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For long term persistence of species, it is important to consider population structure. 28 Traits that hold adaptive advantage such as reproductive timing and stress resilience may differ 29 among locales. Knowledge and consideration of these traits should be integrated into 30 conservation efforts. A reciprocal transplant experiment was carried out monitoring survival, 31 growth, and reproduction using three established populations of Ostrea lurida within Puget 32 Sound, Washington. Performance differed for each population. Ostrea lurida from Dabob Bay 33 had higher survival at all sites but lower reproductive activity and growth. Oysters from Oyster 34 Bay demonstrated greater reproductive activity at all sites with moderate growth and survival. 35 Together these data suggest the existence of O. lurida population structure within Puget Sound 36 and provide information on how broodstock should be selected for restoration purposes.

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Evidence of *Ostrea lurida* (Carpenter 1864) population structure in Puget Sound, WA

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Abstract

For long term persistence of species, it is important to consider population structure. Traits that hold adaptive advantage such as reproductive timing and stress resilience may differ among locales. Knowledge and consideration of these traits should be integrated into conservation efforts. A reciprocal transplant experiment was carried out monitoring survival, growth, and reproduction using three established populations of *Ostrea lurida* within Puget Sound, Washington. Performance differed for each population. *Ostrea lurida* from Dabob Bay had higher survival at all sites but lower reproductive activity and growth. Oysters from Oyster Bay demonstrated greater reproductive activity at all sites with moderate growth and survival. Together these data suggest the existence of *O. lurida* population structure within Puget Sound and provide information on how broodstock should be selected for restoration purposes.

Keywords: *Ostrea lurida*, Restoration, Growth, Reproduction, Mortality, Population Structure

Introduction

Restoration of native oysters is of increasing concern due to ongoing habitat degradation, loss of ecosystem services, and global climate change (Anderson, 1995; Lotze et al., 2011). Resource managers and restoration groups place viable animals into habitats to supplement dwindling populations and encourage persistence. Success of these efforts is highly dependent on the fitness of the transplanted individuals (McKay et al., 2005).

In the marine environment, the assumption of broad-scale fitness among marine invertebrates has been challenged. Palumbi (1997) demonstrated that geographic morphology affected sea urchin population structure and Burford et al. (2014) recently demonstrated a fitness cline in the eastern oyster, *Crassostrea virginica*, along the Atlantic coast. Findings such as these indicate that many similar species have unknown population structures that could hinder restoration efforts.

One species that has received considerable attention with respect to restoration is the Olympia oyster, *Ostrea lurida* Carpenter, 1864. The Olympia oyster is the only native oyster to the west coast of North America. Olympia oysters exist in a variety of habitats within its range from Baja California, Mexico to British Columbia, Canada (Hopkins, 1937). In Puget Sound, oysters experience average temperatures ranging from 5℃ to 20℃ (Hopkins, 1937). They have increased mortality in freezing temperatures (0℃) (Davis, 1955; Baker, 1995) or prolonged exposure to temperatures above 39℃ (LT50) (Brown et al., 2004). *Ostrea lurida* are rhythmical consecutive hermaphrodites (Coe, 1932b), spawning first as males followed by cycling between male and female. Hopkins (1937) observed in south Puget Sound that a maximum of 10-15% of *O. lurida* are brooding at any given time during a spawning season (1932). Peak larval settlement, roughly correlated with peak spawning, occurs twice annually within south Puget

Sound (Hopkins, 1937). Even with the body of information presented by previous research on *O. lurida*, little is known about stock structure.

Despite several studies on Olympia oyster ecology and life history traits in Puget Sound, WA, information on stock structure is limited and nothing is known about adaptive divergence of populations within Puget Sound (Camara and Vadopalas, 2009). Given the size, hydrologic features, and diverse environments of Puget Sound, it is possible that certain populations are adapted to local conditions. Among methods testing for local adaptation, reciprocal transplant experiments are considered robust (Sanford and Kelly, 2011) for investigating fitness. These experiments involve using parent populations from environmentally diverse locales to produce offspring that are placed reciprocally in their home and foreign environments. Population differences in key metrics for fitness can provide evidence of adaptive divergence (Burford et al., 2014).

The main objective of this study was to use a reciprocal transplant experiment to determine whether *O. lurida* populations from geographically diverse areas of Puget Sound, WA exhibit population-level differences in survival, reproduction, and growth in different environments.

Material and Methods

Broodstock Conditioning and Outplanting

Adult oysters (n=600) were collected from three locations in Puget Sound; Fidalgo Bay, Dabob Bay, and Oyster Bay (Figure 1) during November and December 2012. Gametogenesis and maturation occurred after being held for 5 months in common conditions in Port Gamble, Washington. To ensure genetic diversity, each population from each site was subsequently spawned in 24 groups of 20-25 oysters in June 2013. Larvae produced from each population were reared in tanks based on spawning group and settled on microcultch. Post-settlement spat were grown in four replicate screened silos and fed ad libitum until attaining the minimum outplant size (shell length (SL) = 5 mm).

In August 2013, 480 juvenile oysters (5-10 mm) from each population were placed at Fidalgo, Oyster, Dabob, and Clam Bays (Figure 1)**.** At each site, oysters were placed into four 0.61 x 0.61 m grow out trays per population with 12 trays total outplanted. In each tray, oysters (120) were equally distributed in four 10 x 7.5cm mesh (1475 micron) bags containing 30 oysters each. Trays were anchored into substrate using rebar stakes. In late autumn 2013, trays at Fidalgo, Oyster, and Clam Bays were subsequently suspended from floating structures to reduce exposure to extreme temperatures during tidal exchanges and oysters were removed from mesh bags. Trays remained anchored to the substrate submerged in a perched lagoon in Dabob Bay as no suitable floating structure was available and oysters were removed from mesh bags. At each site, temperature loggers (HOBOlogger, OnSet, USA) were deployed.

Site Monitoring

Temperature

Data from temperature loggers were collected at regular intervals and used to calculate minimum and maximum observed temperature for each day using the statistical analysis programming language R (R 3.0.3, R Core Team, 2014) and package *plyr* (Wickham, 2014). The number of days above 20℃ and below 5℃ was calculated for the duration of the project. Degree days (℃D) was calculated by adding the cumulative difference between the daily minimum temperature and the 2014 winter average minimum of 8℃ to determine the amount of energy needed to produce peak brooding activity. Raw temperature data and analysis procedures used are available (Heare et al., 2015).

Mortality

Mortality was determined by counts of live and dead oysters during visits to each site. Survival rates were assessed at all sites in December 2013, January (Dabob Bay only), February, April (Dabob and Clam Bays only), May (Fidalgo and Oyster Bay only), and June 2014. Differences in mortality within sites were determined through a Mantel-Haenszel test performed with the R package *survival* (Therneau, 2014). Mortality data and analysis procedures used are available (Heare et al., 2015).

Growth

Size was determined using ImageJ analysis (Rasband, 2010) of digitized images taken in August 2013 (all sites), April (Dabob Bay), September (Oyster Bay), and October 2014 (Fidalgo and Clam Bays). For each image a size reference was measured along with all oysters. For all oysters, shell length (SL) was determined via a linear measurement of the longest distance from umbo to valve margin. Descriptive statistics were produced by the R package *pastecs* (Grosjean and Ibanez, 2014). Size distributions were tested for normality using the Shapiro-Wilkes test (*stats* package, R Core Team, 2014). Growth was compared using Kruskal-Wallis assuming non-normal distribution (*stats* package, R Core Team, 2014). Pairwise comparisons were performed using the Nemenyi Post Hoc test using Tukey assumptions (*PMCMR* package, Pohlert, 2014). Size data and analysis procedures used are available (Heare et al., 2015).

Reproductive Activity

To assess reproductive activity, individual trays of oysters were anesthetized and each oyster was visually inspected for presence of brooding larvae in the mantle chamber. Specifically, trays were removed from water and exposed to air for 45 minutes then immersed in 0.3M magnesium sulfate (heptahydrate sulfate mineral epsomite ($MgSO_4 \cdot 7H2O$)) (Epsom salt) dissolved in a 50/50 mix freshwater/sea water for 45 minutes. The counts of brooding oysters were determined on weekly basis over three months (May 14th - August 15th, 2014) for a total of 15 time point observations for each site with each brooding female recorded for the day and then measured using calipers. Following Hopkins (1937) observation of the daily minimum temperature spawning threshold for *O. lurida* of 12.5℃, we counted the number of days from the first date which reached this threshold to the date of the first brooding females observed and the maximum proportion of brooding females. The proportion of brooding females per site per visit were arcsine transformed and analyzed via ANOVA (*base* package, R Core Team, 2014). Significant differences among sites and populations, pairwise comparisons were determined using TukeyHSD (*base* package, R Core Team, 2014). Sizes at brooding were likewise compared via ANOVA and TukeyHSD. (*base* package,R Core Team, 2014). Female brooding data and analysis procedures used are available (Heare et al., 2015).

Results

Site Characteristics

Oyster Bay had the highest daily minimum temperature (18.43℃) (Figure 2) in August 2014 while Dabob Bay had the lowest daily minimum temperature (-3.32℃) during February 2014 (Figure 2). The Dabob Bay site experienced the highest amount of temperature variability due to the intertidal placement of samples and the extreme cold weather during low tide events

(Figures 2 & 3). From June to August 2014, Oyster Bay experienced warmer daily temperatures as compared to all other sites (Figures 2 & 3).

Survival

Differences in mortality per population were observed at three of the four sites. Dabob Bay oysters had significantly less mortality by the end of the study period at Dabob (X^2=141, df=2, P=0), Oyster (X^2=76.3, df=2, P=0), and Clam Bays (X^2=13.7, df=2, P=0.00105) (Figures 4A, 4B, & 4C) than other populations.

The Dabob Bay location experienced unexpected elevated mortality, necessitating the premature termination of the Dabob Bay trial in April 2014. There were also significant differences in mortality among populations (X^2=141, df=2, P=0), with the Fidalgo Bay oysters having the lowest survival (21.2% +/- 2.1SD %) (Figure 4C). Limited mortality was observed at both Clam Bay and Fidalgo Bay where at least 80% of oysters remained after 11 months (July 2014) (Figures 4B & 4D).

Growth

Oyster size at outplant was 11.4 (+/-3.2SD) mm. At the end of the experiment the size of oysters among sites were significantly different (X^2=383.4, df=2, P<0.0001), with Oyster Bay producing the largest oysters (Figure 5) and Clam Bay producing the smallest (Figure 7). Oyster size also differed among populations (X^2=196.1, df=2, P<0.0001). At Oyster Bay, Fidalgo Bay oysters were larger than Dabob (Nemenyi Post-Hoc, P=<0.0001) and Oyster Bay (Nemenyi Post-Hoc, P=<0.0001) oysters (Figure 5). At both Fidalgo Bay and Clam Bay, oysters from Dabob Bay broodstock were smaller than Fidalgo Bay (Nemenyi Post-Hoc, P<0.0001 and

P<0.0001, respectively) and Oyster Bay (Nemenyi Post-Hoc, P<0.0001 and P=0.00028, respectively) oysters at the end of the experiment (Figures 6 & 7).

Brooding Females

The proportions of brooding females varied among populations (ANOVA, F=9.1, df=2, P=0.0002) and among sites (ANOVA, F=11.4, df=2, P<0.0001). The greatest proportion of total brooding females present was at Oyster Bay (Figure 8) compared to Fidalgo Bay (P=0.007) and Clam Bay (P<0.0001). The smallest proportion of brooding females was documented at Clam Bay (Figure 10). The Oyster Bay population produced significantly more brooding females than Fidalgo Bay (Tukey's HSD, P=0.001) or Dabob Bays (Tukey's HSD, P=0.0005). The Fidalgo and Dabob Bay populations were not different from one another at all sites (Tukey's HSD, P=0.942).

Oyster Bay reached the spawning temperature threshold of 12.5℃ (as defined by Hopkins, 1937) on May 14th and the first brooding female was observed 15 days later on May 29th (Figure 8). Ambient water temperatures in Oyster Bay rose steadily from late winter reaching the spawning threshold and continuing to increase to the summer maximum of 18.43℃ (Figure 8). At Oyster Bay, Oyster Bay oysters reached the maximum percentage of brooding females on June 19th, 36 days post 12.5℃, equating to 308°D. At this location, Dabob Bay and Fidalgo Bay oyster populations reached the maximum percentage of brooding females on July 10th, 57 days post 12.5℃, 453°D (Figure 8).

At Fidalgo Bay, the 12.5℃ temperature was also reached on May 14th and the first brooding female was observed on June 6th (Figure 9), 23 days later. Fidalgo Bay exhibited a slower, less steady temperature increase throughout the spring season with ambient water temperatures reaching 12.5℃ in mid-May but then dipping into the 10-11℃ range until early

June, after which the site remained above the threshold for the remainder of the summer (Figure 9). The Oyster Bay oysters in Fidalgo Bay reached maximum percentage brooding females by July 11th, 58 days later or 354˚D. Fidalgo Bay and Dabob Bay oysters' populations did not reach maximum percentage brooding females observed until August 8th (Figure 9), 87 days later or 513˚D.

Clam Bay reached 12.5℃ on June 8th and brooding females were observed on June 18th from the Oyster Bay population (Figure 10), 10 days later. Temperatures in Clam Bay reached 12.5℃ in early June but varied above and below this temperature for several days at a time throughout most of summer (Figure 10). Peak spawning could not be determined due to low number of brooding individuals observed at Clam Bay.

Size at brooding varied significantly among populations (ANOVA, F=18.2, df=2, P<0.0001) and sites (ANOVA, F=33.1, df=2, P<0.0001) with the smallest brooding females observed at Clam Bay (Figure 11). Population size at brooding was significantly different between all populations. Size was significantly different between Clam Bay and the other sites (Fidalgo Bay (Tukey's HSD, P=0), Oyster Bay (Tukey's HSD, P=0)). No differences in size of brooding females was observed between Oyster Bay and Fidalgo Bay (P=0.8). The average minimum size at brooding of the ten smallest oysters was 19.1(+/-3.7SD) mm. Two brooding females of 15.0 mm were observed at Clam Bay from the Dabob Bay population. The overall average size of brooding females was 27.1 (+/- 4.5SD) mm.

Discussion

A primary objective for this study was to evaluate population performance in relation to adaptive stock structure of Olympia oysters in Puget Sound, WA. Findings from this study provided new information about *Ostrea lurida* life history as well as distinct phenotypes associated with populations. At the population level, we found some populations favor survival over other traits and some populations favor reproduction suggesting the existence of adaptive structure within Puget Sound, WA. In the remainder of this section, findings from this study are discussed in terms of life history traits, differences in population performance, and implications of these findings with respect to restoration efforts.

Ostrea lurida *Life History*

Mortality

Temperature affected mortality in Olympia oysters. High mortality experienced by all populations at Dabob Bay was attributed to temperature extremes. The Dabob Bay site exceeded the temperature range reported by Baker (1995) on 35% of the total days (85 out of 242 days) with two subfreezing events of -0.78℃ and -3.3℃ in December 2013 and February 2014 respectively (Figure 2). The Oyster Bay site, which also experienced moderate mortality, had a total of 39 days (9% 0f 398 days) outside of the 5-20℃ range. The majority (34 days) were above the upper limit (20℃) but not near the lethal temperature (LT50) of 39℃ reported by Brown et al. (2004). Fidalgo Bay and Clam Bay had fewer days outside of the range (24 days and 0 days respectively) and had low mortality. The role of temperature as a primary determinant of survival when oysters are transplanted outside of their broodstock populations range is similar to its role as found by Burford et al. (2014).

Growth

In the present study, Olympia oysters attained an average size of 35.8 (+/-6.4SD) mm during the first year of growth. Some individuals attained sizes >45 mm. These observations differ from the 2-3 years needed to attain this size in *O. lurida* reported by Hopkins (1937). This discrepancy could be due to changes in environmental conditions present at the site or differences of the population sampled.

A difference in size occurred in relation to site. Oysters from all populations at Oyster Bay grew to the largest size and experienced the warmest temperatures year round. This finding is in accord with other studies (e.g. Malouf and Breese, 1977; Brown and Hartwick, 1988; Shpigel et al., 1992) that demonstrate that warm temperatures improve oyster growth as long as the temperatures are within the tolerable range.

Reproduction

Oysters reproduced as females in Puget Sound at a mean size of 27.1 (+/- 4.5SD) mm. This result contrasts with results of previous research (Hopkins, 1937; Coe, 1932 a&b) that describe *O. lurida* as only reproductive at sizes of 30 mm or greater. The ability to reproduce at smaller sizes is important because it may provide reproductive advantage.

It has been generally accepted that *O. lurida* begin spawning at relatively low temperatures (13℃ Coe, 1931a; 12.5℃ Baker, 1995). Hopkins (1937) suggested that this temperature threshold must occur during high tide, which is related to the daily minimum temperature. In accordance with these earlier studies, we found at all sites brooding only occurred after daily minimum temperatures increased above 12.5℃. The steady increase in temperature as observed in the present study in Oyster Bay may have allowed *O. lurida* to spawn much earlier in the season than at other sites (Figures 8, 9, & 10).

By comparing the reproductive initiation and peak brooding observed to observations by Hopkins (1937) in the same area, it appears that the reproductive period occurred approximately two weeks later in 2014 than in 1932-1933. Further investigation is required to determine if this is simple natural variation or an important change to the spawn timing in the region.

Population Differences

Mortality

Survival differed among populations within a majority of sites. The population derived from Dabob Bay broodstock exhibited better survival than the other two populations (Figure 4). The observed temperature variability (Figures 2 & 3) at Dabob Bay in the present study may be indicative of historic temperature trends to which the parent populations were exposed. If so, the significantly greater survival of the Dabob Bay population at three of the four sites could be a function of increased stress resilience of offspring in response to prevalent temperature extremes. Previous studies on thermal tolerance, (e.g. bay scallops, *Argopecten irradians*, (Brun et al., 2008) and Mediterranean mussels, *Mytilus galloprovincialis*, (Dutton and Hofman, 2009)) demonstrate more frequent exposure to temperature extremes result in elevated heat shock proteins (HSP) and HSP mRNA transcripts. In addition, Sørensen et al. (2004) found that many species exhibit heritable heat shock protein production patterns. The higher survival rates observed in the Dabob Bay population may likewise be related to heritable traits and warrants investigation.

Growth

At all transplant sites, the population derived from Dabob Bay parents exhibited the least growth. This observation is likely related to the fact the Dabob Bay population also had the highest survival. Applebaum et al. (2014) found energetic tradeoffs may improve survival over growth in the Pacific oyster, *C. gigas*. Arendt (1997) suggested that “stress tolerators” exhibit slower intrinsic growth that is relatively unresponsive to improved conditions. Further investigation is required to determine the links between growth, energetic tradeoffs, and environmental variables affecting *O. lurida*.

Reproduction

The Oyster Bay population had a greater proportion of brooding females and reached peak spawning earlier than the other populations, across all sites. One explanation for this is that the relatively rapid water temperature increase and higher temperatures in south Puget Sound may have selected for early spawning oysters in the Oyster Bay population. Evidence for this includes the fact that it took 150 fewer °D for the Oyster Bay population to reach peak spawning compared to the other two populations at two sites. The general rate of temperature increase at a particular locale may influence spawn timing (Lawrence and Soame, 2004). Chávez-Villalba et al. (2002) found place of origin for *C. gigas* broodstock affected the rate of gametogenesis under different temperatures with some populations becoming reproductively active sooner than others. Barber et al. (1991) found gametogenesis and spawn timing were heritable traits within populations of *C. virginica*.

Conclusions

Differences in life history traits among *Ostrea lurida* populations grown in different locations within Puget Sound, WA suggest adaptations possibly linked with environmental cues. High survival, low growth, and low reproductive activity of the Dabob Bay population is likely due to extreme environmental variation at their home site leading to stress resilience adaptations. The greater proportion of brooding females in the Oyster Bay population and reduced environmental energy (°D) needed to induce peak spawning may be related to positive selection pressure for early spawners due to warmer temperature trends at their home site. These findings have important implications for ongoing restoration efforts as well as for future Olympia oyster research.

There are a number of ways that these findings could be used in influencing restoration efforts specific to Puget Sound and elsewhere. Based on the fact that Dabob Bay oysters had the lowest mortality, use of the most robust population for broodstock may increase chances for outplant survival. Generally, this approach would dictate organisms should be used from home environments that experience persistent stressful conditions. An alternative approach managers might take given the current findings is to take the population with the greatest reproductive output (Oyster Bay) and use it as a source of broodstock. This would increase the likelihood of juvenile recruitment and ultimate restoration of the species, while also producing more offspring for outplant. Ultimately, what this study demonstrates is that population structure can and does exist on a relatively small geographic scale and thus moving oyster populations to locations where remnant stocks exist could be disadvantageous. When adaptive structure exists, there should be concern with respect to moving populations as 1) transplanted populations could overwhelm locally adapted remnant populations, and possibly not persist themselves, 2) transplanted populations might not survive in new location and thus wasting resources required for restoration activities, and 3) transplanted populations could interbreed with remnant population and thus result in overall reduced fitness. Many of these implications make assumptions regarding plasticity and adaptive potential, though we still know little about this in marine invertebrates, particularly on the temporal scales involved.

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Figures

Figure 1. Map of Puget Sound with *Ostrea lurida* broodstock and outplant sites. Conditioning site was Port Gamble (G). Broodstock collected from Fidalgo Bay (F), Dabob Bay (D), and Oyster Bay (O). Outplanted at Fidalgo Bay (F), Dabob Bay (D), Clam Bay (C), and Oyster Bay (O).

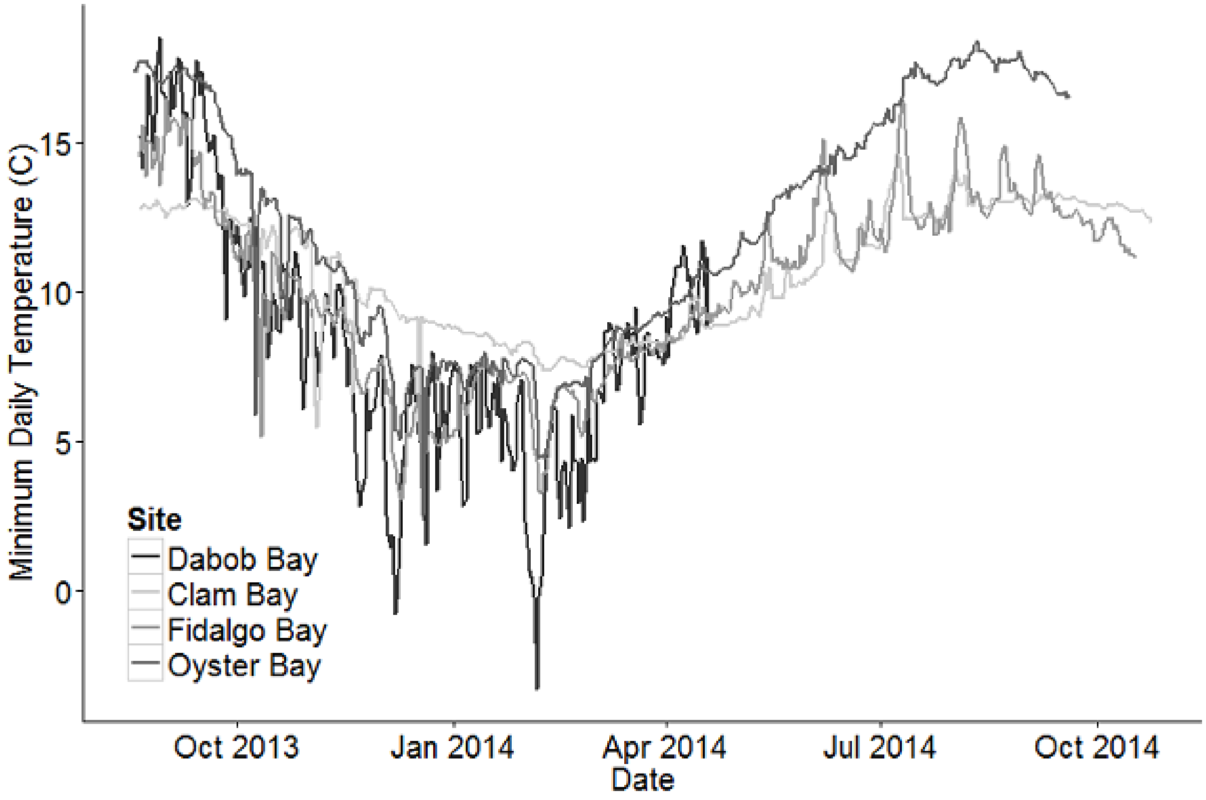
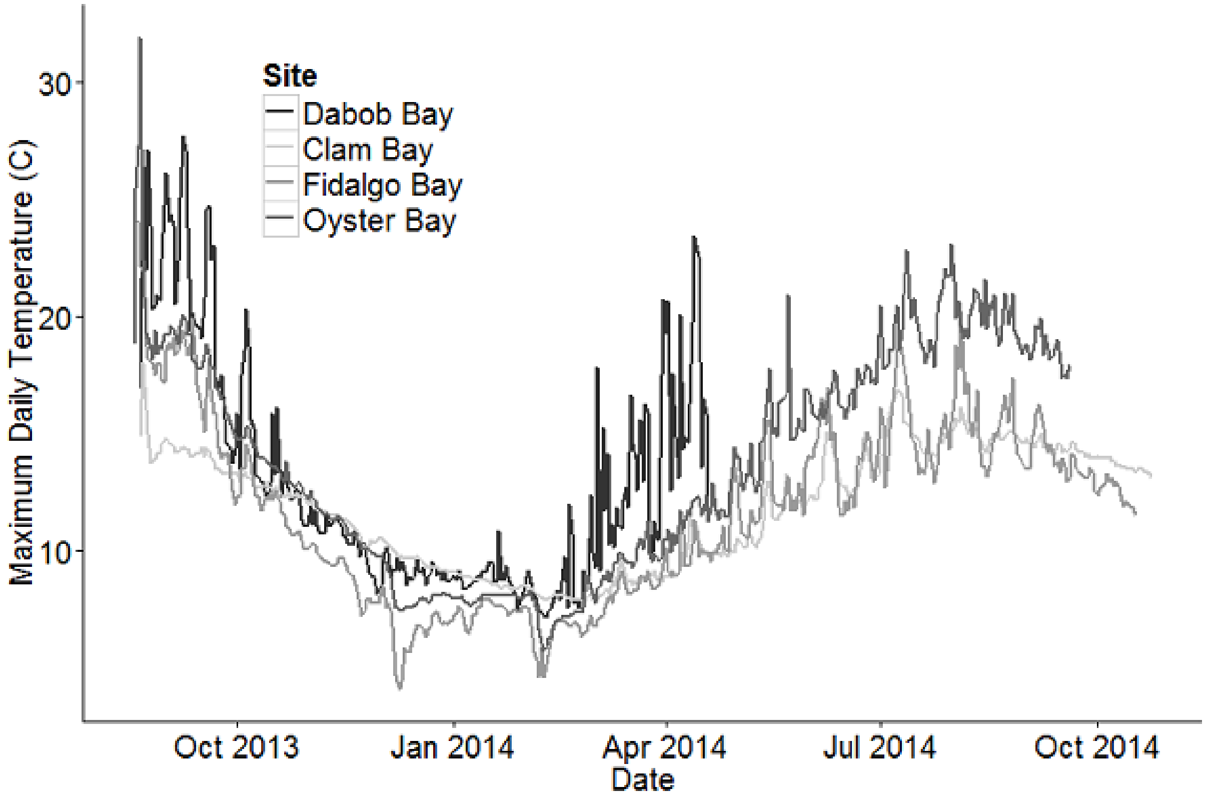


Figure 2. Minimum observed daily temperatures for all sites.



482

483 Figure 3. Maximum observed daily temperatures for all sites.

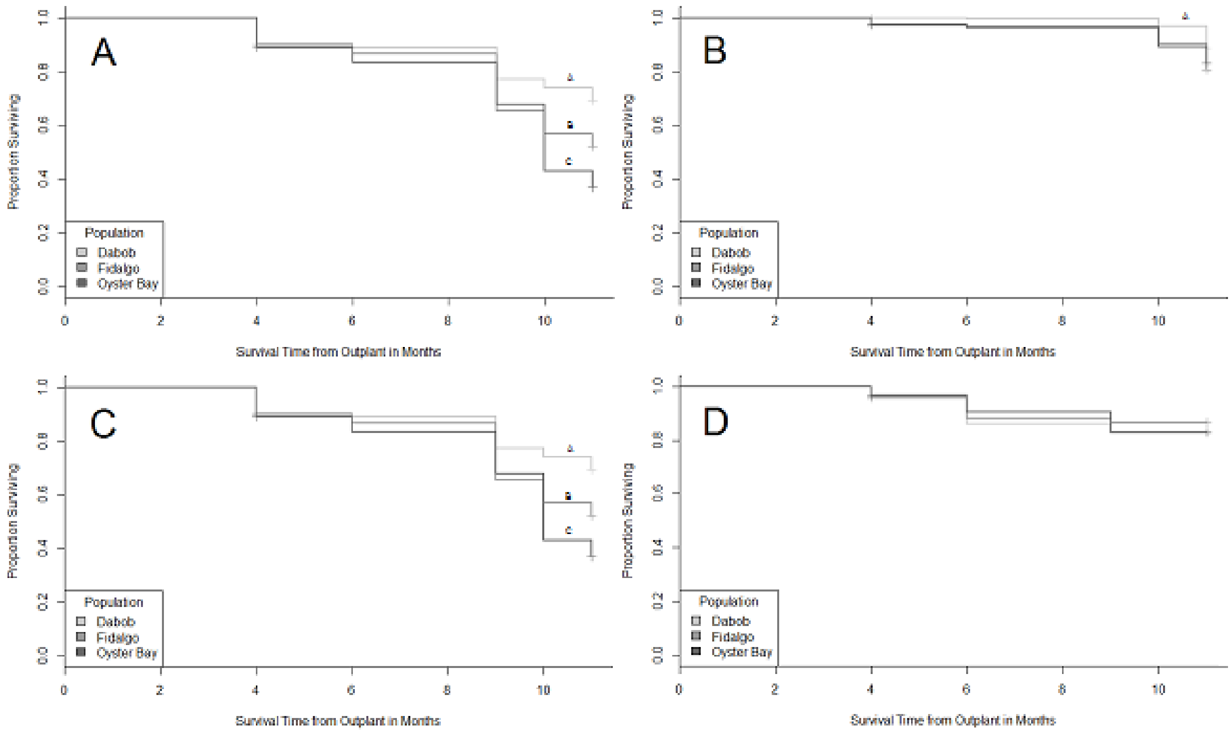


Figure 4. Proportion survival for three *Ostrea lurida* populations at four locations; Oyster Bay (A), Clam Bay (B), Dabob Bay (C), and Fidalgo Bay (D). Lowercase letters (a,b,c) are significant differences.

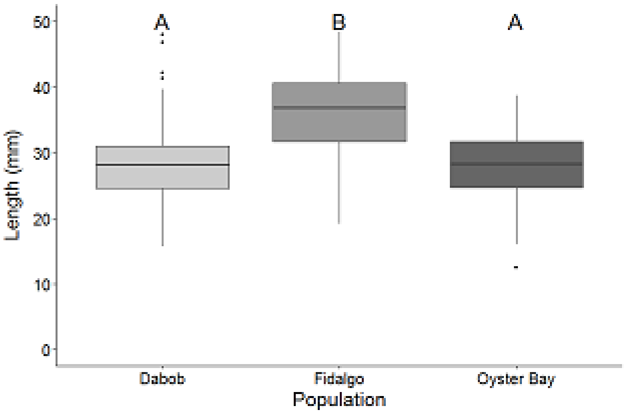


Figure 5. *Ostrea lurida* shell length in September 2014 at Oyster Bay. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Fidalgo Bay oysters were considered different due to Nemenyi Post Hoc test with P=0.0 (Oyster Bay and Dabob Bay oysters).

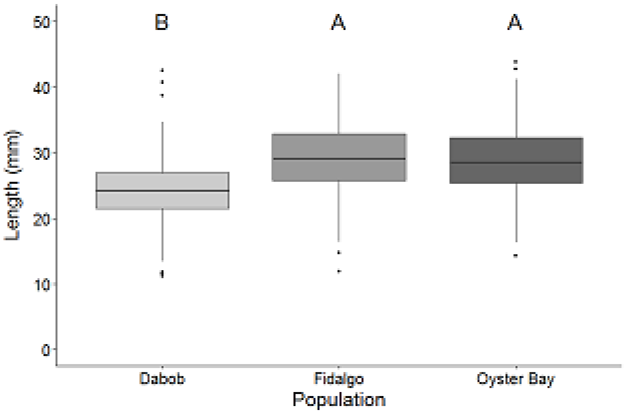


Figure 6. *Ostrea lurida* shell length in October 2014 at Fidalgo Bay. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters were considered different due to Nemenyi Post Hoc test with P=0.0 (Fidalgo Bay and Oyster Bay oysters).

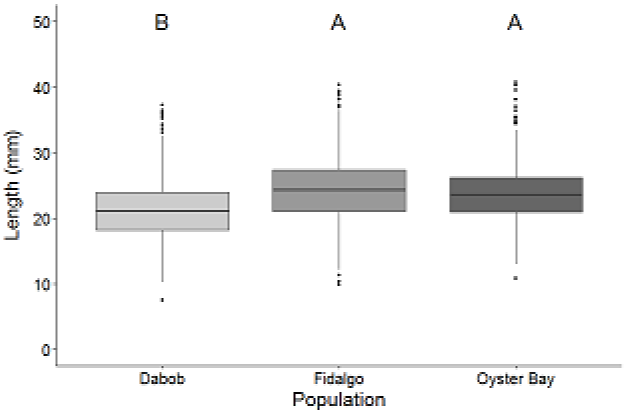


Figure 7. *Ostrea lurida* shell length in October 2014 at Clam Bay. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters were considered different due to Nemenyi Post Hoc test with P=0.00028 (Oyster Bay oysters) and P<0.0001 (Fidalgo Bay oysters).

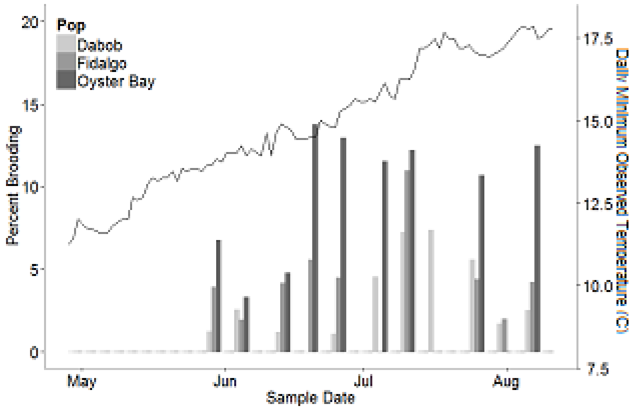


Figure 8. Percent *Ostrea lurida* brooding females from each population at each sample date at Oyster Bay. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or %=(Br/T)*100.

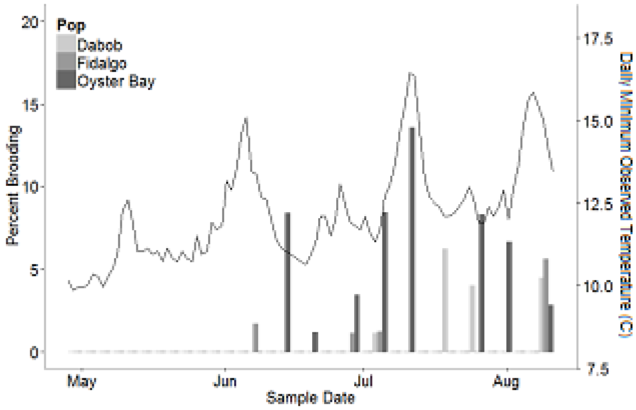


Figure 9. Percent *Ostrea lurida* brooding females from each population at each sample date at Fidalgo Bay. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or %=(Br/T)*100.

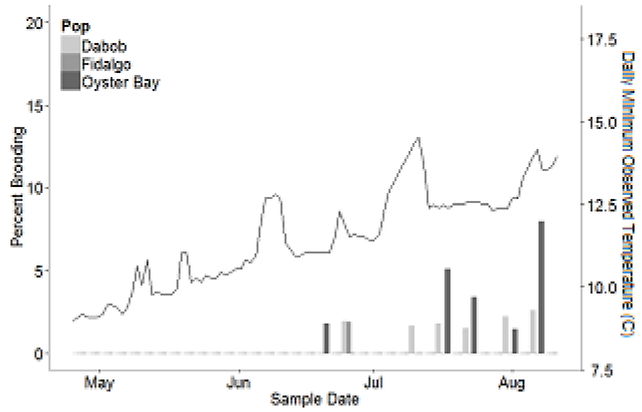


Figure 10. Percent *Ostrea lurida* brooding females from each population at each sample date at Clam Bay. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or %=(Br/T)*100.

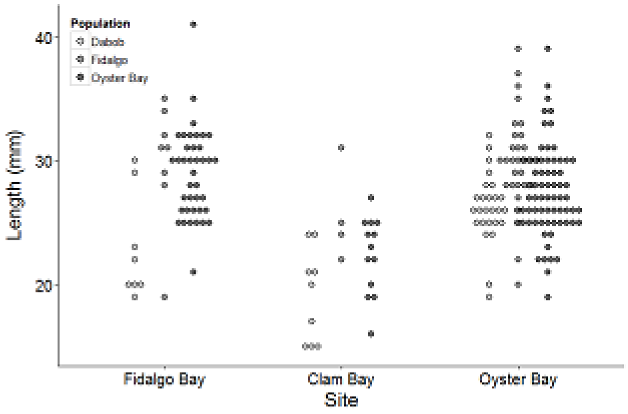


Figure 11. *Ostrea lurida* brooding female shell length comparison among sites.