

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

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Abstract

Species traits that carry adaptive advantage such as reproductive timing and stress resilience may differ among reproductively discrete locales. Knowledge and consideration of these traits should, therefore, be integrated into conservation efforts that include long-term persistence of species. To test for adaptive differences between Olympia oyster, *Ostrea lurida*, populations a reciprocal transplant experiment was carried out monitoring survival, growth, and reproduction using three established populations of *O. lurida* within Puget Sound, Washington. Performance differed for each population. *O. lurida* from Dabob Bay had higher survival at all sites but lower reproductive activity and growth. Oysters from Oyster Bay demonstrated greater proportion of brooding females at a majority of 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.

Introduction

Restoration of native oysters is of increasing importance because of their significant contribution of ecosystem services and the large scale reduction in resident population size caused by ongoing habitat degradation and global climate change (Anderson, 1995; Lotze et al., 2011). The native east coast oyster, *Crassostrea virginica*, has been shown to make large contributions in way of ecosystem of services such as phytoplankton control, refuge creation, and benthic-pelagic coupling (Coen et al., 2007). While *C. virginica* has a greater influence on water quality than the native west coast oyster, *Ostrea lurida*, it is suspected *O. lurida* creates significant habitat value akin to that of the native European oyster, *Ostrea edulis* (zu Ermagassen et al., 2013). In an attempt to restore lost ecosystem services due to population decline, resource managers and restoration groups focus on placing viable animals into habitats to supplement dwindling populations and encourage persistence. Success of these efforts is highly dependent on the survival and reproductive fitness of the transplanted individuals (McKay et al., 2005).

The Olympia oyster, *O. lurida* Carpenter, 1864, is the only native oyster to the west coast of North America and have received considerable attention with respect to restoration. Olympia oysters exist in a variety of habitats within its range from Baja California, Mexico to British Columbia, Canada (Hopkins, 1937; Polson & Zacherl, 2009). In Puget Sound, oysters experience temperatures ranging from 5°C to 20°C (Hopkins, 1937). They have increased mortality in freezing temperatures (0°C) (Davis, 1955; Baker, 1995) or prolonged exposure to temperatures above 39°C (LT50) (Brown et al., 2004). *Ostrea lurida* are rhythmical consecutive hermaphrodites (Coe, 1932b), spawning first as males followed by oscillation between male and female within a spawning season. 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) with the earlier of the two events typically occurring in the latter half of May.

Even with the body of information presented by previous research on *O. lurida*, little is known about existing stock structure.

In the marine environment, the assumption of broad-scale fitness among marine invertebrates has been challenged. Palumbi (1997) demonstrated that geomorphology affected sea urchin population structure and Burford et al. (2014) recently demonstrated a fitness related trait 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 affect restoration efforts.

Despite several studies on Olympia oyster ecology and life history traits in Puget Sound, WA, information on population structure is limited and nothing is known about adaptive divergence, branching out of new and differing fitness related phenotypes from a common ancestor, 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). Alternatively, there are other phenomena such as balanced polymorphism or low effective population size that can present variation phenotypic features that may falsely be attributed to local adaptation (Camara, 2008; Camara and Vadopalas, 2009).

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. We predict that *O. lurida*

populations within the Puget Sound exhibit significant variation in phenotypes that persist under different environmental conditions.

Material and Methods

Reciprocal Transplant Experiment

As previously stated, reciprocal transplant experiments have been shown to be an effective way to measure stock structure in areas of interest. For our project we chose three geographically separated, reproductively discrete groups (which we will refer to as populations for simplicity) of *O. lurida* within Puget Sound. These animals were then brought to a hatchery, spawned, and the offspring from each population was outplanted back into the bays we chose. This way allows us to see how differing natural environments with resident oyster populations affect both local and non local populations over time.

Bays of Origin

Three bays (ie. Fidalgo Bay, Dabob Bay, and Oyster Bay) within Puget Sound were selected for this experiment based on presence of resident *O. lurida* populations, distance from other bays, and latitudinal position. Fidalgo Bay is the most northern site and as such experiences cooler year round conditions. This bay is also directly fed by the Strait of Juan de Fuca, allowing colder sea water directly from the Pacific to mix with bay waters daily. Dabob Bay is located within Hood Canal, an area of Puget Sound distinctly separated from the rest of the sound. The Bay itself is home to many commercial shellfish farms and well as unique tidal flux that can increase or decrease freshwater input from nearby waterways. Oyster Bay is the southern most site and known for its historically large populations of *O. lurida*. Currently there remains at least one large population within the region. It is also home to the

majority of Olympia oyster shellfish aquaculture and harvest. Waters in this bay remain local with little mixing from the rest of the sound and thus remain warmer for the majority of the year. The site also experiences significant effects from effluent waste and logging industries in the area.

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. Oysters were held for 5 months in common conditions in Port Gamble, Washington and spawned in June 2013. To ensure genetic diversity, each population from each site was subsequently spawned in 24 groups of 20-25 oysters. This spawning procedure is based on the findings from previous work within the Roberts lab suggesting that this technique maintains genetic diversity. Larvae produced from each population were reared in tanks based on spawning group and settled on microcultch, ie. very small pieces of oyster shell. 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 (N 48.478252, W 122.574845), Oyster (N 47.131465, W 123.021450), Dabob (N 47.850948, W 122.805694), and Clam Bays (as control site)(N 47.572894, W 122.547425) (Figure 1). For simplicity, we will call these sites Northern site (Fidalgo Bay), Southern site (Oyster Bay), Hood Canal site (Dabob Bay), and Central site (Clam Bay). 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. Size out outplant was similar for all sites except the Central site where the Fidalgo Bay population was larger (see results). Trays were anchored into substrate using rebar stakes. In late autumn 2013, trays at Northern (N 48.496358, W 122.600862),

Southern(N 47.138692, W 123.017387), and Central sites (N 47.573685, W 122.545323) 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 the Hood Canal site (N 47.850948, W 122.805694) as no suitable floating structure was available and oysters were removed from mesh bags.

Environmental Monitoring

At each site, two temperature loggers (HOBOlogger, OnSet, USA) were deployed within separate trays chosen at random. Data from temperature loggers were collected at regular intervals and used for 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°C and below 5°C 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°C to determine the amount of energy needed to produce peak brooding activity. In addition, monthly salinity, chlorophyll a, and dissolved oxygen content was viewed for each site from the Washington Department of Ecology website (<https://fortress.wa.gov/ecy/eap/marinewq/>) for buoys at the Northern site (N 48.5133, W 122.5933, approx. 1.97 km from site), Central site (N 47.6217, W 122.5017, approx. 6.25 km from site), Hood Canal site (N 47.6670, W 122.8200, approx. 20.55 km from site), and Southern site (N 47.1650, W 122.9633, approx. 5.04 km from site). Raw temperature data and analysis procedures used are available (Heare et al., 2015). In addition, analysis procedures used (R code) can be found in Appendix B.

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 (Hood Canal site only due to mortality in December), February, April (Hood Canal and Central sites only), May (Northern and Southern sites only), and June 2014. At Hood Canal, evidence of oyster drill mortalities was observed and accounted for by counting number of shells with holes in them. Differences in mortality within sites were determined through a Mantel-Haenszel test comparing categorical live/dead counts at each sample point in each site for significant differences in the patterns of survival performed with the R package *survival* (Therneau, 2014). To account for oyster drill, *Ocenebrellus* sp. and *Urosalpinx* sp., mortalities we incorporated a general linear model with binomial distribution and corrected for overdispersion with the *dispmod* package (Scrucca, 2012) which corrects P-values based on chisquare values divided by degrees of freedom times the standard error for the factor. Mortality and drill predation data and analysis procedures used are available (Heare et al., 2015). In addition, analysis procedures used (R code) can be found in Appendix B.

Growth

Size was determined using ImageJ analysis (Rasband, 2010) of digitized images taken in August 2013 (all sites), March (Northern, Central, and Southern sites), April (Hood Canal site), May (Northern, Central, and Southern sites), September (Southern site), and October 2014 (Northern and Central sites). 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 (maximum size, minimum size, quartiles, standard deviation) 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). To investigate significant differences between

populations, sites, and population/site interaction we used a linear effects model with fixed effects being population and site and random effects being population by tray using the R package lme4 (Bates et al., 2014) and P-values provided by the mixed function of the *afex* R package (Singmann, Bolker, & Walker, 2015). Shell length data from end of year one was compared using Kruskal-Wallis assuming non-normal distribution based on findings from Shapiro-Wilkes test (*stats* package, R Core Team, 2014). Pairwise comparisons for population by site were performed using the Nemenyi Post Hoc test, a joint rank sum test using information from Kruskal-Wallis to determine significant differences in rank, using Tukey assumptions (*PMCMR* package, Pohlert, 2014). Size data and analysis procedures used are available (Heare et al., 2015). In addition, analysis procedures used (R code) can be found in Appendix B.

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 ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)) (also known as 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. A different tray was checked for each population at each site in a rotation until all four trays for that population at that site had been checked. This was true for all sites except for the Southern site in which several trays were missing, the same tray was checked several weeks in a row until the missing trays were recovered at which point the tray rotation resumed. Following Hopkins (1937) observation of the daily minimum

temperature spawning threshold for *O. lurida* of 12.5°C, 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 to improve normality of proportions and analyzed via Two Way ANOVA (*base* package, R Core Team, 2014). Significant differences among sites, populations, and site/population pairwise comparisons were determined using TukeyHSD (*base* package, R Core Team, 2014). Sizes at brooding were likewise compared via Two Way ANOVA and TukeyHSD to explore population, site, and population by site differences (*base* package, R Core Team, 2014). Female brooding data and analysis procedures used are available (Heare et al., 2015). In addition, analysis procedures used (R code) can be found in Appendix B.

Results

Site Characteristics

The Southern site had the highest daily minimum temperature (18.43°C) (Figure 2) in August 2014 while the Hood Canal site had the lowest daily minimum temperature (-3.32°C) during February 2014 (Figure 2). The Hood Canal 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, the Southern site experienced warmer daily temperatures as compared to all other sites (Figures 2 & 3). Monthly environmental data from the Department of Ecology showed no unusual phenomena outside of the average environmental parameters for *O. lurida*.

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 Hood Canal ($X^2=141$, $df=2$, $P<0.0001$), Southern ($X^2=76.3$, $df=2$, $P<0.0001$), and Central sites ($X^2=13.7$, $df=2$, $P=0.00105$) (Figures 4A, 4B, & 4C) than other populations.

The Hood Canal site location experienced unexpected elevated mortality, necessitating the premature termination of the Hood Canal site trial in April 2014. Evidence of high oyster drill related mortalities was observed at Hood Canal and it was found that the Fidalgo population experienced significantly more drill related mortalities (~48% of Fidalgo population as compared to ~28% of the Dabob population and ~29% of the Oyster Bay population) (GLM, $X^2=6.2$, $df=6$, $P<0.0165$). There were significant differences in mortality among populations ($X^2=141$, $df=2$, $P<0.0001$), with the Fidalgo Bay oysters having the lowest survival (21.2% +/- 2.1SD %) (Figure 4C). Limited mortality was observed at both the Central and Northern site where at least 80% of oysters remained after 11 months (July 2014) (Figures 4B & 4D).

Growth

Oyster mean size at outplant was 11.4 (+/-3.2SD) mm and with no differences in size among population except for the Central site where the Fidalgo population was larger (Figure 9). At the end of the experiment the size of oysters among sites were significantly different (LME $F=268.29$, $df=2$, $P<0.0001$ & Kruskal-Wallis, $X^2=383.4$, $df=2$, $P<0.0001$), with the Southern site producing the largest oysters (Figure 5: Figure 10) and Central site producing the smallest (Figure 7: Figure 9). Oyster size also differed among populations ((LME $F=86.42$, $df=2$, $P=0.007$ & Kruskal-Wallis, $X^2=196.1$, $df=2$, $P<0.0001$). The linear model also indicated that the interaction between populations and sites was significant (LME $F=23.34$, $df=4$, $P<0.0001$). At the Southern site, 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). Based on integrated size

data from throughout the experiment it is apparent this difference arose during Summer months (Figure 10). At the Northern site, oysters from Dabob Bay broodstock were smaller than Fidalgo Bay (Nemenyi Post-Hoc, $P < 0.0001$) and Oyster Bay (Nemenyi Post-Hoc, $P < 0.0001$) oysters at the end of the experiment (Figures 6: Figure 8). Similar results were also observed at the Central site, however as indicated outplant size was different. At the Central site, while the Oyster Bay and Dabob oysters started at the same size, the Oyster Bay oysters did end up larger than the Dabob oysters by the end of the experiment (Nemenyi Post-Hoc, $P = 0.00028$) (Figure 7: Figure 9).

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 the Southern site (Figure 11) compared to the Northern ($P = 0.007$) and Central sites ($P < 0.0001$). The smallest proportion of brooding females was documented at the Central site (Figure 13). The Oyster Bay population produced significantly more brooding females than Fidalgo Bay (Tukey's HSD, $P = 0.001$) or Dabob Bay (Tukey's HSD, $P = 0.0005$) populations. The Fidalgo and Dabob Bay populations were not different from one another at all sites (Tukey's HSD, $P = 0.942$).

The Southern site reached the spawning temperature threshold of 12.5°C (as defined by Hopkins, 1937) on May 14th and the first brooding female was observed 15 days later on May 29th (Figure 11). Ambient water temperatures in the Southern site rose steadily from late winter reaching the spawning threshold and continuing to increase to the summer maximum of 18.43°C (Figure 11). At the Southern site, Oyster Bay oysters reached the maximum percentage of brooding females on June 19th, 36 days post 12.5°C ,

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° C, 453° D (Figure 11).

At the Northern site, the 12.5° C temperature was also reached on May 14th and the first brooding female was observed on June 6th (Figure 12), 23 days later. The Northern site exhibited a slower, less steady temperature increase throughout the spring season with ambient water temperatures reaching 12.5° C in mid-May but then dipping into the 10-11° C range until early June, after which the site remained above the threshold for the remainder of the summer (Figure 12). The Oyster Bay oysters in the Northern site 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 12), 87 days later or 513° D.

The Central site reached 12.5° C on June 8th and brooding females were observed on June 18th from the Oyster Bay population (Figure 13), 10 days later. Temperatures in the Central site reached 12.5° C in early June but varied above and below this temperature for several days at a time throughout most of summer (Figure 13). Peak spawning could not be determined due to low number of brooding individuals observed at the Central site.

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 the Central site (Figure 14). Size at brooding by population was significantly different between all populations. Size was significantly smaller at the Central site compared the other sites (Northern site (Tukey's HSD, $P<0.0001$), Southern site (Tukey's HSD, $P<0.0001$)). No differences in size of brooding females was observed between Southern site and Northern site ($P=0.8$). 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 the

Central site from the Dabob Bay population. The average size of brooding females across populations and sites was 27.1 (+/- 4.5SD) mm.

Discussion

A primary objective for this study was to evaluate population performance in relation to 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 geographically separated, reproductively discrete locales, referred to from here on as populations for simplicity. 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 though these differences may be due to age or size at reproduction and may change annual variation in environmental factors not observed in this study. Due to the distance between the sites and their associated environmental data from the Washington Department of Ecology as well as the monthly resolution of the data, we are unable to comment on fine scale changes within the environment that may have affected our oysters but to what degree is unknown. In the remainder of this section, findings from this study are discussed in terms of differences in sites, differences in population performance, and implications of these findings with respect to restoration efforts.

Site Differences

Mortality

Mortality rates were different across sites, with these differences correlated to temperature and predation. The Hood Canal site experienced the highest mortality rates, experiencing temperature

extremes and predation as evidenced by prevalent holes likely caused by oyster drills, *Urosalpinx* sp. and *Ocenebrellus* sp., and direct observations of these gastropod. Interestingly there was a difference in susceptibility in the population to drill predation (see below). The Hood Canal 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°C and -3.3°C in December 2013 and February 2014 respectively (Figure 2). The Southern site, which also experienced moderate mortality, had a total of 39 days (9% of 398 days) outside of the $5\text{--}20^{\circ}\text{C}$ range. The majority (34 days) were above the upper limit (20°C) but not near the lethal temperature (LT50) of 39°C reported by Brown et al. (2004). The Northern and Central site 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 $35.8 (+/-6.4\text{SD})$ 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 in population density. It should be noted that early studies often sampled from commercial beds where densities were higher, possibly contributing to increased competition and decreased growth. From the WDoE environmental data, there was a clear 10 fold difference in chlorophyll a content between the Northern and Southern sites with the Southern site having the highest primary productivity of all sites.

A difference in size occurred in relation to site. Oysters from all populations at the Southern site 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 by allowing them to reproduce sooner or in harsh environments where growth may be hampered.

It has been generally accepted that *O. lurida* begin spawning at relatively low temperatures (13°C Coe, 1931a; 12.5°C 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°C. The steady increase in temperature as observed in the present study in the Southern site may have allowed *O. lurida* to spawn much earlier in the season than at other sites (Figures 11, 12, & 13). This also seems somewhat correlated to the differences in chlorophyll a content seen between the Northern and Southern sites though to what extent is unknown.

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.

356

357 *Population Differences*

358 *Mortality*

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

371 Predation was also a factor in population specific survival, at least at Hood Canal where oyster drills
372 were prevalent. Interestingly the Fidalgo Bay population had higher mortality attributed to oyster drills
373 at this location. This may be indicative of the population being free of drill predation at their homesite.
374 Related, populations from Dabob and Oyster Bay may have been selected for less susceptibility having
375 persisted in environments with oyster drills. The mechanism associated with susceptibility is not know
376 though could be related to shell thickness or metabolic signatures.

377

Growth

At all transplant sites, the population derived from Dabob Bay parents exhibited the lowest growth. Salinity stress, parasite and disease load, and food availability may have affected size (Brown and Hartwick, 1988; Andrews, 1984) but because of the separation between sites it seems unlikely that the effects seen in this study are primarily due to these factors. 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 (figures 11 – 13), at two sites independent of size which varied between sites (Figures 8 –10, 14). 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 do. 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 improved stress resilience. 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. Findings from this study indicate possible local adaptation in two of the three populations observed but there may be other factors dictating observed phenotypes.

While findings from this study certainly could be indicative of local adaptation, it should be pointed out that there could be other explanations for our observations. Given the nature of larval dispersal, for one, we do not know that the oysters used as broodstock were from parents from that environment. Thus the traits could be a result of selection in a different habitat. Along the same lines of assuming larval dispersion from a separate source population, negative selection could have taken place. For example, barnacle species have shown significant differences in stress tolerance phenotypes related to settlement upon either upper or lower intertidal but this is due to nascent stress tolerance within an individual and not representative of the population as a whole (Sanford and Kelly, 2011). Another possible explanation of the different traits observed for each population is that this could be a result of limited effective population size, or number of successful pairings during spawning. In other words, if too few parents

existed there could be a significant family effect and/or inbreeding depression, a phenomenon that has been reported in aquaculture of *C. gigas* (Camara et al., 2008) and discussed as a potential issue for *O. lurida* restoration by Camara and Vadopalas (2009). Future genotyping and parentage analysis will be able to answer this question.

While we cannot conclusively demonstrate a mechanism of local adaptation in this study, our results certainly have important implications for restoration of *O. lurida* within Puget Sound, WA. There are a number of ways that these findings could be used in generating restoration strategies specific to Puget Sound and in the face of climate change. 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. Interestingly, at this time habitats are facing environmental shifts imposed by climate change and ocean acidification. Having a strong understanding of population related phenotypes creates another option for restoration efforts. An assisted gene flow strategy that incorporates the outplanting of populations known to contain phenotypes fit for the new environmental parameter and have them interbreed with resident populations (Aitken and Whitlock, 2013). It is highly debated whether such a strategy would have benefits that outweigh the drawbacks, such as possible outbreeding depression, but should be considered for restoration efforts facing a variety of climate change scenarios. It should also be pointed out, regardless of the process resulting in the different phenotypes, we do not know whether phenotypes are firmly held in each population. Due to factors including plasticity and epigenetic phenomena, these traits could be lost over time.

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 population structure exists, there should be concern with respect to moving populations as: 1) transplanted populations could overwhelm locally adapted remnant resident populations, and possibly not persist themselves, 2) transplanted populations might not survive in new location and thus wasting resources required for restoration, and 3) transplanted populations could interbreed with remnant population and thus result in overall reduced fitness through outbreeding depression. 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 and geographic scales involved.

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568

569 Figures

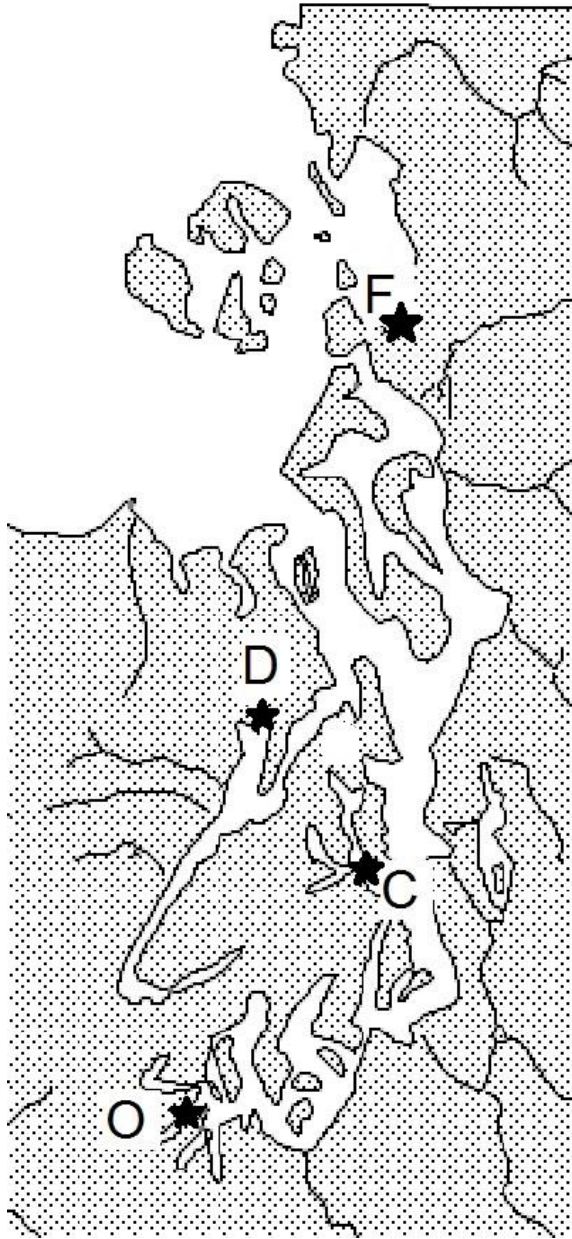


Figure 1. Map of Puget Sound with *Ostrea lurida* broodstock and outplant sites. Conditioning site was Port Gamble (G). Broodstock collected from Fidalgo Bay (F), Dabob Bay (D), and Oyster Bay (O). Outplanted at Fidalgo Bay also known as the Northern site (F), Dabob Bay also known as the Hood Canal Site (D), Clam Bay also known as the Central site (C), and Oyster Bay also known as the Southern site (O).

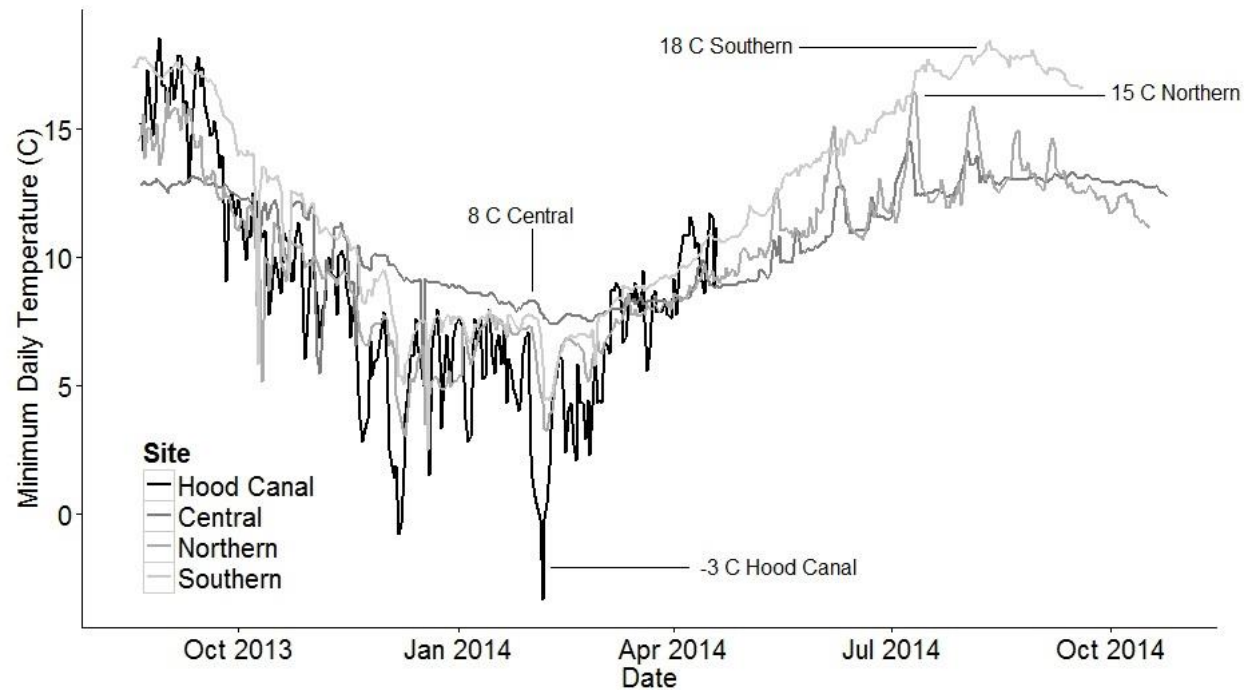


Figure 2. Minimum observed daily temperatures for all sites.

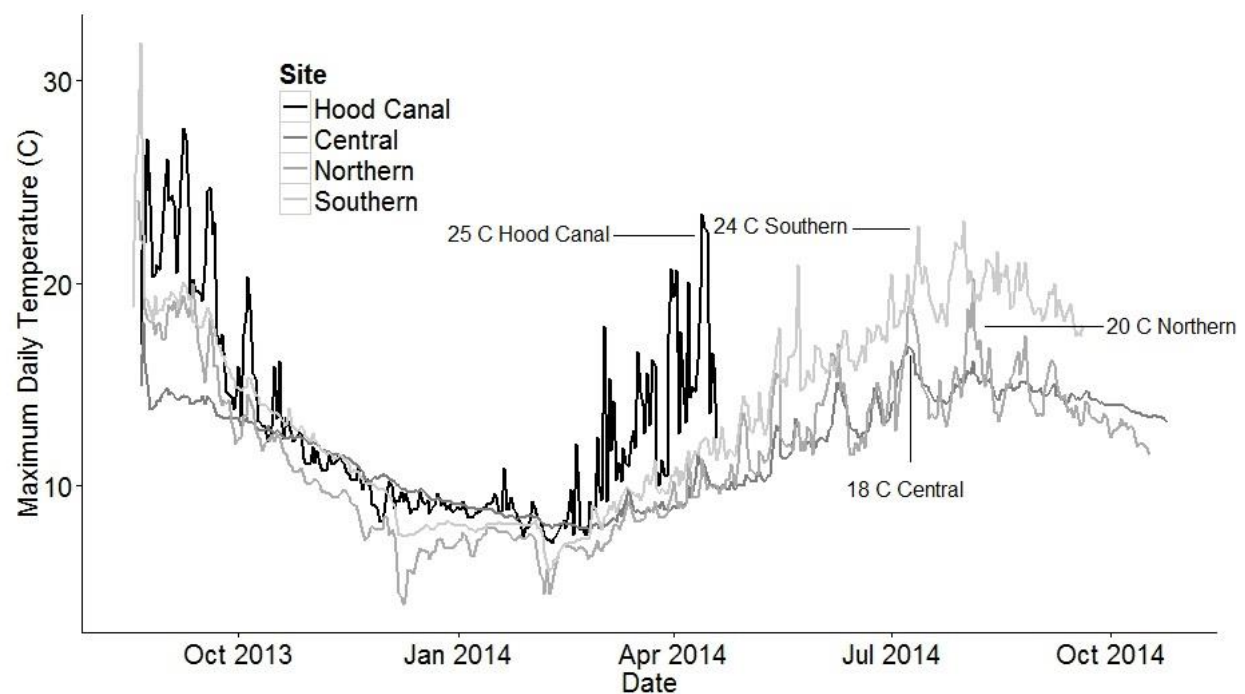


Figure 3. Maximum observed daily temperatures for all sites.

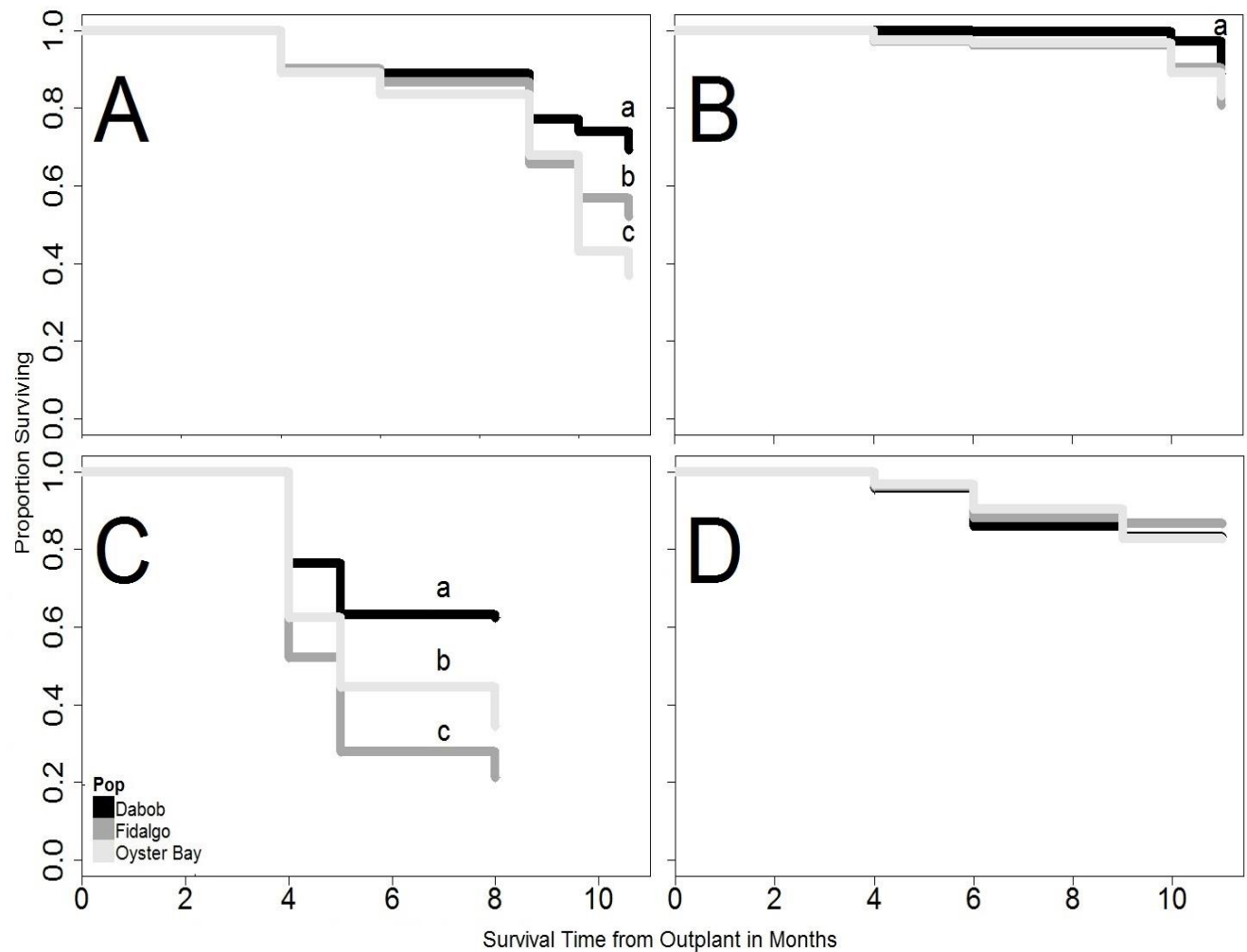


Figure 4. Proportion survival for three *Ostrea lurida* populations at four locations; Southern site (A), Central site (B), Hood Canal site (C), and Northern site (D). Lowercase letters (a, b, c) are significant differences.

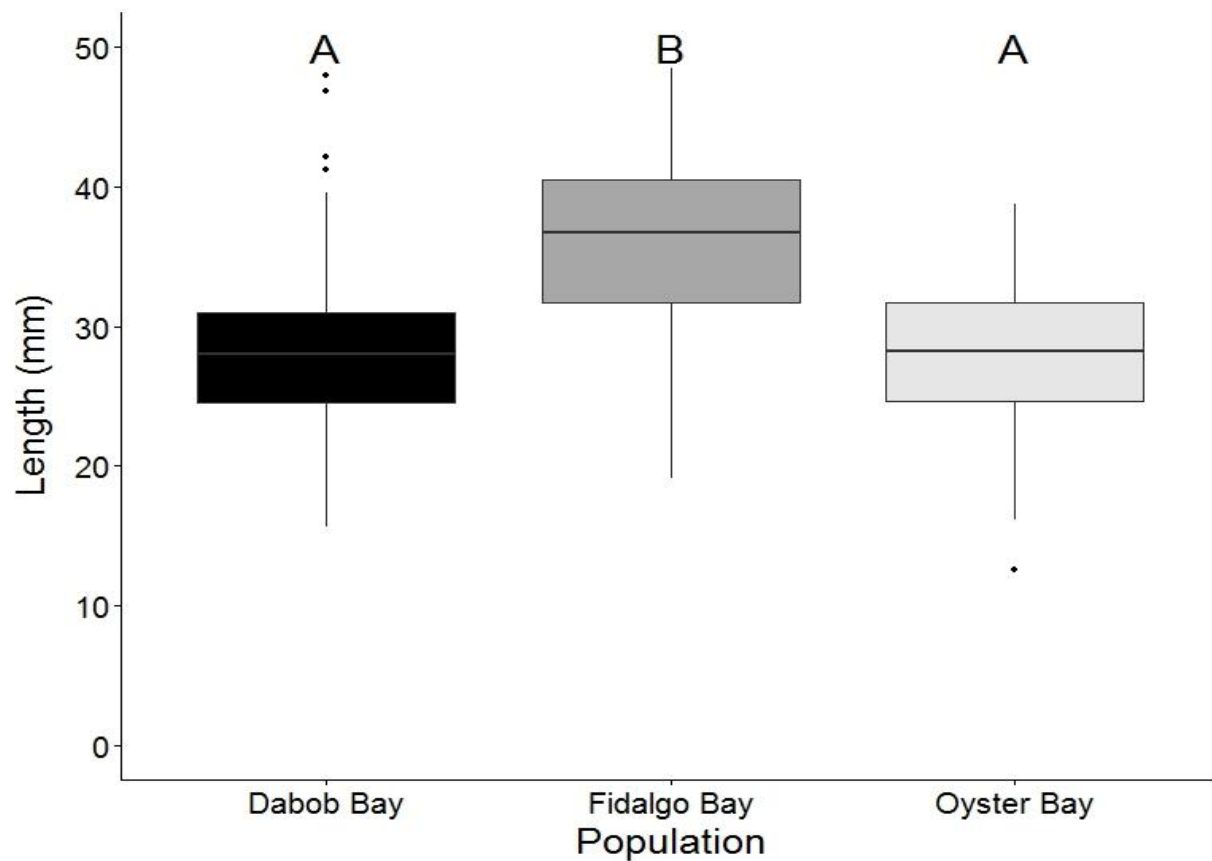


Figure 5. *Ostrea lurida* shell length in September 2014 at Southern site. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Fidalgo Bay oysters were considered different due to Nemenyi Post Hoc test with $P < 0.0001$ (Oyster Bay and Dabob Bay oysters).

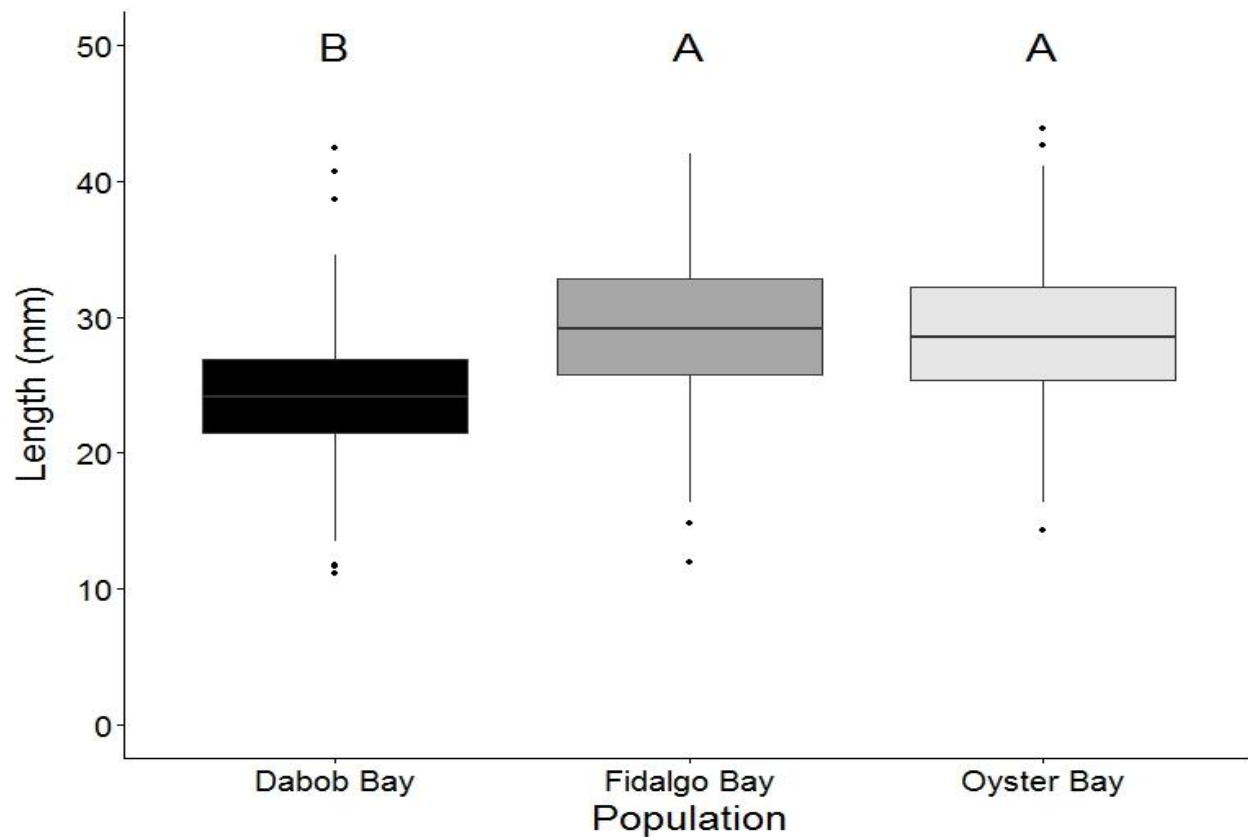


Figure 6. *Ostrea lurida* shell length in October 2014 at Northern site. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters were considered different due to Nemenyi Post Hoc test with $P < 0.0001$ (Fidalgo Bay and Oyster Bay oysters).

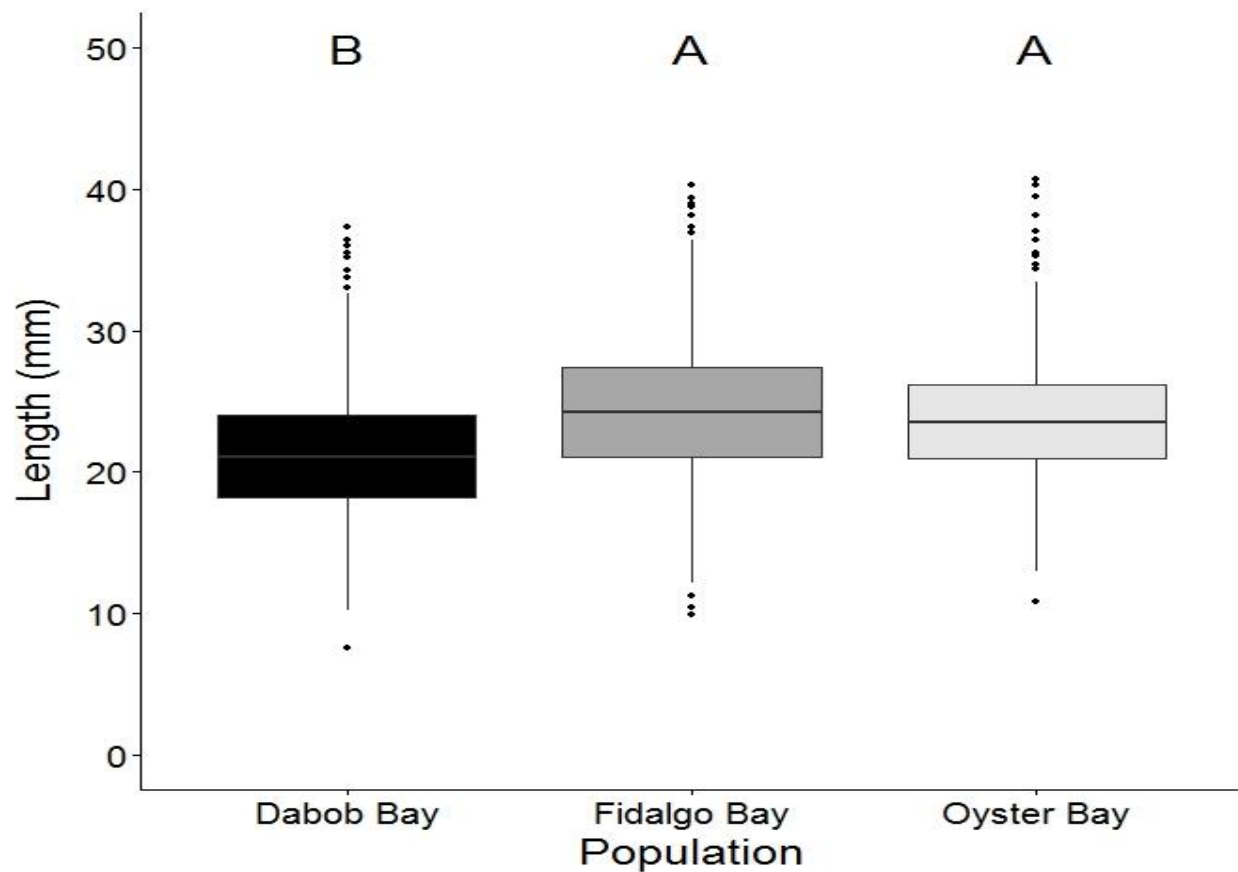


Figure 7. *Ostrea lurida* shell length in October 2014 at Central site. Boxplots with mean SL as central line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters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

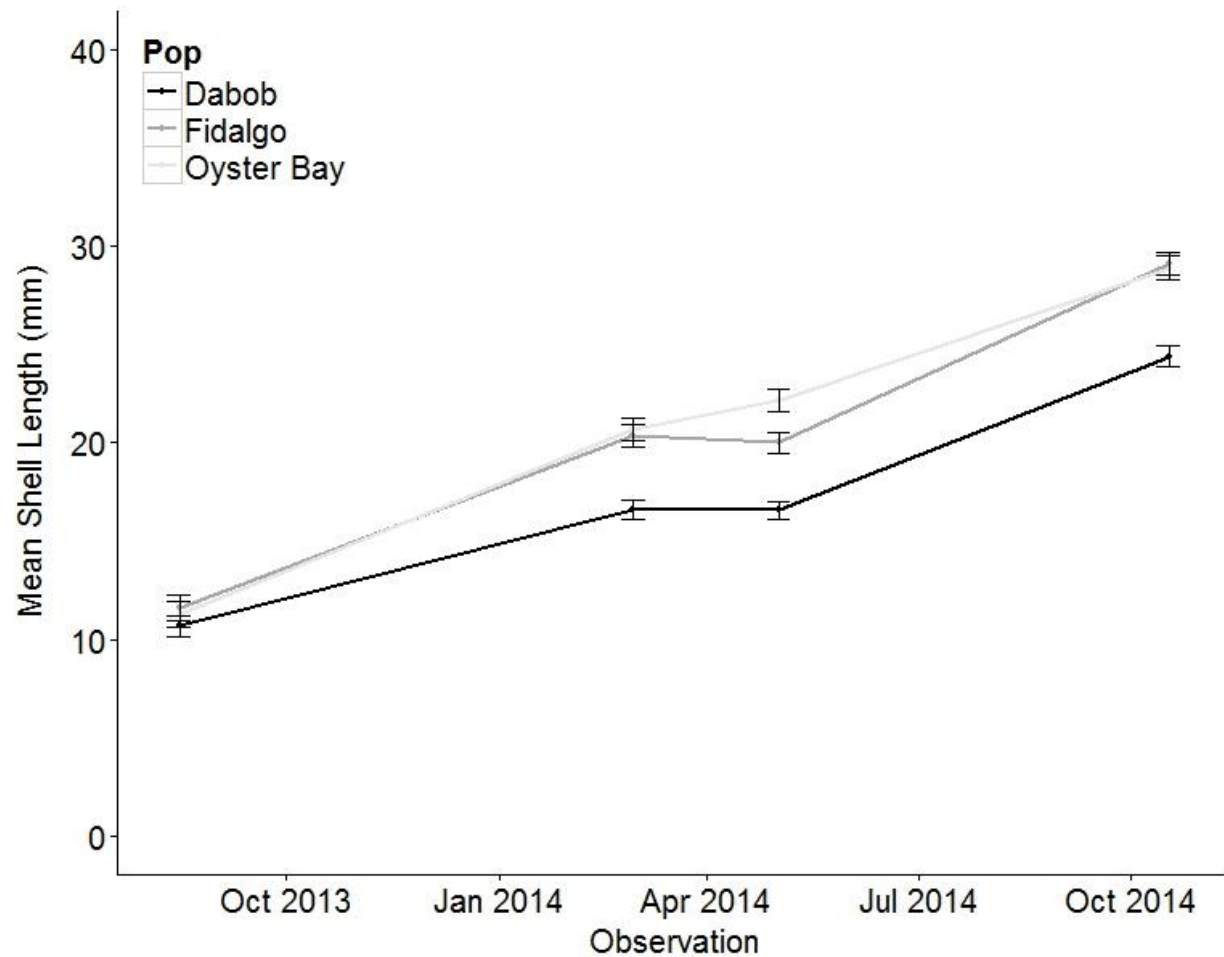


Figure 8. Growth rate of mean shell length in *Ostrea lurida* outplanted at Northern site. Error bars indicate 95% confidence intervals at each time point.

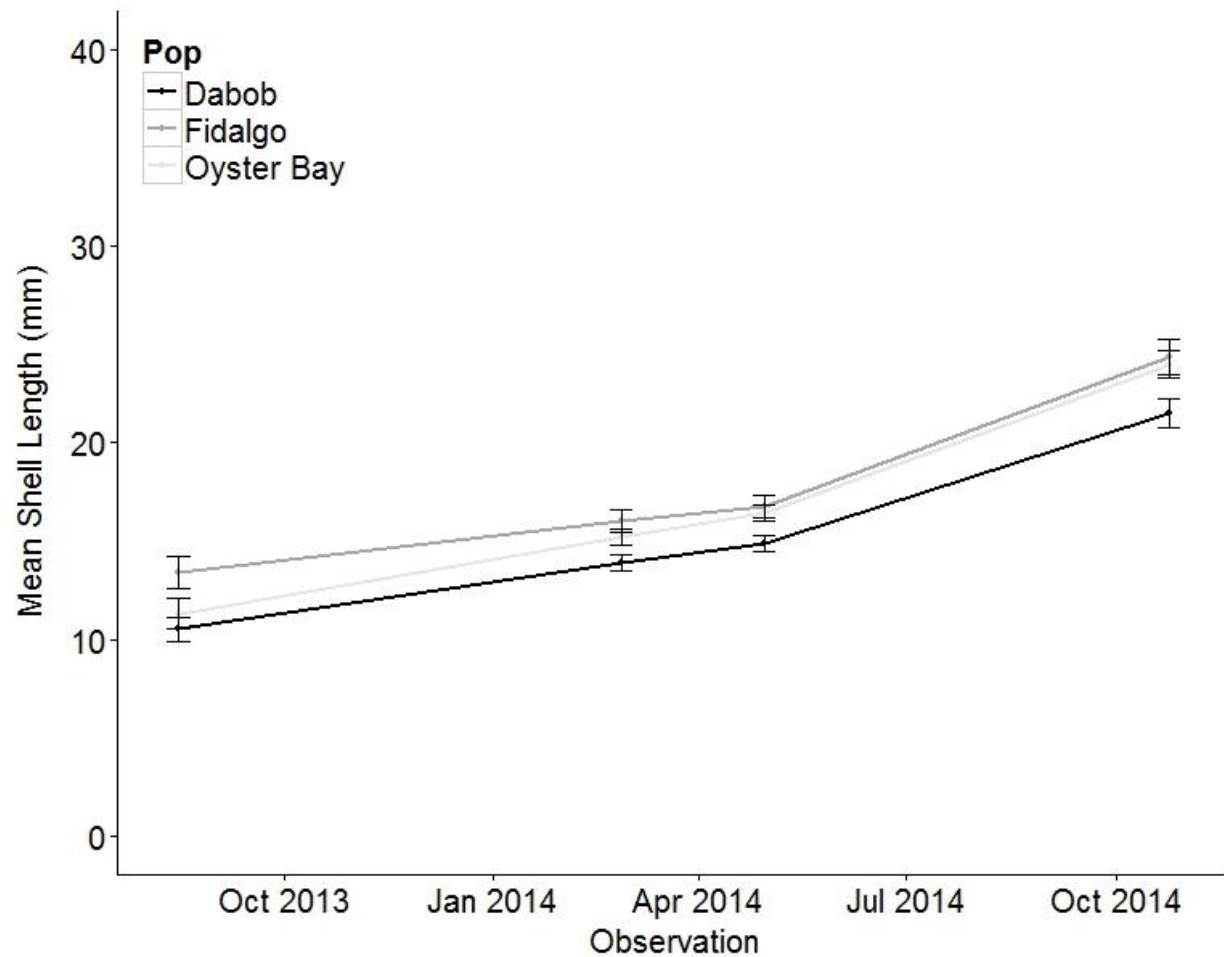
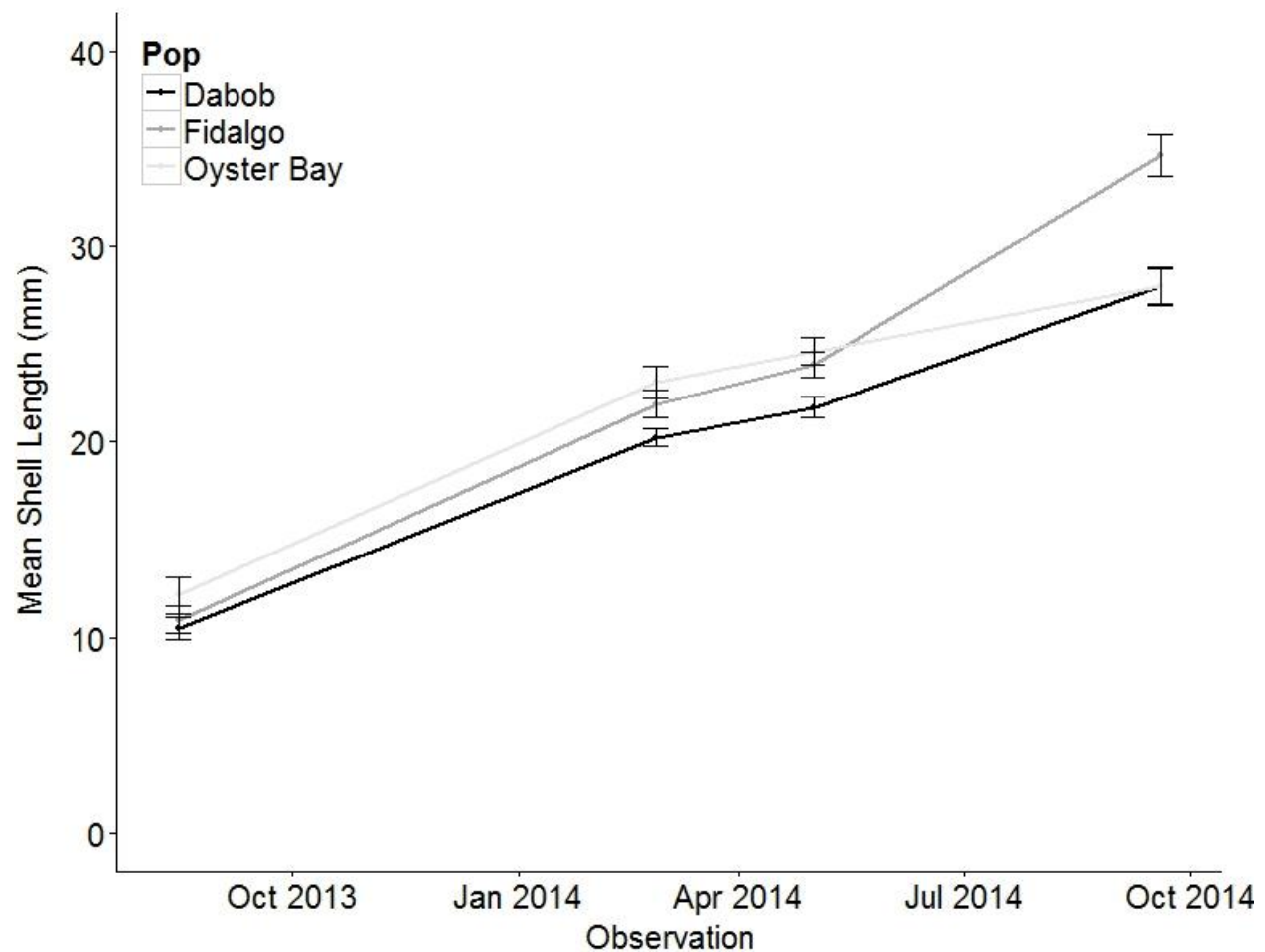


Figure 9. Growth rate of mean shell length in *Ostrea lurida* outplanted at Central site. Error bars indicate 95% confidence intervals at each time point.

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623 Figure 10. Growth rate of mean shell length in *Ostrea lurida* outplanted at Southern site. Error bars
 624 indicate 95% confidence intervals at each time point.

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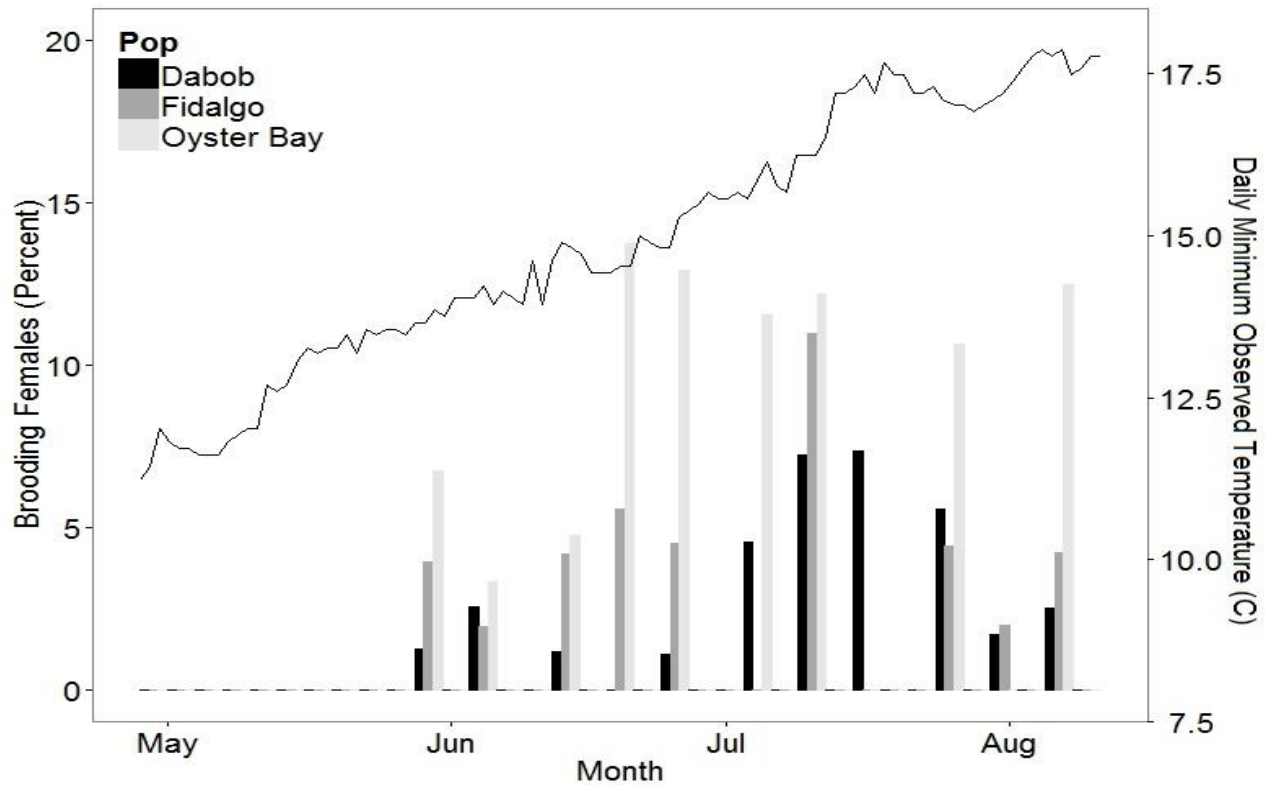


Figure 11. Percent *Ostrea lurida* brooding females from each population at each sample date at Southern site. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or $\% = (Br/T) * 100$.

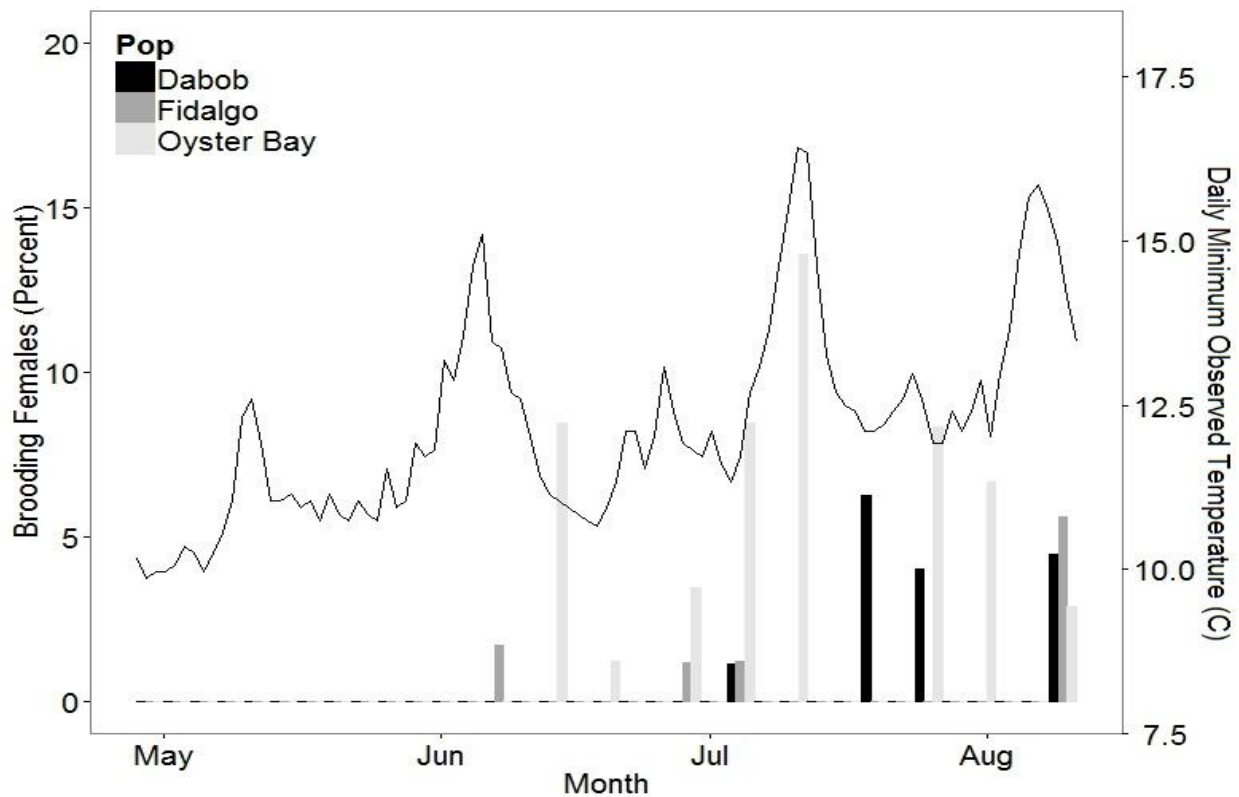


Figure 12. Percent *Ostrea lurida* brooding females from each population at each sample date at Northern site. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or $\% = (Br/T) * 100$.

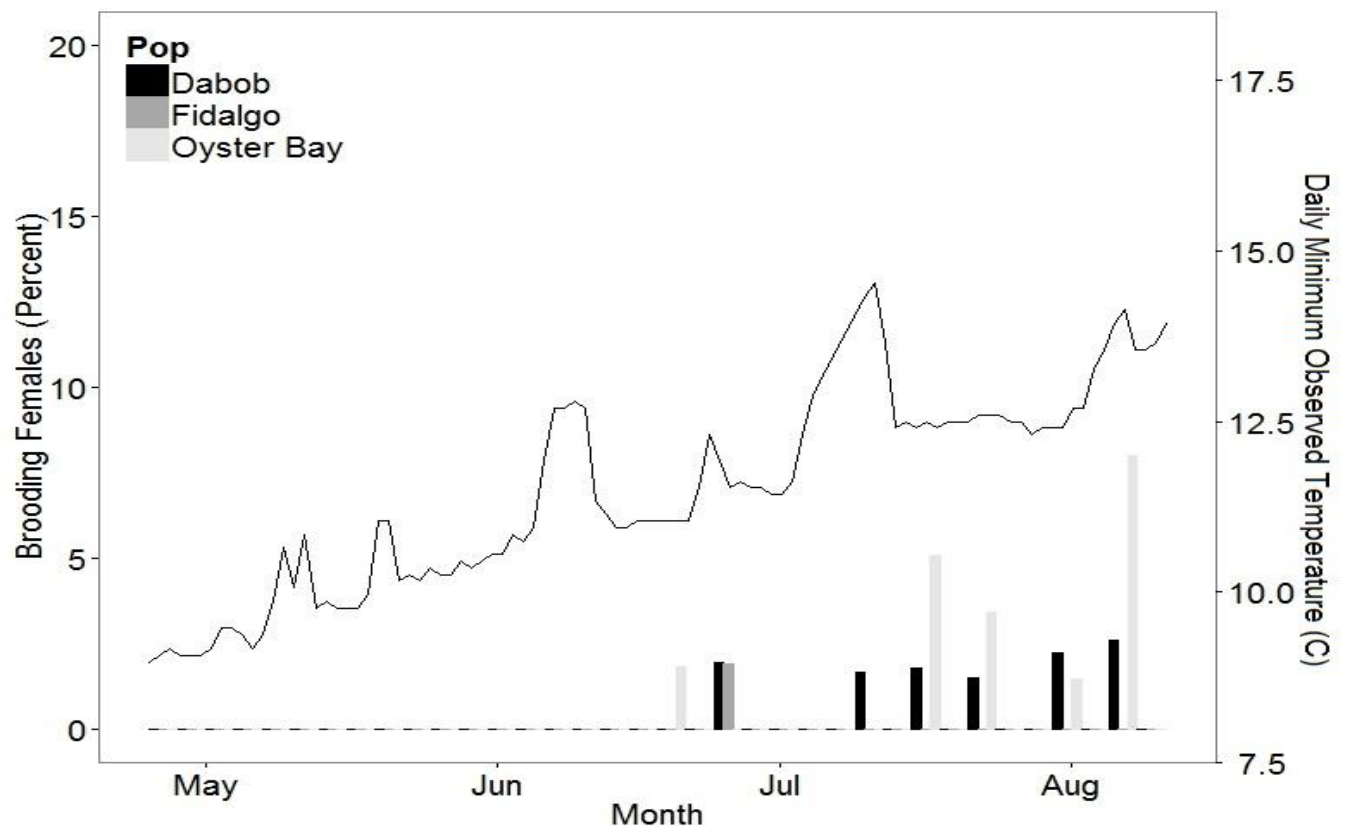


Figure 13. Percent *Ostrea lurida* brooding females from each population at each sample date at Central site. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or $\% = (Br/T) * 100$.

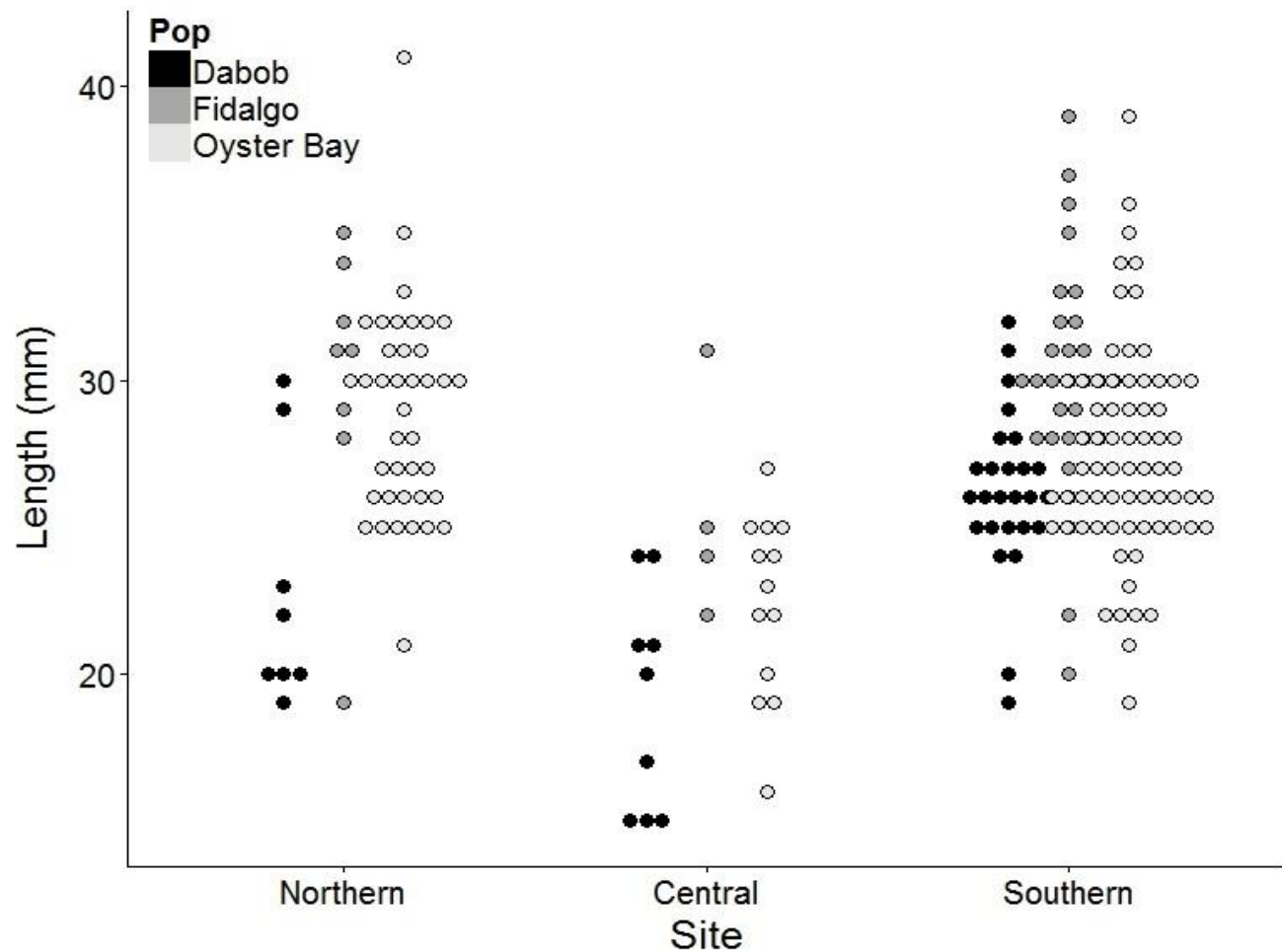


Figure 14. *Ostrea lurida* brooding female shell length comparison among sites.