The Effects of Human-mediated Environmental Change on the Physiology of the Pacific Oyster, *Crassostrea gigas*: Immediate and Long-term Perspectives

Objectives:

1.  To characterize the physiological response of bivalves to stressors of environmental change.

2.  To assay the effects of local environmental parameters and contaminants on wild sets of *Crassostrea gigas.*

3.  To determine the population genetic resources available for potential adaptation to environmental change.

Approach & Significance

*Objective 1: Pacific oyster challenges*

Larval *C. gigas* will be challenged with a variety of environmental stresses (e.g. pH, disease, temperature, metal bioavailability) in a controlled laboratory setting.  Challenges will be accomplished singly and synergistically to determine the breadth of effects on physiology and to best mimic true environmental possibilities.  Regular sampling of the larvae over the time period of the experiment and subsequent molecular analysis will elucidate the physiological pathways that are affected by single and synergistic stressors.  Molecular analysis will include qPCR using a candidate gene approach and an unbiased gene discovery approach (most likely differential display).

*Objective 2: Environmental assays*

During the spawning season, oyster larvae will be sampled at locations inside and in the bay outside of the hatchery.  Water samples from inside and outside the hatchery will also be collected to characterize the local microbial populations and environmental conditions. Sibling juvenile oysters will be placed in bags at sites around Puget Sound will be regularly sampled that fit one of the following criteria: 1) heavy pollution (Seattle Aquarium), 2) heavy agricultural run-off (Samish Bay) and 3) "pristine" environmental conditions (Big Beef Creek). Passive sampling devices will also be placed at these locations to monitor local contaminant profiles. Further lab trials using environmental contaminants and similar trials to Obj. 1 but accomplished with wild *O. lurida* (spawned in the hatchery/lab) will also be achieved.  Gene discovery for *O. lurida* in its response to stressors will be executed using next-generation sequencing.

*Objective 3: Adaptive differences in exposure to environmental stresses*

During periodic sampling of the larval *C. gigas* and *O. lurida* in stress trials, RNA & DNA will be extracted from the live larvae and DNA will be extracted from pre- and post-treatment larvae.  We will use SNP discovery to determine the presence of potentially adaptive SNP markers in comparing the mortalities and the survivors.  This information will give us a better understanding of the genotype-environment interaction of the oysters and will provide genomic resources for future hatchery and management practices.  Genetic analysis of *O. lurida* at different locations in Puget Sound will furnish a more completely understanding of the distribution of adaptive genotypes/phenotypes throughout the area.