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ORIGINAL ARTICLE

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

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Abstract

Species traits that carry adaptive advantages such as reproductive timing and stress resilience may differ among populations. 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, WA, USA. Performance differed for each population. *Ostrea lurida* from Dabob Bay had the highest survival at all sites but the lowest reproductive activity and growth. Oysters from Oyster Bay demonstrated the greatest 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.

KEYWORDS
growth, oyster, reproduction, temperature, transplant

1 | 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, Coll, Magera, Ward-Paige, & Airoldi, 2011). The eastern oyster, *Crassostrea virginica*, has been shown to make large contributions in the way of ecosystem 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 Olympia oyster, *Ostrea lurida*, it is suspected *O. lurida* creates significant habitat value akin to that of the native European oyster, *Ostrea edulis* (zu Ermgassen, Gray, Langdon, Spalding, & Brumbaugh, 2013). In an attempt to restore lost ecosystem services owing to population declines, resource managers and restoration groups often 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, Christian, Harrison, & Rice, 2005).

The Olympia oyster, *O. lurida* Carpenter, 1864, is native to the west coast of North America and has received considerable attention with respect to restoration. The Olympia oyster exists in a variety of habitats within its range from Baja California, Mexico, to British Columbia, Canada (Gillespie, 2009; Hopkins, 1937; Polson & Zacherl, 2009). Olympia oysters experience increased mortality in freezing temperatures (0°C; Baker, 1995; Davis, 1955) or prolonged exposure to temperatures above 39°C (median lethal temperature; Brown, Briden, Stokell, Griffin, & Cherr, 2004). *Ostrea lurida* individuals 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). According to Hopkins (1937), peak larval settlement, roughly correlated with peak spawning, generally occurs twice annually in

South Puget Sound with the earlier of the two events typically occurring in the latter half of May. Carson (2010) likewise observed two settlement peaks (June and August) at two locales in Southern CA. In contrast, Seale and Zacherl (2009) observed only a single settlement peak (June) at two other Southern CA locales.

Despite several studies on Olympia oyster ecology and life-history traits in Puget Sound, WA, USA (e.g. Baker, 1995; Hopkins, 1937; Trimble, Ruesink, & Dumbauld, 2009; White, Ruesink, & Trimble, 2009), peer-reviewed information on genetic population structure is lacking and little is known about adaptive divergence and spatial variation in fitness-related phenotypes (Camara & Vadopalas, 2009). Bible and Sanford (2016) recently focused on adaptive divergence of *O. lurida* among populations in San Francisco Bay, and found evidence that populations might be locally adapted to different salinities. The size, hydrologic features and diverse environments of Puget Sound, and the retention of larvae by *O. lurida* during brooding, coupled with the recent evidence of differential salinity tolerance among San Francisco Bay populations (Bible & Sanford, 2016) suggest the possibility that populations within Puget Sound may likewise be adapted to local conditions. Among methods testing for local adaptation, reciprocal transplant experiments are considered robust (Sanford & Kelly, 2011) for investigating fitness. These experiments involve using parent populations from 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, Scarpa, Cook, & Hare, 2014). Alternatively, there are other phenomena such as balanced polymorphism (Sanford & Kelly, 2011) or low effective population size (genetic drift) that can manifest in phenotypic variation that may be falsely attributed to local adaptation (Camara, Evans, & Langdon, 2008; Camara & Vadopalas, 2009).

The primary 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 | Reciprocal transplant experiment

For this study three geographically separated, discrete groups (which we will refer to as populations for simplicity) of *Ostrea lurida* within Puget Sound were selected. These animals were brought to a hatchery, allowed to spawn and the offspring from each population outplanted back to the bays selected. This approach enables observations about how differing natural environments with resident oyster populations may affect both local and non-local populations over time.

2.2 | Bays of origin

Three bays (i.e. Fidalgo Bay, Dabob Bay and Oyster Bay) within Puget Sound were selected for this experiment based on the presence of resident *Ostrea lurida* populations, distance from other bays, latitudinal position and distinct environmental conditions (Figure 1). Fidalgo Bay is the most northern site and is generally cooler than the other two bays. Fidalgo bay is also more directly influenced by the Strait of Juan de Fuca, allowing colder seawater directly from the Pacific to mix with bay waters. Dabob Bay is located within Hood Canal, a distinctly separate fjord-like arm of Puget Sound, with longer retention and more stratification than the rest of Puget Sound. Oyster Bay is the southernmost site, warmer, highly productive and known for its historical abundance of *O. lurida*. Oyster Bay is also home to several commercial Olympia oyster shellfish aquaculture operations.

2.3 | Broodstock conditioning and outplanting

Adult oysters were collected from the three source locations in Puget Sound; Fidalgo Bay, Dabob Bay and Oyster Bay (n = 600 each; Figure 1) during November and December 2012. Oysters were held for 5 months in common conditions in Port Gamble, WA, and spawned in June 2013. To ensure genetic diversity, each population from each site was allowed to spawn in 24 separate groups of 20–25 oysters.

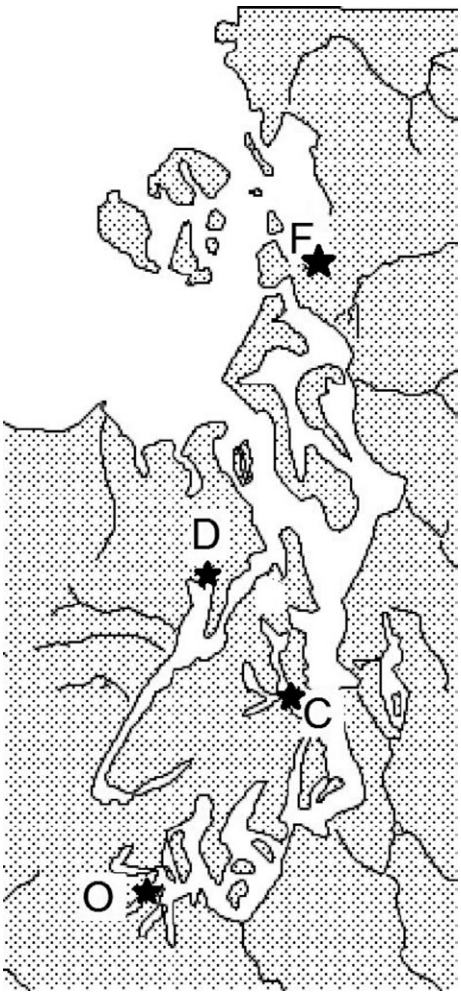

FIGURE 1 Olympia oyster (*Ostrea lurida*) broodstock and outplant sites in Puget Sound, WA. Broodstock collected from Fidalgo Bay (F), Dabob Bay (D) and Oyster Bay (O). Outplanted to Northern (F), Hood Canal (D), Central (C) and Southern (O) sites

Larvae produced from each population were reared in tanks based on spawning group and settled on microcultch (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 SL) from each population were placed at source locations: Fidalgo (48°49'74" N, 122°59'13" W), Oyster (47°34'19" N, 122°40'35" W) and Dabob Bays (47°43'37" N, 122°50'23" W). In addition, oysters from each population were placed at Clam Bay (47°34'16" N, 122°32'48" W), a site in Central Puget Sound with generally moderate environmental conditions relative to the source locations (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 m wide × 0.61 m long grow out trays per population with 12 trays in total outplanted. In each tray, oysters (120) were equally distributed in four 10 × 7.5 cm mesh (1,475 μm) bags containing 30 oysters each. Size at outplant was similar for all sites except the Central site where the Fidalgo Bay population was larger (see Section 3). Trays were anchored into the substrate using rebar stakes. In late fall 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 avoid the risk of mass mortality (not due to selection) by reducing exposure to extreme temperatures during tidal exchanges. Oysters were removed from mesh bags, placed into trays anchored to the substrate and submerged in a perched lagoon in the Hood Canal site (N 47.850948, W 122.805694) as no suitable floating structure was available.

2.4 | 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, 2011). 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 achieve peak brooding activity. In addition, monthly salinity, chlorophyll a and dissolved oxygen content were obtained for each site from the Washington Department of Ecology website (https://fortress.wa.gov/ecy/eap/marinewq/) for buoys at the Northern site (48°51'33" N, 122°59'33" W, approx. 1.97 km from site), Central site (47°62'17" N, 122°50'17" W, approx. 6.25 km from site), Hood Canal site (47°66'70" N, 122°82'00" W, approx. 20.55 km from site) and Southern site (47°16'50" N, 122°96'33" W, approx. 5.04 km from site). Raw temperature data and analysis procedures used are available in Heare, Vadopalas, and Roberts (2015).

Survival

Survival, determined by counts of live and dead oysters, was 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 the 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. This test was performed with the R package *survival* (Therneau, 2014). To account for oyster drill (*Ocenebra inornata* and *Urosalpinx cinerea*) mortalities we incorporated a general linear model with binomial distribution and corrected for over-dispersion with the *dispmod* package (Scrucca, 2012), which corrects *p*-values based on chi-squared values divided by degrees of freedom times the *SE* for the factor. Following Bible and Sanford (2016), survival was also analysed using a generalized linear mixed model (GLMM) approach with Wald χ^2 tests, with population, site and interactions as fixed effects and tray as a random effect. Mortality and drill predation data and analysis procedures used are available in Heare et al. (2015).

2.6 | 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, *SD*) were produced by the R package *pastecs* (Grosjean & Ibanez, 2014). Size distributions were tested for normality using the Shapiro–Wilks test (*stats* package, R Core Team, 2014). To investigate significant differences between populations, sites, and population/site interaction, we used a linear effects model (LME) with population and site as fixed effects and tray as a random effect via the R package *lme4* (Bates, Maechler, Bolker, & Walker, 2014). We calculated *p*-values using the mixed function of the *afex* R package (Singmann, Bolker, & Westfall, 2015). Shell length data from the end of year one was compared using a Kruskal–Wallis test assuming a non-normal distribution based on the findings of the Shapiro–Wilks 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 the Kruskal–Wallis test to determine significant differences in rank, using Tukey assumptions (*PMCMR* package, Pohlert, 2014). Size data and analysis procedures used are available in Heare et al. (2015).

2.7 | Reproductive activity

To assess reproductive activity, individual trays of oysters were anesthetized and each oyster was visually inspected for the presence of brooding larvae in the mantle chamber. Specifically, trays were removed from water and exposed to air for 45 minutes then immersed in 0.3 M magnesium sulfate (heptahydrate sulfate mineral epsomite = Epsom salt; $MgSO_4$ $7H_2O$) dissolved in a 50/50 mix freshwater/seawater for 45 minutes (Hintz et al., 2017). Brooding versus non-brooding oysters were counted weekly from 14 May to 15 August 2014 for a total of 15 time point observations for each site. A different tray was checked weekly for each population at each site in an ongoing rotation. At the Southern site the rotation was disrupted when several trays lost their mooring; thus, the same tray was checked several weeks in a row until the missing trays were recovered at which point the tray rotation resumed. The shell height of each brooding female was measured to the nearest mm using calipers.

Using the daily minimum temperature spawning threshold for *Ostrea lurida* of 12.5°C (Hopkins, 1937), we counted the elapsed days from the threshold temperature date to the date of the first observation of a brooding female and the date of the maximum proportion of brooding females. The proportions of brooding females per site per visit were arcsine transformed to improve normality of proportions and analysed via two way analysis of variance (ANOVA; *base* package, R Core Team, 2014). Significant differences among sites, populations and site/population pairwise comparisons were determined using Tukey's honest significant difference (HSD) test (*base* package, R Core Team, 2014). Sizes at brooding were likewise compared via two-way ANOVA and Tukey's HSD to explore population, site and population by site differences (*base* package, R Core Team, 2014). Female brooding data and analysis procedures used are available in Heare et al. (2015).

3 | RESULTS

3.1 | 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 exhibited high temperature variability due to the inter-tidal placement of samples and the extreme cold weather during low tide events (Figures 2 and 3). From June to August 2014, the Southern site experienced warmer daily temperatures compared to all other sites (Figures 2 and 3).

3.2 | Survival

Differences in mortality per population were observed at three of the four sites. Dabob Bay oysters exhibited significantly lower mortality by the end of the study period at the Hood Canal (χ^2 = 141, *df* = 2, *p* < .0001), Southern (χ^2 = 76.3, *df* = 2, *p* < .0001) and Central sites (χ^2 = 13.7, *df* = 2, *p* = .00105) (Figure 4A–C) than other populations. A significant site × population interaction was detected for the Southern site and Hood Canal and the Southern site and Central site, between oysters derived from Dabob Bay and Fidalgo Bay populations (*p* = .001 and .01, respectively) after 5 months.

High mortality across all populations necessitated the premature termination of the Hood Canal site trial in April 2014. Evidence of high oyster drill related mortalities was observed at this site. The proportion of mortalities due to drills at the Hood Canal Site was significantly greater in the Fidalgo population (48%) compared to the Dabob and Oyster Bay populations (28% and 29%, respectively; GLMM, χ^2 = 6.2, *df* = 6, *p* < .0165). Fidalgo Bay oysters exhibited the lowest overall survival (21.2% ± 2.1 *SD*) at the Hood Canal site (Figure 4C); Oyster Bay oysters exhibited the lowest overall survival at the Southern site (37% ± 2.3 *SD*). Limited mortality was observed at both the Central and Northern sites where ≥80% of oysters remained after 11 months (July 2014; Figure 4B,D).

3.3 | Growth

At the end of the experiment the sizes of oysters among sites were significantly different (LME *F* = 268.29, *df* = 2, *p* < .0001 and Kruskal–Wallis test, χ^2 = 383.4, *df* = 2, *p* < .0001), with the Southern site producing the largest oysters (Figures 5 and 6) and the Central site producing

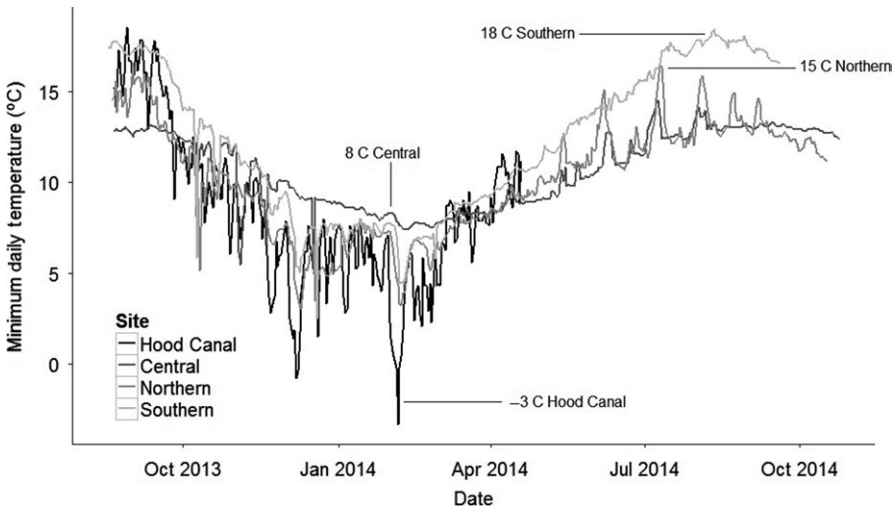

FIGURE 2 Minimum observed daily temperatures for all sites

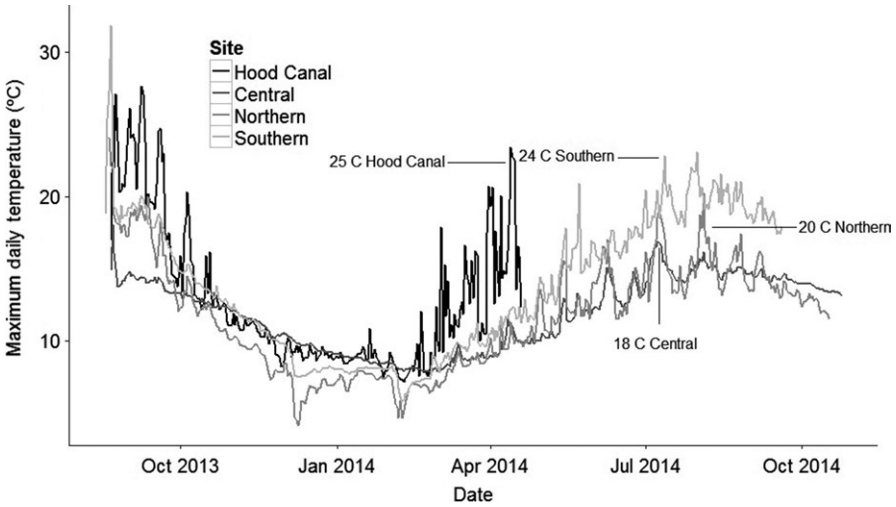

FIGURE 3 Maximum observed daily temperatures for all sites

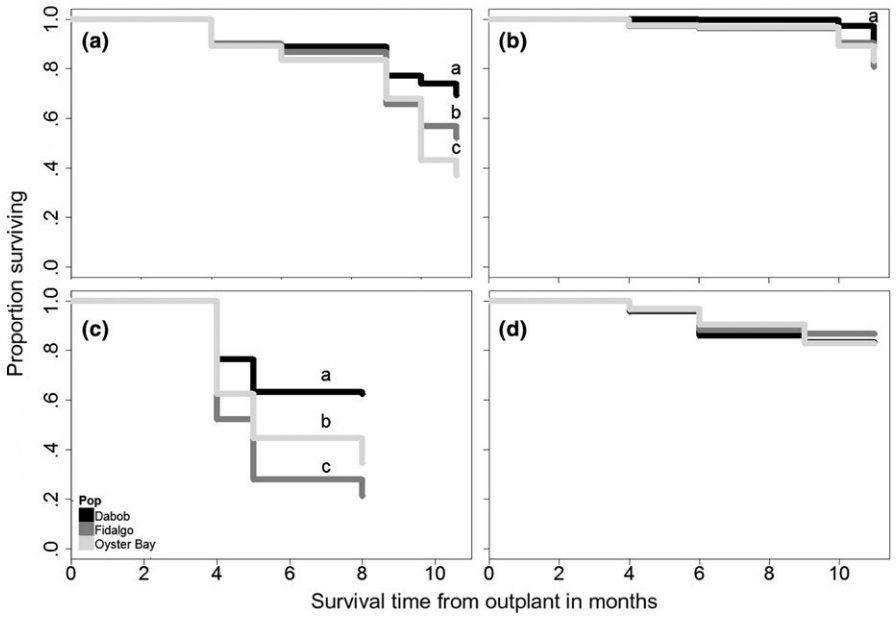

FIGURE 4 Proportion survival for three *Ostrea lurida* populations at four locations; Southern site (A), Central site (B), Hood Canal site (C) and Northern site (D). Lowercase letters (a, b, c) are significant differences

the smallest (Figures 7 and 8). Oyster size also differed among populations (LME $F = 86.42$, $df = 2$, $p = .007$ and Kruskal–Wallis test, $\chi^2 = 196.1$, $df = 2$, $p < .0001$). The linear model also indicated that the interaction between populations and sites was significant (LME $F = 23.34$, $df = 4$, $p < .0001$). At the Southern site, Fidalgo Bay oysters were larger than Dabob (Nemenyi post-hoc, $p < .0001$) and Oyster Bay oysters (Nemenyi post-hoc, $p < .0001$) (Figures 5 and 6). At the Central site, the Oyster Bay oysters were significantly larger than the Dabob oysters by the end of the experiment (Nemenyi post-hoc, $p = .00028$; Figures 7 and 8). At the Northern site, oysters from Dabob Bay broodstock were smaller than Fidalgo Bay (Nemenyi post-hoc, $p < .0001$) and Oyster Bay (Nemenyi post-hoc, $p < .0001$) oysters at the end of the experiment (Figures 9 and 10). Oyster mean size at outplant was 11.4 (±3.2 *SD*) mm and there were no differences in size among populations except for the Central site where the Fidalgo population was larger (Figure 8).

3.4 | Brooding females

The proportions of brooding females varied among populations (ANOVA, $F = 9.1$, $df = 2$, $p = .0002$) and among sites (ANOVA, $F = 11.4$, $df = 2$, $p < .0001$). The greatest overall proportion of total brooding females present was at the Southern site (8.51%; Figure 11) compared to the Northern (1.16%; $p = .007$) and Central sites (0.29%; $p < .0001$). The smallest proportion of brooding females was documented at the Central site (Figure 13). The Oyster Bay population produced significantly more brooding females than the Fidalgo Bay (Tukey's HSD, $p = .001$) or Dabob Bay (Tukey's HSD, $p = .0005$) populations (Figure 14). The Fidalgo and Dabob Bay populations did not differ from one another at any of the sites (Tukey's HSD, $p = .942$). No interaction between site and population was evident (ANOVA, $F = 1.1$, $df = 4$, $p = .3623$).

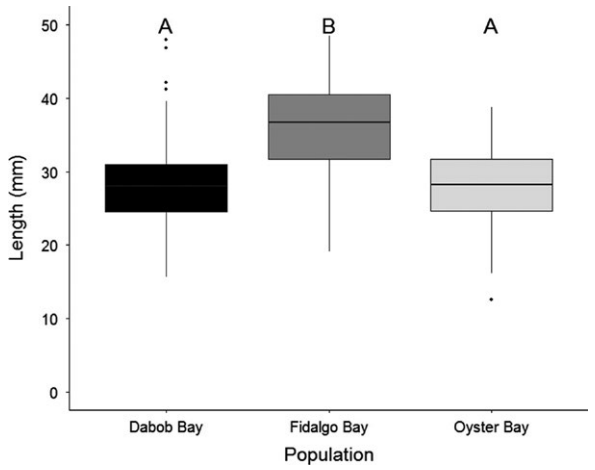

FIGURE 5 *Ostrea lurida* shell length (SL) in September 2014 at Southern site. Boxplots with mean SL as central line and boxes represent second and third quartiles. Horizontal lines are first and fourth quartiles with dots representing outliers from data set. Letters indicate significant differences. Fidalgo Bay oysters were considered different due to Nemenyi post hoc test with $p < .0001$ (Oyster Bay and Dabob Bay oysters)

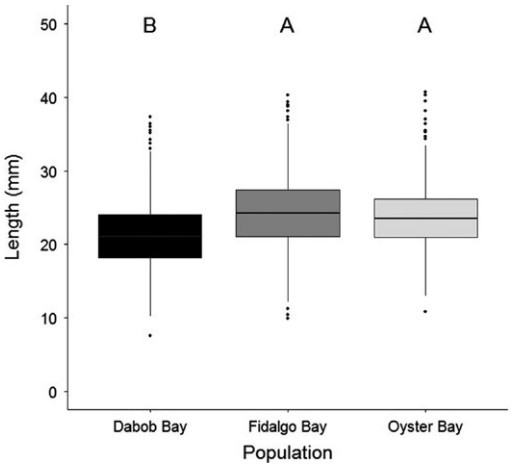

FIGURE 7 *Ostrea lurida* shell length (SL) in October 2014 at Central site. Boxplots with mean SL as central line and boxes represent second and third quartiles. Horizontal lines are first and fourth quartiles with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters were considered different due to Nemenyi post hoc test with $p = .00028$ (Oyster Bay oysters) and $p < .0001$ (Fidalgo Bay oysters)

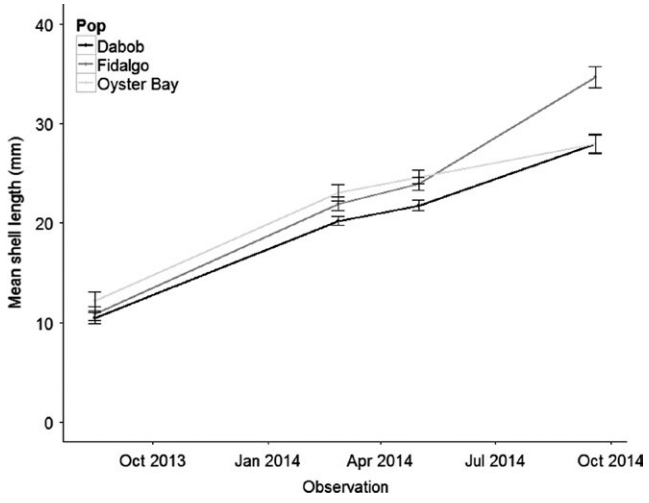

FIGURE 6 Growth rate of mean shell length in *Ostrea lurida* outplanted at Southern site. Error bars indicate 95% confidence intervals at each time point

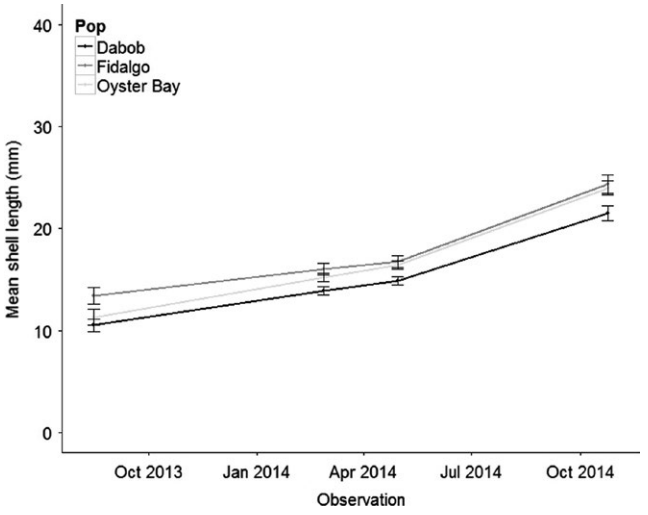

FIGURE 8 Growth rate of mean shell length in *Ostrea lurida* outplanted at Central site. Error bars indicate 95% confidence intervals at each time point

The Southern site reached the spawning temperature threshold of 12.5°C (as defined by Hopkins, 1937) on 14 May and the first brooding female was observed 15 days later on 29 (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 18.4°C (Figure 11). At the Southern site, Oyster Bay oysters reached the maximum percentage of brooding females on 19 June, 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 10 July, 57 days post 12.5°C, 453°D (Figure 11).

At the Northern site, the 12.5°C temperature was also reached on 14 May and the first brooding female was observed on 6 June (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 the maximum percentage of brooding females by 11 July, 58 days later, or 354°D. The Fidalgo Bay and Dabob Bay oyster populations did not reach their maximum percentage of brooding females until 8 August (Figure 12), 87 days later, or 513°D.

The Central site reached 12.5°C on 8 June and brooding females were observed in the Oyster Bay population on 18 June (Figure 13),

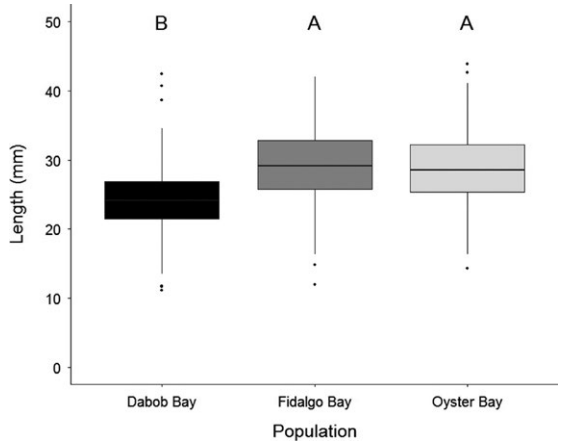

FIGURE 9 *Ostrea lurida* shell length in October 2014 at Northern site. Boxplots with mean SL as central line and boxes represent second and third quartiles. Horizontal lines are first and fourth quartiles with dots representing outliers from data set. Letters indicate significant differences. Dabob Bay oysters were considered different due to Nemenyi post hoc test with $p < .0001$ (Fidalgo Bay and Oyster Bay oysters)

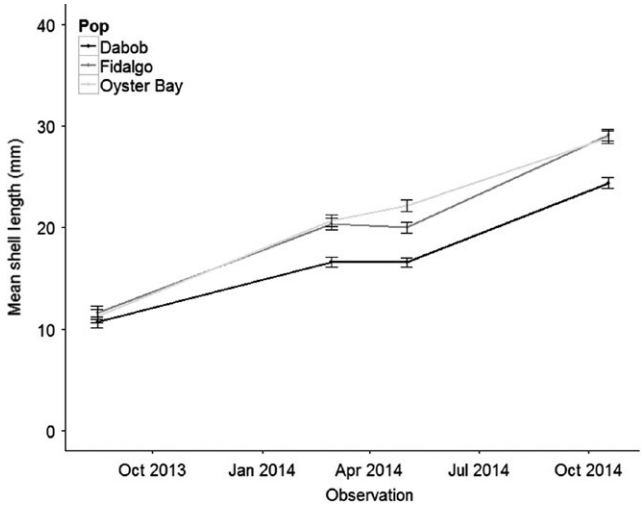

FIGURE 10 Growth rate of mean shell length in *Ostrea lurida* outplanted at Northern site. Error bars indicate 95% confidence intervals at each time point

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 the summer (Figure 13). Peak spawning could not be determined due to the low number of brooding individuals observed at the Central site.

Size at brooding varied significantly among populations (ANOVA, $F = 18.2$, $df = 2$, $p < .0001$) and sites (ANOVA, $F = 33.1$, $df = 2$, $p < .0001$), with the smallest brooding females observed at the Central site (Figure 14). Brooders were significantly smaller at the Central site compared to the Northern site (Tukey's HSD, $p < .0001$) and Southern site (Tukey's HSD, $p < .0001$). No significant size difference in brooders was observed between the Southern and Northern sites ($p = .8$). The average minimum size at brooding of the 10 smallest oysters was

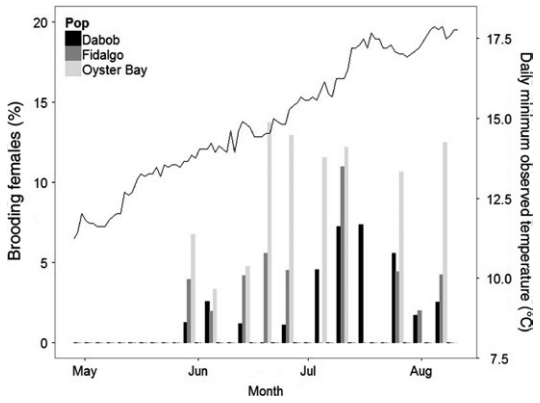

FIGURE 11 Percent *Ostrea lurida* brooding females from each population at each sample date at Southern site. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or % = (Br/T)*100

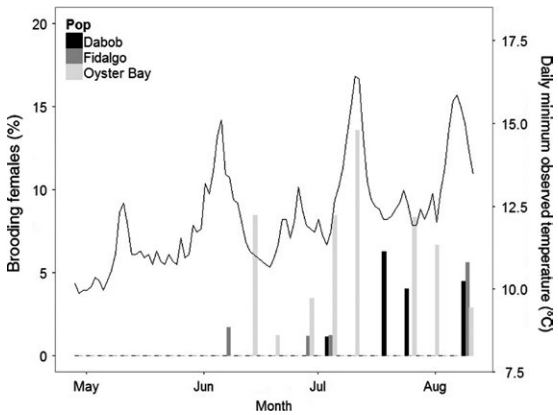

FIGURE 12 Percent *Ostrea lurida* brooding females from each population at each sample date at Northern site. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or % = (Br/T)*100

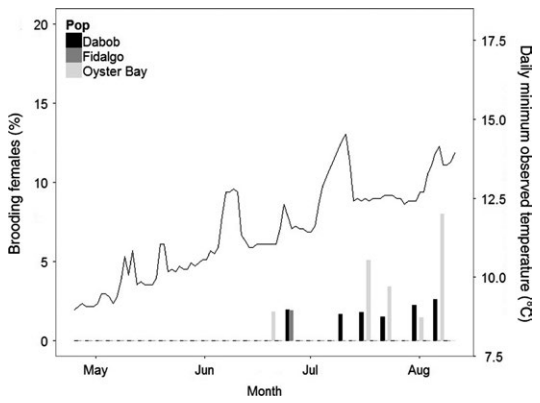

FIGURE 13 Percent *Ostrea lurida* brooding females from each population at each sample date at Central site. Percent determined by number of brooding females (Br) divided by number of open oysters (T) or % = (Br/T)*100

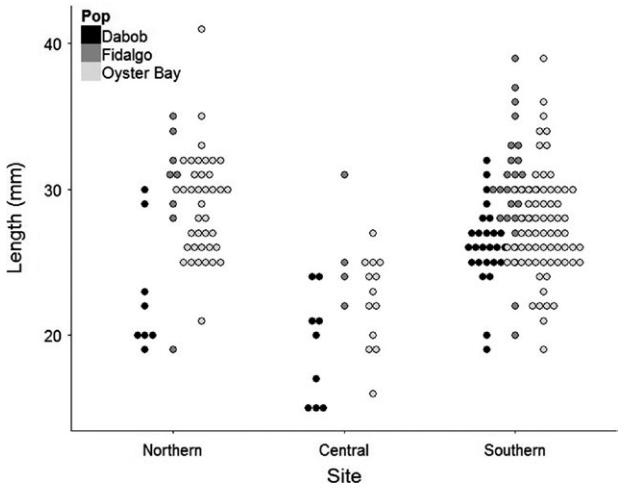

FIGURE 14 *Ostrea lurida* brooding female shell length comparison among sites

19.1 (±3.7 *SD*) mm. Two brooding females of 15.0 mm from the Dabob Bay population were observed at the Central site. The average size of brooding females across populations and sites was 27.1 (±4.5 *SD*) mm.

4 | DISCUSSION

A primary objective for this study was to evaluate population performance in relation to possible 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 separate locales (populations). At the population level, one population exhibited greater survival and one favored reproduction over other traits suggesting the existence of adaptive structure within Puget Sound. In addition, a significant interaction of population and site was detected, an indicator of local adaptation. In the remainder of this section, findings from this study are discussed in terms of differences across sites, differences in population performance and implications of these findings with respect to restoration efforts.

4.1 | Site differences

4.1.1 | Mortality

Mortality rates were different across sites, with these differences correlated to temperature and predation. The oyster populations at the Hood Canal site exhibited high mortality; the site exhibited temperatures outside of the thermal tolerance range reported by Baker (1995) on 35% of the total days (85 out of 242 days) with two subfreezing events of −0.78 and −3.3°C in December 2013 and February 2014, respectively (Figure 2). The Southern site, where populations also experienced moderate mortality, had a total of 39 days (9% of 398 days) outside of the 5–20°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 sites had fewer days outside of the range (24 and 0 days, respectively) and low mortality was observed in all populations. Olympia oysters in Puget Sound naturally occur in seeps, perched lagoons and other habitats that buffer temperature extremes; the deployment of experimental units suspended from floating structures or in a perched lagoon was an attempt to afford similar protection to the outplanted oysters. 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). In addition to the temperature extremes, the oysters at the Hood Canal site experienced predation as evidenced by direct observations of invasive oyster drills and prevalent drill holes. A difference in population susceptibility to drill predation was observed (see below). Factors other than temperature and predation likely affect survival at these sites. Salinity, for example, has been shown to differentially correlate with survival of *Ostrea lurida* populations in San Francisco Bay (Bible & Sanford, 2016). Genetic analyses are necessary to understand causal relationships and determine specific selective forces driving the observed differentiation.

4.1.2 | Growth

In the present study, Olympia oysters attained an average size of 35.8 mm ± 6.4 *SD* during the first year of growth, with some individuals >45 mm. These observations contrast with the 2–3 years Hopkins (1937) estimated was necessary to attain this size. This discrepancy could be due to changes in environmental conditions or differences in the populations sampled.

A difference in size was observed in relation to site. 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. Brown & Hartwick, 1988; Malouf & Breese, 1977; Shpigel, Barber, & Mann, 1992) that demonstrate that warm temperatures improve oyster growth as long as the temperatures are within the tolerable range. The general pattern of productivity in South Puget Sound generally exceeds that of other Puget Sound sub-basins due both to higher temperatures and nutrient levels; for example, Budd Inlet (South Sound) production in 1997 was 6,000 mg C m^{-3} day^{-1}, compared to 2,000–4,500 mg C m^{-3} day^{-1} in Dabob Bay (Newton, Edie, & Summers, 1998).

4.1.3 | Reproduction

Oysters reproduced as females in Puget Sound at a mean size of 27.1 mm (±4.5 *SD*). This result contrasts with results of previous research (Coe, 1932a,b; Hopkins, 1937) that describe *Ostrea 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, 1932a; 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 accord with these earlier studies, 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 11–13). The observed spawn timing also may be correlated with differences in chlorophyll a content between the Northern and Southern sites; the relationship of *O. lurida* spawn timing with primary production warrants further investigation.

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 2 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.

4.2 | Population differences

4.2.1 | Mortality

Survival differed among populations within three out of four sites. The population derived from Dabob Bay broodstock exhibited better survival than the other two populations (Figure 4). The observed temperature variability (Figures 2 and 3) at the Hood Canal site in the present study may be indicative of historic temperature trends to which the parent populations were exposed. If so, the significantly greater survival of the Dabob Bay population at three of the four sites could be a function of increased stress resilience of offspring in response to prevalent temperature extremes. It is important to note that the broodstock collected from Dabob Bay were removed from cobbles that were exposed to only trickles of seawater draining off the beach during low tide periods. Previous studies on thermal tolerance (e.g. bay scallops, *Argopecten irradians*, Brun, Bricelj, MacRae, & Ross, 2008; and Mediterranean mussels, *Mytilus galloprovincialis*, Dutton & Hofmann, 2009) demonstrate that more frequent exposure to temperature extremes results in elevated heat shock proteins (HSPs) and HSP mRNA transcripts. In addition, Sørensen, Kristensen, and Loeschcke (2003) found that many species exhibit heritable HSP production patterns. The higher survival rates observed in the Dabob Bay population may likewise be related to heritable traits.

Predation was also a factor in population-specific survival at the Hood Canal site, where the Fidalgo Bay population had significantly greater mortality attributed to oyster drills. Fidalgo Bay has essentially no drills (B. Blake, unpublished data) to prey upon local oyster populations, whereas populations from Dabob and Oyster Bays may be adapted to environments with oyster drills. The mechanism associated with susceptibility is unknown, but might be related to shell thickness.

4.2.2 | Growth

At all transplant sites, the population derived from Dabob Bay parents exhibited the lowest growth. This observation is likely related to the fact the Dabob Bay population also had the highest survival. Applebaum, Pan, Hedgecock, and Manahan (2014) found energetic tradeoffs may improve survival over growth in the Pacific oyster, *Crassostrea 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 *Ostrea lurida*. For example, salinity stress, parasite and disease load, and food availability may have affected size (Andrews, 1984; Brown & Hartwick, 1988) but because of the separation between sites it seems unlikely that the effects seen in this study are primarily due to these factors.

4.2.3 | 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 and 14). One explanation for this is that the relatively rapid water temperature increase and consistently 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°D less 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 & Soame, 2004). Chávez-Villalba et al. (2002) found place of origin for *Crassostrea gigas* broodstock affected the rate of gametogenesis under different temperatures with some populations becoming reproductively active sooner than others do. Barber, Ford, and Wargo (1991) found gametogenesis and spawn timing were heritable traits within populations of *Crassostrea virginica*. Populations of *Ostrea lurida* at a locale in North Puget Sound were recently found to have initiated brooding at temperatures <11°C (Barber, Dexter, Grossman, Greiner, & McArdle, 2016), further illustrating the variability of this important fitness component on a relatively small spatial scale.

5 | 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 vagaries of larval dispersal, for example, we do not know that the parents of the wild oysters used as broodstock were from that locale. Thus the traits observed could be the result of strong selection in a different habitat. A reciprocal transplant using larvae coupled with molecular screening would be necessary to test the balanced polymorphism hypothesis. The differences observed could also be the result of low effective numbers of breeders in the hatchery, thus indicating a significant family effect and/or inbreeding depression as described in aquaculture of *Crassostrea 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 shed light on the question of genetic drift.

While a mechanism of local adaptation cannot conclusively be demonstrated in this study, the 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. First, based on the fact that Dabob Bay oysters had the lowest mortality overall, use of these more robust oysters for broodstock may increase chances for outplant survival. Second, an alternative approach might be to use the population with the greatest reproductive output (Oyster Bay) 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. Because 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. Third, the assisted gene flow strategy could incorporate the outplanting of populations known to contain fitness phenotypes for the new environmental parameter to inter-breed with resident populations (Aitken & Whitlock, 2013). Whether this strategy would have benefits that outweigh the drawbacks, such as possible outbreeding depression, is unknown, but assisted gene flow may prove a valid strategy for restoration efforts facing a variety of climate change scenarios. Regardless of the process resulting in the different phenotypes, it must be emphasized that the range of phenotypes per population is unknown. 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: (i) transplanted populations could overwhelm locally adapted remnant resident populations, (ii) transplanted populations might not survive in the new location, and thus waste valuable resources required for restoration, and (iii) transplanted populations could inter-breed with remnant populations and thus result in overall reduced fitness through outbreeding depression. Each of these concerns make assumptions regarding plasticity and adaptive potential, although we still know little about this in marine invertebrates, particularly on the temporal and geographic scales involved. Given that population structure was detected among the only three locales examined in this study, more structure might be uncovered upon further investigation. Resource managers and restoration practitioners can minimize disruption of extant population structure, and increase the likelihood of restoration success by restricting transfers of stocks among locales; reliance on habitat restoration and natural recruitment is the most risk-averse strategy. To better understand risks to wild populations, biomarkers of adaptive significance are critical to pursue.

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