**INTRODUCTION**

When an organism experiences cellular stress in the form of xenobiotics, heat shock, or exposure to pollutants, it responds by an increased expression of certain proteins. Many such proteins come from the heat shock protein family, which has often been studied for its importance in cellular stress response (Jackson et al 2011). This family of proteins is ubiquitously expressed, with some showing stress-induced (stress levels have a positive effect on its expression) behavior while others are constitutively expressed (Tutar et al 2006). Its initial discovery in the 1960s by F.M. Ritossa was accidental in nature as a mistakenly increased incubation temperature of Drosophila melanogaster and subsequent analysis of chromosomal “puffing” lead to the finding (Savykoski 2003). The importance of these proteins is that they are universal, being found in both mammals and invertebrates alike. Therefore, the results from these studies done on oyster species can directly be translated to human stress response as well. There have already been various studies done to show that these oysters display increased expression of the hsp70 in the summer relative to winter months (Hamdoun et al 2003). However, the effects on the expression of hsc70 has not been as well covered, with many jumping to the conclusion that they should respond to stress in a similar way since there is homology between hsp70 and hsc70. With my research I hope to determine the actual differences to stress response between the two proteins across two species of oysters, Crassostrea gigas and Ostreola conchapila. Studying the relation between hsp70, hsc70, and temperature rise will help us better understand the mechanics of the stress response of these organisms, which is important because these oysters face constant rise in oceanic temperatures caused by global warming. Both of these species may well go extinct in the future. By comparing the hsp70 and hsc70 expressions of these two species, we can determine which species is better at dealing with heat stress and therefore may be better adapted to survive longer.

**MATERIALS AND METHODS**

*RNA Isolation*

All oysters tissues were first shucked from their shells and stored in plastic weigh boats on ice. Then two separate samples of gill tissue and a section of mantle tissue were separated from the oyster and stored on ice. Next, scissors and tweezers were used to take approximately 50-100 mg of tissue for the RNA, making sure to sterilize the equipment between samples with EtOH, bleach, and water, in that order. These samples were homogenized with 500 µL of TriReagent, with an additional 500 µL of TriReagent added afterwards.

*RNA Extraction*

The homogenized solutions were then incubated for 5 minutes at room temperature before adding 200 µL of chloroform and vortex until it became a milky emulsion. Then another 5-minute incubation at room temperature preceded a 15-minute refrigerated microfuge at max speed (13,300g). Only the aqueous phase was transferred to a fresh tube while the rest was discarded in chloroform waste jar. Adding 500 µL of isopropanol and mixing by inverting the tube several times and vortexing briefly yielded a uniform solution. A 10-minute incubation at room temperature was carried out, then another max speed refrigerated microfuge for 8 minutes led to the formation of a small white pellet. The supernatant was discarded and 1 mL of 75% EtOH was added to the pellet and vortexed. Then the pellet-EtOH mixture was spun in refrigerated microfuge at 7500g for 5 minutes and then the supernatant was removed once again leaving only the pellet intact. As much of the EtOH as possible was removed by pooling any residual EtOH by briefly spinning in the centrifuge then removing with a small pipette tip and also letting the tube sit for approximately 5 minutes with the top open to allow evaporation. After the pellet was isolated from the EtOH, it was dissolved in 100 µL of 0.1% DEPC-H2O and incubated in 55°C water bath for 5 minutes until it became a uniform solution. The tube was taken out and flicked a few times to mix then stored in -80°C.

*RNA Quantification and Reverse Transcription*

The nanodrop machine was first zeroed by pipetting 2 µL of 0.1% DEPC-H2O onto the nanodrop pedestal, lowering the arm, then clicking on “Blank”. After the machine had zeroed, 2 µL of the chosen RNA sample was pipetted onto the nanodrop pedestal and measured for RNA concentration and ratios. Only a couple randomly chosen samples were quantified for RNA just to check that we were getting wanted results. The instrument was wiped off with a KimWipe between samples and after. Rows of PCR tubes were set up and labeled, with 17.75 µL of each RNA sample being poured into each corresponding tube and being stored on ice. The actual reverse transcription methods to go from our RNA samples to the cDNA samples were carried out by Mackenzie Gavery.

*Primer Preparation*

Specific primers were designed for hsp70 and hsc70 genes for both the Pacific oyster and the Olympia oyster. With these primers, stock solutions were made by adding the appropriate amount of nuclease-free water. This amount was calculated by taking the nm measurement of the primer and multiplying that number by 10 to get the appropriate µL to be added (i.e. primer of 28.5 nm gets 285 µL of water added). From these stock solutions, working stocks of 10 µM concentration were made by mixing 10 µL of stock solution to 90 µL of nuclease-free water.

*qPCR and Data Analysis*

A qPCR mastermix was prepared by mixing 12.5 µL of Sensimix, 1 µL of SYBR 25X, 1.25 µL each of the upstream and downstream primer working stocks and 7 µL of PCR water per reaction. The actual amounts needed were calculated by multiplying the above numbers by 24 - 10 control + 10 treatment + 2 blanks + 2 additional to account for pipetting error. The final numbers ended up being 300 µL of Sensimix, 24 µL of SYBR 25X, 30 µL of each primer, and 168 µL of PCR water. After the mastermix had been prepared, 23 µL of it was added to each qPCR tube and 2 µL of each cDNA sample was added to their respective wells, with 2 µL of PCR water instead of cDNA being added to the blanks. These 25-µL mixtures were then submitted to Mackenzie Gavery to be run through qPCR procedures. The qPCR data collected from these samples were put into a program called PCR Miner and we were able to obtain values for CT and the average efficiency of the genes. The equation 1/(1+average efficiency of genes)CT was used to calculate R(0) from these values. Normalized gene expression values were calculated by dividing the R(0) values by each respective normalizing gene R(0) value provided here: <http://128.95.149.81/bivalvia/FISH441_normgene_RFUandMiner.xls>.

**RESULTS**

P value: 0.128665488

Mean: 0.002467631(control), 3.1869\*10-6(treatment)

Standard Deviation: 0.002947699(control), 2.8525\*10-6(treatment)

P value: 0.03301714

Mean: 0.98416464(control), 2.48162768(treatment)

Standard Deviation: 0.63632089(control), 1.93309074(treatment)

P value: 0.004187992

Mean: 0.01296444(control), 0.02701651(treatment)

Standard Deviation: 0.004791894(control), 0.01268495(treatment)

P value: 0.04945965

Mean: 0.59533835(control), 20.9820308(treatment)

Standard Deviation: 0.43805256(control), 30.5720466(treatment)

**DISCUSSION**

From the above graphs it clearly looks like hsc70 expression in Pacific oysters decreased as they were exposed to heat shock whereas in Olympia oysters the expression increased. However, due to unreliable data for most of the Pacific oyster treatment samples, only 4 could even be used for calculations. QPCR procedures were run twice on the hsc70 Pacific treatment samples to make sure that the results were not due to human error. The unreliability of the data, along with the p value being >0.05 make the results inconclusive. The hsc70 expression in Olympia oysters increased when exposed to heat shock, as expected. The hsp70 expression for both species of oysters increased with temperature. In general it looked like Olympia oysters showed a much greater increase in both hsc70 and hsp70 expression in response to heat. Comparing the differences in expression levels, one could lean towards Olympia oysters being better adapted to dealing with heat stress. However, Pacific oysters have been known to better withstand higher temperatures compared to Olympia oysters so how could this be? I suspect that the Olympia oysters showing higher expression of the heat shock proteins was due to the fact that they were closer to their threshold temperatures. If the treatment temperatures were increased further then I believe Pacific oysters would have shown similar increases in their hsc70/hsp70 expression.

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