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**Abstract**

With oysters naturally exposed to varying temperatures, various tests have been performed to see what kind of stresses it can cause. The methods in of some of those tests involve an acute temperature increase, while it is more natural for gradual temperature increase to occur. Here we examine what metabolic effects acute and gradual temperature stresses have on Olympia oysters (Ostreola conchaphila). After obtaining 30 oysters and treating them with varying heat stresses, qPCR was performed on gill tissue to observe gene expression of cytochrome c oxidase and ATP synthase genes. With cytochrome c oxidase gene ~~showing~~ more expression in acute temperature stress than gradual, it could mean future tests are best to be done under a gradual temperature stress.

**Introduction**

Oysters are exposed to temperature change throughout their lives. Due to the temperature stress, there is typically a variance in metabolic rate [1]. A way to measure metabolic rate in an oyster is by measuring the gene expression of cytochrome c oxidase, or ATP synthase beta subunit [2]. Cytochrome c is part of the electron transport chain used to make ATP. The enzyme is specifically used to turn oxygen into water. If a species is stressed, and metabolic rate were to increase, there will be an increase in energy produced and thus more expression of the genes used to make energy. A constant increase in metabolic and respiratory rates due to temperature stress would mean more energy usage for homeostasis. The increased temperature stresses can lead to large mortality rates among populations [3]. With possible increases in temperatures due to global warming, studies have been done to test the effects on many varying oysters, but there are fewer concerning Olympia oysters (Ostreola conchaphila).

2nd paragraph explain what genes do.

The gradual temperature stress is what would be observed in the wild, but studies have been done without taking time to gradually increase the temperature. Therefore, we would like to observe any effects it would have on the metabolic rate of Olympia oysters.

Objectives are to observe the effects of temperature stress on metabolic rate on Olympia oysters, and to see if there is any effect of an acute temperature stress versus a gradual temperature stress. We will use qPCR techniques to measure the gene expression of cytochrome c oxidase and ATP synthase genes

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We obtained 30 Olympia oysters. Ten of them underwent a sudden heat shock at 35 C for one hour, while another ten were exposed to a gradual temperature increase. The last ten were used as a control undergoing no temperature stress. Oysters were held in a controlled tank. The tank included saltwater, a temperature heater, thermometer, and a pump. The control temp was at 14 C. The max temp stress occurred at 35 C. The gradual heat stress occurred by increasing the tank temperature by 3-4 degree increments every two hours until reaching maximum temperature stress (35 C). Due to time issues, the oysters remained around 28 C overnight. After oysters were exposed to stresses, they were opened and dissected for their gill tissue to analyze. To examine the mRNA expression, we isolated RNA from gill tissue. This was done using TriReagent on whole tissue to isolate RNA from other components. After isolation, RNA was washed with ethanol and 0.1%DEPC-H2O. Two of the extracted RNA samples (one from each day samples were being extracted) were then spectro-analyzed using a Nanodrop for purity. The isolated RNA samples were then reverse transcribed into cDNA using M-LV 5X reaction buffer. qPCR was performed with designed primers , sensimix, SYBR dye, water and the cDNA samples. Blanks were included in testing to observe any contamination.

**Results**

Initial size Measurments:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample # | Length (mm) | Width (mm) | Weight of RNA sample (g) | Treatment |
| 51 | 45 | 35 |  | Olympia control |
| 52 | 35 | 29 |  | Olympia control |
| 53 | 35 | 30 |  | Olympia control |
| 54 | 30 | 27 |  | Olympia control |
| 55 | 38 | 28 |  | Olympia control |
| 56 | 38 | 35 | .062 | Olympia control |
| 57 | 38 | 29 |  | Olympia control |
| 58 | 32 | 24 |  | Olympia control |
| 59 | 33 | 25 |  | Olympia control |
| 60 | 30 | 25 |  | Olympia control |
| 61 | 31 | 28 | .045 | Acute shock 35 C |
| 62 | 35 | 29 | .004 | Acute shock 35 C |
| **63** | **35** | **27** |  | **Acute shock 35 C** |
| 64 | 40 | 28 |  | Acute shock 35 C |
| 65 | 35 | 26 |  | Acute shock 35 C |
| 66 | 33 | 27 |  | Acute shock 35 C |
| 67 | 31 | 27 |  | Acute shock 35 C |
| 68 | 31 | 28 |  | Acute shock 35 C |
| 69 | 34 | 26 |  | Acute shock 35 C |
| 70 | 32 | 26 |  | Acute shock 35 C |
| 71 | 39 | 28 |  | Gradual shock 35 C |
| 72 | 37 | 25 |  | Gradual shock 35 C |
| 73 | 44 | 35 |  | Gradual shock 35 C |
| 74 | 39 | 29 |  | Gradual shock 35 C |
| 75 | 42 | 30 |  | Gradual shock 35 C |
| **76** | **32** | **29** |  | **no sample (jingle shell)** |
| 77 | 42 | 29 |  | Gradual shock 35 C |
| 78 | 34 | 29 | .030 | Gradual shock 35 C |
| 79 | 30 | 28 | .062 | Gradual shock 35 C |
| 80 | 37 | 27 |  | Gradual shock 35 C |

\*samples in **BOLD** did not give data for analysis

\*\*All other weights not noted were estimated between .04g -.07g

Spectro-analysis:

|  |  |  |  |
| --- | --- | --- | --- |
| Sample # | Concentration | 260/280 ratio | 260/230 ratio |
| 56 | 679.4 | 1.98 | .97 |
| 79 | 700.1 | 1.94 | 1.91 |

Primers used:

Cytochrome c oxidase subunit 3:
Forward: GCATAGAAGACTACGCCACTCT : 34.9nm
Reverse: CACAACCATAGCCGCAATCAC : 40.1nm

ATP synthase beta subunit
Forward: TACCACTCGACCACTAGCCA : 38.6nm
Reverse: AATGCAGCAAGGAAAGCGTG : 29.2nm

Primers were tested with proven cDNA samples and were found to amplify with cDNA samples and not in blanks, also showed only one peak on melt curve.



T-test results:

|  |  |  |  |
| --- | --- | --- | --- |
|  | control vs. acute | control vs. gradual | acute vs. gradual |
| p-Value | 0.321374 | 0.747123 | 0.58748 |
| standard deviations | 6.827053 | 15.9496 | 14.44051 |

Graph of the mean expression values:

**Disscussion**

**Start with general importnance**

The spectro-analysis results were generally what were expected. The normal ranges are: 260/280=1.8-2.0 and 260/230=1.5-2.0. The in-range A260/280 ratios indicate the samples were clean of proteins. Sample #56 result of a low 260/230 ratio indicating carryover of phenol, ethanol or high salt in the sample. Since the samples were all gill tissues, there is a possibility that salt concentrations could be higher and thus the cause for the low A260/230 ratio. As seen with sample #79, the ratios were in range, and can generalize that the rest of the samples were clean and pure, with the possibility of high salt concentration.

From the graph of all the mean normalized gene expression values it does show that the acute and gradual treatments did cause the oysters to express the cytochrome c oxidase gene more. This also shows that the acute treatment seemed to stress out the oysters more than the gradual treatment. Looking at individual values, the acute treatment was boosted greatly by two values over 30 in samples 66 and 68, while the gradual treatment only had one of those values from #79. The p-values over .05 do indicate that the results are insignificant, but this can be from a lower sample size or only doing one run on each sample. If this is also true for ATP Synthase gene, then we would get results opposite of what was expected. This could mean that oysters’ metabolic rates would be higher when treated with acute heat shock. If trying to perform experiments using temperature shock, it would be better to test using a gradual temperature increase when trying to simulate natural stresses. Past tests results should not be completely nullified since acute temperature stress does cause an increase in metabolic rate like a gradual increase, but just preference it with that the results could be slightly exaggerated.

References:

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