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

1. Introduction

Restoration of native species is of increasing concern due to habitat degradation, loss of ecosystem services, and global climate change (Anderson, 1995; Lotze et al., 2011). Resource managers and conservation groups place viable animals into habitats to supplement dwindling populations and encourage persistence. Theory suggests that the success of these efforts is highly dependent on the fitness of the transplanted individuals. 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 may 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), which spawn first as male 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 spawning season. 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.

While there have been several studies on Olympia oyster ecology and life history traits in Puget Sound, WA, information on stock structure, particularly with regard to any adaptive structure, is lacking (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. To better predict the success of *O. lurida* supplementation efforts within Puget Sound, more extensive research needs to be performed to determine whether local populations vary in performance metrics.

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 indicate an adaptive advantage (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.

2. Material and Methods

2.1 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 four replicate screened silos, settled on microcultch and fed ad libitum until attaining the minimum outplant size (shell length (SL) = 5 mm).

In August 2013, 480 oysters (5-10 mm) from each population were planted at Fidalgo, Oyster, Dabob, and Clam Bays (Figure 1)**.** At each site, oysters from each population were placed into four 0.61 x 0.61 m grow out trays 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. Trays remained anchored to the substrate in Dabob Bay as no suitable floating structure was available. At each site, HOBOlogger temperature sensors (OnSet, USA) were deployed.

2.2 Site Monitoring

2.2.1 Temperature

Data from temperature loggers were collected at regular intervals and used to calculate minimum and maximum observed temperature for each day using the R 3.0.3 (R Core Team, 2014, 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 environmental energy needed to produce peak brooding activity. Raw temperature data and analysis procedures used are available (Heare et al., 2014).

2.2.2 Mortality

Mortality was determined by counts of live and dead oysters during visits to each site. Survival was 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. All trays were examined during winter visits and a single tray was counted each week during June. Differences in mortality within sites were determined through survdiff tests performed in the R 3.0.3 (R Core Team, 2014) with the R package "*survival*" (Therneau, 2014). Mortality data and analysis procedures used are available (Heare et al., 2014).

2.2.3 Growth

Size was determined using digital images of oysters via ImageJ analysis (Rasband, 2010). Images used were 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 PostHoc test using Tukey assumptions (R package "PMCMR", Pohlert, 2014). Size data and analysis procedures used are available (Heare et al., 2014).

2.2.4 Reproductive Activity

To assess reproductive activity the number 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. 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. Each brooding female was recorded for the day and then measured using calipers. The date of maximum brooding activity was consider to be the date with the highest proportion of brooding females observed. 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 female observed and the maximum proportion of brooding females. The proportion of brooding females per site per visit was arcsine transformed and analyzed via ANOVA (‘base’ package, R 3.0.3 R Core Team, 2014). Significant differences among sites, populations, and pairwise comparisons were determined using TukeyHSD (‘base’ package, R 3.0.3, R Core Team, 2014). Minimum average SL at brooding was calculated by averaging the 10 smallest brooding females observed. Sizes at brooding were likewise compared via ANOVA and TukeyHSD. (R 3.0.3, R Core Team, 2014, ‘base’ package). Female brooding data and analysis procedures used are available (Heare et al., 2014).

3. Results

3.1 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).

3.2 Survival

Differences in mortality were observed at three of the four sites. Dabob Bay oysters had more individuals survive 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 very high overall mortality, necessitating the premature termination of the Dabob Bay trial in April 2014. There was also significant differences in mortality across 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 Clam Bay and Fidalgo Bay where at least 80% of oysters remained after 11 months (July 2014) (Figures 4B & 4D).

3.3 Growth

Mean oyster SL at outplant was 11.36 +/-3.15SD mm. Using the Kruskal-Wallis test due to non-normal size distributions, we found the mean SLs of oysters by site were different

(X^2=383.4411, df=2, P<0.0001), with Oyster Bay producing the largest oysters (Figure 5) and Clam Bay producing the smallest (Figure 7). Mean SL of oysters also differed among populations (X^2=196.062, df=2, P<0.0001). A Nemenyi post hoc test showed at Oyster Bay, Fidalgo Bay oysters were larger than Dabob (P=1.2e-12) and Oyster Bay (P=6.0e-12) oysters (Figure 5). At both Fidalgo Bay and Clam Bay, oysters from Dabob Bay were smaller than Fidalgo Bay (P<0.0001 and P<0.0001, respectively) and Oyster Bay (P<0.0001 and P=0.00028, respectively) oysters at the end of the experiment (Figures 6 & 7).

3.4 Brooding Females

The proportions of brooding females varied among populations (ANOVA, F=9.146, df=2, P=0.0002) and among sites (ANOVA, F=11.424, df=2, P<0.0001). Using Tukey's Honestly Significant Difference test we found that 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 produced at Clam Bay (Figure 10).

Tukey's test also showed that the Oyster Bay population produced more brooding females than Fidalgo Bay (P=0.001) or Dabob Bays (P=0.0005). This trend was evident at Clam Bay (Figure 10) but non-significant. The Fidalgo and Dabob Bay populations were not different from one another at all sites (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.

Size at brooding varied among populations (ANOVA, F=18.263, df=2, P<0.0001) and sites (ANOVA, F=33.111, df=2, P<0.0001) with the smallest brooding females observed at Clam Bay (Figure 11). The average minimum SL 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 SL of brooding females was 27.14 +/- 4.46SD mm. A Tukey's post hoc test was used to compare SL at brooding. Shell length was different between Clam Bay and the other sites (Fidalgo Bay (P=0), Oyster Bays (P=0)), the smallest occurred at Clam Bay and the largest SLs at the other two sites. There was no difference between Oyster Bay and Fidalgo Bay (P=0.8).

4. Discussion

Our primary objective for this study was to evaluate population performance 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 population. We found that *O. lurida* reproduce within the first year in Puget Sound. On a population scale we found some populations favor survival over other traits while other populations favor reproduction. These findings suggest the existence of adaptive *O. lurida* stock structure within Puget Sound, WA.

4.1 Ostrea lurida *Life History*

4.1.1 Mortality

Temperature appears to have affected mortality in Olympia oysters. High mortality experienced by all populations at Dabob Bay is likely attributed to temperature. The Dabob Bay site exceeded the temperature range reported by Hopkins (1937) 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. Overall, temperature appears to affect mortality. This finding is similar to Burford et al.'s (2014) mortality findings.

4.1.2 Growth

Using Hopkins (1937) description of growth in *O. lurida*, we were surprised to find that our oysters, in the same region as the Hopkins experiment, grew faster than expected. In the present study, Olympia oysters attained an average SL of 35.8 +/-6.4SD mm during the first year of growth. Some individuals attained SL >45 mm. These findings are not in accordance with the observations Hopkins (1937) reported: *O. lurida* reach 35-45 mm in size in 2-3 years in south Puget Sound. This discrepancy could be due to the limited ability of Hopkins (1937) to determine with certainty the age of the oysters used in the study. Growth was affected by site and produced expected results.

Sites showed a difference in size, oysters from all populations at Oyster Bay grew to the largest size and experienced the warmest temperatures year round. This is not surprising as studies (Malouf and Breese, 1977; Brown and Hartwick, 1988; Shpigel et al., 1992) have shown that increased temperatures improve growth as long as the temperatures are within the tolerable range for oyster species (*Crassostrea virginica* and *Crassostrea gigas*). Overall growth in each area may have been influenced by the amount of available energy within the environment for consumption and growth.

4.1.3 Reproduction

In this study, *O. lurida* reproduce as females in the first year in Puget Sound, at a mean SL of 27.14 +/- 4.46SD mm. This result contrasts with results of previous research (Hopkins, 1937; Coe, 1932a&b) that describe *O. lurida* as only reproductive at sizes of 30 mm or greater.

This contrast may be due to the techniques used at the time which required the use of field collected oysters and limited the researchers' ability to determine age of each oyster examined. The ability to reproduce within the first year is important because it may provide reproductive advantage to first season spawners as compared to oysters which do not spawn until their second season, and thus must survive environmental threats for twice as long before beginning reproductive activities.

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. We found at all sites brooding only occurred after daily minimum temperatures increased above 12.5℃. The steady increase in temperature observed in Oyster Bay may have allowed *O. lurida* to spawn much earlier in the season than at other sites (Figures 8, 9, & 10).

Also, comparing the timing of the reproductive period to that observed by Hopkins (1937) in the same area, it appears that the reproductive period has been delayed by two weeks or more in south Puget Sound. Further investigation is required to determine if this is simple natural variation or an important change to the spawn timing in the region.

4.2 Population Differences

4.2.1 Mortality

Survival differed among populations within each site. At all transplant sites, the population derived from Dabob Bay parents 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 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 in response to prevalent temperature extremes. In previous studies on thermal tolerance, it has been shown that species such as Bay scallops, *Argopecten irradians*, (Brun et al., 2008) and Mediterranean mussels, *Mytilus galloprovincialis*, (Dutton and Hofman, 2009) that experience more frequent exposure to temperature extremes produce more 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.

4.2.2 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 *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*.

4.2.3 Reproduction

At all transplant sites, the Oyster Bay population had a greater proportion of brooding females and reached peak spawning earlier than the other populations. The relatively rapid water temperature increase to sustained warm periods in south Puget Sound may have selected for early spawning oysters in the Oyster Bay population, which required 150 fewer °D than the other

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two populations to reach peak spawning. 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*. The possibility of adaptive advantage of different spawn timing in *O. lurida* warrants investigation.

4.3 Conclusion

The differences in life history traits among *Ostrea lurida* populations grown in different locations within Puget Sound, WA suggest adaptations possibly linked with environmental cues. The high survival, low growth, and low reproductive effort of the Dabob Bay population may be 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 implications for ongoing restoration efforts as well as for future plans of study on the life history of Olympia oysters.

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7. Figures

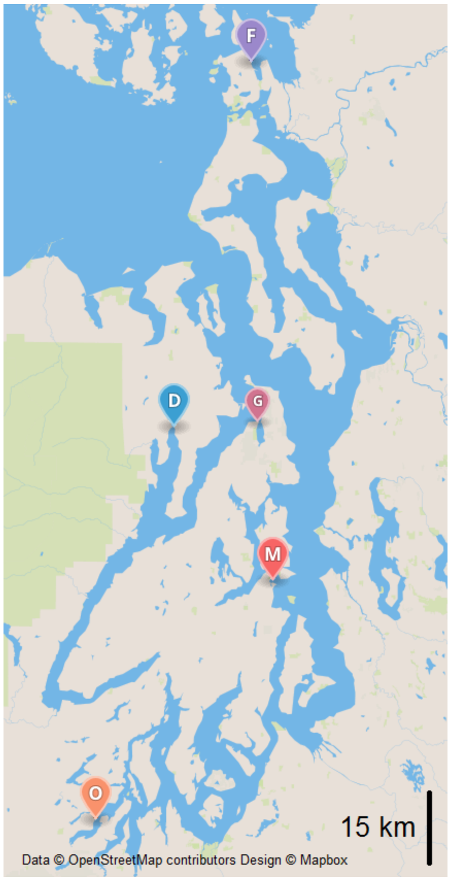
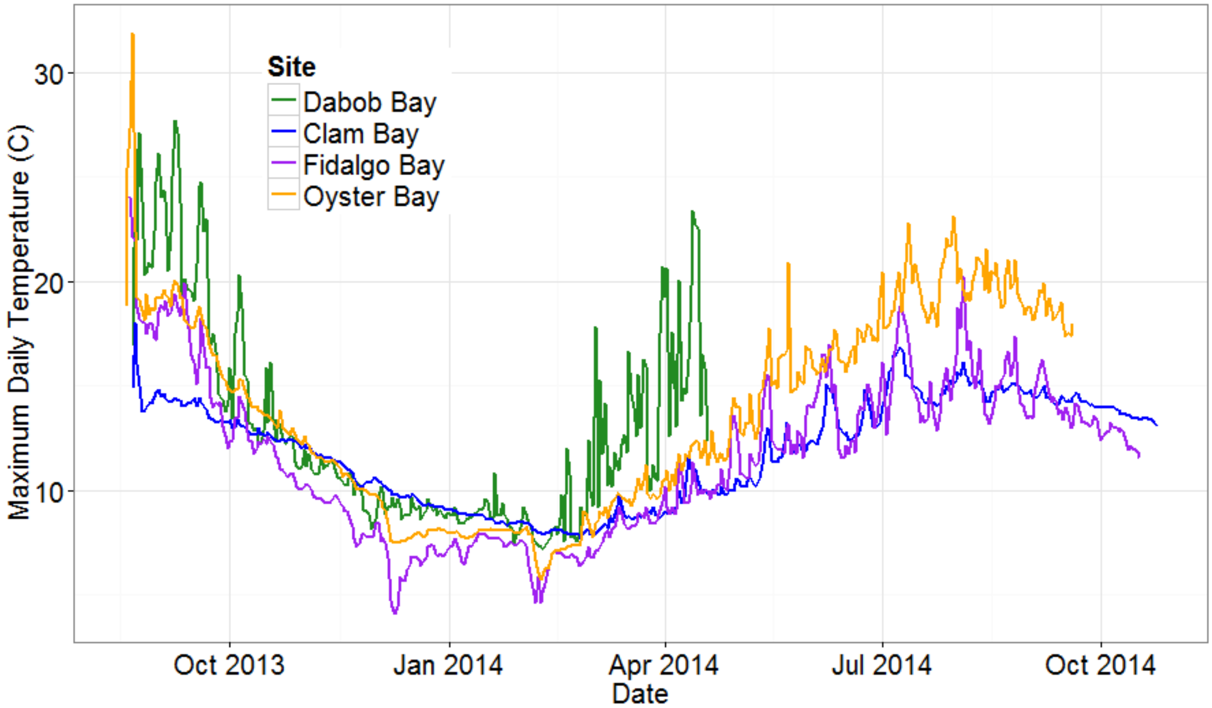


Figure 1. Map of Puget Sound with *Ostrea lurida* Broodstock and Outplant Sites. Conditioning site was Port Gamble (G). Broodstock collected from Fidalgo Bay (F), Dabob Bay (D), Oyster Bay (O). Outplanted at Fidalgo Bay (F), Dabob Bay (D), Clam Bay (C), Oyster Bay (O).



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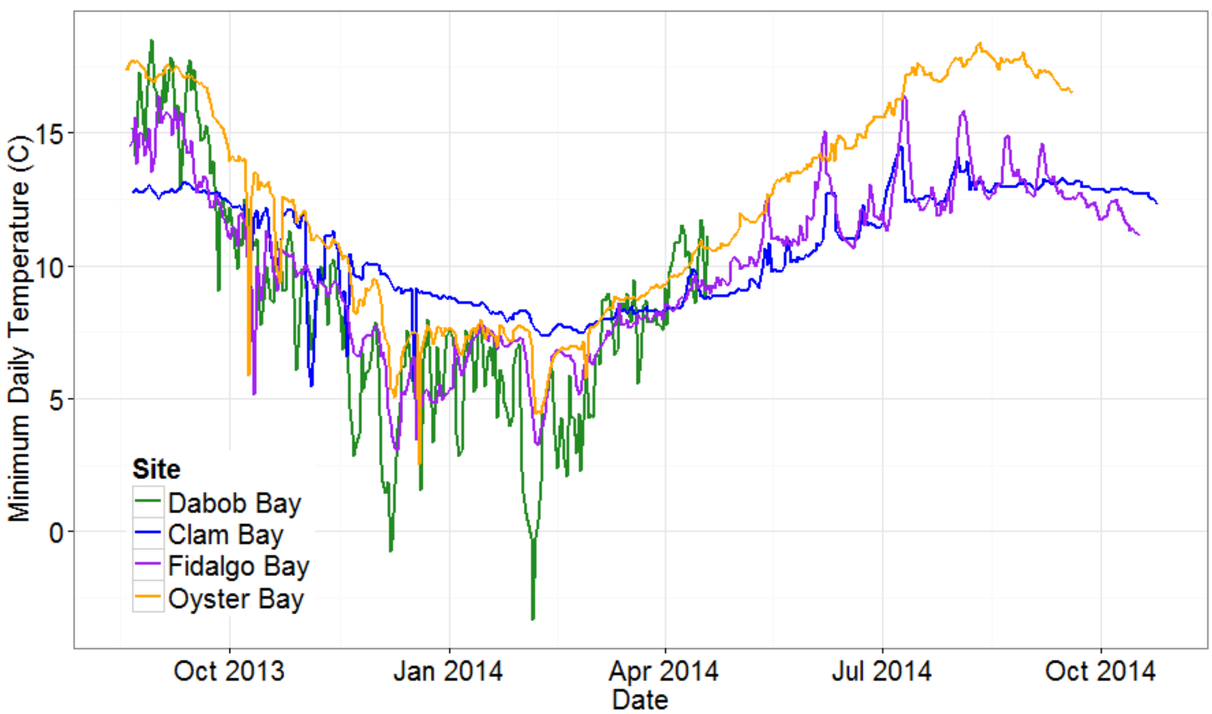
453 Figure 2. Minimum Observed Daily Temperatures for All Sites.



454

455 Figure 3. Maximum Observed Daily Temperatures for All Sites.

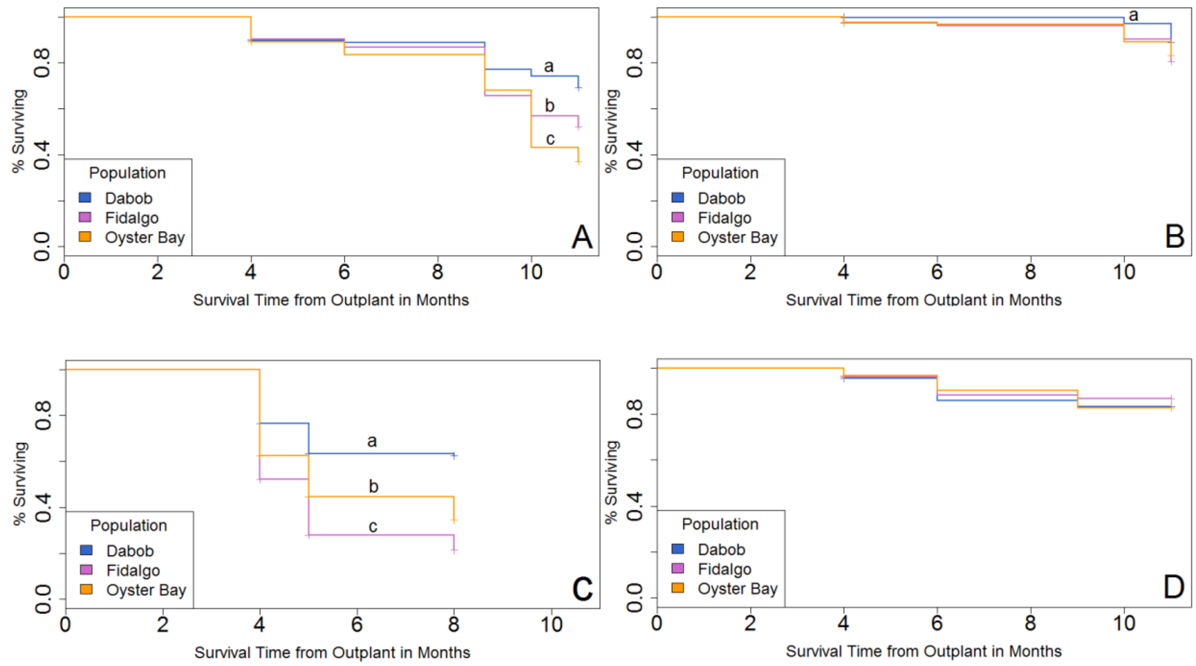


Figure 4. Proportion Survival for Three *Ostrea lurida* Populations at Four Locations; Oyster Bay (A), Clam Bay (B), Dabob Bay (C), and Fidalgo Bay (D). Lowercase letters (a,b,c) are significant differences.

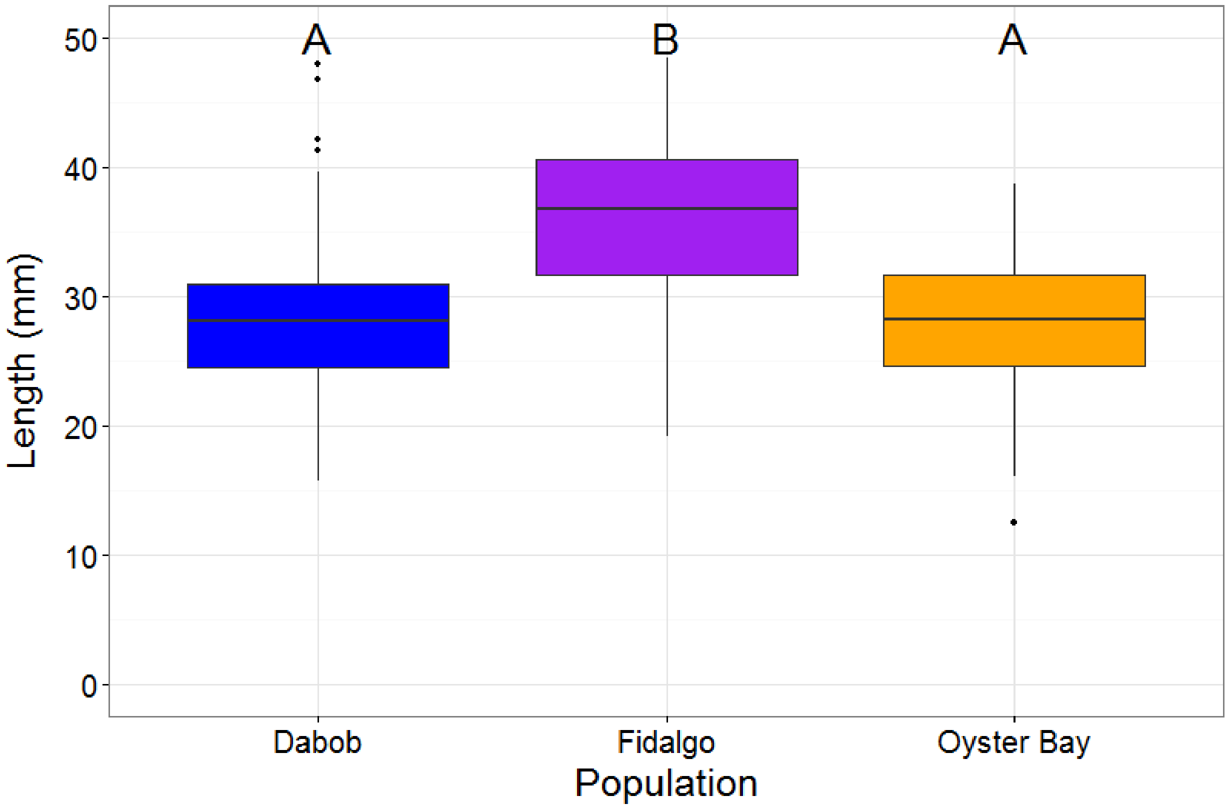


Figure 5. *Ostrea lurida* Shell Length in September 2014 at Oyster Bay. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Fidalgo Bay oysters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

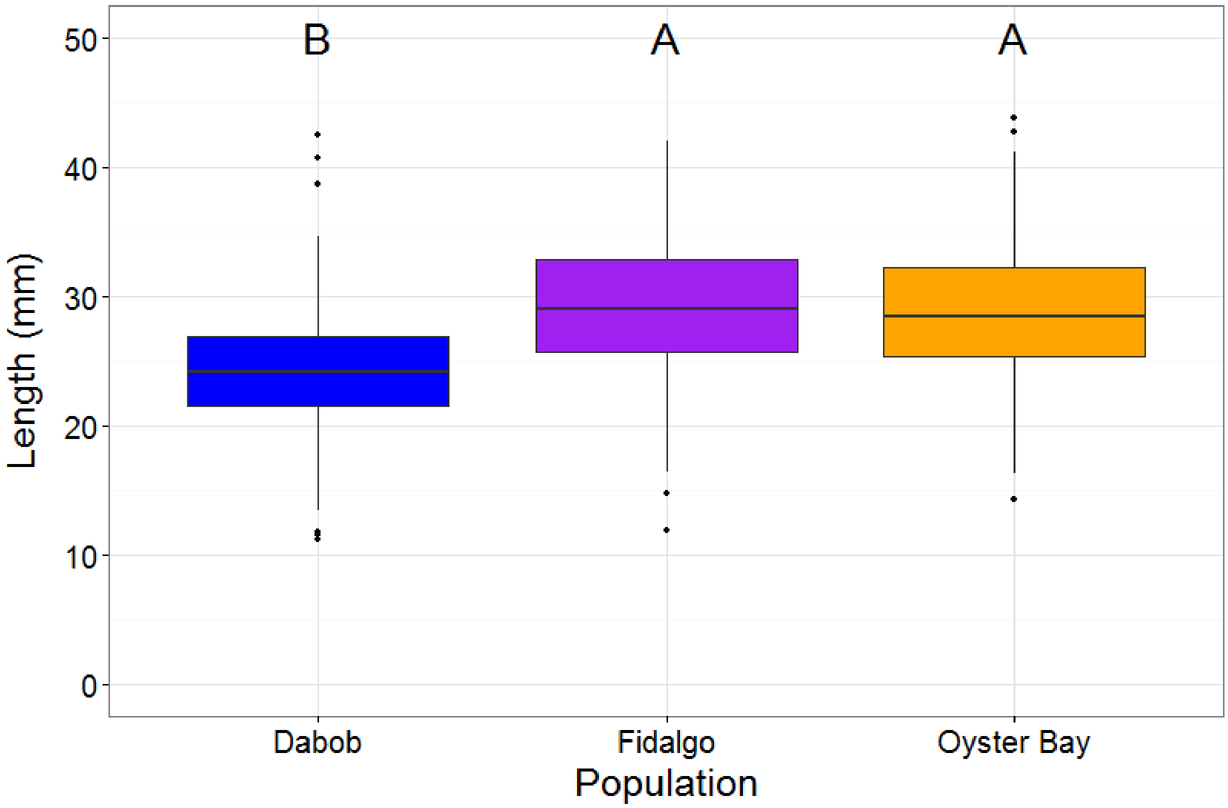


Figure 6. *Ostrea lurida* Shell Length in October 2014 at Fidalgo Bay. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters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

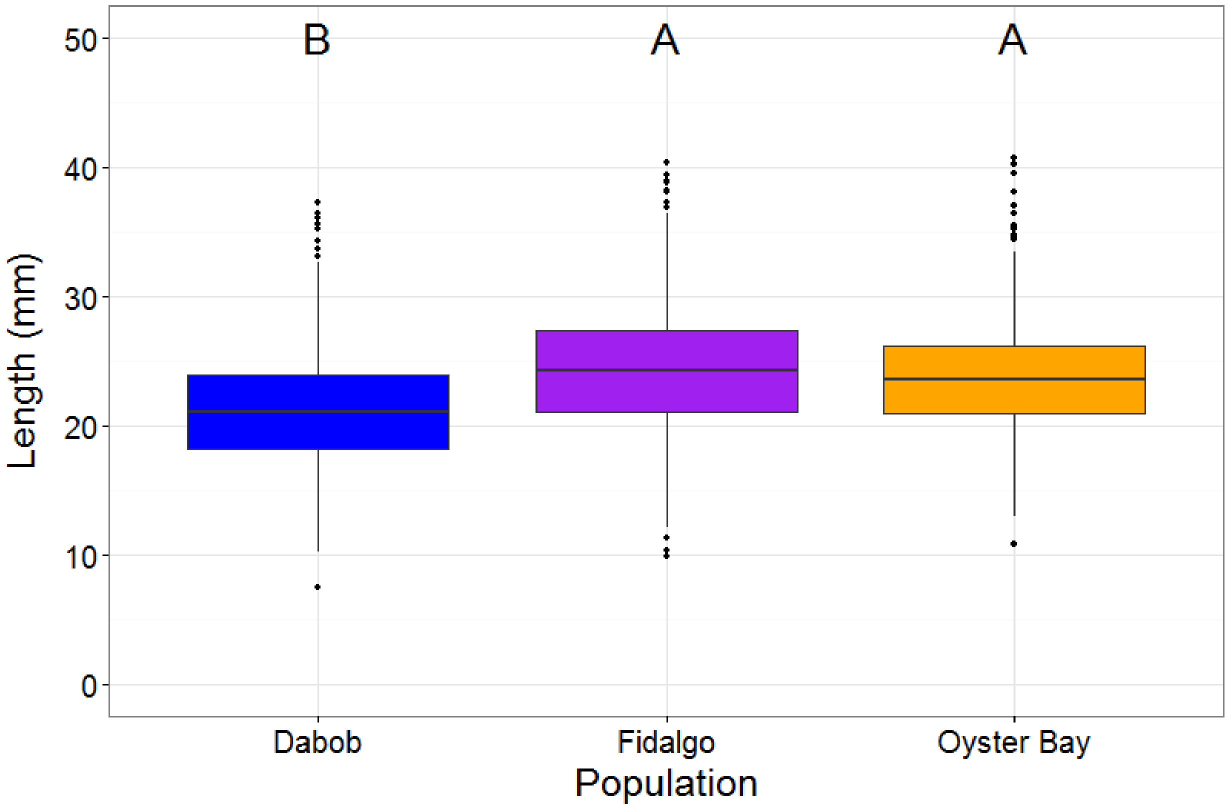


Figure 7. *Ostrea lurida* Shell Length in October 2014 at Clam Bay. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters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

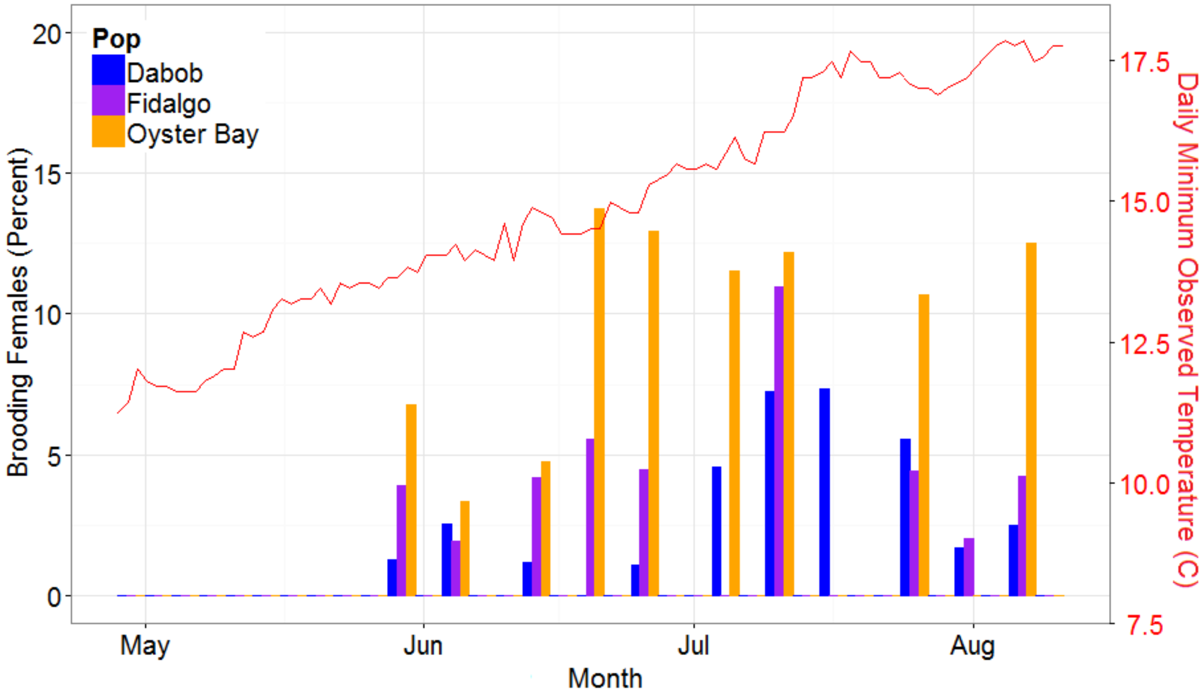


Figure 8. Percent *Ostrea lurida* Brooding Females from Each Population at Each Sample Date at Oyster Bay. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or %=(Br/T)*100.

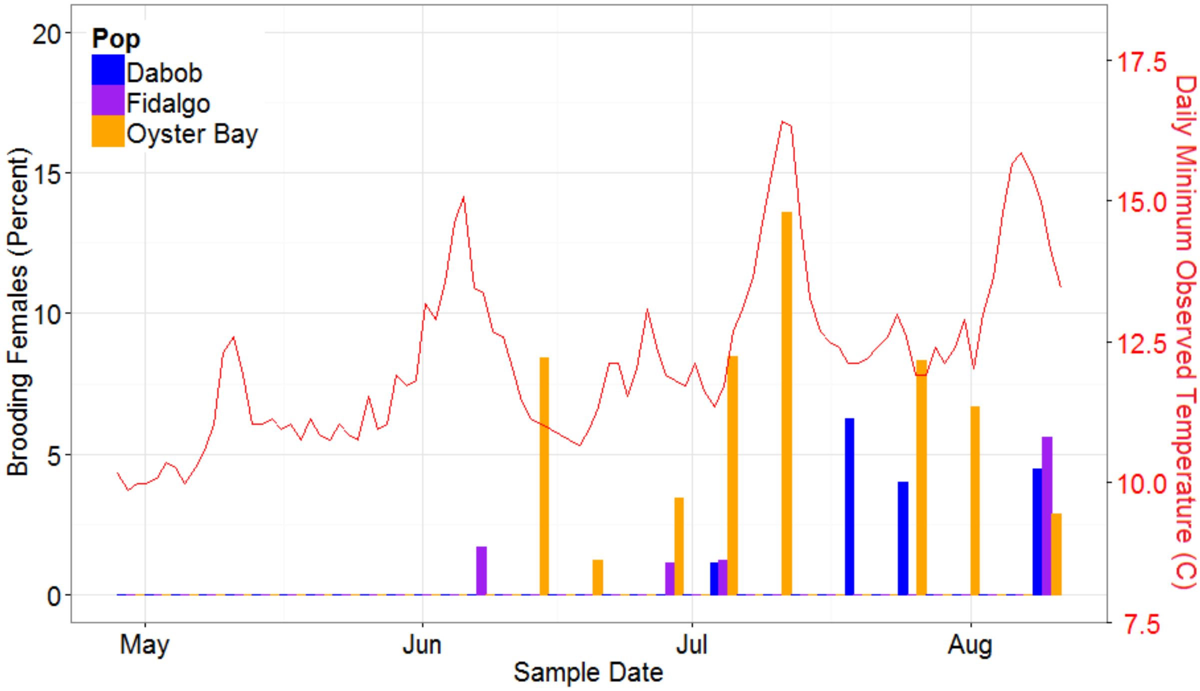


Figure 9. Percent *Ostrea lurida* Brooding Females from Each Population at Each Sample Date at Fidalgo Bay. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or %=(Br/T)*100.

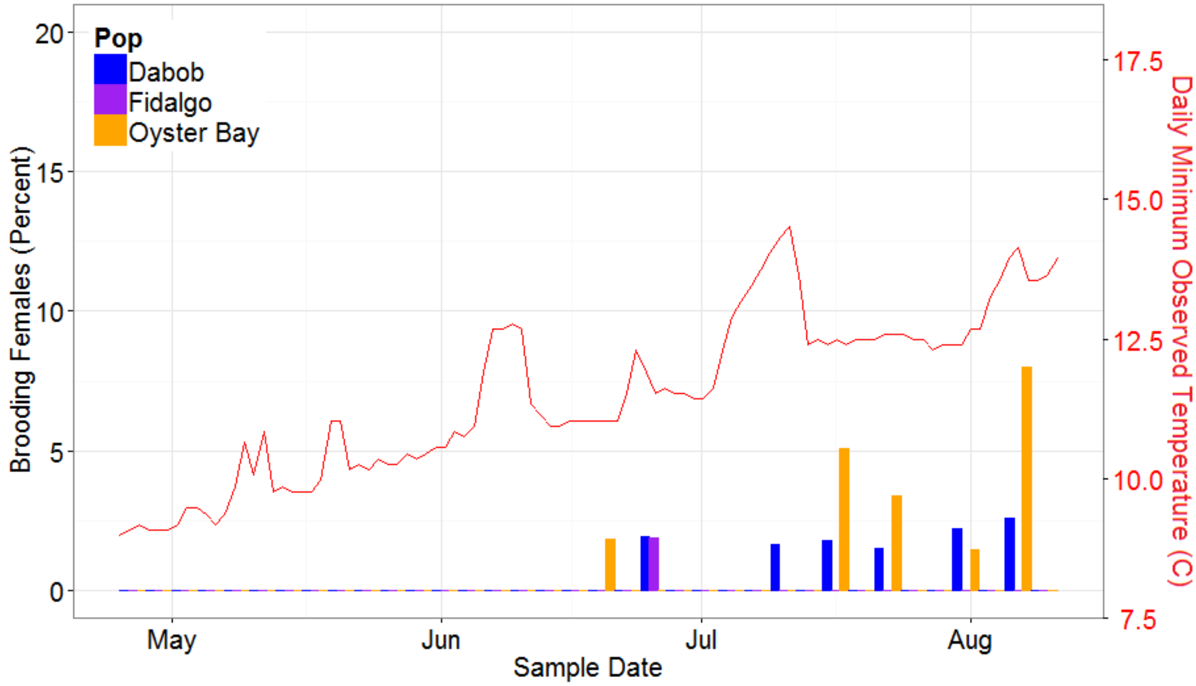


Figure 10. Percent *Ostrea lurida* Brooding Females from Each Population at Each Sample Date at Clam Bay. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or %=(Br/T)*100.

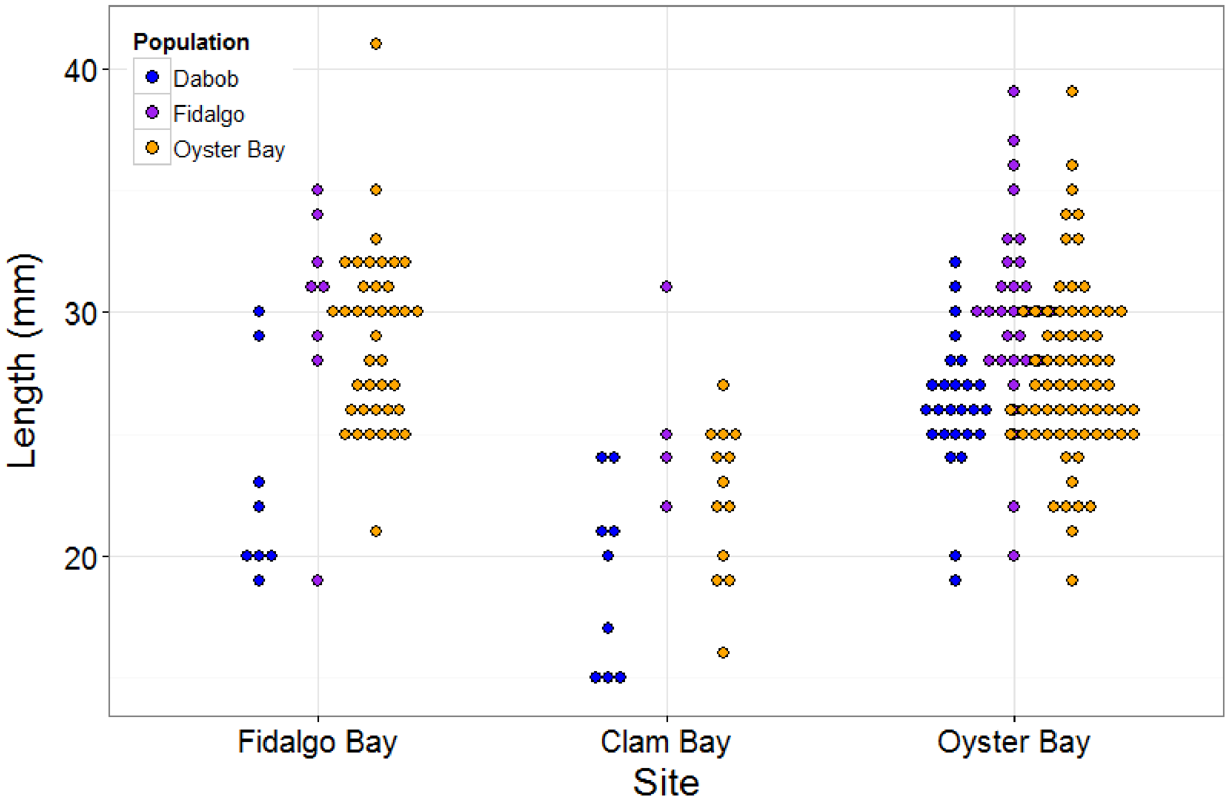


Figure 11. *Ostrea lurida* Brooding Female Shell Length Comparison Among Sites.