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

Jolee Thirtyacre

Introduction

As humanity continues to grow in population, advance in technology, and fine-tune medicine, we have to continually be aware of the effects these advances have on the environment around us. One of the biggest topics of discussion around human development and environmental impacts is the increase in greenhouse gases and temperature across the planet (Wang et al. 2023). However, it is not the only emerging global environmental concern. Pharmaceuticals are now considered an emerging global contaminant with over 600 detected substances in the environment (Adeleye et al. 2022; Fent et al. 2006). Of these drugs, analgesics, specifically non-steroidal anti-inflammatory drugs (NSAIDs), are some of the most common drugs in the aquatic ecosystems around the world (Aguirre-Martinez et al. 2013; Blasco and Trombini 2023).

Concern over rising NSAID levels in the aquatic environment stems from our understanding of what these drugs do to the human body to reduce inflammation (Fent et al. 2006; Lofrano et al. 2022). Ibuprofen, an NSAID, targets the COX enzyme in humans, but this same enzyme is known to cause sub-lethal effects in species of aquatic invertebrates and fishes (Adeleye et al. 2022; Blasco and Trombini 2023; Lofrano et al. 2022). Wastewater treatment facilities pull out ibuprofen from raw wastewater, with a range of 90-99% efficiency (Adeleye et al. 2022). However, due to how common this drug is, residual ibuprofen still makes its way to the aquatic environment and has accumulated to levels of 0.1-1.0 ug/L in surface waters throughout the United States (Adeleye et al. 2022; Fent et al. 2006). To further exacerbate the problem, the effects of these drugs on organisms could be compounded with the increases in global temperature as temperature can alter drug toxicity (Nieto et al. 2016).

Invertebrates make up the majority of marine animal biomass and have a large role to play in ecosystem functions such as being prey, nutrient recyclers, and filter feeders (Aguirre-Martinez et al. 2013; Blasco and Trombini 2023). They also play an important role as indicator species for the overall health on an ecosystem (Eades and Waring 2010). However, little research has gone into how these organisms are affected by NSAIDs.

This study strives to help answer three questions by [1] investigating how different dosages of ibuprofen impact the physiology of *Hemigrapsus oregonensis* (the hairy shore crab), [2] how these crabs are impacted by increasing water temperatures, and [3] how the combined effects of temperature and ibuprofen impact this species in Puget Sound, Washington USA. We hypothesized that crabs exposed to warmer temperatures or higher doses of ibuprofen would have higher oxygen consumption rates and lower levels of glucose than crabs exposed to ambient water temperatures due to stress-induced energy production. Therefore, crabs exposed to the high temperature and high dosage of ibuprofen would experience the highest oxygen consumption and the lowest glucose levels of our treatments.

Methods

General Information

Crabs were collected from a boat launch at Lions Park in Bremerton, Washington, USA. Of the crabs collected, 18 crabs were randomly selected for usage in this experiment. There were six tanks total,

two control tanks and four ibuprofen treatment tanks. Each was filled to 2 L with artificial seawater which was created in-lab. Each treatment and control tank had three crabs. The study lasted two weeks and the crabs were not fed during the duration of the study. Tanks were changed every week. Prior to water changes, crab survival was recorded. All analyses, graphing, and descriptive statistics were run in Microsoft Excel.

Water Temperature Treatments

The experiment consisted of two temperature treatment groups with three different drug treatments in each temperature [see "Ibuprofen Treatments" below]. An ambient temperature of 13 °C was used to mimic the temperature of the water where the hairy shore crabs were collected. A high temperature of 27 °C was used to mimic a potential rise in seawater temperature in Puget Sound. Temperatures were maintained using a large secondary bath which was set to the designated temperature and the treatment tanks were set within.

Ibuprofen Treatments

For the Ibuprofen treatment, one Kroger Coated Ibuprofen USP 200 mg tablet was crushed using a mortar and pestle. The resulting powder was put into 20 mL of artificial seawater. The solution was shaken to try and dissolve the ibuprofen particulates. However, the powder never dissolved. Therefore, during the times were ibuprofen was taken up to be put in the tanks, the vial was shaken to make a relatively evenly distributed mixture of the drug in solution. The low dose, due to instrument constraints, contained 5 uL of solution resulted in a total of 12.5 ug of ibuprofen injected into each low-dose 2 liter tank. For the high dose, 25 uL of solution were used resulting in a total of 62.5 ug of ibuprofen injected into each high-dose 2 liter tank. After water changes, these same dosages were re-administered using the initially created stock solution. This resulted in ambient and high temperature, no-drug; ambient and high temperature, low-drug; and ambient and high temperature, high-drug groups.

Oxygen Consumption

Oxygen consumption rates were collected using a resazurin assay each week. A 10 mL resazurin stock solution was made each week using common resazurin steps. After creating the stock solution, a working solution was created. Stock solution and working solution ingredient values changed depending on the amount of sample runs occurring across all lab groups that week.

One crab was selected at random from each of the six tanks, was patted dry, and weighed for normalization. After, each crab was placed in its own sealed container which contained 35 mL of resazurin working solution. The crabs remained completely submerged in these containers for an hour and a half. Every half hour, 200 uL of the resazurin solution were sampled from each container and placed in labeled wells for analysis. After sampling concluded, each crab was rinsed off with artificial seawater and placed back into their respective treatment tanks.

Resazurin plates were run through an Agilent BioTek Synergy HTX Multi-Mode Microplate Reader at an excitation of 530 and emission of 590 to obtain fluorescence values. After gathering fluorescence values, all measurements were divided by crab weight to normalize values and then divided by 60 to get oxygen consumption per minute.

Glucose Levels

Hemolymph was collected at the end of the two-week study. All crabs that were still alive had a portion of their hemolymph extracted through the crab's legs. All extracted glucose was placed in individually labeled vials for glucose analysis. Standard operating procedures for the use of a Cayman Chemical Glucose Colorimetric Assay Kit were used to create the assay buffers, standards, and enzyme mixtures for glucose analysis. Glucose assays were run by placing 85 uL of diluted assay buffer and 15 uL of all the created standards into two labeled wells. Labeled sample wells received 85 uL of diluted assay buffer and 15 uL of sample. Reactions were initiated by adding 100 uL of the enzyme mixture. The plate was covered and incubated at a temperature of 37 °C for ten minutes. Then, samples were uncovered, and absorbance was read at 500-520 nm using a plate reader. Each hemolymph sample was run twice for replication if enough sample remained after the first run.

Results

Ibuprofen and Temperature

After the first week of the experiment within the ambient group, oxygen consumption rates revealed that crabs in the no drug treatment had the highest oxygen consumption (Fig. 1A). This group was followed by the consumption rate of crabs in the high dose group, and finally by the low dose treatment (Fig. 1A). However, during the second week of measurements, the crab in the high dose had the highest oxygen consumption rate (Fig. 1B). The control saw the second highest oxygen consumption, and again, the crab in the low dose treatment had the lowest rate. Oxygen rates across all three groups had a smaller overall spread during week two's analysis.

The first week of the experiment within the high temperature treatments saw the highest oxygen consumption rate for the crab in the high temperature control (Fig. 1A). The high dose treatment had the second highest oxygen consumption rate, followed by the low-dose crab, who had relatively minimal oxygen consumption through time (slope of 0.19 fluorescence/g/min) (Fig. 1A). The second week showed a narrower spread of rates for the three dosage groups (Fig. 1B). The crab in the high temperature control continued to have the highest oxygen consumption rate (Fig. 1B). The low and high dosage crabs showed similar oxygen rates, with the low dosage crab consuming slightly more oxygen (Fig. 1B).

Week one showed that the crab in the high temperature control had the highest overall oxygen consumption across all groups and the crab in the high temperature, low drug treatment experienced the lowest oxygen consumption rate (Fig. 1A). During week two, the high and ambient temperature groups distinguished themselves with all ambient temperature crabs showing higher oxygen consumption rates than all the tested crabs exposed to the higher temperature (Fig. 1B).

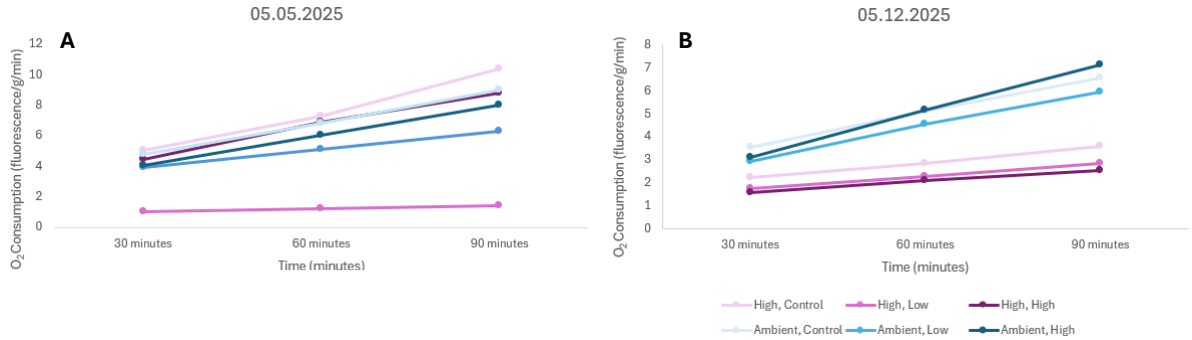

Figure 1. The rate of oxygen consumption (fluorescence/g/min) at ambient (13 °C) and high temperature (27 °C) drug treatment groups, where the dose "control" is 0 ug/L of ibuprofen, "low" is 6.25 ug/L, and "high" is 31.25 ug/L. (**A.**) Oxygen rates were taken after one week of treatment exposure (05.05.2025) and (**B.**) two weeks after treatment exposure (05.12.2025).

Glucose

Average hemolymph glucose levels were highest for the crabs living in the ambient control tank (0.31 ± 0.22), followed by the crabs in the high temperature, high dose (0.20 ± 0.063); high temperature, low dose (0.19 ± 0.016); ambient, high dose (0.13 ± 0.035); high temperature control (0.075 ± 0.041); and ambient temperature, low dose (0.071 ± 0.058) treatments (Fig. 2). Across temperature treatments, the high temperature saw hemolymph glucose levels decrease with decreased levels of ibuprofen exposure (Fig. 2). Conversely, the ambient temperature saw the lowest glucose levels in the crabs exposed to the low dose of ibuprofen and the highest glucose levels in the no-drug group (Fig. 2).

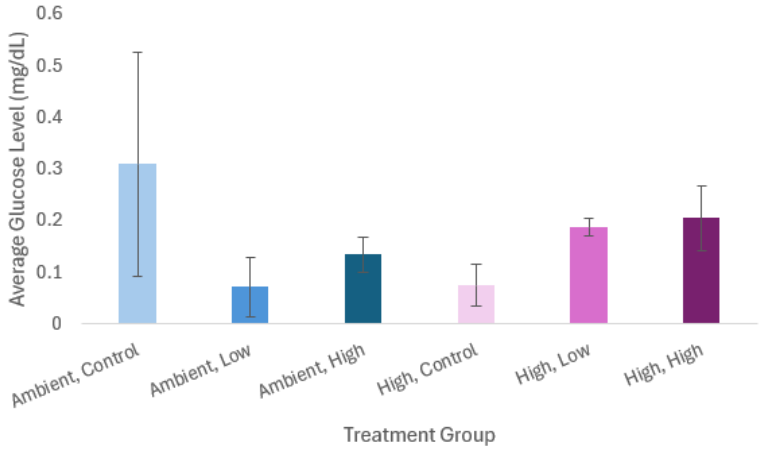

Figure 2. The average glucose levels in the hemolymph of *H. oregonensis* after two weeks of treatment. Treatment groups are designated as "water temperature (°C), ibuprofen dose (ug/L)." Error bars are standard deviations.

Discussion

NSAIDs are some of the most common drugs purchased today, where ibuprofen sees consumption rates of around 180 tons per year in Germany alone (Cleuvers 2004). This has led to the rising concern around the impacts of NSAIDs in the aquatic environment and their impact on marine life. This study looked at addressing the question of physiological responses of hairy shore crabs to increased levels of ibuprofen along with increased temperatures.

After two weeks, we found overall lower oxygen consumption rates in crabs exposed to the high temperature treatment groups as compared to crabs exposed to the ambient temperature. This did not align with our hypothesis and contradicted the study by Nieto et al. (2016) that found increases in oxygen consumption when exposed to higher temperatures for *Atyaephyra desmarestii*. This difference could be explained by the compounding impacts of ibuprofen exposure on oxygen consumption and metabolic demands, as well as an expression of metabolic depression after a long exposure to high temperature as Nieto et al.'s (2016) experiment only lasted seven days in comparison to our twelve-day study.

In the high temperature treatments, crabs that were not exposed to ibuprofen had the highest oxygen consumption. This did not match our hypothesis but aligns with the idea that crabs exposed to ibuprofen were experiencing levels of metabolic depression and subsequently showed lower oxygen consumption. Aguirre-Martinez et al. (2013) similarly saw a "diminished health status" for *Carcinus maenas* (European green crab) exposed to increased levels of ibuprofen when compared to control groups, which may provide evidence for the possible metabolic depression seen here.

Week one saw the lowest oxygen consumption rates in crabs exposed to high temperature and low levels of ibuprofen. This could present a possible acute ibuprofen threshold where short periods of low-level exposure suppress crab physiological responses, but after longer exposure, the rates go back up. This can be seen by the increased oxygen consumption during week two. However, Cleuvers (2004) hypothesized acute effects are less likely than chronic ones, but Blasco and Trombini (2023) argue that drug impacts are species, dosage, and seasonally dependent. This result could also be from our low sampling size.

Previous studies have found no impact of temperature on glucose and lactate levels (Eades et al. 2010), while some have found higher levels of glucose in species exposed to high stress environments (Soares et al. 2022). These results are unlike the ones found in this study, where exposure to stressors (temperature and/or ibuprofen) saw a reduction in glucose levels across crabs sampled. This overall decrease mirrored our hypothesis, but the specific levels of decrease did not. The results could be due to the confounding effects of multiple stressors on the crabs. Nieto et al. (2016) stated that ibuprofen was designated in the European Union's Directive 93/67/ECC as "harmful" to aquatic species, but that the Directive does not account for other compounding stressors. In their study, they showed that a similar NSAID also designated as "harmful", when combined with increased temperature, resulted in the mortality of the study species, aligning it with the Directive's "toxic" status definition (Nieto et al. 2016). Similarly, we could be seeing severe reductions in metabolic proficiency via these low glucose levels when combining ibuprofen exposure and temperature increases.

Arrigo et al. (2025) also poses that European green crab sexes respond differently to increasing temperatures. Particularly, males stored glycogen while females depleted their energy stores when exposed to temperature stressors (Arrigo et al. 2025). We did not determine the sexes of our crabs for this experiment. Therefore, sex may be playing a role in glucose levels and causing the relatively low levels across all groups except for our control, but we are unable to decipher these specific interactions.

Pharmaceutical contamination within the aquatic environment has become an increasingly important area of study, especially when trying to understand bioaccumulation in the food web starting at lower trophic levels. Of the drugs spilling into the environment, NSAIDs are one of the most prevalent. There have been relatively few studies designed to understand the impacts of such drugs on indicator species of overall aquatic ecosystem health like invertebrates. Though some studies have shown adverse physiological effects of NSAIDs on the functioning of invertebrates and fishes. Here, we saw how increasing temperature and different dosages of ibuprofen can have negative impacts on oxygen consumption rates and glucose level in hairy shore crabs. However, our study was not able to get at the underlying physiological mechanisms behind the decreased oxygen consumption and decreased glucose levels. Future studies should look into cellular and tissue responses to these stressors to get at underlying physiological mechanisms (Aguirre-Martinez et al. 2013; Parolini et al. 2011). Future studies should also examine how chronic exposure to drugs impacts these species to evaluate possible dosage thresholds of NSAIDs and other drugs on aquatic species (Cleuvers 2004; Fent et al. 2006). Combining stressors will further help us understand the real-world application of these studies on the environment as no stressor is felt alone. Further studies like this will help shine light on how pharmaceuticals and increasing temperatures are projected to collectively impact our ecosystems as society continues to grow and develop.

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