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The Ecophysiological Effects of Water Temperature and Ibuprofen on *Hemigrapsus oregonensis*

Introduction:

Although the presence of pharmaceuticals in the environment has been occurring for decades, only recently has there been an effort to closely monitor the concentrations of these pollutants in the marine environment (Fernandes et al. 2021). NSAIDs (non-steroidal anti-inflammatory drugs), such as ibuprofen, are among the many studied pharmaceuticals for potential risk to marine organisms (Blasco & Trombini 2023). Additionally, the rise of temperature due to climate change is heavily researched for concern to marine organism physiology. *Hemigrapsus oregonensis*, commonly known as the hairy shore crab, serves as an ideal model species for understanding physiological stress in response to these environmental changes. *H. oregonensis* reside on the intertidal where they are constantly being exposed to elevated temperatures during low tides, as well as possible pharmaceutical contamination not only in the water but also within sediment (Blasco & Trombini 2023). Despite the numerous research done on the effects of either elevated temperatures or ibuprofen individually, little has been done to analyze the combined effects of these two environmental factors on crab physiology. Therefore, in this study, we aimed to evaluate the physiological response of *H. oregonensis* to the combined effects of elevated water temperatures and ibuprofen exposure through a resazurin assay for oxygen consumption and physiology assay in hemolymph glucose levels.

Methods:

Sampling and Collection of Specimens:

H. oregonensis was collected from Lion's Park boat launch/trestle (47° 35' 07" N 122° 38' 42" W) between 11:30 to 13:34 on April 27th, 2025. The tide during collection was -1.68 feet while the substrate of collection sites was mixed (shell, sand, pebble). Crabs were then placed in the UW class control tank at 16:21.

Experimental Design:

A total of 18 *H. oregonensis* organisms were used for the experiment, 3 crabs for each treatment. In total, there were six treatments used to analyze the effect of temperature and ibuprofen on the oxygen consumption and glucose levels of the crab: 1) high temperature – no dose, 2) high temperature – low dose, 3) high temperature – high dose, 4) ambient temperature – no dose (the control), 5) ambient temperature – low dose, and 6) ambient temperature – high dose. The high temperature was set at 27 ℃, while the ambient temperature was set at 13℃. High dose tanks had an ibuprofen concentration of 31.25 μg/L, while the low dose tanks had 6.25 μg/L.

Kroger coated tablet USP of 200 mg of Ibuprofen was used to create a solution for the treatment with doses. Once grinded, a stock solution of an Ibuprofen concentration of 2500 mg/L (2.5 μg/μL) was made. Within each tank held 2 liters of water, in high dose treatments we added 25 μL of the stock ibuprofen solution, while in the low dose treatments we added 5 μL into the tank. Through previous research, ibuprofen concentrations found within the Salish Sea were at a much smaller concentration of 21.70 ng/L (Blasco & Trombini 2023). However, this concentration could not be applied within the study due to limitations of sensitive measuring.

Respirometry:

Cellular respiration and oxygen consumption of *H. oregonensis* was measured with resazurin. The resazurin stock solution (10 mL) was created by mixing 0.5 g resazurin salt, 10 mL DI water, and 10 µL DMSO. The working resazurin solution (150 mL) was prepared by mixing 148 mL seawater (DI water with Instant Ocean adjusted to 23-25 ppt), 333 µL resazurin stock solution, 150 µL DMSO, and 1.5 mL antibiotic solution 100x Penn/Strep & 100x Fungizone.

Oxygen consumption for each treatment was determined by using a representative crab from each tank. Resazurins assays were taken weekly during the experiment. Six 100 mL beakers were filled with 35 mL of working resazurin solution. Once patted dry and weighed, one crab from each treatment was placed into an individual beaker, with a timer quickly started once placed. Every 30 minutes, 200 µL of working resazurin solution from each beaker was withdrawn and placed in the wells of the 96 well plates. A duration of 90 minutes total was taken for each treatment. The well plate containing the trial samples was run in a plate reader at Excitation 530, Emission 590 to obtain fluorescence values. At the end of the trials, crabs were withdrawn from their chambers and rinsed off with 33-35 ppt saltwater.

Glucose levels in hemolymph:

Glucose levels of *H. oregonensis* was measured by extracting hemolymph from each crab within each treatment at the end of the experiment. Once values of the hemolymph glucose levels were obtained, an average for each treatment was calculated. To perform the assay, at least two sample wells were created for each treatment. This was created by adding 85 µl of diluted Assay Buffer and 15 µl of a sample together then placed into a well. The reaction would then be initiated by adding 100 µl of Enzyme Mixture to all sample wells. Once the plate of sample wells was covered, the samples were incubated for 10 minutes at 37℃. When finished with incubation, the absorbance at 500-520 nm was determined with a plate reader.

Results:

Within the first week of oxygen consumption analysis, the high temperature - no dose treatment showed to have the highest rate of oxygen consumption while the high temperature - low dose treatment showed to have the lowest rate of oxygen consumption. By the second week of oxygen consumption analysis, the ambient temperature - high dose treatment had the highest rate of oxygen consumption, while the high temperature - high dose treatment had the lowest rate of oxygen consumption. General patterns seen from week 1 to week 2 in the high temperature treatments showed that the no dose and high dose treatments had a decrease in oxygen consumption rate, but the low dose's rate slightly increased. The patterns seen in the ambient temperature treatments showed that both the treatments with doses of ibuprofen increased in oxygen consumption rate, while the control seemed to decrease.

Glucose levels at the end of the experiment showed that the control/ambient - no dose treatment had the highest average glucose level, while the ambient - low dose and high - no dose treatments had the lowest average glucose level. In both high and ambient temperature treatments, the treatments with high doses of ibuprofen had a higher glucose level than the treatments with low doses of ibuprofen. Additionally, both high temperature treatments that did have doses of ibuprofen showed to have higher glucose levels than the corresponding ibuprofen dose in the ambient temperature treatments.

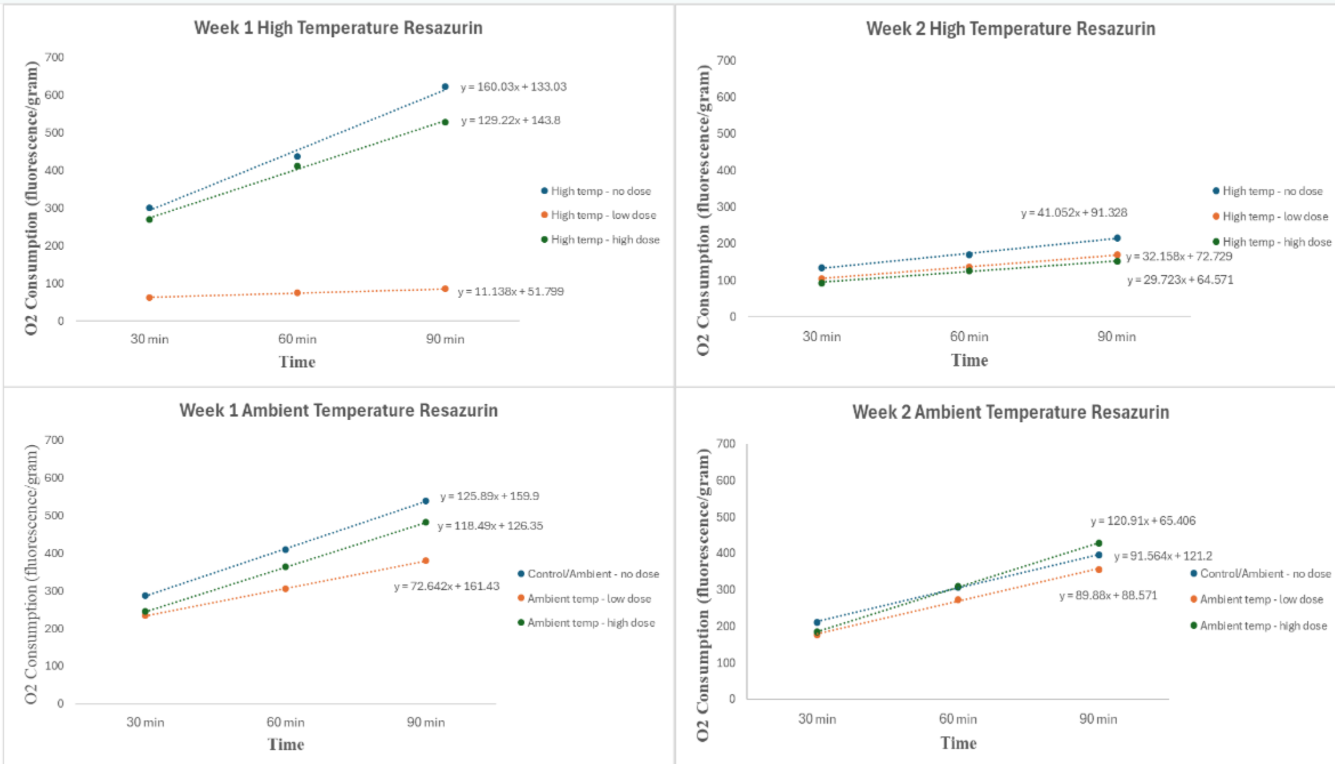

Figure 1. Results of resazurin assay for each treatment at 30 minutes, 60 minutes, and 90 minutes in week 1 and week 2 of data collection. The top row shows the results for high temperature (27℃) and varied ibuprofen dose treatments. Bottom row reveals the results for ambient temperature (13℃) and varied ibuprofen dose treatments. The left column are results for week 1, while the right column are results for week 2.

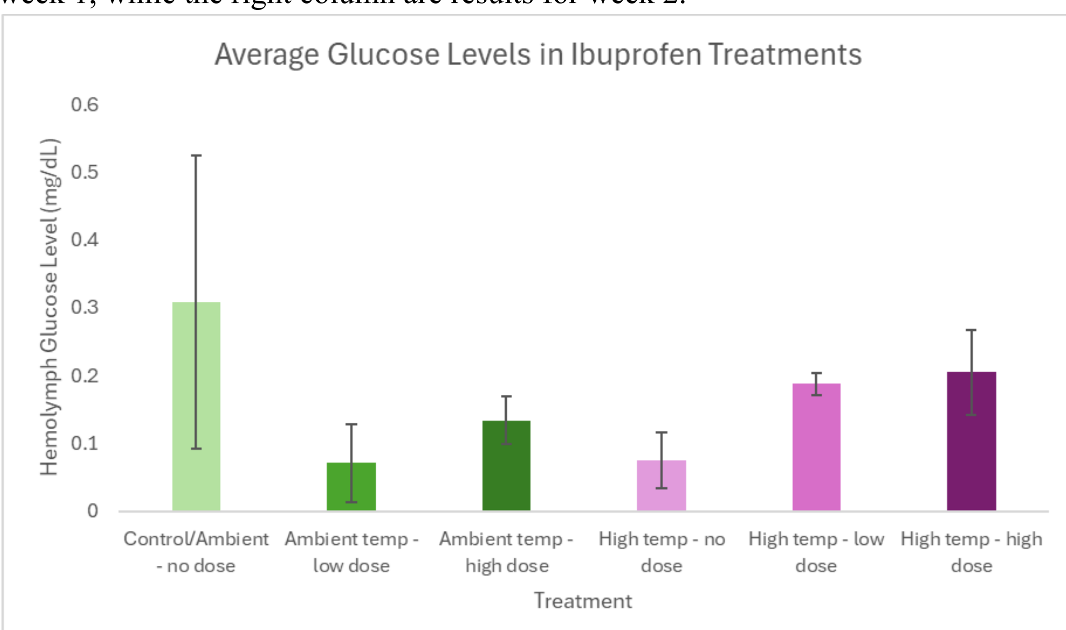

Figure 2. Results for the average glucose levels within each treatment.

Discussion:

Both the high temp - no dose and the high temp - high dose treatments showed the expected relationship of increased oxygen consumption rates to their ambient temperature counterparts in week 1. This is in agreement with another study that has shown that increased temperatures resulted in an increased oxygen consumption rate in shrimp residing in ibuprofen contaminated water (Nieto et al. 2016). However, the high temp - low dose treatment's oxygen consumption rate in week 1 falls below any other treatment's rate within the experiment. This result did not follow any articles that focussed on either ibuprofen exposure or increased temperatures. A likely explanation for such a low oxygen consumption rate present however may have been that the individual crab analyzed was above the resazurin liquid line during analyzation, thus disrupting the process of the respirometry measurement.

Interestingly enough, by week 2 all high temperature treatment oxygen consumption rates decreased below any of the ambient temperature treatments. One possible explanation for this would be that the prolonged exposure (past 1 week) to increased temperature had disrupted the crabs' ability to effectively consume oxygen in the water. Results from the ambient temperature treatments showed unexpected results from week 1 to week 2 as the treatments with doses of ibuprofen showed a slight increase in oxygen consumption rate but the no dose treatment had a decrease in oxygen consumption rate. An explanation could be that without the additional stressor of ibuprofen in the water, the crabs were able to follow the expected results of a decreased metabolic rate, thus decreased oxygen consumption rate in the ambient temperature (Rivers et al. 2024).

Previous research has shown that it is expected for high temperature treatments to result in higher hemolymph glucose level due to a higher metabolic rate in warmer temperatures in any ectotherms, such as a crab (Su et al. 2020). Results of a decreased glucose level in the low and high dose treatments of the ambient temperatures when compared to their high temperature parallels was as expected. The control treatment is set as the baseline for representing what occurs when the crabs are not stressed by either temperature or ibuprofen exposure. Of the glucose levels identified, the control - no dose treatment had the highest amount, whereas the rest of the treatments had much lower levels of glucose in the hemolymph. Other studies have shown contradicting results when comparing the crabs in control treatments to treatments exposed to some other pollutants such as copper, chloroform, and adrenaline (Qyli et al. 2020). Their results showed an increase in hemolymph glucose in treatments with pollutants compared to the control treatments.

Looking closer at the results of figure 2 in the high temperature treatments we can see that the treatments containing ibuprofen doses had higher glucose levels than the treatments that were simply exposed to only an increase in temperature. An interpretation of this result could be that the exposure to ibuprofen aided in decreasing the energetic costs for the crab in a high temperature environment. This hypothesis could also be applied to the ambient temperature treatments as the treatment with the high dose seems to have a slightly higher hemolymph glucose level than the low dose treatment.

The significance of this study was to bridge together an understanding of how the combined effects of increased temperature and ibuprofen exposure affect the physiology of *H. oregonensi*s. Although much research has been done to understand how both these environmental stressors individually affect the physiology of marine organisms, not much has been done to understand how the organisms will be affected with the factors combined. The results of this study are ecologically relevant as *H. oregonensis* and many other shore crabs are often exposed to multiple stressors at once in their natural habitats. Thus, the combined effects of pharmaceutical exposure and environmental conditions in this study more accurately reflect the complications that the crabs face in the wild.

Future work that will involve the combined effects of temperature and pharmaceuticals may want to assess the physiology more closely, not only in additional physiology assays being tested but also examination of internal morphology of the crabs. Additionally, it would benefit the future scientists to find access to a liquid form of ibuprofen if this experiment were to be repeated. Not only would a liquid form more likely represent the form that is present in aquatic environments, but it would also dissolve better into the water and equally diffuse through the treatment tanks. Lastly, one limitation of the present study was the number of organisms that could be used to analyze within each treatment which caused large variations within the data (i.e. SD bars in figure 2). For future work, it would be beneficial to have a larger sample size to further prove significance within the results.

References:

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