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Characterizing the expression of HSP-70 in response to elevated concentrations of environmental copper sulfate and carbon dioxide in *Mytilus trossulus*

*Abstract*

 Examining the effects of multiple stressors acting synergistically can provide an improved understanding of an organism’s stress response physiology. Mussels were exposed to four different treatments, each with a different combination of high and/or low concentrations of copper sulfate and carbon dioxide. The gill tissue of these organisms was then analyzed to determine protein concentration, particularly that of HSP-70. Although the abundance of HSP-70 was indeterminate, the relative protein concentrations between treatments suggested that exposure to copper actually impaired the efficacy of stress response. However, protein concentrations alone cannot give an adequate indication of the overall health of an organism.

*Introduction*

 Aquatic ecosystems, especially those that are in proximity to human settlements, are becoming increasingly polluted due to both climate change and human activities. Rising global temperatures are causing a wide variety of effects in aquatic environments, most notably higher CO2 concentrations as well as lower environmental pH (Doney et al. 2009), the latter of which increases the difficulty that organisms with calcareous shells have in forming these shells (Orr et al. 2005). Furthermore, heavy metals, such as copper, have been found in increasing concentrations in aquatic environments adjacent to anthropogenic settlements (Valavanidis et al. 2006). For this reason, it is important to examine the effect that such stressors have on cellular biology.

 Heat-shock proteins activate in a wide variety of ways in response to several different environmental stressors, including thermal stress, foreign contaminants, immune response, etc. (Li & Srivastrava 2004). In particular, the HSP-70 family, the focus of this study, are molecular chaperones that function by aiding in the folding or unfolding of newly translated or damaged proteins (Feder & Hoffmann 1999). HSP-70 is one of the most thoroughly characterized and understood cellular stress response mechanisms, and as such is commonly used in studies observing how organisms react to stressors introduced to their environment. As such, these proteins are ideal indicators of the degree to which organisms can cope in stressful environments.

 This experiment focuses on how mussels are affected by the introduction of stressful concentrations of copper and CO2 to their environment. As sessile filter feeders, mussels are good indicators of the severity of adverse environmental conditions. While many studies have previously examined HSP-70 expression in organisms as it depends on various environmental metrics, very few have examined its expression when exposed to multiple stressors simultaneously. Environmental contaminants can have compounding effects, and as such, analyzing an organism’s stress response in the presence of multiple stressors provides a better understanding of that organism’s cellular physiology. Our hypothesis states that HSP-70 will be approximately equally expressed in the presence of either copper or high CO2, but be much higher (more than double the expression) in the presence of both stressors.

*Methods*

 40 mussels (*Mytilus trossulus*) were divided evenly between four different treatments, each testing a combination of either high or low copper concentration (5 or 0 mg/L Copper Sulfate) and pCO2 (approximately 1900 or 800 μatm CO2 (see Table 1)). For the duration of this paper, the low copper / high pCO2 treatment will be referred to as tank A; high copper / high pCO2 will be tank B; high copper / low pCO2 will be tank C; and low copper / low pCO2 (control treatment) will be tank D. In tanks A and B, CO2 was administrated via an aerator. At the beginning of the experiment, a copper sulfate solution was administered to tanks B and C that brought the total copper concentration to roughly 5 mg/L. Tank pH was frequently recorded across all tanks, and pCO2 was calculated from this value. Water was circulated using an aerator in each tank. Water changing procedures were deemed unnecessary, as mussels were not fed for the duration of the experiment, and the tanks used were of sufficiently large volume (50 gallons each). Mussels were suspended off the bottom in perforated bags. All tanks were maintained at 16.5°C and approximately 30.00 PSU.

The experiment lasted for a total of two weeks. Each week, one individual from each treatment was removed, and its gill tissue extracted for later analysis. Only one mussel was sampled per treatment per week due mainly to time constraints; with extra time, replicates would have been included. The sampling period was initially planned to extend an extra week, but was cut short due to unexpected organism mortality. A baseline sample were also obtained at the beginning of the experiment. The obtained gill samples were used to extract and quantify protein concentrations. Both procedures were performed to the specifications of Steven Roberts’ FISH 441 lab manuals. For the purposes of protein quantification, the standard curve calculation was determined to be:

[Sample (μg/mL)] = (average wavelength + 0.0003) / 0.00007

Additionally, an SDS-page and western blot procedure were performed as per the procedures in Steven Robert’s lab manuals in order to determine HSP-70 protein presence in the sample. The aforementioned extracted protein samples were used for these procedure. In order to obtain equal protein concentrations, the extracted samples were diluted with DI water until their concentrations were all equivalent prior to performing the SDS-page using the equation:

Dilution (μL) = 15 / ( [sample concentration] / 490)

where 490 was the smallest extracted protein concentration.

*Results*

Table 1: Recorded values for pH, and the corresponding calculated pCO2, in all treatments across the duration of the experiment.

|  |  |  |
| --- | --- | --- |
|  | Tanks A & B | Tanks C & D |
| Date Sampled | measured pH | pCO2 (μatm) | measured pH | pCO2 (μatm)  |
| 8-Nov | 7.50 | 2045.33 | 7.84 | 893.0 |
| 11-Nov | 7.72 | 1198.92 | 7.88 | 806.6 |
| 12-Nov | 7.56 | 1765.80 | 7.89 | 786.0 |
| 13-Nov | 7.53 | 1901.54 | 7.87 | 827.8 |
| 15-Nov | 7.49 | 2094.86 | 7.84 | 893.0 |
| 18-Nov | 7.54 | 1860.12 | 7.87 | 829.2 |
| 25-Nov | 7.52 | 1945.75 | 7.81 | 960.5 |
| 27-Nov | 7.54 | 1858.28 | 7.87 | 828.5 |

Table 2: Wavelengths and concentrations of samples obtained from protein quantification. Concentration denotes the total amount of protein in the sample (including, but not exclusively, HSP-70). The volumes for SDS-page were diluted with 0.1% DEPC water to get 15 μL in order to achieve equal protein concentrations between every sample. The resulting solutions were used as protein stocks in the SDS-page protocol.

|  |  |  |  |
| --- | --- | --- | --- |
| Date / Treatment | Avg. Quantification Wavelength (nm) | Concentration (μg/mL) | Volume for SDS-Page (μL) |
| 11/5 Baseline | 0.152 | 2175.71 | 3.3782 |
| 11/12 A | 0.203667 | 2913.81 | 2.522471 |
| 11/12 B | 0.054333 | 780.4762 | 9.417328 |
| 11/12 C | 0.055667 | 799.5238 | 9.192972 |
| 11/12 D | 0.315667 | 4513.81 | 1.628336 |
| 11/19 A | 0.373333 | 5337.619 | 1.377018 |
| 11/19 B | 0.034 | 490 | 15.0 |
| 11/19 C | 0.078 | 1118.571 | 6.570881 |
| 11/19 D | 0.376 | 5375.714 | 1.36726 |

 Based on the results of protein quantification, in almost every treatment (except treatment C), total protein concentration consistently either increased or decreased away from the baseline (treatment C initially decreased in week 1, then increased slightly in week 2). For every sampling date, both high-copper treatments (B and C) exhibited lower protein concentrations than baseline – at the end of the experiment, treatment B was about 22% that of baseline, and treatment C was about 51%. In contrast, the low-copper treatments (A and D) both exhibited higher concentrations than baseline – at the end of the experiment, the concentrations of both treatments were more than double that of the baseline.

Unfortunately, the SDS-page / Western blot procedure did not yield any results. It’s possible that the protein concentrations used However, the most likely explanation is that the voltage of the Western blot was initially well below 20V, and didn’t reach this value until several minutes into the procedure. As a result, the wells failed to expand, and no definitive results were observable. However, the initial starting position of these wells were in fact observable, indicating that the HSP-70 antibody solution had successfully bound to the HSP-70 in the samples. From this, we can at least infer that there was a significant (visible) amount of HSP-70 in the protein stocks.

*Discussion*

 Due to the failure of the SDS-page and Western blot procedure to produce distinct results, the initial hypothesis cannot be absolutely confirmed nor denied. However, the fact that HSP-70 was present in some amount is an encouraging result, if nothing else. Fortunately, there are still other parts of our experiment from which we can extrapolate results- namely, using data from protein extraction and quantification. For example, the fact that the final protein concentration of treatment B was lower than the other treatments at any point in the experiment is in direct opposition to the hypothesis. This observation implies that if an organism is subjected to multiple significantly damaging environmental stressors simultaneously for an extended period (>1 week), the stress could be so great that the organism’s overall metabolism would be severely weakened. This would cause a general reduction in metabolism, resulting in extremely reduced rates of transcription and translation (Brauer et al. 2008). This is further supported by the fact that the final protein concentration of Treatment B was a fraction of both treatments A and C; the combination of stressors was likely too much for the mussels to cope with. The rate of unanticipated mortality also reflected this observation, as it was much higher in treatment B than in the other three treatments.

 Total protein concentration seemed to be primarily dependent on the presence or absence of copper. Relative to the baseline protein concentration, both of the low copper treatments (A and D) were much higher, whereas the high copper treatments (B and C) were both much lower. This indicates a few possibilities: copper is inherently more damaging as an environmental stressor than increased pCO2; 5 mg/L copper sulfate is much too high a concentration for mussels to tolerate; or increasing the external copper concentration so abruptly shocked the mussels to a point beyond recovery. In any case, if this experiment were to be repeated, the copper would be better administered slowly over the course of several days, increasing by 1 mg/L per day at most, and probably be at a much lower concentration as well.

 In contrast to the copper treatments, protein concentration increased in the low pH / high pCO2 treatment (tank A). This indicates that the mussels in this treatment were able to tolerate the effects of increased CO2 concentration. That said, these concentrations were approximately comparable to that of the control treatment (tank D), which not only had a protein concentration twice as high as that of the baseline at the end of the experiment, but was also higher than any other treatment at any time. Unfortunately, the data available is insufficient to determine exact cause of this phenomenon. However, it’s important to bear in mind that, while the concentration of protein correlates with overall metabolic activity, rates of translation do not necessarily directly relate to rates of translation (Greenbaum et al. 2003). Additionally, there may have been a higher concentration of HSP-70 in the experimental tanks, but a greater total protein concentration in the control group. The only way to know for certain would be to repeat the experiment and successfully obtain data from the SDS-page / Western blot protocols.

*Literature cited*

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