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Fish 441

Research Proposal

**Background:**

 Oysters are a valuable resource in multiple dimensions. They form a vital component of the filter feeding base of any ecosystem that help remove excess nutrients from the environment, serve as a bio-indicating species regarding the health of an ecosystem, and a valuable aquaculture species for human consumption. Being able to determine the health of an ecosystem and the animals therein would be a valuable tool for conservation biologists. One way ecosystem health can be assessed is via the measurement of stress related biomarkers. Previous research has shown that heat shock proteins, including HSP70, are an often used protein group in response to multiple environmental stressors including heat, anoxia, and xenobiotic contamination. There has been little research into the effects of mechanical stress as well as the effects of multiple stressors in quick succession in relation to the heat shock protein family.

**Objectives:**

 Our objective is to determine if there are physiological responses to stress by measuring specific biomarker in *Crassostrea gigas* and *Crassostrea virginica*. The stresses we have chosen are two-fold for *C. gigas*, physical and heat shock, and singular for *C. virginica*, exposure to crude oil contaminated water. The biomarkers we will look at are anti-oxidant proteins, superoxide dismutase and metallothionein respectively.

**Methods:**

 Twenty *C. gigas* animals will be split into two groups, 10 control and 10 experimental animals. We will first spin the experimental animals in a centrifuge at 100 rpm for three minutes. After centrifugation we will allow them to rest in room temperature seawater for 30 minutes before exposing them to 22\* C seawater for a 20 minute exposure. We will sample three animals right after heat shock exposure, three more one hour after exposure, and the last four 3 hours after. Tissue samples will be taken from the adductor muscle and digestive gland of each animal. DNA will be extracted and qPCR using superoxide dismutase primers will be run on the products, with elongation factor used as a comparison housekeeping gene. The *C. virginica* samples have already been excised, both sample and control, and stored in ethanol at -80\*C, they will be extracted in the same manner and have qPCR run using metallothionein primers and compared against the same housekeeping gene.

**Timeline:**

 Animals will be procured the week of 11-2-14 and allowed to acclimate to tank temperatures. Stress tests and sampling will be conducting the week of 11-9-14. PCR assays will be conducted and analyzed either the week of 11-9 or 11-16.

**Expected Results:**

 We expect that to see upregulation of the stress biomarkers in animals that have undergone stress. Heat Shock proteins have been shown to be used in response to multiple stressors but there is also a limit to the ability of an animal to acclimate so it is possible that after two stressors in quick succession there will be some mortality in the sample.