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

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

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3 J. Emerson Heare[1], Brady Blake[2], Jonathan P. Davis[3], Brent Vadopalas[1], Steven B. Roberts[1]

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5 School of Aquatic and Fishery Sciences, University of Washington[1]

6 Washington Department of Fish and Wildlife[2]

7 Puget Sound Restoration Fund[3]

8

9 Corresponding Author:

10 Steven B. Roberts

11 1122 Boat St.

12 Seattle, WA 98105

13 sr320@uw.edu

14

15 **Abstract**

16   Species traits that carry adaptive advantage such as reproductive timing and stress resilience

17 may differ among populations. Knowledge and consideration of these traits should, therefore, be

18 integrated into conservation efforts that include long-term persistence of species. To test for adaptive

19 differences between Olympia oyster, *Ostrea lurida*, populations a reciprocal transplant experiment was

20 carried out monitoring survival, growth, and reproduction using three established populations of *O.*

21 *lurida* within Puget Sound, Washington. Performance differed for each population. *O. lurida* from Dabob

22 Bay had higher survival at all sites but lower reproductive activity and growth. Oysters from Oyster Bay

23 demonstrated greater proportion of brooding females at a majority of sites with moderate growth and

24   survival. Together these data suggest the existence of *O. lurida* population structure within Puget Sound

25   and provide information on how broodstock should be selected for restoration purposes.

26

27   **Key words:** oyster, reproduction, growth, transplant, temperature

28

29   **Introduction**

30   Restoration of native oysters is of increasing importance because of their significant

31   contribution of ecosystem services and the large scale reduction in resident population size caused by

32   ongoing habitat degradation and global climate change (Anderson, 1995; Lotze *et al.,* 2011). The eastern

33   oyster, *Crassostrea virginica*, has been shown to make large contributions in way of ecosystem of

34   services such as phytoplankton control, refuge creation, and benthic-pelagic coupling (Coen *et al*., 2007).

35   While *C. virginica* has a greater influence on water quality than the native west coast oyster, *Ostrea*

36   *lurida*, it is suspected *O. lurida* creates significant habitat value akin to that of the native European

37   oyster, *Ostrea edulis* (zu Ermgassen *et al.,* 2013). In an attempt to restore lost ecosystem services due to

38   population decline, resource managers and restoration groups may focus on placing viable animals into

39   habitats to supplement dwindling populations and encourage persistence. Success of these efforts is

40   highly dependent on the survival and reproductive fitness of the transplanted individuals (McKay *et al*.,

41   2005).

42   The Olympia oyster, *O. lurida* Carpenter, 1864, is native to the west coast of North America and

43   has received considerable attention with respect to restoration. Olympia oysters exist in a variety of

44   habitats within its range from Baja California, Mexico to British Columbia, Canada (Hopkins, 1937;

45   Polson & Zacherl, 2009; Gillespie 2009). Olympia oysters experience increased mortality in freezing

46   temperatures (0 ˚C) (Davis, 1955; Baker, 1995) or prolonged exposure to temperatures above 39 ˚C

47   (LT50) (Brown *et al.,* 2004). *Ostrea lurida* are rhythmical consecutive hermaphrodites (Coe, 1932b),

48   spawning first as males followed by oscillation between male and female within a spawning season.

49   Hopkins (1937) observed in south Puget Sound that a maximum of 10-15% of *O. lurida* are brooding at

50   any given time during a spawning season (1932). According to Hopkins (1937), peak larval settlement,

51   roughly correlated with peak spawning, generally occurs twice annually within south Puget Sound with

52   the earlier of the two events typically occurring in the latter half of May. In contrast, Seale & Zacherl

53   (2009) observed only single settlement peaks in June in two southern California estuaries.

54   Despite several studies on Olympia oyster ecology and life history traits in Puget Sound, WA (e.g.

55   Hopkins 1937; Baker, 1995; Trimble et al. 2009; White *et al*., 2009), peer-reviewed information on

56   genetic population structure is lacking and little is known about adaptive divergence and spatial

57   variation in fitness related phenotypes (Camara & Vadopalas, 2009). Bible and Sanford (2016) recently

58   focused on adaptive divergence of *O. lurida* among populations in San Francisco Bay, and found

59   evidence that populations might be locally adapted to different salinities. The size, hydrologic features,

60   and diverse environments of Puget Sound, and the retention of larvae by *O. lurida* during brooding,

61   coupled with the recent evidence of differential salinity tolerance among San Francisco bay populations

62   (Bible and Sanford 2016) suggests the possibility that populations within Puget Sound may likewise be

63   adapted to local conditions. Among methods testing for local adaptation, reciprocal transplant

64   experiments are considered robust (Sanford & Kelly, 2011) for investigating fitness. These experiments

65   involve using parent populations from diverse locales to produce offspring that are placed reciprocally in

66   their home and foreign environments. Population differences in key metrics for fitness can provide

67   evidence of adaptive divergence (Burford *et al.,* 2014). Alternatively, there are other phenomena such as

68   balanced polymorphism (Sanford & Kelly, 2011) or low effective population size (genetic drift) that can

69   manifest in phenotypic variation that may be falsely attributed to local adaptation (Camara *et al.,* 2008;

70   Camara & Vadopalas, 2009).

71   The primary objective of this study was to use a reciprocal transplant experiment to determine

72   whether *O. lurida* populations from geographically diverse areas of Puget Sound, Washington exhibit

73   population-level differences in survival, reproduction, and growth in different environments.

74

75   **Materials and Methods**

76   *Reciprocal Transplant Experiment*

77   For this study three geographically separated, discrete groups (which we will refer to as

78   populations for simplicity) of *O. lurida* within Puget Sound were selected. These animals were brought to

79   a hatchery, spawned, and the offspring from each population outplanted back to the bays selected. This

80   approach enables observations about how differing natural environments with resident oyster

81   populations may affect both local and non local populations over time.

82

83   *Bays of Origin*

84   Three bays (ie. Fidalgo Bay, Dabob Bay, and Oyster Bay) within Puget Sound were selected for

85   this experiment based on presence of resident *O. lurida* populations, distance from other bays,

86   latitudinal position, and distinct environmental conditions. Fidalgo Bay is the most northern site and is

87   generally cooler. This bay is also more directly influenced by the Strait of Juan de Fuca, allowing colder

88   sea water directly from the Pacific to mix with bay waters. Dabob Bay is located within Hood Canal, a

89   distinctly separate fjord with longer retention and more stratification than the rest of Puget Sound.

90   Oyster Bay is the southernmost site, warmer, highly productive, and known for its historically

91   abundance of *O. lurida*. Oyster Bay is also home to several commercial Olympia oyster shellfish

92   aquaculture operations.

93

94   *Broodstock Conditioning and Outplanting*

95   Adult oysters were collected from three locations in Puget Sound; Fidalgo Bay, Dabob Bay, and

96   Oyster Bay (n=600 each; Figure 1) during November and December 2012. Oysters were held for 5

97   months in common conditions in Port Gamble, Washington and spawned in June 2013. To ensure

98   genetic diversity, each population from each site was allowed to spawn in 24 separate groups of 20-25

99   oysters. Larvae produced from each population were reared in tanks based on spawning group and

100   settled on microcultch (very small pieces of oyster shell). Post-settlement spat were grown in four

101   replicate screened silos and fed ad libitum until attaining the minimum outplant size (shell length (SL) =

102   5 mm).

103   In August 2013, 480 juvenile oysters (5-10 mm) from each population were placed at Fidalgo (N

104   48.478252, W 122.574845), Oyster (N 47.131465, W 123.021450), Dabob (N 47.850948, W 122.805694),

105   and Clam Bays (as control site) (N 47.572894, W 122.547425) (Figure 1). For simplicity, we will call these

106   sites Northern site (Fidalgo Bay), Southern site (Oyster Bay), Hood Canal site (Dabob Bay), and Central

107   site (Clam Bay). At each site, oysters were placed into four 0.61W x 0.61L m grow out trays per

108   population with 12 trays total outplanted. In each tray, oysters (120) were equally distributed in four 10

109   x 7.5cm mesh (1475 micron) bags containing 30 oysters each. Size at outplant was similar for all sites

110   except the Central site where the Fidalgo Bay population was larger (see results). Trays were anchored

111   into the substrate using rebar stakes. In late autumn 2013, trays at Northern (N 48.496358, W

112   122.600862), Southern (N 47.138692, W 123.017387), and Central sites (N 47.573685, W 122.545323)

113   were subsequently suspended from floating structures to reduce exposure to extreme temperatures

114   during tidal exchanges and oysters were removed from mesh bags. Oysters were removed from mesh

115   bags, placed into trays anchored to the substrate and submerged in a perched lagoon in the Hood Canal

116   site (N 47.850948, W 122.805694) as no suitable floating structure was available.

117

118   *Environmental Monitoring*

119   At each site, two temperature loggers (HOBOlogger, OnSet, USA) were deployed within separate

120   trays chosen at random. Data from temperature loggers were collected at regular intervals and used for

121   minimum and maximum observed temperature for each day using the statistical analysis programming

122   language R (R 3.0.3, R Core Team, 2014) and package *plyr* (Wickham, 2014). The number of days above

123   20°C and below 5 °C was calculated for the duration of the project. Degree days (°D) was calculated by

124   adding the cumulative difference between the daily minimum temperature and the 2014 winter average

125   minimum of 8 °C to determine the amount of energy needed to achieve peak brooding activity. In

126   addition, monthly salinity, chlorophyll a, and dissolved oxygen content was viewed for each site from

127   the Washington Department of Ecology website (https://fortress.wa.gov/ecy/eap/marinewq/) for buoys

128   at the Northern site (N 48.5133, W 122.5933, approx. 1.97 km from site), Central site (N 47.6217, W

129   122.5017, approx. 6.25 km from site), Hood Canal site (N 47.6670, W 122.8200, approx. 20.55 km from

130   site), and Southern site (N 47.1650, W 122.9633, approx. 5.04 km from site). Raw temperature data and

131   analysis procedures used are available (Heare *et al.,* 2015).

132

133   *Survival*

134   Survival, determined by counts of live and dead oysters, was assessed at all sites in December

135   2013, January (Hood Canal site only due to mortality in December), February, April (Hood Canal and

136   Central sites only), May (Northern and Southern sites only), and June 2014. At Hood Canal, evidence of

137   oyster drill mortalities was observed and accounted for by counting number of shells with holes in them.

138   Differences in mortality within sites were determined through a Mantel-Haenszel test comparing

139   categorical live/dead counts at each sample point in each site for significant differences in the patterns

140   of survival performed with the R package *survival* (Therneau, 2014). To account for oyster drill

141   (*Ocenebra inornata* and *Urosalpinx cinerea*) mortalities we incorporated a general linear model with

142   binomial distribution and corrected for overdispersion with the *dispmod* package (Scrucca, 2012) which

143   corrects P-values based on chi square values divided by degrees of freedom times the standard error for

144   the factor. Following Bible and Sanford (2016), survival was also analyzed using a generalized linear

145   mixed model approach with Wald $\chi2$ tests, with population, site, and interactions as fixed effects and

146   tray as a random effect. Mortality and drill predation data and analysis procedures used are available

147   (Heare *et al.,* 2015).

148

149   *Growth*

150   Size was determined using ImageJ analysis (Rasband, 2010) of digitized images taken in August

151   2013 (all sites), March (Northern, Central, and Southern sites), April (Hood Canal site), May (Northern,

152   Central, and Southern sites), September (Southern site), and October 2014 (Northern and Central sites).

153   For each image, a size reference was measured along with all oysters. For all oysters, shell length (SL)

154   was determined via a linear measurement of the longest distance from umbo to valve margin.

155   Descriptive statistics (maximum size, minimum size, quartiles, standard deviation) were produced by the

156   R package *pastecs* (Grosjean and Ibanez, 2014). Size distributions were tested for normality using the

157   Shapiro-Wilkes test (*stats* package, R Core Team, 2014). To investigate significant differences between

158   populations, sites, and population/site interaction we used a linear effects model with fixed effects

159   being population and site and random effects being population by tray using the R package lme4 (Bates

160   *et al*., 2014) and P-values provided by the mixed function of the *afex* R package (Singmann, Bolker, &

161   Walker, 2015). Shell length data from end of year one was compared using Kruskal-Wallis assuming non-

162   normal distribution based on findings from Shapiro-Wilkes test (*stats* package, R Core Team, 2014).

163   Pairwise comparisons for population by site were performed using the Nemenyi Post Hoc test, a joint

164   rank sum test using information from Kruskal-Wallis to determine significant differences in rank, using

165   Tukey assumptions (*PMCMR* package, Pohlert, 2014). Size data and analysis procedures used are

166   available (Heare *et al.,* 2015).

167

Reproductive Activity

169   To assess reproductive activity, individual trays of oysters were anesthetized and each oyster

170 was visually inspected for presence of brooding larvae in the mantle chamber. Specifically, trays were

171 removed from water and exposed to air for 45 minutes then immersed in 0.3M magnesium sulfate

172 (heptahydrate sulfate mineral epsomite=Epsom salt; $MgSO_4 \cdot 7H2O$) dissolved in a 50/50 mix

173 freshwater/seawater for 45 minutes. Brooding vs non-brooding oysters were counted weekly from May

174 14th - August 15th, 2014 for a total of 15 time point observations for each site. A different tray was

175 checked weekly for each population at each site in an ongoing rotation. At the Southern site the rotation

176 was disrupted when several trays lost their mooring, thus the same tray was checked several weeks in a

177 row until the missing trays were recovered at which point the tray rotation resumed. The shell height of

178 each brooding females was measured to the nearest mm using calipers.

179   Using the daily minimum temperature spawning threshold for *O. lurida* of 12.5 ˚C (Hopkins

180 1937), we counted the elapsed days from the threshold temperature date to the date of the first

181 observation of a brooding female and the date of the maximum proportion of brooding females. The

182 proportions of brooding females per site per visit were arcsine transformed to improve normality of

183 proportions and analyzed via Two Way ANOVA (*base* package, R Core Team, 2014). Significant

184 differences among sites, populations, and site/population pairwise comparisons were determined using

185 TukeyHSD (*base* package, R Core Team, 2014). Sizes at brooding were likewise compared via Two Way

186 ANOVA and TukeyHSD to explore population, site, and population by site differences (*base* package, R

187 Core Team, 2014). Female brooding data and analysis procedures used are available (Heare *et al.,*

188 2015).

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190 **Results**

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191   *Site Characteristics*

192   The Southern site had the highest daily minimum temperature (18.43 ˚C) (Figure 2) in August

193   2014 while the Hood Canal site had the lowest daily minimum temperature (-3.32 ˚C) during February

194   2014 (Figure 2). The Hood Canal site exhibited high temperature variability due to the intertidal

195   placement of samples and the extreme cold weather during low tide events (Figures 2 & 3). From June

196   to August 2014, the Southern site experienced warmer daily temperatures compared to all other sites

197   (Figures 2 & 3).

198

199   *Survival*

200   Differences in mortality per population were observed at three of the four sites. Dabob Bay

201   oysters exhibited significantly lower mortality by the end of the study period at Hood Canal (X^2=141,

202   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)

203   (Figures 4A, 4B, & 4C) than other populations. A significant site x population interaction was detected

204   for the Southern site and Hood Canal and the Southern site and Central site, between oysters derived

205   from Dabob Bay and Fidalgo Bay populations (P=0.001 and 0.01, respectively) after 5 mo.

206   High mortality across all populations necessitated the premature termination of the Hood Canal site trial

207   in April 2014. Evidence of high oyster drill related mortalities was observed at this site. The proportion

208   of mortalities due to drills was significantly greater in the Fidalgo population (48%) compared to Dabob

209   and Oyster Bay populations (28% and 29%, respectively; GLM, X^2 =6.2, df=6, P<0.0165). Fidalgo Bay

210   oysters exhibited the lowest overall survival (21.2% ± 2.1 SD) at the Hood Canal site (Figure 4C), Oyster

211   Bay oysters exhibited the lowest overall survival at the Oyster Bay site (XY%, ± YZ SD). Limited mortality

212   was observed at both the Central and Northern site where ≥ 80% of oysters remained after 11 months

213   (July 2014) (Figures 4B & 4D).

214

215   *Growth*

216   Oyster mean size at outplant was 11.4 (+/-3.2SD) mm and with no differences in size among

217   population except for the Central site where the Fidalgo population was larger (Figure 9). At the end of

218   the experiment the size of oysters among sites were significantly different (LME F=268.29, df=2,

219   P<0.0001 & Kruskal-Wallis, X^2=383.4, df=2, P<0.0001), with the Southern site producing the largest

220   oysters (Figure 5: Figure 10) and Central site producing the smallest (Figure 7: Figure 9). Oyster size also

221   differed among populations ((LME F=86.42, df=2, P=0.007 & Kruskal-Wallis, X^2=196.1, df=2, P<0.0001).

222   The linear model also indicated that the interaction between populations and sites was significant (LME

223   F=23.34, df=4, P<0.0001). At the Southern site, Fidalgo Bay oysters were larger than Dabob (Nemenyi

224   Post-Hoc, P=<0.0001) and Oyster Bay (Nemenyi Post-Hoc, P=<0.0001) oysters (Figs. 5 and 10). At the

225   Northern site, oysters from Dabob Bay broodstock were smaller than Fidalgo Bay (Nemenyi Post-Hoc,

226   P<0.0001) and Oyster Bay (Nemenyi Post-Hoc, P<0.0001) oysters at the end of the experiment (Figures

227   6: Figure 8). At the Central site, the Oyster Bay oysters were significantly larger than the Dabob oysters

228   by the end of the experiment (Nemenyi Post-Hoc, P=0.00028) (Figure 7: Figure 9).

229

230   *Brooding Females*

231   The proportions of brooding females varied among populations (ANOVA, F=9.1, df=2, P=0.0002)

232   and among sites (ANOVA, F=11.4, df=2, P<0.0001). The greatest proportion of total brooding females

233   present was at the Southern site (Figure 11) compared to the Northern (P=0.007) and Central sites

234   (P<0.0001). The smallest proportion of brooding females was documented at the Central site (Figure

235   13). The Oyster Bay population produced significantly more brooding females than Fidalgo Bay (Tukey's

236   HSD, P=0.001) or Dabob Bay (Tukey's HSD, P=0.0005) populations. The Fidalgo and Dabob Bay

237   populations were not different from one another at all sites (Tukey's HSD, P=0.942). No interaction

238   between site and population was evident (ANOVA, F=1.1, df=4, P=0.3623).

239   The Southern site reached the spawning temperature threshold of 12.5 ˚C (as defined by

240   Hopkins, 1937) on May 14th and the first brooding female was observed 15 days later on May 29th

241   (Figure 11). Ambient water temperatures in the Southern site rose steadily from late winter reaching the

242   spawning threshold and continuing to increase to the summer maximum of 18.4 ˚C (Figure 11). At the

243   Southern site, Oyster Bay oysters reached the maximum percentage of brooding females on June 19th,

244   36 days post 12.5 ˚C, equating to 308 ˚D. At this location, Dabob Bay and Fidalgo Bay oyster populations

245   reached the maximum percentage of brooding females on July 10th, 57 days post 12.5 ˚C, 453 ˚D (Figure

246   11).

247   At the Northern site, the 12.5˚C temperature was also reached on May 14th and the first

248   brooding female was observed on June 6th (Figure 12), 23 days later. The Northern site exhibited a

249   slower, less steady temperature increase throughout the spring season with ambient water

250   temperatures reaching 12.5 ˚C in mid-May but then dipping into the 10-11 ˚C range until early June,

251   after which the site remained above the threshold for the remainder of the summer (Figure 12). The

252   Oyster Bay oysters in the Northern site reached maximum percentage brooding females by July 11th, 58

253   days later or 354 ˚D. Fidalgo Bay and Dabob Bay oysters' populations did not reach maximum

254   percentage brooding females observed until August 8th (Figure 12), 87 days later or 513 ˚D.

255   The Central site reached 12.5˚C on June 8th and brooding females were observed on June 18th

256   from the Oyster Bay population (Figure 13), 10 days later. Temperatures in the Central site reached 12.5

257   ˚C in early June but varied above and below this temperature for several days at a time throughout most

258   of summer (Figure 13). Peak spawning could not be determined due to low number of brooding

259   individuals observed at the Central site.

260   Size at brooding varied significantly among populations (ANOVA, F=18.2, df=2, P<0.0001) and

261   sites (ANOVA, F=33.1, df=2, P<0.0001) with the smallest brooding females observed at the Central site

262   (Figure 14). Size at brooding by population was significantly different among all populations. Brooders

263   were significantly smaller at the Central site compared to the Northern site (Tukey's HSD, P<0.0001),

264   and Southern site (Tukey's HSD, P<0.0001)). No significant size difference in brooders was observed

265   between Southern site and Northern site (P=0.8). The average minimum size at brooding of the ten

266   smallest oysters was 19.1(+/-3.7SD) mm. Two brooding females of 15.0 mm were observed at the

267   Central site from the Dabob Bay population. The average size of brooding females across populations

268   and sites was 27.1 (+/- 4.5SD) mm.

269

270   **Discussion**

271   A primary objective for this study was to evaluate population performance in relation to

272   possible stock structure of Olympia oysters in Puget Sound, WA. Findings from this study provided new

273   information about *Ostrea lurida* life history as well as distinct phenotypes associated with geographically

274   separated, reproductively discrete locales referred to from here on as populations for simplicity. At the

275   population level, one population exhibited greater survival and one favored reproduction over other

276   traits suggesting the existence of adaptive structure within Puget Sound, WA. In addition, a significant

277   interaction of population and site was detected, an indicator local adaptation. In the remainder of this

278   section, findings from this study are discussed in terms of differences in sites, differences in population

279   performance, and implications of these findings with respect to restoration efforts.

280

281   Site Differences

282   *Mortality*

283   Mortality rates were different across sites, with these differences correlated to temperature and

284   predation. The oyster populations at the Hood Canal site exhibited high mortality; the site exceeded the

285   temperature range reported by Baker (1995) on 35% of the total days (85 out of 242 days) with two

286   subfreezing events of -0.78 ˚C and -3.3 ˚C in December 2013 and February 2014 respectively (Figure 2).

287   The Southern site, where populations also experienced moderate mortality, had a total of 39 days (9%

288   0f 398 days) outside of the 5-20 ˚C range. The majority (34 days) were above the upper limit (20˚C) but

289   not near the lethal temperature (LT50) of 39 ˚C reported by Brown et al. (2004). The Northern and

290   Central site had fewer days outside of the range (24 days and 0 days respectively) and low mortality was

291   observed in all populations. The role of temperature as a primary determinant of survival when oysters

292   are transplanted outside of their broodstock populations range is similar to its role as found by Burford

293   *et al*. (2014). In addition to the temperature extremes, the oysters at the Hood Canal site experienced

294   predation as evidenced by direct observations of invasive oyster drills and prevalent drill holes. A

295   difference in population susceptibility to drill predation was observed (see below). Factors other than

296   temperature and predation likely affect survival at these sites. Salinity, for example, has been shown to

297   differentially correlate with survival of *O. lurida* populations in San Francisco Bay (Bible and Sanford,

298   2016). Genetic analyses are necessary to understand causal relationships and determine specific

299   selective forces driving the observed differentiation.

300

301   *Growth*

302   In the present study, Olympia oysters attained an average size of 35.8 mm +/-6.4SD) mm during

303   the first year of growth, with some individuals >45 mm. These observations contrast with the 2-3 years

304   Hopkins (1937) estimated was necessary to attain this size. This discrepancy could be due to changes in

305   environmental conditions or differences in the populations sampled.

306   A difference in size was observed in relation to site. All populations at the Southern site grew to

307   the largest size and experienced the warmest temperatures year round. This finding is in accord with

308   other studies (e.g. Malouf & Breese, 1977; Brown & Hartwick, 1988; Shpigel *et al.,* 1992) that

309   demonstrate that warm temperatures improve oyster growth as long as the temperatures are within

310   the tolerable range. The general pattern of productivity in South Puget Sound generally exceeds that of

311   other Puget Sound subbasins due both to higher temperatures and nutrient levels; for example, Budd

312   Inlet (South Sound) production in 1997 was 6000 mg C m^{-3} d^{-1}, compared to 2000-4500 mg C m^{-3} d^{-1} in

313   Dabob Bay (Newton et al. 1998).

314

315   *Reproduction*

316   Oysters reproduced as females in Puget Sound at a mean size of 27.1 mm (+/- 4.5SD). This result

317   contrasts with results of previous research (Hopkins, 1937; Coe, 1932a,b) that describe *O. lurida* as only

318   reproductive at sizes of 30 mm or greater. The ability to reproduce at smaller sizes is important because

319   it may provide reproductive advantage by allowing them to reproduce sooner or in harsh environments

320   where growth may be hampered.

321   It has been generally accepted that *O. lurida* begin spawning at relatively low temperatures (13

322   ˚C Coe, 1932a; 12.5 ˚C Baker, 1995). Hopkins (1937) suggested that this temperature threshold must

323   occur during high tide, which is related to the daily minimum temperature. In accord with these earlier

324   studies, at all sites brooding only occurred after daily minimum temperatures increased above 12.5 ˚C.

325   The steady increase in temperature as observed in the present study in the Southern site may have

326   allowed *O. lurida* to spawn much earlier in the season than at other sites (Figures 11, 12, & 13). This also

327   seems somewhat correlated to the differences in chlorophyll a content seen between the Northern and

328   Southern sites though to what extent is unknown.

329   By comparing the reproductive initiation and peak brooding observed to observations by

330   Hopkins (1937) in the same area, it appears that the reproductive period occurred approximately two

331   weeks later in 2014 than in 1932-1933. Further investigation is required to determine if this is simple

332   natural variation or an important change to the spawn timing in the region.

333

334   *Population Differences*

335

Mortality

337   Survival differed among populations within 3 out of 4 sites. The population derived from Dabob

338   Bay broodstock exhibited better survival than the other two populations (Figure 4). The observed

339   temperature variability (Figures 2 & 3) at the Hood Canal site in the present study may be indicative of

340   historic temperature trends to which the parent populations were exposed. If so, the significantly

341   greater survival of the Dabob Bay population at three of the four sites could be a function of increased

342   stress resilience of offspring in response to prevalent temperature extremes. Previous studies on

343   thermal tolerance, (e.g. bay scallops, *Argopecten irradians*, Brun *et al.,* 2008, and Mediterranean

344   mussels, *Mytilus galloprovincialis*, Dutton & Hofman, 2009) demonstrate more frequent exposure to

345   temperature extremes result in elevated heat shock proteins (HSP) and HSP mRNA transcripts. In

346   addition, Sørensen *et al.* (2004) found that many species exhibit heritable heat shock protein production

347   patterns. The higher survival rates observed in the Dabob Bay population may likewise be related to

348   heritable traits.

349   Predation was also a factor in population specific survival at the Hood Canal site. The Fidalgo Bay

350   population had significantly greater mortality attributed to oyster drills. Oysters in Fidalgo Bay are

351   effectively naïve to drills, populations from Dabob and Oyster Bays may be adapted to environments

352   with oyster drills. The mechanism associated with susceptibility is unknown, but might be related to

353   shell thickness.

354

Growth

356   At all transplant sites, the population derived from Dabob Bay parents exhibited the lowest

357   growth. This observation is likely related to the fact the Dabob Bay population also had the highest

358   survival. Applebaum *et al*. (2014) found energetic tradeoffs may improve survival over growth in the

359   Pacific oyster, *C. gigas*. Arendt (1997) suggested that "stress tolerators" exhibit slower intrinsic growth

360   that is relatively unresponsive to improved conditions. Further investigation is required to determine the

361   links between growth, energetic tradeoffs, and environmental variables affecting *O. lurida*. For example,

362   salinity stress, parasite and disease load, and food availability may have affected size (Brown and

363   Hartwick, 1988; Andrews, 1984) but because of the separation between sites it seems unlikely that the

364   effects seen in this study are primarily due to these factors.

365

366   *Reproduction*

367   The Oyster Bay population had a greater proportion of brooding females and reached peak

368   spawning earlier than the other populations (figures 11 – 13), at two sites independent of size which

369   varied between sites (Figures 8 –10, 14). One explanation for this is that the relatively rapid water

370   temperature increase and consistently higher temperatures in south Puget Sound may have selected for

371   early spawning oysters in the Oyster Bay population. Evidence for this includes the fact that it took

372   150 ˚D less for the Oyster Bay population to reach peak spawning compared to the other two

373   populations at two sites. The general rate of temperature increase at a particular locale may influence

374   spawn timing (Lawrence & Soame, 2004). Chávez-Villalba *et al.* (2002) found place of origin for *C. gigas*

375   broodstock affected the rate of gametogenesis under different temperatures with some populations

376   becoming reproductively active sooner than others do. Barber *et al*. (1991) found gametogenesis and

377   spawn timing were heritable traits within populations of *C. virginica*. Populations of *O. lurida* at a locale

378   in North Puget Sound were recently found to have initiated brooding at temperatures < 11 ˚C (Barber *et*

379   *al.* in press), further illustrating the variability of this important fitness component on a relatively small

380   spatial scale.

381

382   *Conclusions*

383   Differences in life history traits among *Ostrea lurida* populations grown in different locations

384   within Puget Sound, WA suggest adaptations possibly linked with environmental cues. High survival, low

385   growth, and low reproductive activity of the Dabob Bay population is likely due to extreme

386   environmental variation at their home site leading to improved stress resilience. The greater proportion

387   of brooding females in the Oyster Bay population and reduced environmental energy (˚D) needed to

388   induce peak spawning may be related to positive selection pressure for early spawners due to warmer

389   temperature trends at their home site. Findings from this study indicate possible local adaptation in two

390   of the three populations observed but there may be other factors dictating observed phenotypes.

391   While findings from this study certainly could be indicative of local adaptation, it should be pointed out

392   that there could be other explanations for our observations. Given the vagaries of larval dispersal, for

393   example, we do not know that the parents of the wild oysters used as broodstock were from that locale.

394   Thus the traits observed could be the result of strong selection in a different habitat. The differences

395   observed could also be the result of low effective numbers of breeders in the hatchery, thus indicating a

396   significant family effect and/or inbreeding depression as described in aquaculture of *C. gigas* (Camara *et*

397   *al.*, 2008) and discussed as a potential issue for *O. lurida* restoration by Camara & Vadopalas (2009).

398   Future genotyping and parentage analysis will shed light on this question.

399   While a mechanism of local adaptation cannot conclusively be demonstrated in this study, the

400   results certainly have important implications for restoration of *O. lurida* within Puget Sound, WA. There

401   are a number of ways that these findings could be used in generating restoration strategies specific to

402   Puget Sound and in the face of climate change. First, based on the fact that Dabob Bay oysters had the

403   lowest mortality overall, use of these more robust oysters for broodstock may increase chances for

404   outplant survival. Second, an alternative approach might be to use the population with the greatest

405   reproductive output (Oyster Bay) and use it as a source of broodstock. This would increase the likelihood

406   of juvenile recruitment and ultimate restoration of the species, while also producing more offspring for

407   outplant. Because habitats are facing environmental shifts imposed by climate change and ocean

408   acidification, having a strong understanding of population related phenotypes creates another option

409   for restoration efforts. Third, the assisted gene flow strategy could incorporate the outplanting of

410   populations known to contain fitness phenotypes for the new environmental parameter to interbreed

411   with resident populations (Aitken & Whitlock, 2013). Whether this strategy would have benefits that

412   outweigh the drawbacks, such as possible outbreeding depression, is unknown, but assisted gene flow

413   may prove a valid strategy for restoration efforts facing a variety of climate change scenarios. Regardless

414   of the process resulting in the different phenotypes, it must be emphasized that the range of

415   phenotypes per population is unknown. Due to factors including plasticity and epigenetic phenomena,

416   these traits could be lost over time.

417   Ultimately, what this study demonstrates is that population structure can and does exist on a

418   relatively small geographic scale and thus moving oyster populations to locations where remnant stocks

419   exist could be disadvantageous. When population structure exists, there should be concern with respect

420   to moving populations as: 1) transplanted populations could overwhelm locally adapted remnant

421   resident populations, 2) transplanted populations might not survive in the new location, and thus waste

422   valuable resources required for restoration, and 3) transplanted populations could interbreed with

423   remnant population and thus result in overall reduced fitness through outbreeding depression. Each of

424   these concerns make assumptions regarding plasticity and adaptive potential, though we still know little

425   about this in marine invertebrates, particularly on the temporal and geographic scales involved.

426

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437 R/LME/N-3. The views expressed herein are those of the authors and do not necessarily reflect the

438 views of NOAA or any of its sub-agencies.

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551 **Figures**
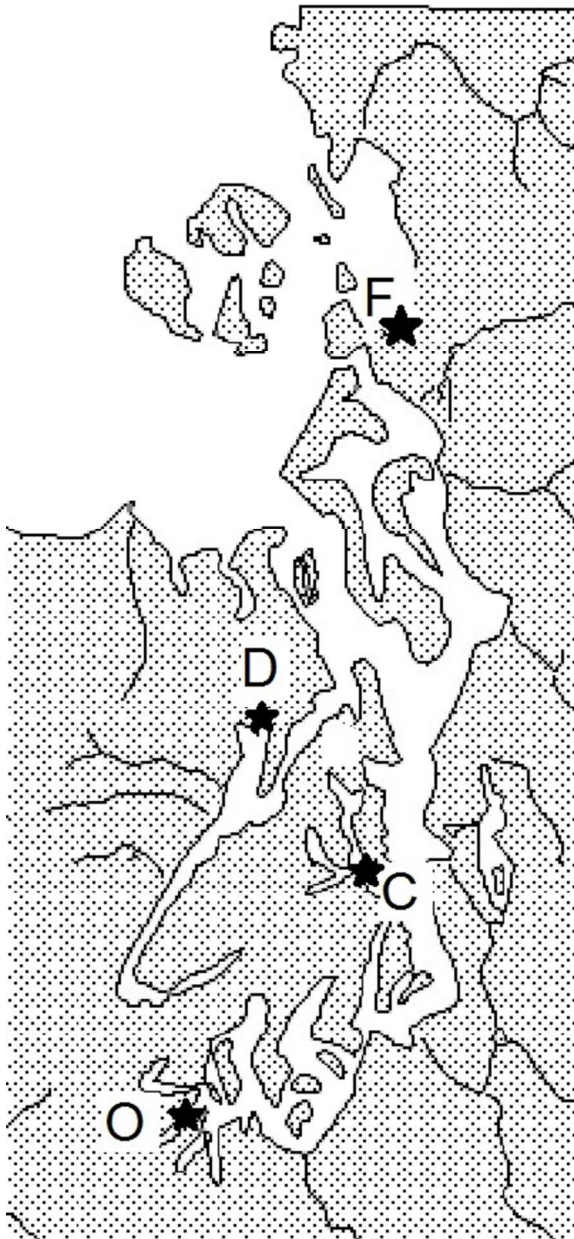
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553   Figure 1. Map of Puget Sound with *Ostrea lurida* broodstock and outplant sites. Broodstock collected

554   from Fidalgo Bay (F), Dabob Bay (D), and Oyster Bay (O). Outplanted at Fidalgo Bay, also known as the

555   Northern site (F), Dabob Bay, also known as the Hood Canal Site (D), Clam Bay, also known as the

556   Central site (C), and Oyster Bay, also known as the Southern site (O).

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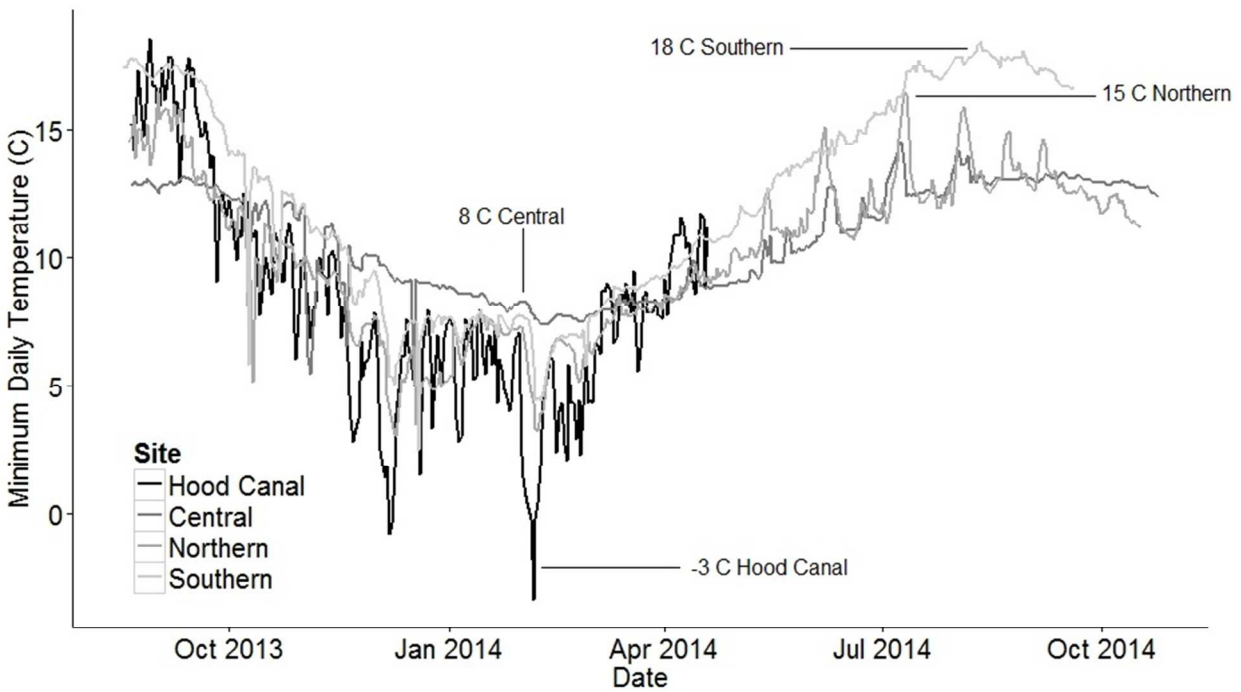
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559   Figure 2. Minimum observed daily temperatures for all sites.
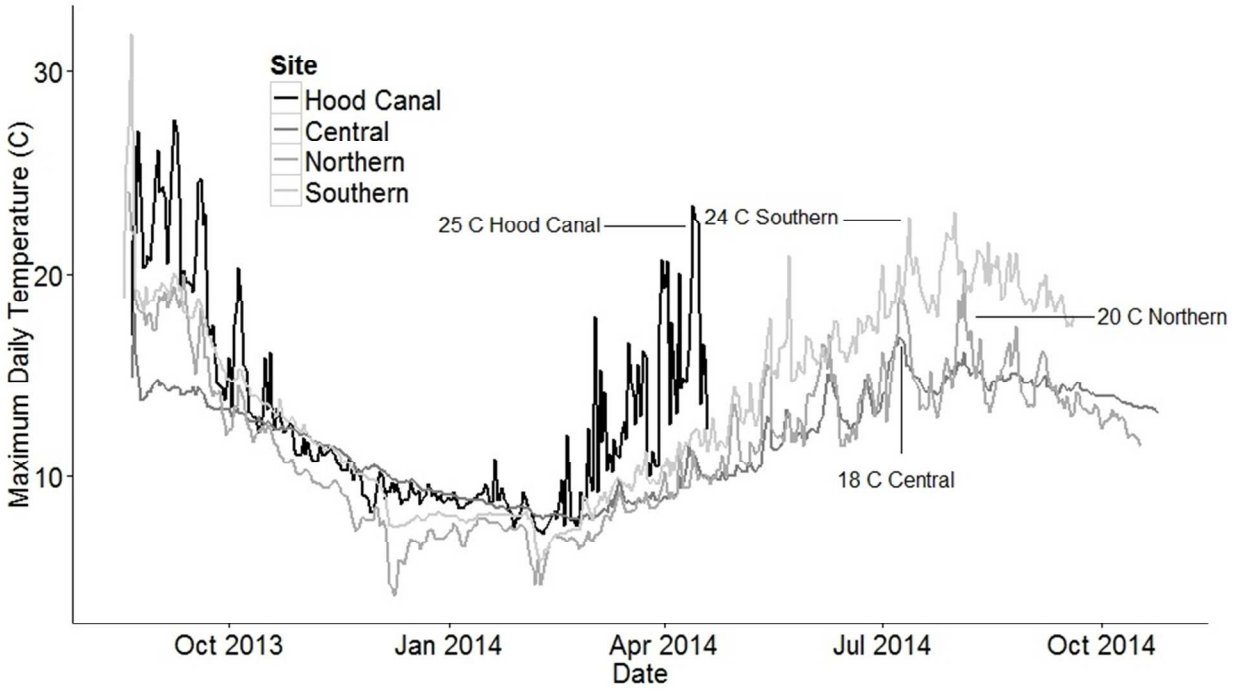
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561   Figure 3. Maximum observed daily temperatures for all sites.

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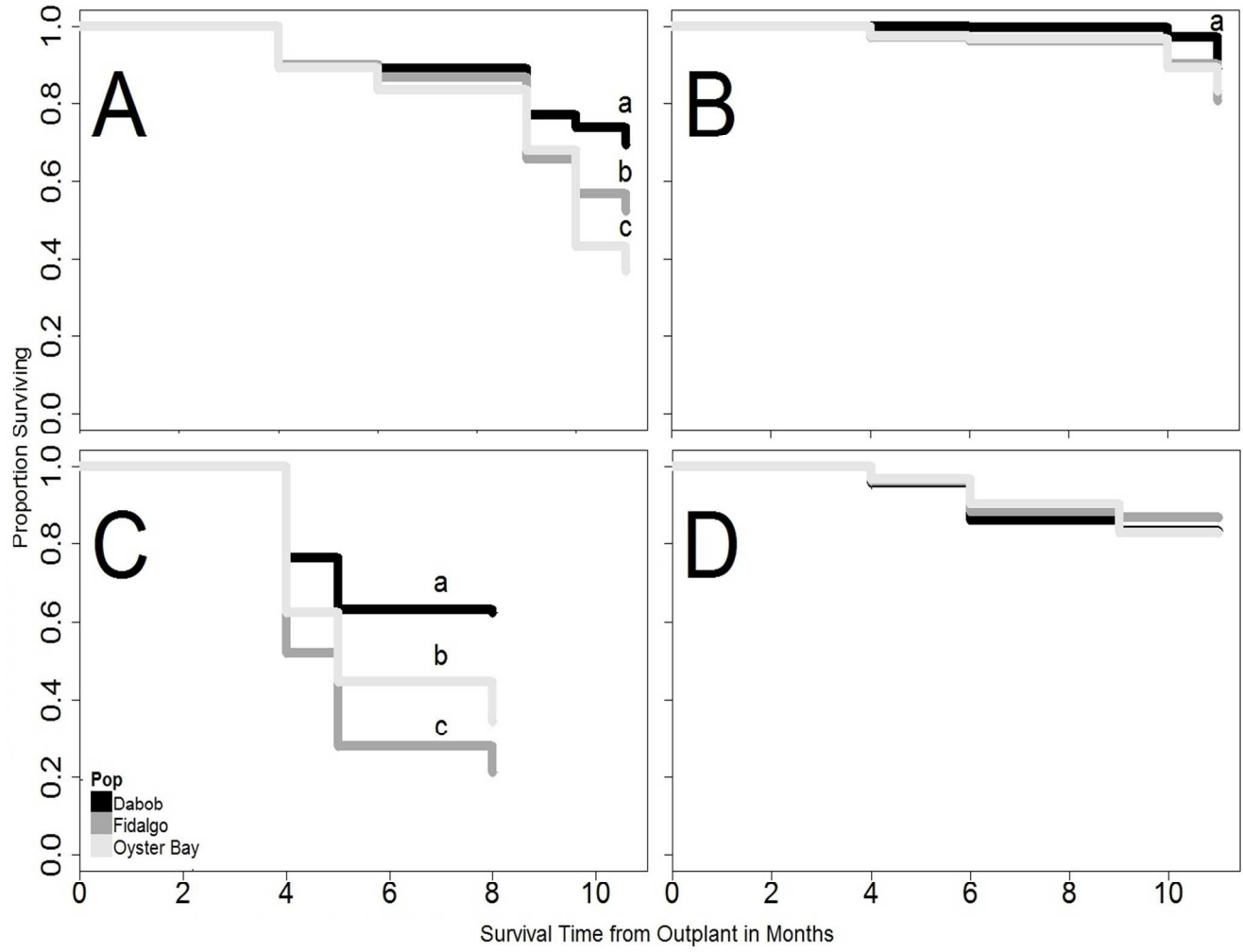
Figure 4. Proportion survival for three *Ostrea lurida* populations at four locations; Southern site (A),

Central site (B), Hood Canal site (C), and Northern site (D). Lowercase letters (a, b, c) are significant

differences.

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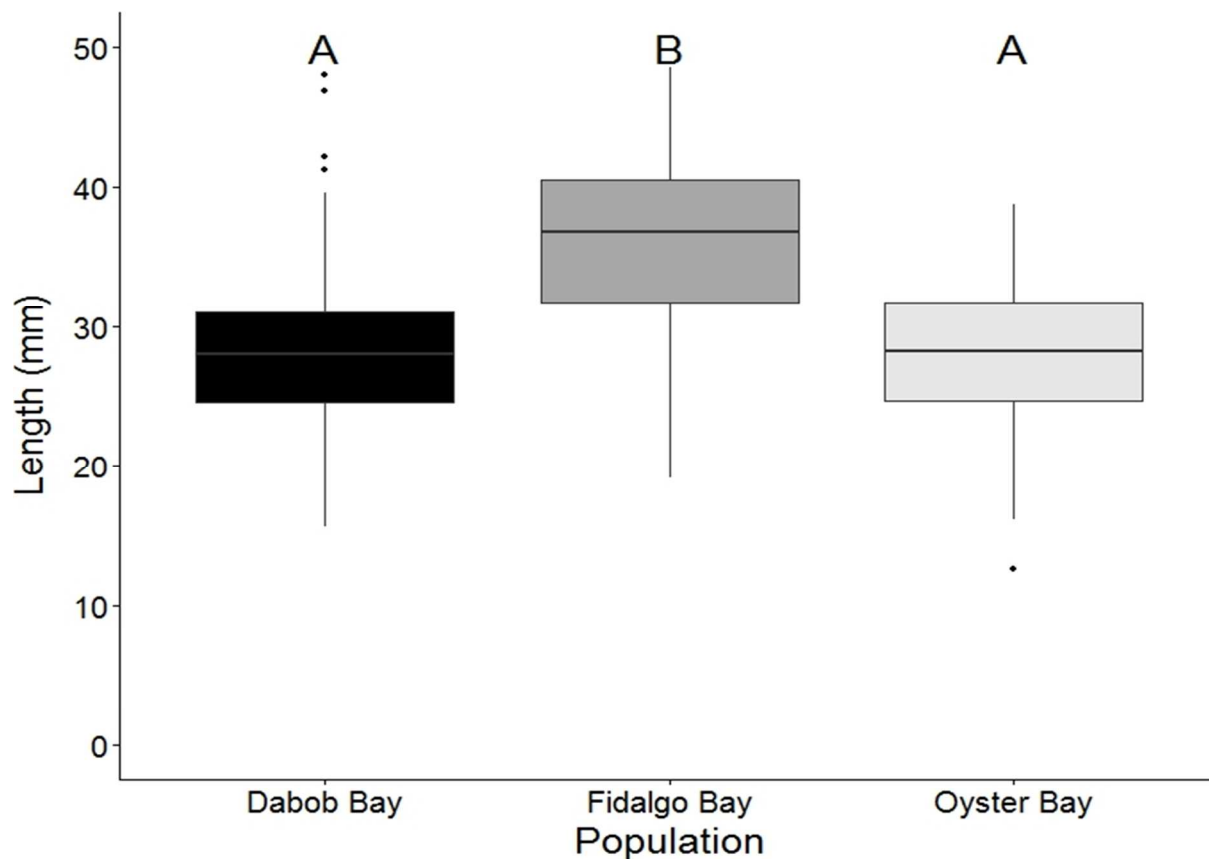
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570   Figure 5. *Ostrea lurida* shell length in September 2014 at Southern site. Boxplots with mean SL as central

571   line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots

572   representing outliers from data set. Letters indicate significant differences. Fidalgo Bay oysters were

573   considered different due to Nemenyi Post Hoc test with P<0.0001 (Oyster Bay and Dabob Bay oysters).
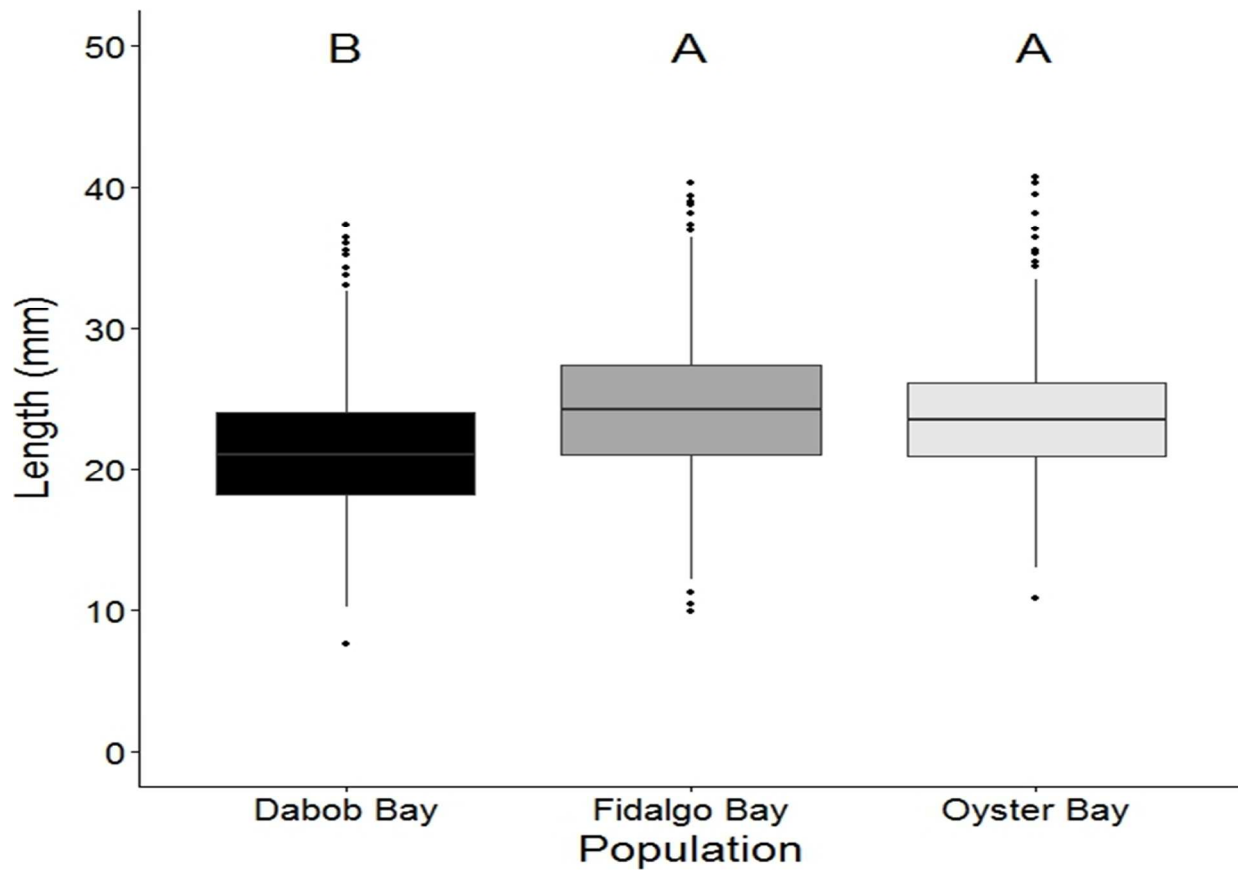
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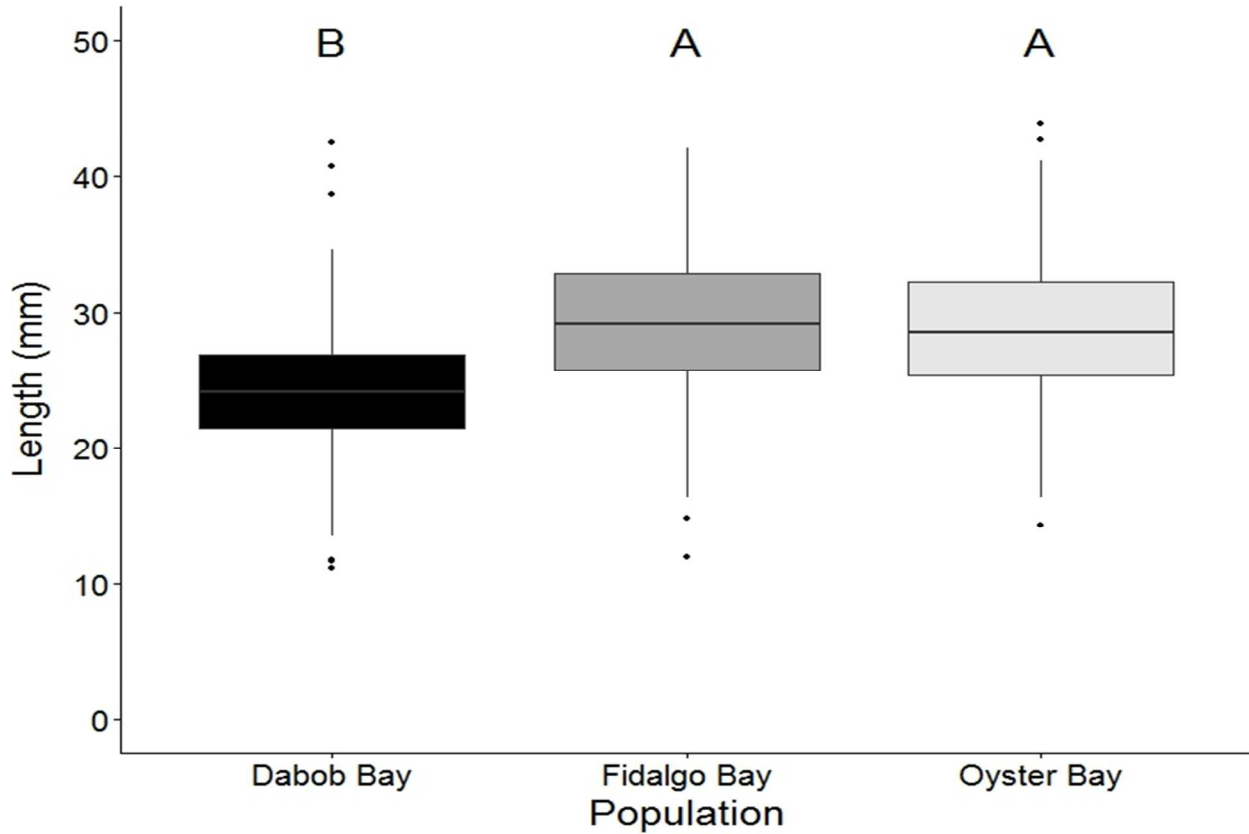
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576 Figure 6. *Ostrea lurida* shell length in October 2014 at Northern site. Boxplots with mean SL as central

577 line and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots

578 representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters were

579 considered different due to Nemenyi Post Hoc test with P<0.0001 (Fidalgo Bay and Oyster Bay oysters).

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582   Figure 7. *Ostrea lurida* shell length in October 2014 at Central site. Boxplots with mean SL as central line

583   and boxes represent second and third quartile. Horizontal lines are 1st and 4th quartile with dots

584   representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters were

585   considered different due to Nemenyi Post Hoc test with P=0.00028 (Oyster Bay oysters) and P<0.0001

586   (Fidalgo Bay oysters).
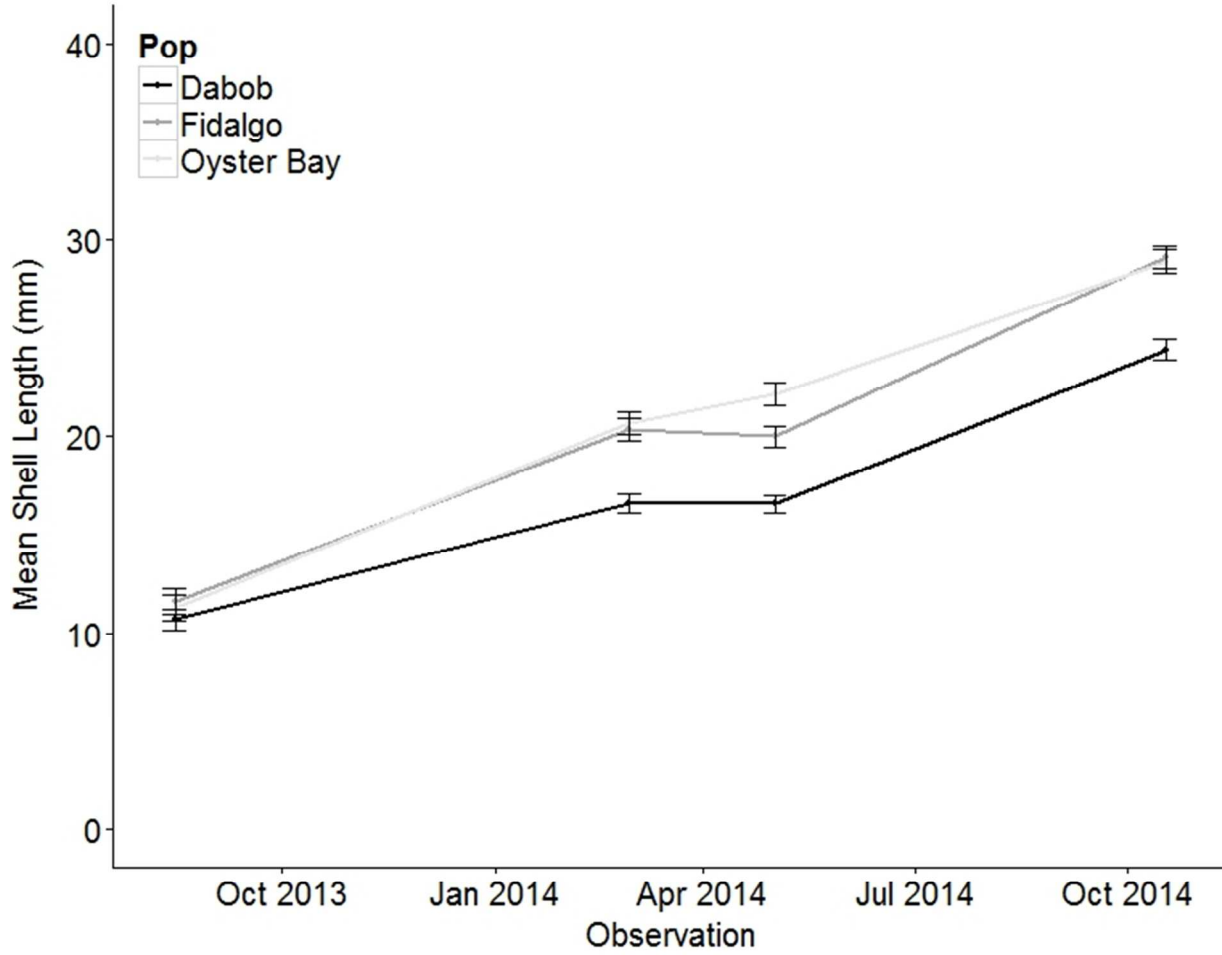
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588　Figure 8. Growth rate of mean shell length in *Ostrea lurida* outplanted at Northern site. Error bars

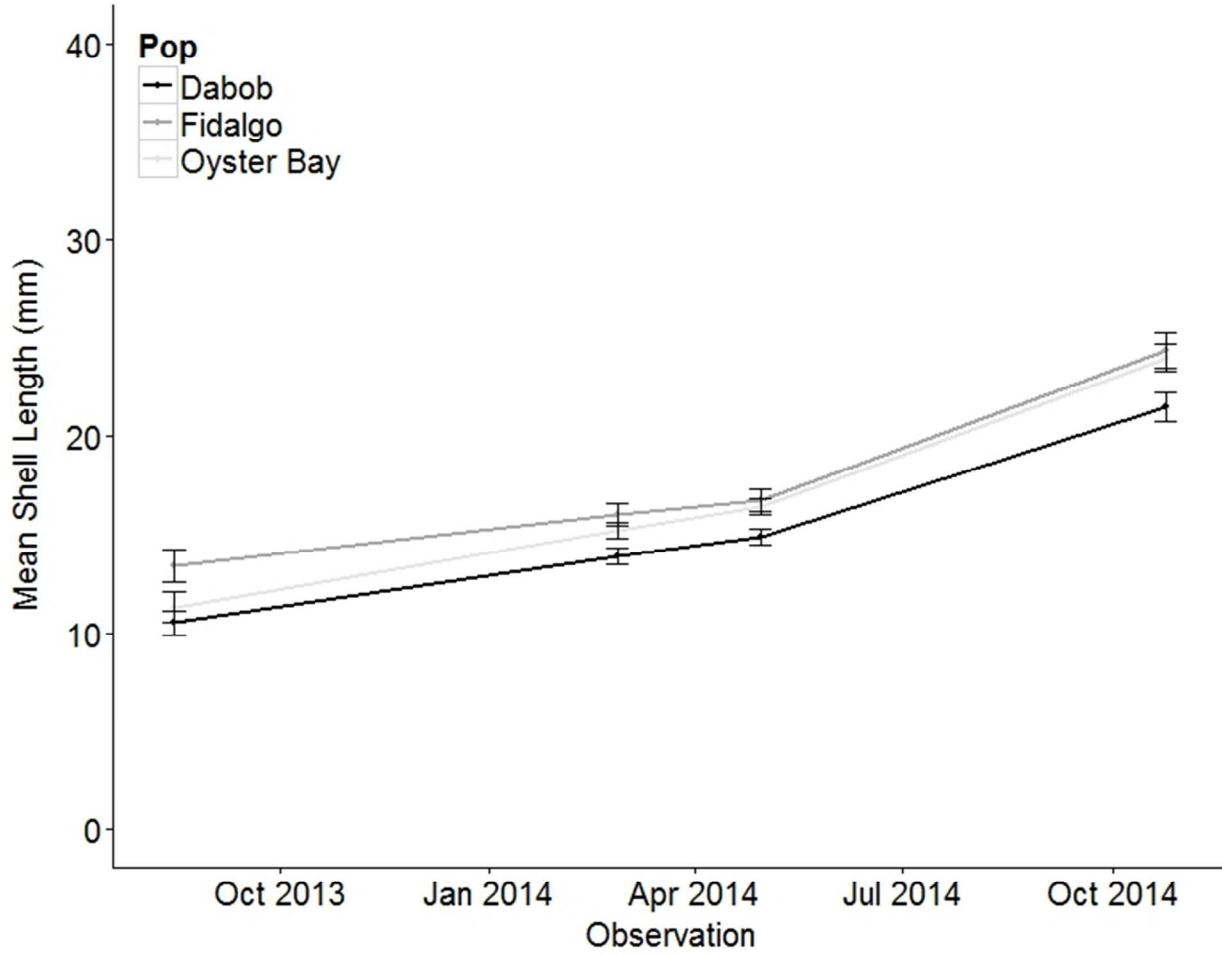589　indicate 95% confidence intervals at each time point.
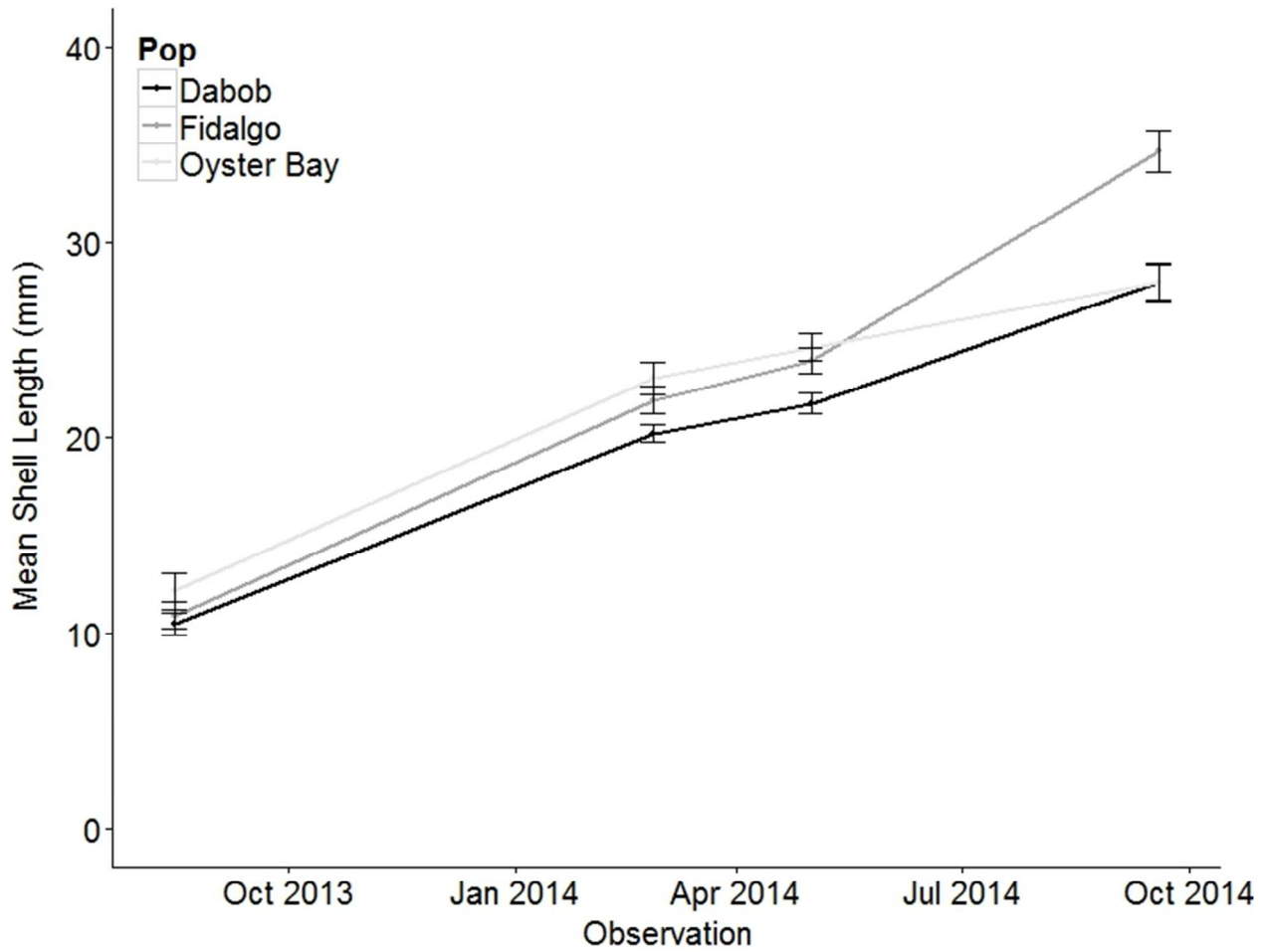
Figure 9. Growth rate of mean shell length in *Ostrea lurida* outplanted at Central site. Error bars indicate

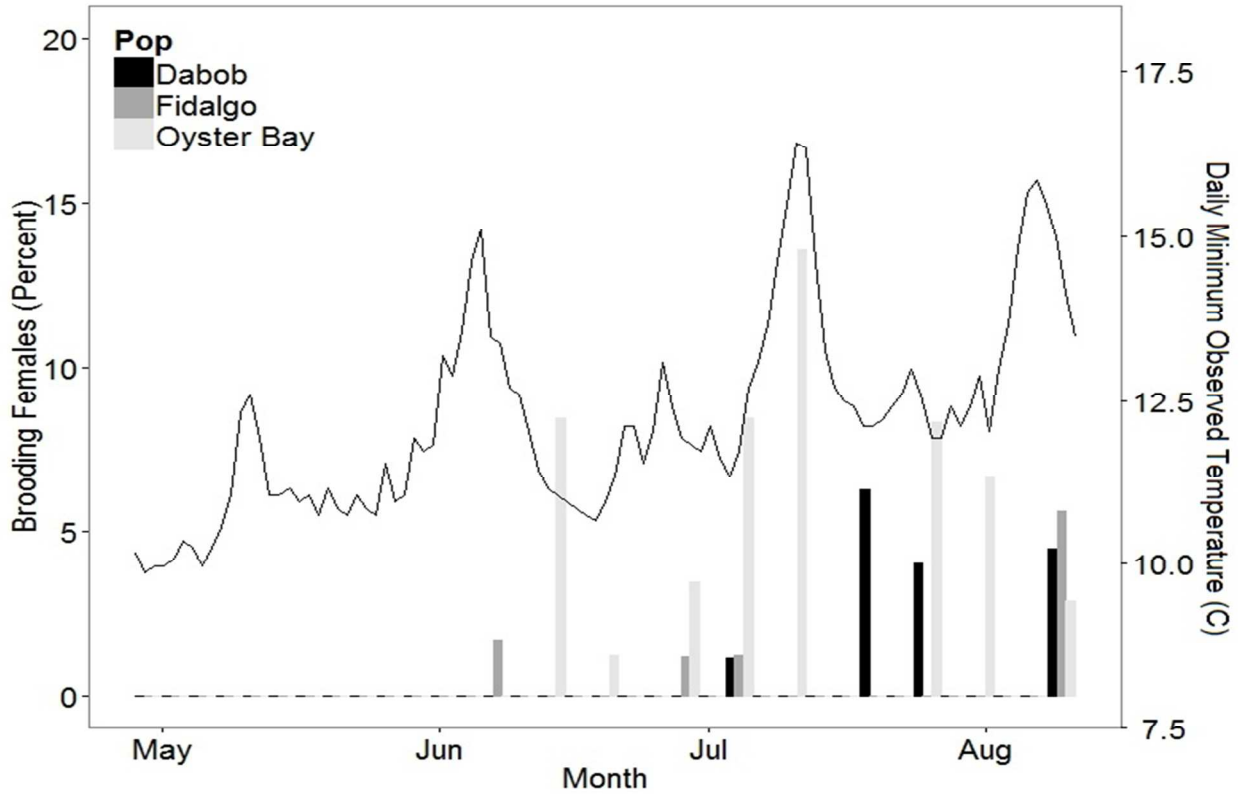95% confidence intervals at each time point.

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605   Figure 10. Growth rate of mean shell length in *Ostrea lurida* outplanted at Southern site. Error bars

606   indicate 95% confidence intervals at each time point.

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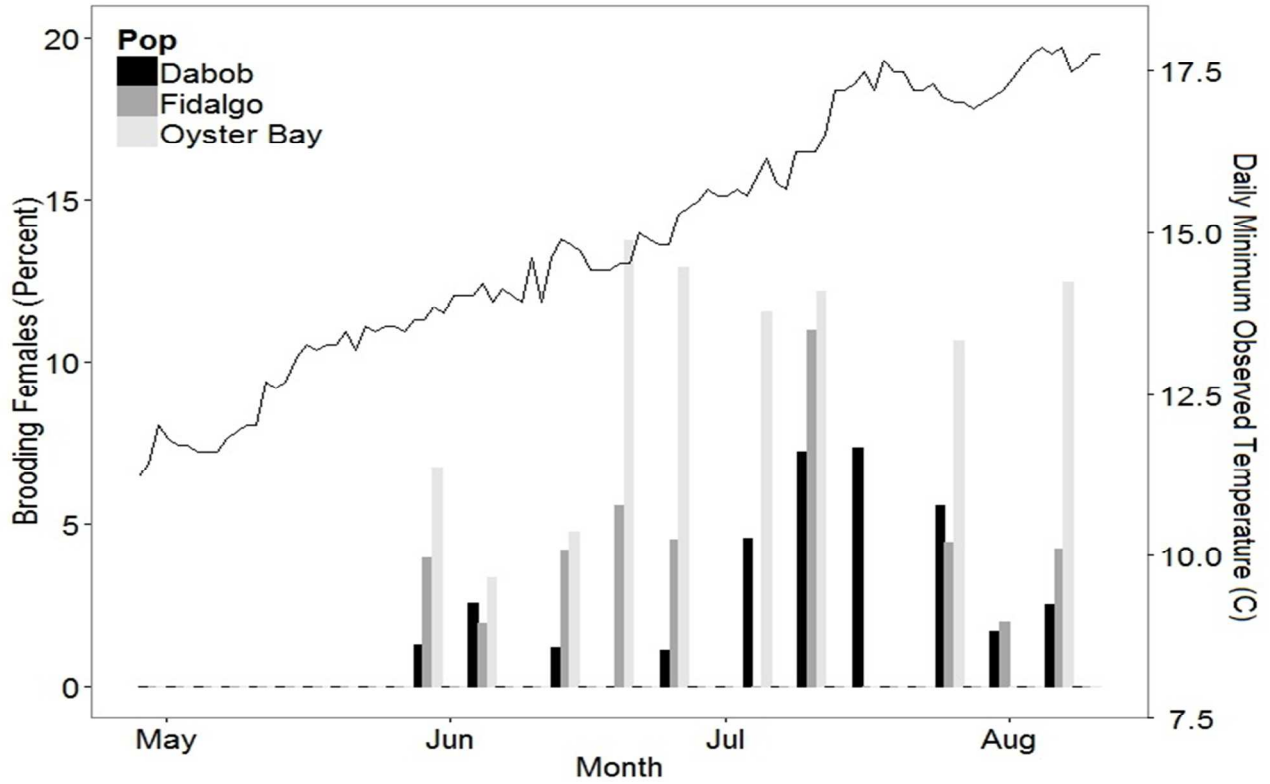
610   Figure 11. Percent *Ostrea lurida* brooding females from each population at each sample date at

611   Southern site. Percent determined by number of brooding females (Br) divided by number of open

612   oysters (T) or %=(Br/T)*100.

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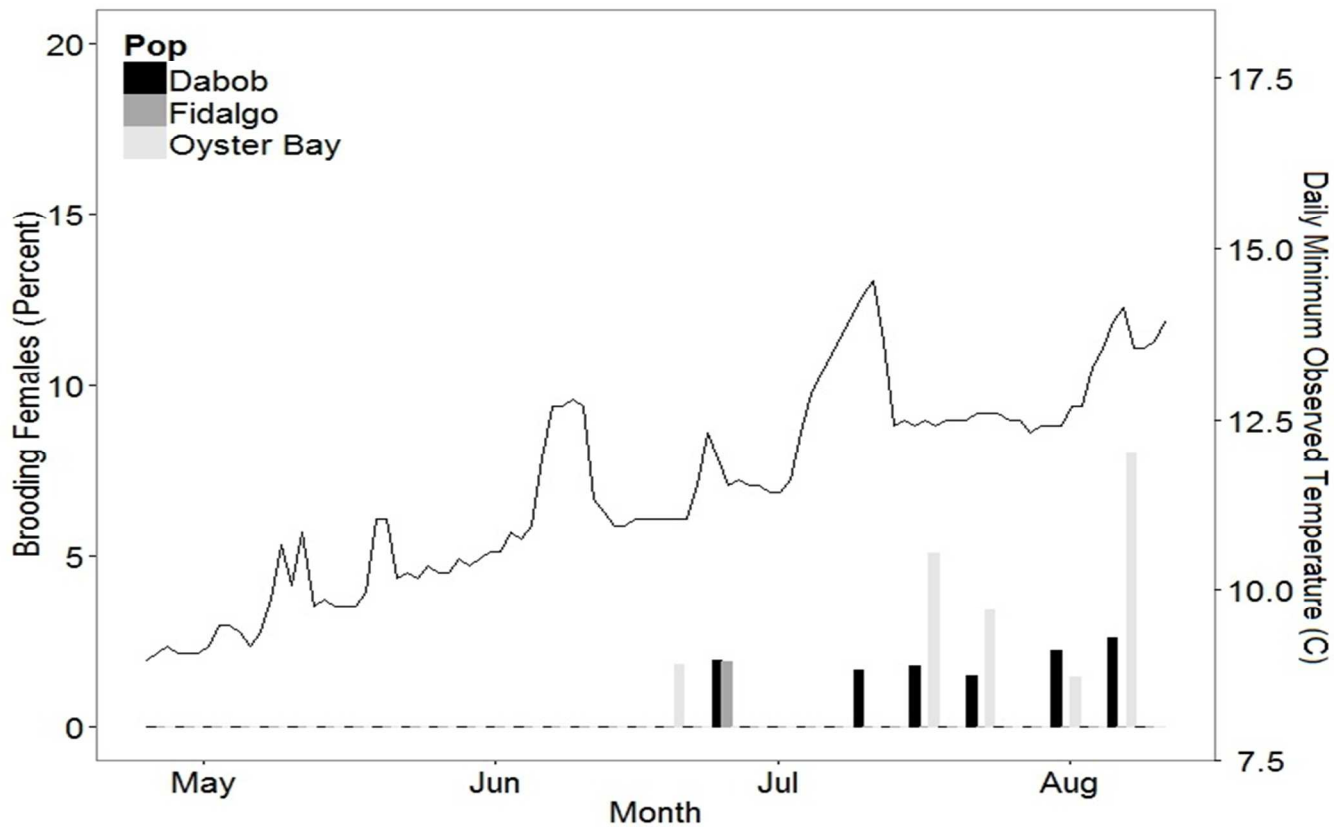21
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616   Figure 12. Percent *Ostrea lurida* brooding females from each population at each sample date at

617   Northern site. Percent determined by number of brooding females (Br) divided by number of open

618   oysters (T) or %=(Br/T)*100.

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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.

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

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