Climate adaptation in Ostrea lurida via transgenerational epigenetic inheritance

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Overview: This project will examine the phenotypic plasticity via epigenetic modifications of the Northeast Pacific Ocean's only native oyster, the Olympia oyster, in response to multiple environmental stressors & across multiple generations. By examining how changes in epigenetic markers over three generations correspond to climate resilience, I will reveal whether *O. lurida* populations may be able to persist in our rapidly changing world. I will condition *O. lurida* broodstock of known lineages in elevated temperature and dissolved CO_2, breed them, rear the progeny in a common garden, then repeat the previous steps with the progeny, and continue through a third generation. I will compare overall larval quality, production, development, and ultimately fitness. I will measure differential gene expression and DNA methylation in broodstock and larvae for each generation. I anticipate that *O. lurida* broodstock conditioned in elevated pCO_2 and temperature will: differentially express genes compared to oysters reared in ambient conditions; present different rates and loci of DNA methylation, particularly on transposable elements; transfer heritable, climate stress related epigenetic markers to successive generation; and produce a greater percentage of progeny that are more tolerant to the dual climate stressors, as determined by growth rate, survival and fitness.

Intellectual Merit: Results will have implications on future community-level resilience in *O. lurida*, and other invertebrates with similar genetic polymorphism and epigenetic mechanisms. A broadening body of work indicates that low pH and high temperature negatively affect fertilization and early life stages of many marine invertebrates. Oysters may, however, contain a unique capacity to keep pace with rapidly shifting climate stressors via epigenetic plasticity. The oyster genome is highly polymorphic, with a diverse set of genes that respond to environmental stress. While natural selection operates on times scales not relevant to projected changes in climate, the newly emerging field of epigenetics suggests a potential for expedited adaptation. Epigenetic transgenerational plasticity is the concept that the environment can alter gene expression without modifying DNA sequence, and these modifications are hereditary. Adult oysters in poor environmental conditions may acclimate via epigenetic modifications, which are then inherited by their progeny. Indeed, urchins, mussels and oysters exposed to low pH have produced more pH-tolerant larvae. For example, offspring of Sydney rock oysters (*Saccostrea glomerata*) exposed to elevated pCO_2 during reproductive conditioning performed better in OA conditions compared to larvae of broodstock conditioned in ambient pCO_2 levels. To date there has been no direct confirmation that observed transgenerational plasticity is due to epigenetic changes. Also unknown are the persistence of these adaptations through the generations, and if degree of epigenetic change differs between families. This project seeks to answer these questions, and will be the first to explore mechanisms underlying potential differential gene expression and transgenerational inheritance in oysters reared in dual climate stressors.

Broader Impact: Our shorelines have the power to feed us, and to teach us importance of ocean health. Harvesting shellfish provides communities with a poignant and tangible connection to marine species conservation and water quality. The ultimate goal of my *O. lurida* research is to re-establish healthy, self-sustaining populations of the Northeast Pacific Ocean's only native oyster. Results from this project could directly inform restoration and commercial hatchery breeders to select for climate change-tolerant oysters, or to induce a multi-generational "immune-like" response by exposing broodstock to future climate conditions. Similarly, restoration groups could refine selection processes for shoreline enhancement and seeding processes, or amplify efforts in locations with variable pH and temperature swings to allow for maximum adaptability. Additionally, resource managers and commercial growers could apply lessons from *O. lurida* to other economically vital species, such as *Crassostrea gigas* and *Crassostrea virginica* (oysters), *Mytilus edulis* (mussel), and *Venerupis philippinarum* (manila clam). Understanding the resiliency of intertidal communities is vital to maintaining rich and healthy shorelines. By predicting how marine communities will change in the future we can better manage them now, ensuring that communities remain connected to their shorelines.

Background

Olympia oysters & the threat of a changing ocean: The ecologic and economic void left by the near collapse of the Olympia oyster, *Ostrea lurida,* has spurred major efforts to restore populations along its historical distribution from Alaska to Baja California (Pritchard et al. 2015). Oysters provide essential services via attachment substrate, habitat for intertidal and juvenile species, biofiltration and denitrification, and as a key food source for birds and intertidal predators (Ruesink et al. 2005). *O. lurida* also has enormous economic potential, as indicated by historical harvest records in Willapa Bay, WA, which at its peak exported over one hundred million oysters annually compared to less than twelve hundred today (Trimble, Ruesink, and Dumbauld 2009). Although shoreline enhancement and seeding projects are making headway, there is growing concern that changing ocean conditions further threatens existing populations and may stymie restoration investments (Wasson et al., 2015).

A broadening body of research indicates that dual climate stressors, increasing temperature and ocean acidification, will negatively affect marine invertebrates (Kurihara 2008; Ross et al. 2011; Byrne 2011), with evidence that farmed oysters are already exhibiting negative effects (Barton et al. 2012). Temperature is the critical driver of gametogenesis and spawning in *O. lurida* (Oates 2013; Coe 1932), and warming is hypothesized to impact reproductive timing and synchronicity in this sequential hermaphrodite (Barber et al. 2016). To predict effects of ocean acidification (OA) on *O. lurida*, laboratory experiments have explored shifts in larval growth rates and survival, observing that larvae exposed to low pH display poor shell development in multiple life stages (Hettinger et al. 2013), and larvae reared at low pH are more susceptible to predation by invasive snails (Sanford et al. 2014). There may, however, be cause for optimism with insight from the emerging field of epigenetics. Epigenetic suggests the environment can trigger changes in genomic markers, which can be passed to offspring, thus transferring acclimatization between generations (e.g. Anway et al. 2005).

Exploring epigenetics in oysters: The Pacific Oyster, *Crassostrea gigas,* is an emerging model organism and provides insight into the oyster epigenome. Recent work by the Roberts Lab has explored the potential role that epigenetic plasticity, the concept that poor environmental conditions alter gene expression and phenotype without modifying DNA sequence, may play in the *C. gigas* environmental tolerance range. Methylation, a primary epigenetic mechanism, is the covalent bond of a methyl group to a cytosine in the dinucleotide pair CpG which "silences" protein coding at that site (Feil and Fraga 2012). Recent findings suggest that DNA methylation patterning in *C. gigas* appears to act as a control switch for expression, as methylation is primarily intragenic and correlates with transcriptomic expression. Additionally, methylation patterns differ between families, primarily at transposable element loci (Gavery and Roberts, 2013, Olson and Roberts 2014, Olson and Roberts 2015).

As a result of their work, Gavery and Roberts (2014) theorized that gene body methylation in *C. gigas* depends on the gene's function: if a gene is crucial to cell function, methylation occurs and protects the gene from transcriptional variation, or "noise." If stochastic variation is beneficial then it is not methylated, thus allowing for more transcriptional variation opportunities. To date, we have yet to observe a correlation between changes in methylation patterns resulting in a phenotypic change that is beneficial to fitness.

Climate-change induced epigenetic plasticity: While responses to climate stressors have yet to be observed on the epigenetic molecular level in Mollusca, recent studies have observed transgenerational phenotypic response to OA such as increased egg size, larval growth and survival in an oysters, mussels, urchins, and sea stars (Parker et al. 2012; Fitzer et al. 2014; Evans and Watson-Wynn 2014; Dupont et al. 2012; Uthicke et al. 2013). For example, the offspring of Sydney rock oysters (*Saccostrea glomerata*) exposed to OA during reproductive conditioning performed better in OA compared to larvae of broodstock conditioned in ambient levels (Parker et al. 2012). While the phenotypic carry-over effects in these studies suggest genetic mechanisms may be at play, they exclusively represent larval response and indicators, with no extended monitoring of phenotypes into juvenile stages and

maturity. Changes that are beneficial at larval stages could be deleterious in other stages. Alternatively, acclimation could be ephemeral, not persisting through an animal's lifetime, or through successive generations (Ross, Parker, and Byrne 2016). To identify the potential for persistent acclimation, we must understand the underlying mechanisms by which inheritance may occur, if the degree of epigenetic change differs between families, and if they persist into reproductive maturity.

In response to these data gaps, I seek to elucidate the potential for beneficial transgenerational plasticity in *O. lurida.* ***This project will be the first to explore differential gene expression and transgenerational inheritance in molluscs reared in dual climate stressors, and to directly connect changes in epigenetic markers to beneficial phenotypic plasticity.***

Study specimens: A unique aspect of this project is that I have access to 1,200 *O. lurida* individuals from four separate lineages. All were born in the same season and hatchery, and are from wild broodstock (F0) collected at different sites in Puget Sound. While these individuals are from genetically isolated populations, they were grown and conditioned in the same locations and manner. As such, they represent four distinct sibling populations with different genotypes but identical life histories; therefore we can directly compare epigenetic changes between families. Conveniently, I can also leverage comprehensive genetic, phenotypic, and epigenetic surveys already performed on these population from a previous experiment, allowing for a rich analysis of this project's results.

Conceptual Framework, Figure 1:

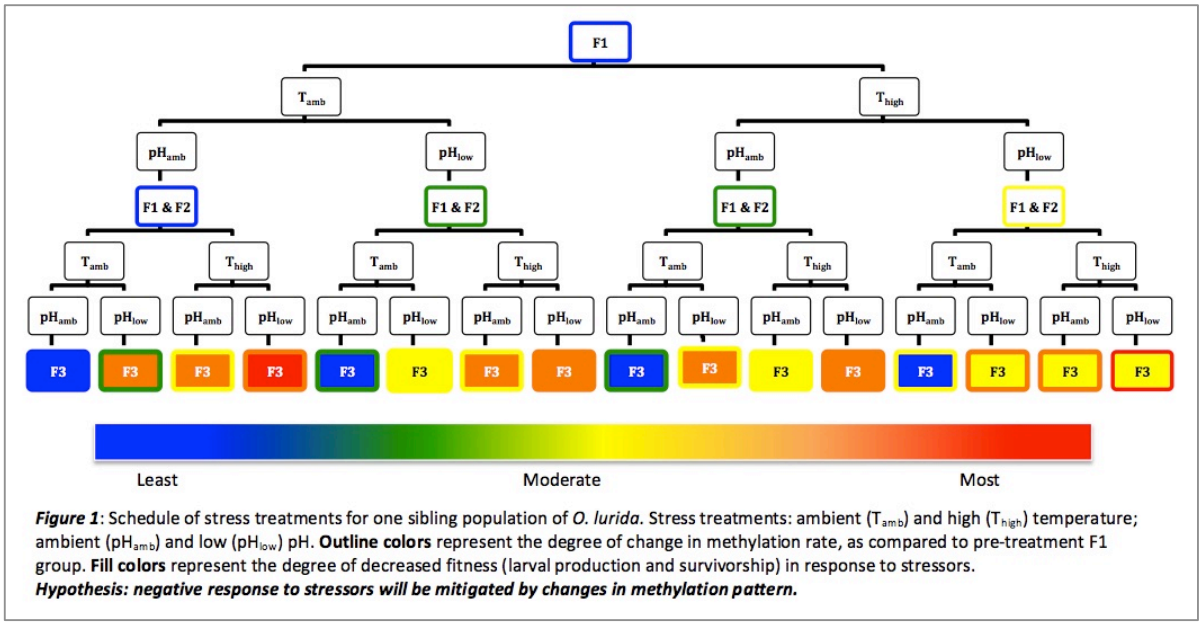

Figure 1: Schedule of stress treatments for one sibling population of *O. lurida*. Stress treatments: ambient (T_{amb}) and high (T_{high}) temperature; ambient (pH_{amb}) and low (pH_{low}) pH. **Outline colors** represent the degree of change in methylation rate, as compared to pre-treatment F1 group. **Fill colors** represent the degree of decreased fitness (larval production and survivorship) in response to stressors.
Hypothesis: negative response to stressors will be mitigated by changes in methylation pattern.

Objectives: The overall objective of this project is to forecast whether *O. lurida* populations have the capability to persist through changes in ocean conditions. In performing this multi-year, multi-generation experiment I seek to examine *O. lurida's* potential adaptability to environmental stressors (temperature, pH) via changes in the epigenome. By executing this project with four distinct populations of *O. lurida*, I seek to tease out the family effect (genotype) on epigenetic plasticity. Ultimately, I hypothesize that fitness under environmental stress is influenced by the ancestral environment and epigenetic "memory." Specific questions include:

- Do methylation patterns in *O. lurida* broodstock (F1) tissue change after exposure to stressors?

- Are changes observed in F1's epigenetic patterns transferred to progeny (F2)? If so, do they persist through maturation and to the third generation (F3)?
- Are rates of epigenetic changes different between basin/population? If so, does this correspond to rates of overall epigenetic patterns?
- When F2 and F3 groups are exposed to environmental stressors, do they respond differently if their F1 ancestors were exposed to the same stressors? I.e., do they have an inherited "memory," and does this improve their growth, survival and/or fecundity?

Methods: This is a full factorial experiment with four *O. lurida* Puget Sound populations, two temperature treatments and two pH treatments.

In December 2016, 1,200 oysters were moved from Clam Bay to the Kenneth K. Chew Shellfish Hatchery and overwintered in two groups at ambient and elevated temperatures. In mid February, they were divided and moved into two pH treatments: ambient and elevated pCO_2, where they will undergo reproductive conditioning for two months. In April I will breed them within treatment groups, rear the progeny in a common garden (Clam Bay in Manchester, Washington) until November 2017, then repeat the previous steps with the progeny to produce a third generation in February 2018.

Ctenidia and mantle tissue samples will be collected for epigenetic analyses; the visceral mass will be fixed for histology and analyzed for gonad maturation. In all generations I will compare overall larval quality, production, development, and ultimately fitness via survival. Using the methyl-CpG binding domain protein-enriched genome sequencing method (MBD-seq) I will measure differentially methylated regions in broodstock and larvae for each generation in relation to their line's exposures and phenotypic response.

I anticipate that *O. lurida* broodstock conditioned in elevated pCO_2 and temperature will: produce a greater percentage of progeny that are more tolerant to the dual climate stressors, as determined by growth rate, survival and fitness; differentially express genes compared to oysters reared in ambient conditions; present different rates and loci of DNA methylation and expression in non-coding RNA or transposable elements; transfer heritable, climate stress related epigenetic markers to successive generation.

Intellectual Merit: This will be the first study exploring a direct connection between epigenetic changes, transgenerational inheritance, and phenotypic plasticity in molluscs under dual climate stressors. Results will have implications on future community-level resilience in *O. lurida*, as well as other molluscs with similar epigenomic patterns. Recent literature suggests that transgenerational carryover effects occur from adult to larvae, however persistence of these changes is unknown. By rearing multiple generations, I will understand whether epigenetic changes are simply acclimatory and ephemeral, or if they persist through progeny. Additionally, the use of four distinct sibling populations with different genotypes but identical life histories will allow for an analysis of family effects on epigenetic plasticity.

This project will also be the first to examine transgenerational responses to two stressors, temperature and OA; climate models predict both warming ocean surface temperatures and declining pH, therefore multi-stressor experiments are most representative of the real-world situation (IPCC, 2014). The timing of the temperature treatments simulates an unusually warm winter, which we are already experiencing in Puget Sound, WA. The animals will also be exposed to pH treatment during gametogenesis, which will likely provide interesting results regarding fecundity and sex ratios, as *O. lurida* are sequential hermaphrodites (Coe, 1932).

Epigenetic plasticity is not limited to oysters. Mussels, scallops, clams, among other important marine organisms may also be capable of inheriting acclimatization. If I identify a direct link between transgenerational plasticity and epigenetic mechanisms in *O. lurida*, this will significantly modify our understanding of environmentally induced phenotypic change, which will apply to marine species worldwide.

With results from this project I will argue whether oysters are capable of epigenetic and phenotypic changes faster than traditional evolutionary mutation and selection allows. This fundamental understanding of adaptation will become increasingly important as our oceans warm and acidify, and sessile species that are ecologically and economically vital will become increasingly threatened.

Broader Impacts: Project results will have profound implications for wild community-level population dynamics in *O. lurida*. Correlations between environmental exposure and epigenetic adaptation will help identify important relationships between geographic sources of larvae and the communities they seed. As a result, restoration groups could refine selection processes for shoreline enhancement and seeding processes, or amplify efforts in bays with variable pH and temperature swings to allow for natural selection. To support effective conservation and restoration, I will report project results to West Coast resource managers and *O. lurida* restoration organizations such as the Puget Sound Restoration Fund.

This research will support a limber and resilient wild and farmed shellfish industry. This fits into a global need to develop more sustainable protein sources. As promulgated in the 2015 NOAA Marine Aquaculture Strategic Plan, marine aquaculture's full potential is yet to be realized, but is identified as a resource-efficient and sustainable seafood source (NOAA, October 2015). If we are able to identify mechanisms by which the oyster is predisposed to withstand rapid changes, hatchery managers can ensure conservation or enrichment of these traits by modifying their practices. For example, breeding programs are attentive to genetic diversity, however many treat their water by buffering or modifying temperature to maximize larval survival. Controlling chemistry in this manner might limit acclimatization, a potential crucial step if we hope to rebuild wild populations resilient to climate change. To support informed aquaculture practices, I will disseminate results from this project to aquaculture professionals at annual meetings of the Pacific Coast Shellfish Grower's Association and the National Shellfish Association, in addition to connecting directly with the Puget Sound Restoration Fund and with local shellfish growers.

Ultimately, when coastal communities are able to harvest wild and farmed oysters and other bivalves directly from their local waters, it breeds a reliance on, appreciation of, and conservation of healthy marine systems and clean waterways. To serve my overall goal of connecting communities to their shorelines via harvestable shellfish, I will conduct outreach at local community events (e.g. Oyster Fest in Shelton, WA) to support a message of hope for the future of sustainable and healthy seafood.

Budget Justification: Funds are requested for faculty and hatchery staff time, materials, comprehensive sample processing and analysis, publication and conference attendance. No funds are requested for graduate student time or tuition, as I am fully funded through 2019 by the National Science Foundation Graduate Research Fellowship Program. The Kenneth K. Chew Center for Shellfish Research and Restoration staff labor supplies live algae (daily), cleaning, monitoring, and general facility maintenance, in addition to a hatchery facility fee for overhead costs. By performing this experiment at the Ken Chew center, I will have access to the flow-through ocean acidification system with marginal modifications, leveraging previous project materials and installation labor. Weekly trips to the hatchery during treatments and daily trips during spawning include mileage (50 miles/trip, personal vehicle) and ferry tolls ($25/trip). Materials are needed for 8 sampling events over 2 years, with an estimated 800 samples. I will perform all laboratory DNA, RNA & protein extractions with UW Roberts Lab and Genome Sciences instruments, but support is needed for tissue sampling materials, preservation, extraction kits and associated materials. Sequencing services (DNA & RNA) will be conducted by the Washington University St. Louis Genomics Center. I will analyze protein samples at the University of Washington Proteomics Resource via the Lumos mass spectrometer, which is available to UW-affiliates for marginal costs (~$200/day, ~2 hrs/sample). Support is needed for publication fees and attendance at the annual Pacific Coast Shellfish Growers Association conference travel, lodging and registration.

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