Title: Local adaptation in an estuarine foundation species: implications for restoration

Authors: Jillian M. Bible (jmbible@ucdavis.edu)^{a,b*}, Eric Sanford (edsanford@ucdavis.edu)^{a,b}

^aDepartment of Evolution and Ecology, University of California Davis, One Shields Ave, Davis, California 95616, USA

^bBodega Marine Laboratory, 2099 Westside Road, Bodega Bay, California 94923, USA

* Corresponding author phone: +1-707-776-7368

Abstract:

Understanding the extent of local adaptation is critical for conservation and restoration planning in an era of environmental change. Adaptive differentiation among populations may mediate a species' response to environmental stress, yet these evolutionary processes are seldom studied in marine systems. We used native Olympia oysters (*Ostrea lurida*) as a model organism for studying local adaptation with applications to marine restoration. We tested whether populations of oysters in California estuaries are locally adapted to their home sites and to stressful low salinity events, which are predicted to increase in frequency with climate change. We spawned oysters from three sites in San Francisco Bay and raised their offspring under common laboratory conditions. These oysters were then reciprocally transplanted among the three field sites. At two of the three sites, oysters of local origin tended to survive better than those from other populations, suggesting that local adaptation may occur even within a single estuary. In a subsequent experiment, we raised oysters from two sites in San Francisco Bay and one site in Tomales Bay through two generations under common conditions and then subjected these oysters to a low salinity challenge in the laboratory. Oysters with the highest survival originated from the site with the lowest field salinity, suggesting that natural selection may favor stress tolerant phenotypes in certain regions. As interest grows in restoring heavily impacted native species, our results indicate that considering local adaptation may be essential to deciding how and where to conserve and restore species faced with changing conditions.

Key words: Olympia oyster; environmental stress; conservation; salinity; common garden; reciprocal transplant

1. Introduction

Anthropogenic impacts including habitat destruction, overharvesting, and climate change have severely degraded many of the planet's ecosystems (Vitousek et al. 1997), with estuaries among the most severely affected (Lotze et al. 2006). These widespread impacts require humans to take a central management role in conserving and restoring ecosystems (Vitousek et al. 1997). Many early restoration guidelines focused on choosing appropriate species (e.g., native or non-native), but it has become increasingly apparent that genetic differentiation within a species can have substantial influence on restoration success, especially during a time of rapid environmental change (Montalvo et al. 1997, Rice and Emery 2003, McKay et al. 2005).

Selecting the source of genetic material is important in restoration planning (Montalvo et al. 1997) and should be informed by whether populations are locally adapted to their home environments (McKay et al. 2005). Local adaptation occurs when populations become fine-tuned to their local environments through natural selection. In general, selection imposed by strong environmental gradients promotes adaptive differentiation, whereas the homogenizing effect of high gene flow from distant populations can swamp out local adaptation (Kawecki and Ebert 2004, Sanford and Kelly 2011). The outcome of local adaptation is that on average local genotypes have higher fitness than genotypes from foreign habitats (Kawecki and Ebert 2004). In a restoration context, non-local genotypes might compromise a project's success through maladaptation and other processes (McKay et al. 2005). However, using local genotypes for restoration is not always possible and can result in low genetic diversity if remnant populations are small and relatively homogeneous, decreasing a population's evolutionary potential and its chance of surviving environmental change (Montalvo et al. 1997, Rice and Emery 2003, McKay et al. 2005, Harris et al. 2006, Jones 2013). Given that climate change is already causing unprecedented impacts (Walther et al. 2002), restoration projects need to balance rebuilding past ecosystems with building ecosystems for future resilience (Harris et al. 2006, Jones 2013). To choose source populations for a restoration project it is critical to understand (1) whether populations are locally adapted to their home environments, (2) if so, the spatial scale of that adaptive differentiation, and (3) whether this variation makes populations more or less robust against predicted change. This information will enable source material selection that protects current genetic structure while striving for sufficient diversity to allow for future adaptation (McKay et al. 2005, Sanford and Kelly 2011).

Although local adaptation is frequently considered in restoration planning for terrestrial ecosystems (Rice and Emery 2003, McKay et al. 2005, Leimu and Fischer 2008), it has often been neglected in marine restoration (Baums 2008; for exceptions, see Hammerli and Reusch 2002, Camara and Vadopalas 2009, Fraser et al. 2011). This is at least in part because local adaptation has been understudied in marine ecosystems, where planktonic life stages and long-range dispersal have long been assumed to homogenize marine populations (reviewed by Conover 1998, Sotka 2005, Conover et al. 2006, Baums 2008, Sanford and Kelly 2011, Sotka 2012). Even for comparatively well-studied taxa like salmonids, empirical data regarding local adaptation are sparse and most management approaches are based on general theoretical principles as opposed to species-specific data (Fraser et al. 2011). In recent years, data from neutral genetic markers have in some cases been used to identify marine management units (e.g., Fraser et al. 2011). However, differences in neutral genetic markers may provide an incomplete

view of the importance of evolutionary processes to management because these markers do not necessarily correlate with quantitative genetic variation in traits that are important to organismal performance (McKay and Latta 2002). As evidence grows that adaptive differentiation in these traits can arise even in the face of high rates of gene flow if selection is adequately strong (e.g., Koehn et al. 1980, Hice et al. 2012, Fitzpatrick et al. 2015), there is increasing interest in assessing local adaptation in marine species (Conover et al. 2006, Sanford and Kelly 2011), and applying the results to management (Baums 2008, Camara and Vadopalas 2009).

We chose Olympia oysters (*Ostrea lurida*) in Northern California, USA, as a model system for studying local adaptation with applications to marine restoration. Olympia oysters are the only native oyster on the west coast of the United States and Canada. They inhabit intertidal or shallow subtidal regions of bays and estuaries from British Columbia, Canada, to Baja, Mexico (Polson and Zacherl 2009). Olympia oysters are common in the fossil record of Native American shellmounds (Gifford 1916) and they supported a thriving commercial industry in the 19th century until overfishing and pollution led to its collapse in the early 20th century (Baker 1995). There is growing interest in restoring Olympia oysters (Grosholz et al. 2007, Camara and Vadopalas 2009, Trimble et al. 2009), a foundation species that serves as habitat (Kimbrow and Grosholz 2006) and like other oyster species, likely provides ecosystem services such as water filtration and nutrient cycling (Newell 2004). One key question in restoration planning is whether populations are locally adapted to their environments, leading to differential susceptibility to stressors (Camara and Vadopalas 2009). If Olympia oyster populations are locally adapted, restoration projects involving population supplementation could swamp out local genotypes and reduce population fitness. However, if extant populations are not locally adapted or are adapted to historic conditions that are not likely to recur, leaving them without supplementation could lead to local extinctions (Camara and Vadopalas 2009).

Olympia oysters are capable of withstanding low salinities, but extreme low salinity events (e.g., 5 ppt for eight days), which occur episodically in northern San Francisco Bay (B. S. Cheng, unpublished data), can cause mass mortality (Cheng et al. 2015). Rising global temperatures are expected to result in higher evaporation rates and a nearly exponential increase in atmospheric water-holding capacity leading to heavier precipitation events (Min et al. 2011). Climate change models predict more frequent extreme precipitation events throughout most of the Northern Hemisphere (Singh et al. 2013) and downscaled models forecast more frequent high precipitation years in the San Francisco Bay Area, which will result in low salinity events (Cloern et al. 2011). This predicted shift in extreme precipitation events is a substantial threat to Olympia oysters.

We tested the hypothesis that Olympia oyster populations in two Northern California estuaries (Tomales Bay and San Francisco Bay; Fig. 1) are locally adapted to their home environments and the salinity regimes therein. Given that (1) freshwater input and salinity regimes vary strongly within and among California estuaries (Conomos 1979, Cheng et al. 2015), and (2) larval dispersal and connectivity among estuaries can be restricted (Stick 2011), we hypothesized that Olympia oyster populations might experience divergent selection for low salinity tolerance. Here, we conducted two experiments to assess whether local adaptation to home sites and to low salinity events varied within San Francisco Bay and between San Francisco and Tomales Bays, a phenomenon that may play an important role in mediating the response of oyster populations to environmental changes, with important consequences for restoration.

2. Methods

2.1 General approach

We chose sites (Fig. 1) with substantial oyster populations and differing salinity conditions. We spawned oysters in the laboratory and then raised first-generation (F1) and second-generation (F2) oysters under common garden conditions in the laboratory. To assess whether oyster populations are locally adapted to their sites, we conducted a reciprocal transplant experiment in the field with F1 oysters. Although this design controlled for lasting influences of environmental exposure, it cannot rule out the influence of parental effects (Kawecki and Ebert 2004). Thus, we also conducted a follow-up experiment to test for differences in salinity tolerance among populations of laboratory-reared F2 oysters. Raising organisms through two generations under common garden conditions is generally considered sufficient to test for local adaptation (Kawecki and Ebert 2004, Sanford and Kelly 2011), although transgenerational plasticity has persisted for more than two generations in some laboratory studies (e.g., Hercus and Hoffmann 2000).

To obtain broodstock, we collected adult *Olympia* oysters ($n = 60\text{--}170/\text{site}$) from approximately mean lower low water at four intertidal sites (Fig. 1) and transported them to Bodega Marine Laboratory (Bodega Bay, California, USA). Oysters from each site were evenly distributed among ten 19 L tanks (4 sites $\times$ 10 tanks = 40 tanks). We visually checked each tank daily for release of brooded larvae, and larvae were collected from multiple tanks (i.e., multiple females) from each site within a sixteen-day period. Larvae were collected using a 37 μm nylon mesh filter and pooled by site into 100 L culturing cylinders that were bubbled with air to maintain oxygen levels and stimulate water movement and lined with 10 $\times$ 10 cm PVC ‘tiles’ for settlement (Cheng et al. 2015). When settled oysters were approximately six weeks old, tiles were culled to approximately 10–20 oysters/tile to avoid overgrowth interactions. A subset of tiles was randomly chosen for reciprocal transplants. The remaining tiles ($n = 50\text{--}76$ tiles/site) were transferred to 75 L tanks for long-term growth and eventual use as broodstock to generate F2 oysters. (For oyster husbandry details see Appendix A.)

2.2 Reciprocal transplant field experiment

To test whether variation in oyster performance within San Francisco Bay was consistent with local adaptation, we reciprocally transplanted two-month old F1 juvenile oysters to the three sites in San Francisco Bay (Fig. 1). Tomales Bay was excluded from the reciprocal transplant experiment to minimize risk of transferring non-local genotypes. We conducted this experiment during the rainy season (December 2011–March 2012) to maximize the chances of low salinity events. Outplanted oysters were small (mean $\pm$ SE = 11.54 ± 0.15 mm²; see Appendix B for initial size ANOVA) and were removed before becoming reproductively mature to minimize the risk of introducing non-local genotypes. Twenty-four tiles were randomly chosen from each site ($n = 10\text{--}21$ oysters/tile; 1158 oysters in total) and were assigned to an outplant location (either back to their home site or to one of the other two sites) using stratified random assignments based on the number of oysters per tile. Tiles were outplanted at mean lower low water (attached to bricks with cable ties threaded through two holes in each tile so that the cable ties did not contact oysters) and arranged along the shoreline in eight blocks at each location. To assess

survival, tiles were photographed before outplanting and thereafter every two weeks (following removal of dead oysters) for three months. Salinity was measured every two weeks at each site.

2.3 Salinity tolerance laboratory experiment

To test whether oysters from different estuaries and from different sites within a single estuary vary in their responses to extreme low salinity, we performed a laboratory experiment with F2 oysters from two sites in San Francisco Bay (Loch Lomond and Oyster Point; Berkeley was excluded due to insufficient spawning) and one site in Tomales Bay (Marshall). Using F2 oysters reared in a common laboratory environment allowed us to minimize the potential influences of environmental history and maternal effects, increasing the likelihood that detected differences among populations were genetically based (Kawecki and Ebert 2004). We used five-month old oysters (mean $\pm$ SE = 201.1 ± 4.03 mm²; see Appendix B for initial size ANOVA; see Appendix A for husbandry details), the earliest age at which they would experience low salinity in San Francisco Bay since spawning occurs in summer and early fall and low salinity pulses follow in winter or spring (A. L. Chang, unpublished data). We chose our low salinity treatment level and duration (5 ppt for eight days) to mimic extreme events that have occurred in northern San Francisco Bay and because previous experiments identified this level and duration as the threshold for oyster mortality (Cheng et al. 2015).

We exposed F2 oysters to either low or control salinity treatments (5 and 34 ppt, respectively) for eight days in a room held at 12–13°C, a typical field temperature when low salinity events are most frequent (Cheng et al. 2015). Tiles from each population were placed in 8 L tanks (15 tanks x 2 treatments for Oyster Point and Loch Lomond and 9 tanks x 2 treatments for Marshall due to fewer oysters). Each tank contained 9–10 oysters and tanks were randomly assigned to one of the two treatments and one of four wet tables. Oysters in the 5 ppt treatment were ramped down from 34 ppt over the course of five days. Salinities were maintained by pre-mixing seawater and distilled water before performing daily water changes. Oysters were fed Shellfish Diet on a daily basis (Appendix A). Water temperatures (range = 9.7–14.8°C; mean $\pm$ SD = 12.4 ± 0.85 °C) and salinities (range = 5.0–5.7 ppt; mean $\pm$ SD = 5.35 ± 0.17 ppt and range = 33.3–34.4 ppt; mean $\pm$ SD = 33.9 ± 0.24 ppt) were checked in a random subset of tanks (9 tanks/day). At the end of the eight-day salinity treatment, dead oysters (gaping and no reaction to probing) were removed. Surviving oysters were given a two-week recovery period (Appendix C), after which all dead oysters were removed and survivors were counted.

2.4 Statistical analyses

We analyzed results from both the reciprocal transplant and laboratory experiments using generalized linear mixed models with binomial error distributions and logit link functions. In the reciprocal transplant experiment, individual oyster survival (a binary response) was analyzed separately at two time points: after one month and after three months. Here, we included source population, outplant site, and their interaction as fixed effects and block along the shoreline and tile nested within block as random effects. We also included initial size as a covariate. For our laboratory experiment, individual oyster survival (a binary response) was analyzed with population, salinity, and their interaction as fixed effects and table, tank nested within table, and tile nested within tank as random effects.

For both experiments, model overdispersion was not possible because we used binary response data (McCullagh and Nelder 1989). We therefore compared treatment effects using Wald χ^2 tests (Bolker et al. 2009). Post-hoc multiple comparisons were carried out using Tukey-Kramer tests. All analyses and graphics were produced using R (Version 3.1.0) and the lme4, car, multcomp, optimx, and ggplot2 packages (Appendix D). Data and R code are available (Bible 2015).

3. Results

3.1 Reciprocal transplant field experiment

We analyzed survival data collected in early January, after oysters were in the field for one month and approximately 50% of the oysters remained (Fig. 2A–C). There were significant differences in survival with source population (Wald $\chi^2 = 16.062$, $p < 0.001$) and outplant site (Wald $\chi^2 = 15.871$, $p < 0.001$). Regardless of outplant site, oysters from the Berkeley source population had 14.8% lower survival than oysters from Loch Lomond (Z value = 2.703, $p = 0.019$) and 16.2% lower survival than oysters from Oyster Point (Z value = 3.673, $p < 0.001$) (Fig. 2D, Appendix E). Regardless of source population, oysters outplanted to Loch Lomond had 23% lower survival than oysters outplanted to Berkeley (Z value = 3.931, $p < 0.001$) and 20.2% lower survival than oysters outplanted to Oyster Point (Z value = 3.104, $p = 0.005$) (Fig. 2D, Appendix E). We found suggestive evidence of a population by site interaction (Wald $\chi^2 = 8.211$, $p = 0.084$) with patterns consistent with local adaptation at two of the three sites (Fig. 2D). At Loch Lomond and Oyster Point, the local populations had higher survival rates than the other populations. At Loch Lomond, oysters originating from Loch Lomond had higher survival rates than oysters from Berkeley and Oyster Point (by 22.6 and 12.5%, respectively). At Oyster Point, oysters originating from Oyster Point had higher survival rates than oysters from Loch Lomond and Berkeley (by 13.5 and 14.8%, respectively). At Berkeley, however, the Berkeley population had lower survival than oysters from Loch Lomond and Oyster Point (by 20.7 and 24.3%, respectively).

After three months in the field, there was a significant effect of source population (Wald $\chi^2 = 7.300$, $p = 0.026$) and of outplant site (Wald $\chi^2 = 10.066$, $p = 0.006$) (Fig. 2E). Regardless of outplant site, oysters originating from Oyster Point had 4% higher survival than oysters originating from Berkeley (Z value = 2.303, $p = 0.055$; Fig. 2E, Appendix E). Regardless of source population, oysters outplanted to Berkeley had 9.2% higher survival than those outplanted to Oyster Point (Z value = 3.377, $p = 0.002$; Fig. 2E, Appendix E). There was no evidence for a site by population interaction (Wald $\chi^2 = 6.159$, $p = 0.188$). After three months, earlier signals of local adaptation (Fig. 2D) may have been obscured because mortality in all groups was high (mean ~95%; Fig. 2A–C). Low survival resulted in low power to detect differences and caused all treatment outcomes to converge despite differences in the preceding survival trajectories.

Salinity differed among the three San Francisco sites, with consistently lower salinity at Loch Lomond than the other two sites. In addition, Marshall in Tomales Bay had consistently higher salinity than all three San Francisco Bay sites (Fig. 3A). Conditions during our field experiment (Winter 2011–2012) were relatively dry and there were no extreme low salinity events (below 10ppt) in northern San Francisco Bay (Fig. 3B).

3.2 Salinity tolerance laboratory experiment

Second-generation laboratory-reared oysters exposed to low salinity (5 ppt) in the laboratory for eight days had 67.5% lower survival than those exposed to 34 ppt (Wald $\chi^2 = 133.032$, $p < 0.0001$; Fig. 4). Evidence that survival rates depended on the source population bordered on significant (Wald $\chi^2 = 5.683$, $p = 0.058$) with oysters from Loch Lomond, the site with the lowest recorded salinity in the field, exhibiting higher survival rates when challenged with low salinity than oysters from Oyster Point and Marshall (by 14.7 and 14%, respectively), suggesting differences in low salinity tolerance among populations (Fig. 4).

4. Discussion

Our results provide evidence of differences in environmental tolerance among *Olympia* oyster populations, likely reflecting local adaptation to spatial variation in salinity. These phenotypic differences suggest that populations of *Olympia* oysters may vary in their response to both current and future ocean conditions, with important implications for restoration planning.

4.1 Evidence for local adaptation among *Olympia* oyster populations

Although survival after three months in the field was too low (mean ~5%; Fig. 2A–C) to detect differences in our reciprocal transplant experiment, earlier stages in the experiment provided suggestive evidence for spatial differentiation consistent with local adaptation at two of the three sites. After four weeks in the field, Loch Lomond and Oyster Point oysters generally had higher survival at their home sites than oysters from the other sites (Fig. 2D). Although other studies have found similarly high mortality rates (Baker 1995, Trimble et al. 2009), increased survival has been noted when oysters are sufficiently large when outplanted (Heare et al. 2015). We expect that the number of survivors would have been higher in our experiment had outplanted oysters been larger or had there been more individuals. Salinity differed consistently among sites, but the correlative nature of this field experiment prevented us from definitively attributing survival differences to a specific stressor. Nonetheless, results from the field experiment provided suggestive evidence of adaptive differences to salinity, which motivated our laboratory experiment using F2 oysters raised under common garden conditions. Findings from our controlled laboratory experiment suggested that *Olympia* oyster populations differed in their tolerance of extreme low salinity between estuaries and even among sites within the same estuary (<20km apart) (Fig. 4). When challenged with low salinity, oysters from Loch Lomond, the site with the lowest field salinity, showed higher survival than oysters from the other sites. Differences among populations could reflect genetic drift or historical effects. However, given that the observed differences in salinity tolerance were in the predicted direction and persisted through multiple generations, available evidence is most consistent with a pattern of local adaptation among oyster populations.

4.2 Factors promoting local adaptation

Multiple factors could contribute to local adaptation in these oyster populations. First, environmental differences among sites may promote divergent selection. After three months in the field, oysters outplanted to Loch Lomond and Oyster Point exhibited higher mortality than those outplanted to Berkeley (Fig. 2E). These differences in mortality rates show that Loch Lomond and Oyster Point were more stressful environments for oysters than Berkeley. A recent report on oyster restoration also found that Berkeley is less stressful for oysters than Loch Lomond or Oyster Point (Wasson et al. 2014). Thus, oysters at Loch Lomond and Oyster Point

are likely subject to particularly harsh stressors potentially leading to intense selection for tolerant genotypes. Although some studies have found that increased stressor tolerance comes with a cost and stress-tolerant individuals perform worse in benign conditions (Piola and Johnston 2006), we found that stress-tolerant oysters had higher survival even when stressors were not present. Oysters from our least stressful site may be subject to weaker selection and therefore may be less robust, even at their home site (Fig. 2). Importantly, when present, local adaptation often occurs in only a subset of populations since the strength of selection can vary strongly among sites. This pattern of local adaptation that is limited to more extreme sites has been observed in organisms ranging from terrestrial plants to marine invertebrates (e.g., Rice and Mack 1991, Piola and Johnston 2006).

Exposure to low salinity differs consistently among our field sites (Fig. 3A), contributing to variation in environmental stress. San Francisco Bay receives runoff from approximately 40% of California (Conomos 1979), resulting in lower salinities than in Tomales Bay, a much smaller estuary with less freshwater inflow (Cheng et al. 2015). In addition, 90% of the freshwater comes into San Francisco Bay through the delta in the northeast (Conomos 1979), resulting in North Bay sites (e.g., Loch Lomond) experiencing low salinity more often than those in the Central and South Bay. As predicted, oysters from Loch Lomond were more tolerant of extreme low salinity (Fig. 4), consistent with the hypothesis that these contrasting salinity regimes have imposed divergent selection on oyster populations.

In addition to environmental variation, differences in gene flow shape patterns of local adaptation. Although data on Olympia oyster larval dispersal in Northern California estuaries are not currently available, water circulation patterns provide useful information about potential larval transport among sites. Estuaries can have long retention times (Conomos 1979) and bivalve larvae can take advantage of circulation patterns to migrate vertically and remain within their natal estuary (Wood and Hargis 1971), decreasing dispersal among estuaries. Within San Francisco Bay, density-driven exchanges between the northern and southern branches occur in winter (Walters et al. 1985). However, in summer, when Olympia oyster larvae are in the water (Grosholz et al. 2007), flow between the two branches is diminished and residence times in each branch can be on the order of months (Walters et al. 1985), which is considerably longer than the 1–3 week pelagic larval duration of Olympia oysters (Baker 1995, Hettinger et al. 2012). Long residence times may limit population connectivity, especially between Loch Lomond (in the north) and Oyster Point (in the south). Since Berkeley is in the center of the bay, it is more likely connected to larval sources from both branches. Thus, any potential for local adaptation at Berkeley may be swamped out by gene flow from other sites. Consistent with restricted gene flow, microsatellite work suggested genetic structure among Olympia oyster populations from different California estuaries and even within San Francisco Bay (Stick 2011) and recent work revealed differences in survival, growth, and reproduction among Olympia oyster populations within Puget Sound (Heare et al. 2015).

4.3 Balanced genetic polymorphism

One open question is whether the survival differences in our experiments reflect true local adaptation arising in relatively isolated populations (Kawecki and Ebert 2004), or whether they reflect a balanced genetic polymorphism (Sotka 2005, Sanford and Kelly 2011). Balanced polymorphism occurs when populations are connected via dispersal but non-random, post-

settlement mortality selectively eliminates maladapted genotypes at each destination site. This decreases realized connectivity (survival to reproduction at the destination site) and maintains differences among populations (reviewed by Marshall et al. 2010, Sanford and Kelly 2011).

This type of “phenotype-environment mismatch” (Marshall et al. 2010) can occur when the spatial scale of gene flow is larger than the spatial scale of the selective gradient (Sanford and Kelly 2011). For example, genetic differences in salinity tolerance of mussels in Long Island Sound were maintained through differential mortality of genotypes after substantial larval dispersal and gene flow among sites with different salinity regimes (Koehn et al. 1980). Although gene flow might be somewhat restricted between the northern and southern parts of San Francisco Bay (Stick 2011), there is some evidence that *Olympia* oyster larvae can travel within and among estuaries in Southern California (Carson 2010). Hence, it is possible that oyster larvae disperse among our sites during many years and that recurrent selection contributes to maintaining the patterns documented here.

4.4 Temporal variability in selection

Temporal variation in the environment can lead to fluctuating selection that counteracts local adaptation (Leimu and Fischer 2008). If the contrasting salinity regimes at our sites are persistent, divergent selection should maintain stable differences among populations in low salinity tolerance. However, if salinity patterns are variable and impose intermittent selection, a complex pattern of temporal variation in the degree to which local populations are differentiated may occur (e.g., Rice and Mack 1991). Historical records indicate considerable and increasing annual variability in minimum salinity within San Francisco Bay (Chang 2009). Indeed, the relatively dry conditions during our field experiment (Fig. 3A–B) were preceded by a wet period. We collected broodstock in September 2011, approximately five months after an extreme low salinity pulse in northern San Francisco Bay (Appendix F), which caused near 100% oyster mortality at some sites (A. L. Chang, unpublished data). Thus, our Loch Lomond broodstock may have experienced a selection event resulting in the survival of only low salinity tolerant genotypes that produced laboratory offspring tolerant of low salinity. If the Loch Lomond population experiences high levels of self-recruitment, even periodic low salinity events in the region might lead to oysters that have evolved consistently higher tolerance for low salinity. However, if Loch Lomond oysters are connected more strongly via dispersal to other populations and post-settlement mortality of non-adapted genotypes only occurs during the years with extreme low salinity events, then Loch Lomond oysters may have varying degrees of low salinity tolerance depending on the year. Understanding this variability could be important for the timing of broodstock collection for restoration efforts.

4.5 The spatial scale of local adaptation

The scale of local adaptation remains uncertain across both marine and terrestrial ecosystems (Conover et al. 2006, Sanford and Kelly 2011, Bischoff and Hurault 2013). In the marine environment, it was long assumed that local adaptation would be evident only in comparisons among distant populations, due to the homogenizing effects of long-range dispersal. Indeed, reviews of local adaptation in salmonids and marine invertebrates reveal that local adaptation is most common over large regional scales (Fraser et al. 2011, Sanford and Kelly 2011) although it does exist at finer spatial scales, ranging from 10s of kilometers to 1000s of meters (Hammerli and Reusch 2002, Fraser et al. 2011, Sanford and Kelly 2011). Tests of the spatial scale of local

adaptation within a metapopulation are strongest with a large number of populations (Blanquart et al. 2013). Thus, a design with more populations would have been required in our study to quantify the exact spatial scale of local adaptation (e.g., Hice et al. 2012). However, experiments are often limited by the number of populations that can be tested due to the need for common garden rearing for multiple generations before outplanting (Kawecki and Ebert 2004), which is especially rare among populations of planktonic marine organisms like oysters (see Lonsdale and Levinton 1985, Dittman et al. 1998, Colin and Dam 2004). Although future studies are needed to determine the specific spatial scale of differentiation among populations, our study suggests adaptive divergence in *Olympia* oysters within and between estuaries (approximately 20–100 km between sites; Fig. 1).

Interestingly, geographic distance did not predict whether populations were adaptively differentiated from one another. Oysters from two nearby sites (Loch Lomond and Oyster Point, 35 km apart within San Francisco Bay) differed in salinity tolerance, whereas no differences in tolerance were seen between more distant populations in two different estuaries (Oyster Point and Marshall; Fig. 4). Although it is generally true that adaptive differentiation increases with geographic distance (Fraser et al. 2011, Sanford and Kelly 2011), “environmental distance” (McKay et al. 2005) may be more important in predicting differentiation among populations (Marshall et al. 2010, Bischoff and Hurault 2013). Here, oysters from the higher salinity sites (Marshall and Oyster Point) had more similar responses to low salinity in the laboratory than those from the lowest salinity site (Loch Lomond) despite their geographic separation (Fig. 4). Geographic distance may fail to predict adaptive differentiation patterns as a result of both true local adaptation (i.e., organisms from similar habitats undergo similar evolutionary trajectories) and phenotype-environment mismatches that create barriers to realized connectivity (Marshall et al. 2010).

4.6 Restoration implications

If populations are locally adapted, local sources may survive better at their home sites and may be favored for restoration projects. However, in some cases, the convention of using local sources to return ecosystems to their previous state may not be feasible nor provide the genetic diversity needed for success given dramatic anthropogenic changes (Montalvo et al. 1997, Rice and Emery 2003, McKay et al. 2005, Harris et al. 2006, Jones 2013). In *Olympia* oyster restoration, being aware that some populations may harbor stress-tolerant genotypes (e.g., Loch Lomond oysters are more tolerant of low salinity) can help managers decide what mix of source material to use for balancing historical faithfulness, current success, and projected future resilience (Sotka 2012). Since *Olympia* oyster populations may be locally adapted within and among estuaries, all source material is not necessarily equivalent. McKay et al. (2005) advise collecting locally for plant restoration with the assumption that nearby populations are more likely connected by gene flow and experience similar environments. However, because similarities in environmental conditions that drive local adaptation may not correlate with geographic distance, matching the environmental conditions of collection and restoration sites may be more important for source material selection (McKay et al. 2005, Bischoff and Hurault 2013). Also, because patterns of stress tolerance are not necessarily temporally consistent (Rice and Mack 1991), it is prudent to consider the selective forces that have recently acted on a population before choosing broodstock since collection timing may influence restoration projects.

In addition to aiding in source selection, the results from this study can assist in prioritizing sites for restoration and conservation. Because survival rates were higher at Berkeley than at the other two San Francisco sites, Berkeley might be considered for restoration projects. Loch Lomond might be prioritized for conservation of its stress-tolerant genotypes, analogous to suggestions for corals that are locally adapted to elevated temperatures (West and Salm 2003). Because the degree of gene flow among sites is uncertain, it is unclear whether the low salinity tolerant genotypes from Loch Lomond have a restricted origin (arising via true local adaptation) or whether those genotypes are widespread throughout the estuary and are merely concentrated at Loch Lomond via post-settlement mortality of non-adapted genotypes. If the former is true, then this population might merit special consideration for conservation (Sanford and Kelly 2011).

Much as the local adaptation literature is biased toward terrestrial studies (Sotka 2012), applying local adaptation theory and practices to restoration has been dominated by terrestrial viewpoints (but see Conover 1998, Conover et al. 2006, Baums 2008, Camara and Vadopalas 2009, and Sanford and Kelly 2011). Although some terrestrial lessons can be directly transferred to marine systems, fundamental differences may exist. For example, collecting source material based on “environmental distance” (McKay et al. 2005) might be particularly important for marine restoration because environmental variation across small geographic scales is common in marine systems (Helmuth et al. 2006) and phenotype-environment mismatches result in decreased realized connectivity due to mortality shortly after larval dispersal (Marshall et al. 2010). This is in contrast to many terrestrial taxa (e.g., insects, birds, and mammals) where phenotype-environment mismatches often do not result in mortality and decreased connectivity, but rather in reduced reproductive success of dispersing adults (Marshall et al. 2010). Although some restoration decisions will need to be made with imperfect information, greater success will be achieved by striving to inform ecological restoration with species-specific studies of local adaptation.

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Figures:

Figure 1. Field sites in California, USA, for collection of Olympia oyster (*Ostrea lurida*) broodstock (M:Marshall, LL:Loch Lomond, B:Berkeley, and OP:Oyster Point). Sites in San Francisco Bay were used as outplant sites in the reciprocal transplant experiment. Marshall, Loch Lomond, and Oyster Point oysters were used in the laboratory experiment that tested low salinity tolerance.

Figure 2. Variation in survival among source populations of Olympia oysters reciprocally transplanted among three sites in San Francisco Bay, 2011–2012. (A–C) Data are mean percent oyster survival ($\pm$ SE) over time for oysters outplanted to (A) Loch Lomond (LL), (B) Berkeley (B), and (C) Oyster Point (OP). (D–E) Data are mean percent oyster survival ($\pm$ SE) after (D) one month in the field (January 2012), and (E) three months in the field (March 2012).

Figure 3. (A) Winter 2011–2012 salinity data at our four study sites. Data for San Francisco Bay sites were collected by the authors. Data for Marshall, Tomales Bay are from Sacramento Landing (approx. 2 km from the study site) and were provided by the Grosholz Lab, UC Davis. (B) Winter 2011–2012 time series salinity data at China Camp (approximately 8 km around a headland from our Loch Lomond site). Data are from the National Estuarine Research Reserve System Monitoring Program, China Camp Station, San Francisco Bay, CA, USA.

Figure 4. Variation in low salinity tolerance among populations of laboratory-reared, second-generation Olympia oysters. Bars are mean percent oyster survival ($\pm$ SE) in replicate laboratory tanks ($n=9-15$ per population $\times$ treatment) for oysters exposed for eight days to control (34 ppt) or low salinity (5 ppt) conditions.