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Paper Draft (only have the methods section completed so far)

Thirty Olympia Oysters (*Ostrea lurida*) were harvested from \_\_\_\_\_\_ and placed into temperature regulated tubs with airstones to circulate oxygen throughout. A control group of ten oysteres was then exposed to temperature of 14C. A treatment group of 10 oysters was expossed to a gradual temperature increase from 14-35C, and a second treatment group of the remaining 10 oysters was placed directly into 35C. All groups were exposed to treatment over a 26 hour period. Directly following treatment, measurments of each individual were made before being shucked. 25-50mg of gill tissue from each oyster was extracted and placed into separate pre-labeled 1.5 ml microcentrifuge tubes with specimin IDs. Specimin IDs corrilated with the type of treatment they received. RNA was then extracted from entire tissue sample using TriReagent, which allow RNA to separate from cellular tissue and DNA. Next, issolated RNA was reverse transcribed into complementry DNA or cDNA by a DNA polymerase enzyme commonly refered to as reverse transcriptase. Each cDNA sample was added to a master mix of varius ingrediance, including HIF-1 primer, which is required for qPCR (quantitative polymerase chain reaction); the process which exponentially amplifiys complementary DNA . All 30 cDNA samples were ran through 40 PCR cycles, after which raw gene expression data was generated and anylized using a program called PCR Minor. The normalizing gene “actin” was used to create a baseline for HIF-1 expression, allowing for gene expression to be compared between treatment groups.