Fecundity and fitness the native oyster *Ostrea lurida* in varying temperatures

Olympia oysters & economic opportunity: 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[1]. 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[2]. *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[3]. Although enhancement and seeding projects are making headway, there is growing concern that shoreline modifications and changing ocean conditions further threaten existing populations and may stymie restoration investments[4]. A broadening body of research indicates that environmental stressors will negatively affect marine invertebrates[5,6], with evidence that farmed oysters are already exhibiting negative effects[7]. Temperature is the critical driver of gametogenesis and spawning in *O. lurida*[8,9], and warming is hypothesized to impact reproductive timing and synchronicity in this sequential hermaphrodite[10]. There may, however, be cause for optimism with insight from the emerging field of epigenetics. Epigenetics suggests the environment can trigger changes in genomic markers, which can be passed to offspring, thus transferring acclimatization between generations[11].

Exploring epigenetics in oysters: Recent work by the Roberts Lab has explored the potential role that epigenetic plasticity, the concept that the environmental induces gene expression and phenotype changes without modifying DNA sequence, may play in oyster environmental tolerance range. Recent findings suggest that DNA methylation, a primary epigenetic mechanism, appears to act as a control switch for expression in a key aquaculture oyster, *Crassostrea gigas*[12,13]. Gavery and Roberts[14] posit that methylation 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.

Environmentally induced epigenetic plasticity: While responses to environmental stressors have yet to be observed on the epigenetic molecular level in Mollusca, recent studies have observed transgenerational phenotypic response to varying ocean chemistry such as increased egg size, larval growth and survival in an oysters, mussels, urchins, and sea stars[15-20]. While 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[21]. 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 in varying temperatures, 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. Conveniently, I can also leverage comprehensive genetic, phenotypic, and epigenetic surveys already performed on these populations.

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 varying winter temperatures 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. Specific questions include:

- Do methylation patterns in *O. lurida* broodstock (F1) gonad tissue differ between temperatures?
- 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?
- When F2 and F3 groups are exposed to higher temperature, 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: In late autumn oysters will be moved from Clam Bay to the NOAA Manchester Research Station and overwintered at three temperatures (6°, 9°, 12°) for three months, conditioned for one months, then bred. I will rear the progeny in a common garden until the following November, then repeat the previous steps with the progeny to produce a third generation. Gonad tissues will be collected for gonad maturation and epigenetic analyses. In all generations I will compare overall larval quality, production, development, and ultimately fitness via survival to juvenile stage. I will also measure methylation using the methyl-CpG binding domain protein-enriched genome sequencing method (MBD-seq).

I anticipate *O. lurida* broodstock conditioned in elevated temperature will: produce progeny that are more tolerant to the temperature stressor, 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, 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. 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 effects occur from adult to larvae, however persistence of these changes is unknown. By rearing multiple generations, I will understand whether epigenetic changes are 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 high winter temperature. The timing of the temperature treatments simulates an unusually warm winter, which we are already experiencing in Puget Sound, WA.

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 important shellfish species 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 nation's basins warm, 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 temperature swings to allow for natural selection. To support effective conservation and restoration, I will report results to West Coast resource managers and *O. lurida* restoration organizations.

This research will support a limber and resilient wild and farmed shellfish industry. This fits into a national need to invest in sustainable and diverse protein sources to ensure food security. 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[22]. If we are able to identify mechanisms by which the oyster is predisposed to withstand environmental stressors, 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 modifying temperature to maximize larval survival. Controlling conditions in this manner might limit acclimatization, a potential crucial step if we hope maintain robust aquaculture production. To support informed 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, an investment in this research will refine the practices of culturing a native and underutilized shellfish species. A thriving shellfish aquaculture industry maximizes our coastal resources, augments the economies of coastal communities, diversifies our agricultural industries, and safeguards our nation's food security.

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