**The epigenetic and subcellular response of diploid and triploid Pacific Oysters to desiccation and heat-stress**

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**Abstract**

**Keywords:**

**1. Introduction**

Background:

DNA Methyltransferase Expression (possibly marine-organism related, what is their function in other organisms, possibly even higher order organisms)

Ploidy differences (possibly looking at plants,

**Materials and Methods:**

**Experimental Design:**

Adult Pacific oysters (*Crassostrea gigas*) were obtained from (not sure where from..) and held at the University of Washington. Diploid and triploid oysters were subject to desiccation (need to clarify?) and elevated temperature exposure at 27°C for 24 hours (*n=8 per ploidy)*, while others were kept as controls at (*n=8 per ploidy*). Ctenidia, mantle, and adductor tissue was resected from all individuals and stored at -80°C for subsequent analysis.

**RNA Isolation:**

Ctenidia tissue was homogenized in 500 μL RNAzol RT (Molecular Research Center, Inc., Cincinnati, OH, USA), volume was brought up to 1 mL with additional RNAzol RT, and samples were vortexed vigorously for 10 s. Samples were then incubated at room temperature (RT) for 10 min. 400 μL of 0.1% DEPC-H2O was added and samples were centrifuged for 15 min at 12,000 g, at RT. 750 μL of the supernatant was transferred to a new tube, an equal volume of isopropanol was added, vortexed vigorously for 10 s, and incubated at RT for 5 min. Samples were then centrifuged for 15 min at 12,000 g, at RT. The supernatant was discarded and 400 μL of 75% ethanol (made with 0.1% DEPC-H2O) was added to the samples. Samples were centrifuged for 1.5 min at 4,000 g and the wash step was repeated. Ethanol was removed and pellets were resuspended in 50 μL of 0.1% DEPC-H2O. Samples were quantified using a Qubit 3.0 with the RNA High Sensitivity assay and were subsequently stored at -80°C.

**DNAse treatment and reverse transcription:**

**Quantitative PCR:**

Sample cDNA was diluted 1:5 with molecular-grade water. Reaction volumes were 20 μL and were run in white qPCR plates (USA Scientific) with clear lids (USA Scientific, Ocala, FL, USA). One microliter of diluted cDNA was used as a template. Cycling conditions were: one cycle of 98.0°C for 2 minutes; 40 cycles of 98°C for 2 seconds and 55.0°C for 5 seconds. Two qPCR replicates were run for each sample, for each primer set.

**Global DNA Methylation Analysis:**

Global DNA methylation levels of samples were quantified by measuring the amount of 5-methyl-cytosines (5-mC) using the MethylFlashTM Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric) (Epigentek, Farmingdale, NY). All samples were first diluted to 5 ng/μL. Eight genomic DNA samples per treatment were loaded (4 mL) in duplicate to ELISA plates, along with a set of controls (0.1%, 0.2%, 0.5%, 1%, 2%, 5% 5-mC), all with binding solution. Capture and detection 5-methylcytosine antibodies were added to the samples, and the binding of this antibody was quantified using a colorimetric reaction.  The absorbance resulting from this colorimetric reaction was quantified at 450 nm in a plate reader. The amounts of 5-mC in the samples were subsequently calculated by comparing sample absorbance values to a 5-mC standard curve.

Tables and Figures

Figure 1:

