Intertidal responses to heat shock—an analysis of heat shock protein transcription in green sea urchins, *Strongylocentrotus droebacheiensis*

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**Abstract**

Synthesis of heat shock proteins and factors are crucial in protecting organisms from cellular damage from thermal stress. Thermotolerance in green sea urchins, *Strongylocentrotus droebacheiensis*, was examined by viewing cases of sudden extreme heat and reduced water coverage by exposing specimens to 23°C with 1 cm water. Findings indicate ­­­­­­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_. These results may have larger implications in green sea urchin survival tactics in daily tidal fluctuations \_\_\_\_\_\_\_\_\_\_\_\_\_\_.

**Introduction**

Heat and exposure stresses are a part of the average life of most intertidal marine species. Daily tidal fluctuations result in varying environments from an ideal habitable oasis to an exposed and relatively hostile area within a matter of hours. Compensating for drastic changes in environmental conditions must, therefore, be engrained in the organisms that inhabit the intertidal zone in order to survive. One such organism is a key species in intertidal food webs and a crucial part of maintaining a steady state ecosystem.

Sea urchins are a source of food for various top predators such as otters, birds, and octopuses as well as meticulous controllers of kelp abundance (source). With daily ebb and flow of tides, urchins run the risk of exposure to sunlight and heat stress and must have the ability to survive such stress. One known mechanism is the synthesis of heat shock proteins when cellular stress is induced. Widely studied in urchin embryos, heat shock proteins are translated due to the chaperone heat shock factor acting as a transcriptional element. It has been established that sea urchins utilize heat shock proteins to prevent further thermal stress while developing.

As an extreme case, the urchins could be left exposed to summer heat as high as 23°C for varying amounts of time. Cases like this may be more easily overcome if the organism has a predisposition to generate heat shock proteins. The idea of “priming” be an anticipatory mechanism for surviving thermal stress. The goal of this study is to monitor heat shock protein gene expression by analyzing RNA products via reverse transcription and qPCR.

**Materials and Methods**

**Experimental Set-Up**
Collection of urchin samples proceeded in Autumn 2013 at the Shilshole Bay Marina; specimens were collected from the southernmost dock of the north bay. Urchins were removed from pilings and below the surface from the same dock either by netting or in a moderately gentle pulling fashion. Four specimens were then acclimated to a sea table in two separate containers allowing flow with an air stone in each. Samples were taken by removing tube feet from each individual and the tanks were categorized such that two urchins were in a “control” tank, and one was in a “treatment” tank. Both the control and treatment groups were fed every few days and each had consistent amounts of kelp, rocks, water, and space. Tubefeet were held in 1.5 ml microcentrifuge tubes as baseline data and stored in a -80° C freezer. The treatment group urchins was exposed to 23° C in approximately 1 cm of seawater for two hours once a day for one week. The final sample was taken from the subjects on the eighth day and stored in the -80° C freezer to be analyzed.

**RNA isolation**

Expression was analyzed via quantitation Polymerase Chain Reaction (qPCR) after reverse transcription. RNA was extracted from baseline and post-treatment samples using 1 ml TriReagent to lyse the cell tissue and 200 ul chloroform; the solution was mixed for 30 sec and incubated at room temperature for 5 minutes, then centrifuged for 15 minutes at approximately 16.3 rpm. The aqueous solution above the organic solute was washed with 500 ul isopropanol, mixed, incubated at room temperature for 10 minutes and centrifuged for 8 minutes. Next, the supernatant was pipetted off and each pellet was washed with 75% EtOH, spun in refrigerated microfuge at 7500 g for 5 minutes. After removing the supernatant the pellet was allowed to dry and resuspended in 100 uL 0.1%DEPC-H2O, incubated at 55°C for 5 minutes. After quantifying the RNA in a NanoDrop Spectrophotometer (brand?) with the Beer-Lambert law, the samples were stored at -80°C.

**Reverse transcription**

Reverse transcription to cDNA took place starting with 5 ul of each RNA sample combined with 12.75 ul of a master mix consisting of 0.5 ul oligoT and 12.25 ul of nuclease free H2O. All 8 samples were then transferred to a thermalcycler to incubate for 5 minutes at 70°C and immediately transferred to ice. Next, 7.25 ul of a mix of 5 ul M-MLV 5 X Reaction Buffer, 1.25 ul dNTPs, 0.5 ul M-MLV RT, and 0.5 nuclease-free H2O was added to each PCR tube—total volume of the tube at this point is 25 ul. PCR strip tubes were centrifuged for 10 seconds and incubated for 60 minutes at 42°C, heated to 70°C for 3 minutes, then stored on ice.

**Quantitative Polymerase Chain Reaction (qPCR)**

In a strip PCR plate with clear tops we added 1 ul of cDNA and 25 ul of a master mix containing 12.5 ul SsoFast EvaGreen supermix, 0.5 ul of each primer (forward and reverse), and 10.5 ul Ultra Pure Water to each tube. Primers used were for the purple sea urchin, *Strongylocentrotus purpuratus*, from NCBI as follows:

Forward: (designing new primers this weekend)

Reverse:

The mixture was then placed in a thermalcycler (brand) to proceed with denaturation, annealing and extension cycles. The thermal profile began with a 10 minute denaturing at 95°C followed by an additional 15 seconds. Next, the temperature decreased to 55>C for 15 seconds, then rose to 72°C for another 15 seconds before a reading was taken. This process repeated for 40 cycles and finally stopped at 95°C for 10 seconds to prepare for a melt curve. The melt curve was read from 65°C to 95°C at 0.5°C for 5 seconds before the plate was finalized.

**Results**

Quantification of RNA resulted in significant\* increases in concentration from the beginning of the experiment to end in the controls, but the opposite effects in the treatment group. In the urchins exposed to heat we saw a decrease in concentration for the post-treatment group. Similar results were seen for cDNA, with no significant differences in A 260/280 ratio.

Table . RNA quantification via concentration and absorbance ratios using a NanoDrop Spectrophotometer \_\_\_ series. UX(2) indicates the post-treatment sampling from both control and treatment conditions.

|  |
| --- |
| **RNA quantification** |
|   | **Label** | **Sample** | **Concentration** | **A 260/280** | **A 260/230** |
| Control | U1 | U1 | 19.5 | 1.67 | 0.32 |
| Control | U2 | U2 | 31 | 1.56 | 0.36 |
| Control | U7 | U1 (2) | 26.7 | 1.52 | 0.27 |
| Control | U8 | U2 (2) | 38.8 | 1.43 | 0.26 |
| Treatment | U3 | U3 | 32.8 | 1.64 | 0.43 |
| Treatment | U4 | U4 | 51.6 | 1.62 | 0.58 |
| Treatment | U6 | U3 (2) | 20.7 | 1.66 | 0.82 |
| Treatment | U5 | U4 (2) | 35.1 | 1.55 | 0.31 |

Table . Post-reverse transcription, cDNA quantification via concentrationa dn absorbance ratios using a NanoDrop Spectrophotometer \_\_\_ series. UX(2) indicates the post-treatment sampling from both control and treatment conditions.

|  |
| --- |
| **cDNA quantification** |
|   | **Label** | **Sample** | **Concentration** | **A 260/280** | **A 260/230** |
| Control | U1 | U1 | 1455.8 | 1.59 | 1.93 |
| Control | U2 | U2 | 1548.3 | 1.59 | 1.95 |
| Control | U7 | U1 (2) | 1581.4 | 1.60 | 1.96 |
| Control | U8 | U2 (2) | 1630.8 | 1.60 | 1.91 |
| Treatment | U3 | U3 | 1620.5 | 1.60 | 1.97 |
| Treatment | U4 | U4 | 1648.9 | 1.60 | 1.97 |
| Treatment | U6 | U3 (2) | 1607.5 | 1.59 | 1.93 |
| Treatment | U5 | U4 (2) | 1607.4 | 1.60 | 2.01 |

\*\*\* As of right now results are that there was no amplification in quantitative PCR. My primers didn’t work and I will be designing new ones to order on Monday.

Theoretically, if our hypothesis is correct, we should see amplification in the post-treatment urchins that were heat shocked for two hours a day. The post-treatment control urchins should have the “middle” amount of expression taking tank conditions into account; and the pre-treatment urchins should have about the same amount of expression in both the control and experimental groups with either no amplification or some at the end of the cycling.

Include screenshot of qpcr output (quantification and melt curve)

**Discussion**

Sources of error:

Small sample sizes—definitely see biological differences; baseline was different across treatments

Primers not exact species

Difficulty getting tissue from tube feet-especially not without adding stress

May have been tank effects—differences in salinity and temperature

Was definite differences in treatments though—spines and feces in treatment tanks

**Works Cited—need formatting**

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