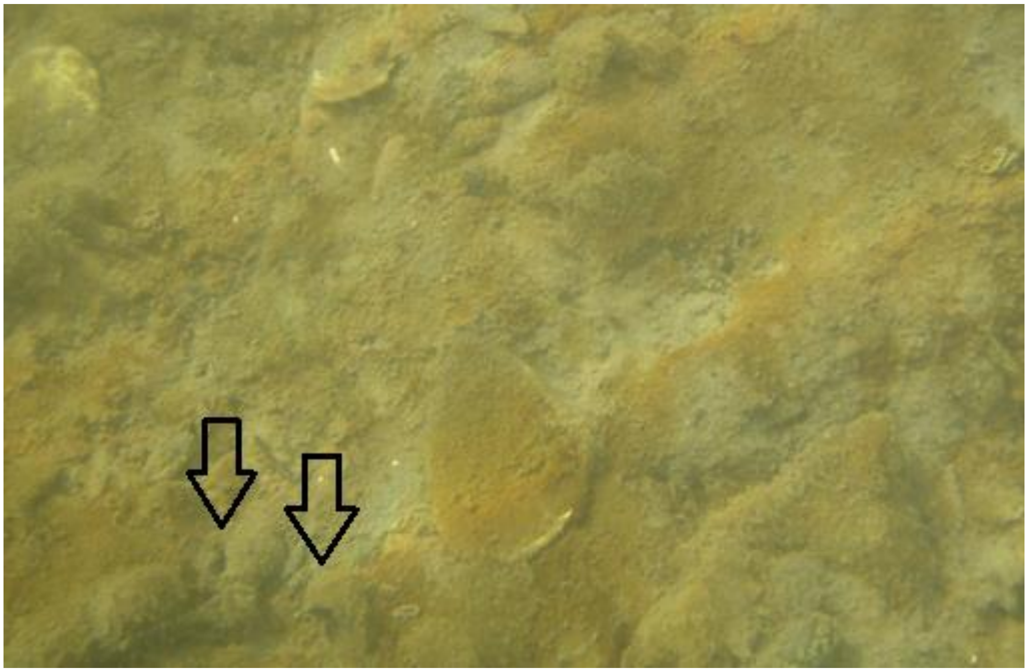

Figure 2. The typical muddy substrate in the Gorge Waterway. Arrows indicate live Olympia oysters (Ostrea lurida).

The Craigflower Site (48˚27'04" N; 123˚25'20" W) is located south of the Craigflower Bridge on the west side of the Gorge Waterway behind Portage West Apartments (Figure 3). The "biweekly" stakes were deployed and collected every 2 weeks throughout the survey period. As in previous years, three stakes were used at the Craigflower site for each two-week time period. All biweekly stakes were removed and replaced at the same locations in 2019 for consistency (Figure 3); comparable with the stake locations of all previous survey years (2014-2018).

To identify the stake locations, a tape measure was run from a fence post on the edge of the Portage West Apartments property (baseline 0 m location) at an angle of 40° for 11.8 m (Figure 4). A transect line was then run from the baseline end location at an angle of 154°. Stakes were placed every 5 m and hammered into the sediment so that the distances from the lowest stake shells to the benthos averaged around 20 cm. There was a larger gap between B1B and B2B (Figure 4).

Figure 3. The location of the Craigflower site. The red line to the south of Craigflower Bridge illustrates the exact position where we placed the biweekly settlement stakes.

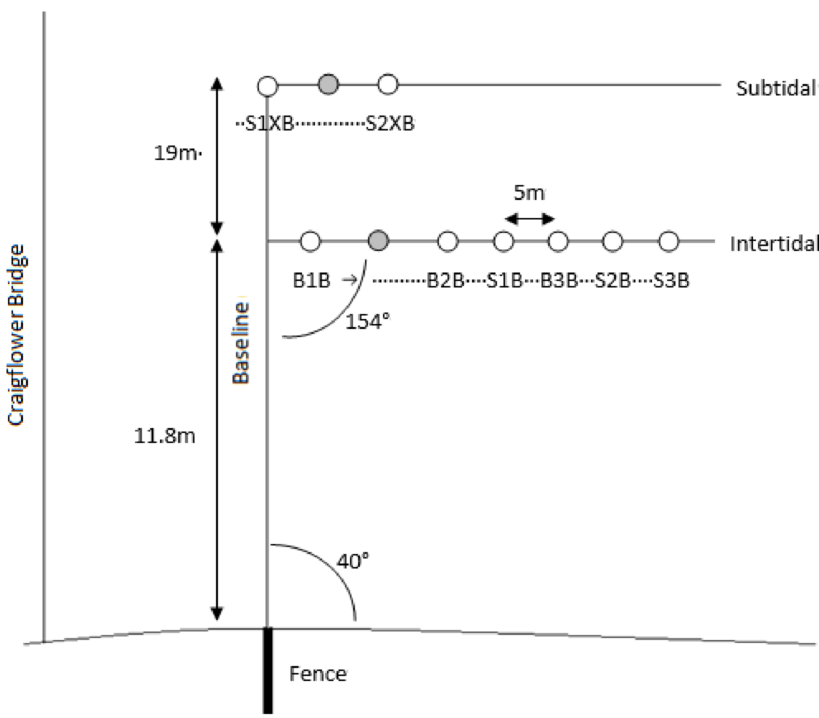

Figure 4. Settling stake placement framework at the Craigflower site. Filled circles = 2017 overwintering stakes.

2.2.1 Bi-weekly

Bi-weekly settling stakes were deployed on May 21st, 2019 and were switched out every two weeks until September 25th, 2019.

2.3 Processing and Analysis: 2019 Data

Once collected, the stakes were immediately brought back to the lab for processing. After removal from the stakes, the individual Pacific oyster shells were photographed with their labels and a scale bar (Figure 1B). These shells were then carefully wrapped in heavy-duty Scott paper shop towels and individually labelled. The shells were placed in Ziploc bags with an additional label. Once bagged, we froze all the shells to preserve them for later analysis.

For analysis, a shell was thawed and carefully unwrapped to avoid dislodging of any spat. The total oyster spat present on the inner surface of the shells were counted with the aid of a dissecting microscope using delineation sticks, as needed, to ensure accurate counts (Figure 5). For quality assurance, counters were first trained to recognize oyster spat and other species under the microscope. Experienced staff double-checked several shells to ensure spat counts were consistent between counters. Spat were assumed to be that of *O. lurida*, based on morphological evaluation in 2012 and the low, relative abundance of Pacific oysters (*Crassostrea gigas*) in the Gorge Waterway.

Figure 5. Typical set-up for counting spat on settling stake shells. Delineation sticks placed on shell were used to ensure accurate counts. Count data were recorded on the data sheet to the right of the dissection microscope.

Total surface area of the settling shells was calculated using *ImageJ* software (Wilkie et al. 2013). The total shell surface area was calculated, and the surface area of the central hole was subtracted from the total area. The total spat observed per shell was converted to spat/m^2, dividing the spat count by the calculated surface area of the inner shell surface. Spat densities are reported as the average over all the shells of the three settling stakes (n = 15) for each two-week time category (n = 9). Using *R* software (R Core Team, 2019), paired Wilcoxin tests were used to determine the significance of the differences in spat densities between each time category; this was used instead of an ANOVA and post-hoc t-tests because the data are not normally distributed.

2.4 Processing and Analysis: 2014 - 2019 Data Comparison

Spat density counts from survey years 2014 to 2019 are compiled for comparison over a six-year timeframe. Methodological details from previous survey years are outlined in their respective reports, but they are consistent across all survey years. All the spat density data combined in this document come from settlement stakes placed in the same locations at the Craigflower site for the six survey years. Data processing and analysis is consistent throughout these years except that surface area of settlement shells in 2017-2019 were calculated using *ImageJ* software (Wilkie et al. 2013), whereas in 2014-2016 the area was estimated as an oval approximation from shell length and width (less the area of the central hole).

The surface area of the 2017-2019 shells was also estimated with the oval approximation, generating a linear regression model (Adjusted $R^2 = 0.556$, $p<0.01$). Using the equation of this regression line, we were able to estimate what the 2014 – 2016 settlement shell areas would be if they were calculated using ImageJ based on their Oval area calculations. All the assumptions of a linear regression were tested, and none were violated, so we are confident that these 'ImageJ' area estimates are accurate. To further ensure the accuracy of these estimates, we compared the 2014 – 2019 spat density data using both the 'Oval' and the 'ImageJ' calculations. Although the magnitude of the spat density estimates differs between the two area measurement methods, the relative differences in spat density between each survey year are consistent across both methods. Since 'ImageJ' is the more accurate method to calculate shell area, we present the results that use this method, including the estimated 'ImageJ' values for 2014 – 2016 settlement shells.

Temperature of the Gorge Waterway was monitored by dataloggers at the Gorge Waterway Nature House, located about 500m downstream of the spat collection site. A Davis horticultural wireless weather station was used, part of the UVic School Weather station network (http://www.victoriaweather.ca/). Two submersible temperature probes were deployed – one at high tide and the other at lowest low tide levels. Only the lower probe results are reported here.

3.0 Results

3.1 Data from 2019

Most of the two-week time categories significantly differ in oyster spat densities (Table 1). However, the greatest differences occur between the stakes collected in the first half of the survey period (June 4th to July 16th) and those collected in the second half of the survey period (July30th to September 24th) with a notable drop in spat densities between July 16th and July 30th (Figure 6). The greatest average spat density occurred on the first oyster settlement stakes placed in the Gorge Waterway – removed June 4th; this maximum value is 6421 ± 832 spat/m^2 and may represent the spawning peak of 2019. However, this suggests that adult *O. lurida* began to release their veliger larvae well before May 21st. The lowest average spat density was 149 ± 42 spat/m^2 and occurred on the settlement stakes retrieved on August 27th. However, the stakes retrieved from the Craigflower bridge site during the two following two-week time-intervals after August contained greater average densities of spat; the spat densities on the September 24th stakes are significantly higher than those on the August 27th stakes (Figure 6). This suggests that spat settlement likely continues into October at low frequencies.

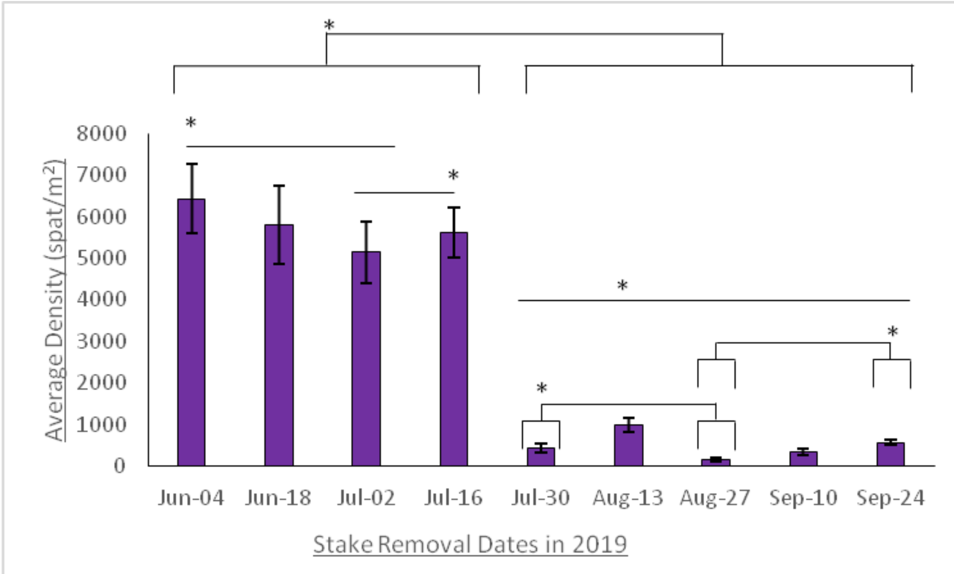

Figure 6. The average (± SE) Olympia oyster (*Ostrea lurida*) spat density across nine two-week time-intervals from May to September 2019 at the Craigflower site in the Gorge Waterway. The average and SE values are calculated from three oyster settlement stakes, each holding five Pacific oyster shells (n=15). For each time-interval, the three oyster settlement stakes were placed in the water two weeks prior to the stake removal dates. Spat densities calculated in m^2 are extrapolated from the number of spat accumulated on each shell, divided by the shell area (in cm^2), as calculated using ImageJ software. Asterisks indicate the time intervals that collected significantly more spat than those included under each bracket or line.

3.2 Comparative Results for 2014 – 2019

Among the six survey years, the 2017 settlement stakes supported the highest average spat density with 7914 ± 1407 spat/m^2 (Figure 7). In contrast, the 2018 settlement stakes supported the lowest average spat density with only 730 ± 116 spat/m^2. Generally, *O. lurida* spat settlement frequency appears to have gradually increased from 2014 to 2017, followed by the enormous decrease in 2018. However, settlement in 2019 has increased from 2018 to an average spat density like that of 2014.

Among the survey years, some spat settlement patterns across survey periods are similar, but major differences are also present (Figure 7). The peak *O. lurida* spat settlement events of 2014 to 2017 survey years occurred between June 17th and July 7th; coincidentally, these events occur in the two-week time-intervals that include the summer solstice – June 21st. The peak spat settlement of 2018 seems to have occurred a couple weeks later than previous survey years (July 4th to 18th). Peak spat settlement in 2019 occurred much earlier than the previous five years, but there is more of a plateau in the spat settlement frequency distribution around the peak than previous survey years. It is unclear if the peak spat settlement event occurred after May 21st, since the apparent peak settlement occurred during the first time-interval of the 2019 survey season; it is possible that a greater spat settlement event occurred prior to May 21st. The reason peak settlement in 2019 occurs at notably earlier time period than the previous five years is also unclear.

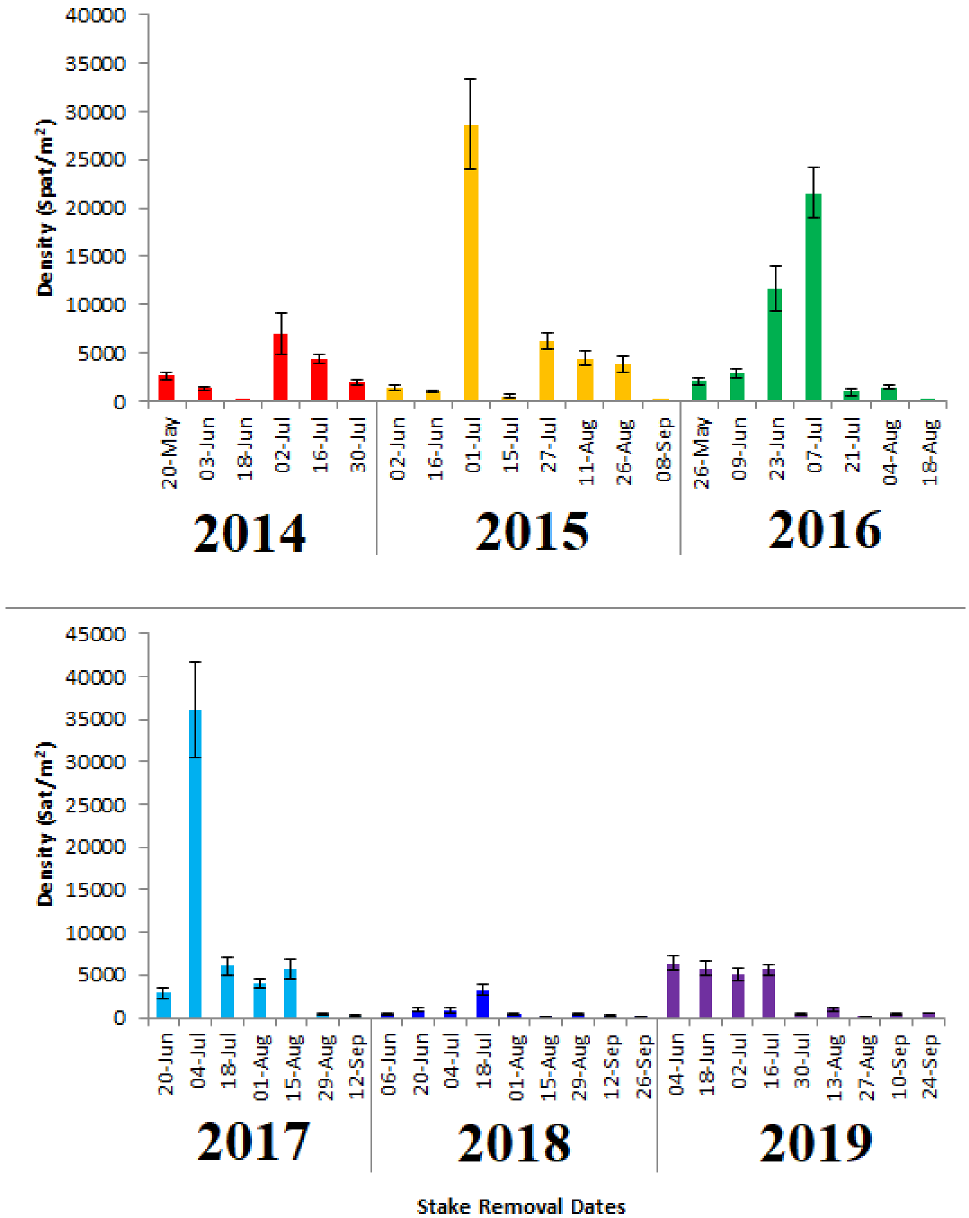

Figure 7. The average (± SE) Olympia oyster (*Ostrea lurida*) spat density present on settlement stakes after two weeks in the Gorge waterway across the last six years. The average and SE values are calculated from three oyster settlement stakes, each holding five Pacific oyster shells (n=15). The dates indicate the day each set of settlement stakes were removed for each two-week time-interval; the survey methodology is consistent across every year. However, the date of removal and the number of two-week time intervals surveyed differ across the years. Spat densities are calculated using ImageJ software from the number of spat accumulated on each shell, divided by the shell area.

3.3 Temperature datalogging

Data logger malfunction was common but thankfully there were multiple and these malfunctions occurred at different times. The superimposition of data from different years provided a fairly complete picture (Fig. 8).

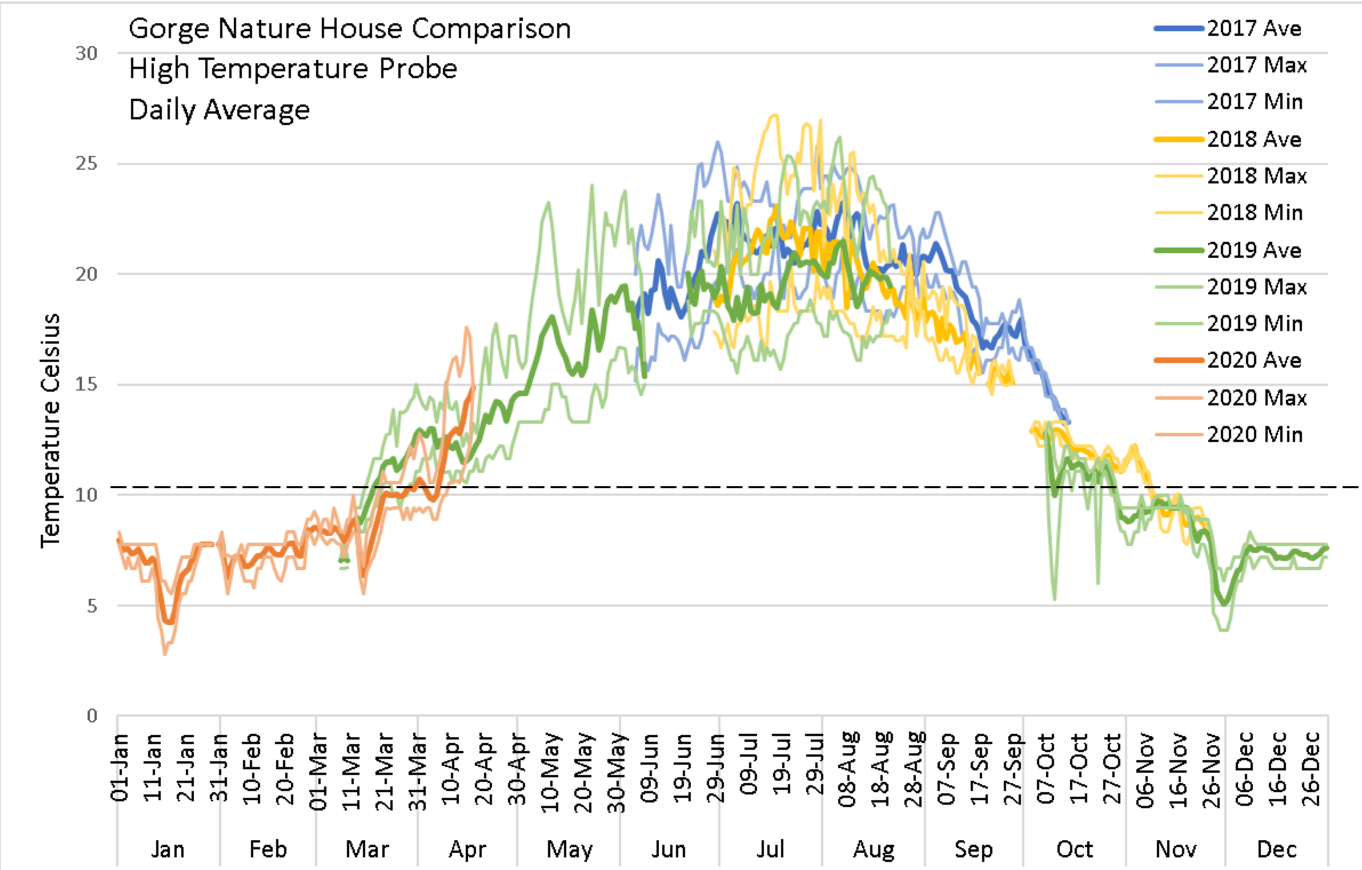

Figure 8. Multi-year bottom water temperature of the Gorge Waterway at Esquimalt Gorge Park. Dashed line indicates 13 ^{o}C, the nominal threshold for Olympia oyster reproduction. Daily means, maximum and minimum values are graphed, leaving out periods when the datalogger malfunctioned.

4.0 Discussion

Over the 6-year monitoring period (2014-2019), settlement occurred as a quite distinct peak in July from 2014-2018, while in 2019 there was a broader peak that was already underway when monitoring started in early June. Monitoring started earlier only in 2014, showing some early spat settlement, but also a peak in June. Olympia oyster reproduction is reported to be mostly temperature dependent, with a threshold temperature of 13^{o}C in the Puget Sound (Pritchard et al. 2015), however, there are likely other factors besides that influence peak settlement (Seale & Zacherl, 2009).

The Gorge average temperature reaches the 13^{o}C threshold in mid-April. Given that gonad development, brooding of eggs, and larval dispersion may take two or more weeks, we could expect spat settlement to start in early or mid-May. Unfortunately, our sampling did not generally capture this period, except in 2014 and 2016. In both of these years, spat settlement occurred at the start of the monitoring period, indicating reproduction was already underway – though it seems to generally increase for early June. However, in 2019 this early reproduction appears to have been generally more active – possibly corresponding with slightly elevated water temperatures in this year. Gillespie (2009) reports that oysters can spawn once or more each year. This is likely happening in the Gorge, though in a less synchronous fashion later in the year, with spat collected in smaller numbers at least into September. As temperature is still above the reproductive threshold at this time, it is likely that the oysters have expended all potential gametes by this time. Future work could include monitoring of reproductive state and larval presence, as well as starting spat collection earlier.

Recruitment varied over the years, as has been reported for Olympia oysters throughout its range (Wasson et al., 2016). In the case of the monitoring period in the Gorge, best recruitment occurred between 2015 and 2017, as indicated by peak recruitment levels. A steep drop in recruitment in 2018 and 2019 followed, approaching that of 2014. This may, in part, be due to the construction of the Craigflower bridge in late summer of 2013. The concrete footings of this bridge were immediately colonized by a large number of native oysters. As these are believed to become reproductive at two years of age (Gillespie, 2006), this would correspond to increased larval supply starting in 2015. Cumulative mortality of these oysters, including through erosion by the water currents (see bridge report), could explain reduced larval supply by 2018.

5.0 Acknowledgements

We thank the many volunteers that assisted in the collection and deployment of settling stakes, as well as the tedious work of counting spat. We also thank the research associates that carried out the spat monitoring in past years and Edward Wiebe who set up and maintained the datalogging weather station.

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