The Ecophysiological Effects of Water Temperature and Ibuprofen on Hemigrapsus oregonensis

Introduction

Marine ecosystems' ecological health is increasingly threatened by pharmaceutical drugs in wastewater, raising concerns regarding their physiological impacts on marine organisms. Pharmaceuticals, notably ibuprofen—a commonly used nonsteroidal anti-inflammatory drug (NSAID)—have been documented to disrupt biological functions in aquatic invertebrates (Crane et al., 2006; Ericson et al., 2010). Aguirre-Martínez et al. (2013) demonstrated significant oxidative stress and DNA damage in marine crabs that were chronically exposed to pharmaceutical contamination.

Simultaneously, marine species are facing rising physiological stress from increases in water temperature due to climate change (Rodrigues et al., 2015). *Hemigrapsus oregonensis*, an ecologically important species of crab, serves as a reputable bioindicator for evaluating these environmental stressors due to its resilience. While previous research has independently studied pharmaceutical pollution and temperature stress, their combined effects remain inadequately explored. Additional studies, such as Trombini et al. (2021) and Sumpter & Margiotta-Casaluci (2022) emphasize the need for examining physiological endpoints under multiple stressors.

This study aims to investigate how ibuprofen exposure and different temperatures influence the physiological processes of glucose metabolism and respiration rates in *H. oregonensis*. We hypothesized that crabs exposed to higher water temperatures and higher doses of ibuprofen would show increased physiological stress, measured by increased oxygen consumption and depleted glucose levels. Understanding these impacts can help predict broader ecological consequences on more economically important crab species and inform policymakers.

Methods

A controlled laboratory experiment was designed using 18 *H.oregonensis* crabs randomly divided into treatment and control groups. Artificial saltwater (2 liters per container) was prepared in six containers, maintained at two temperature settings: ambient (13°C) and elevated (27°C). Ibuprofen concentrations were set at an environmental dose (6.25 µg/L) and a high dose (31.25 µg/L), based on prior literature (Eades & Waring, 2010; Ericson et al. 2010). The experiment comprised six distinct treatments: (1) ambient temperature without ibuprofen, (2) ambient temperature with environmental ibuprofen dose, (3) ambient temperature with high ibuprofen dose, (4) elevated temperature without ibuprofen, (5)

elevated temperature with environmental ibuprofen dose, and (6) elevated temperature with high ibuprofen dose.

Oxygen consumption was measured weekly using a resazurin-based assay to quantify metabolic rates. Crabs were weighed to the nearest hundredth of a gram to normalize metabolic data. Each crab was placed in an individual chamber with 35 mL of resazurin working solution (prepared with Instant Ocean seawater, DMSO, and antibiotics), and 200 µL samples were withdrawn every 30 minutes over a 90-minute period. These were plated in a 96-well plate and read on a plate reader at Excitation 530 nm and Emission 590 nm. Fluorescence values were normalized to crab mass.

Glucose concentrations were measured from hemolymph extracted at the end of the experiment. Samples were analyzed using the Cayman Glucose Colorimetric Assay Kit (Item No. 10009582). This assay detects glucose via enzymatic oxidation and a subsequent peroxidase-mediated colorimetric reaction, measured at 514 nm. Each sample was diluted in assay buffer and analyzed in duplicate using a microplate reader. A glucose standard curve was prepared to convert absorbance to concentration (mg/dL), and results were normalized per individual crab.

Results

This study tested the hypothesis that higher temperatures and higher ibuprofen doses would increase physiological stress in *H. oregonensis*, as measured by elevated oxygen consumption and depleted glucose levels. During week one, the lowest oxygen consumption rates were recorded in crabs exposed to environmental doses of ibuprofen, regardless of temperature. In contrast, the highest oxygen consumption rates occurred under elevated temperature conditions without ibuprofen exposure during week 1 (Figure 1a). Notably, two mortalities occurred: one in the ambient temperature-high ibuprofen dose treatment and one in the elevated temperature-no ibuprofen treatment. During week 2 (Figure 1b), all three high temperature treatments showed the lowest oxygen consumption, while the ambient temperature high dose and the control group showed the highest oxygen consumption.

The hemolymph glucose assay showed our control group had the highest glucose level at 0.31 Mg/dL. The ambient temperature, environmental dose, and high temperature no dose had the lowest glucose levels, which were both below 0.1 Mg/dl. The next highest glucose level was ambient temperature high dose, high temperature environmental dose, and high temperature high dose at 0.13, 0.07, and 0.2 Mg/dL, respectively.

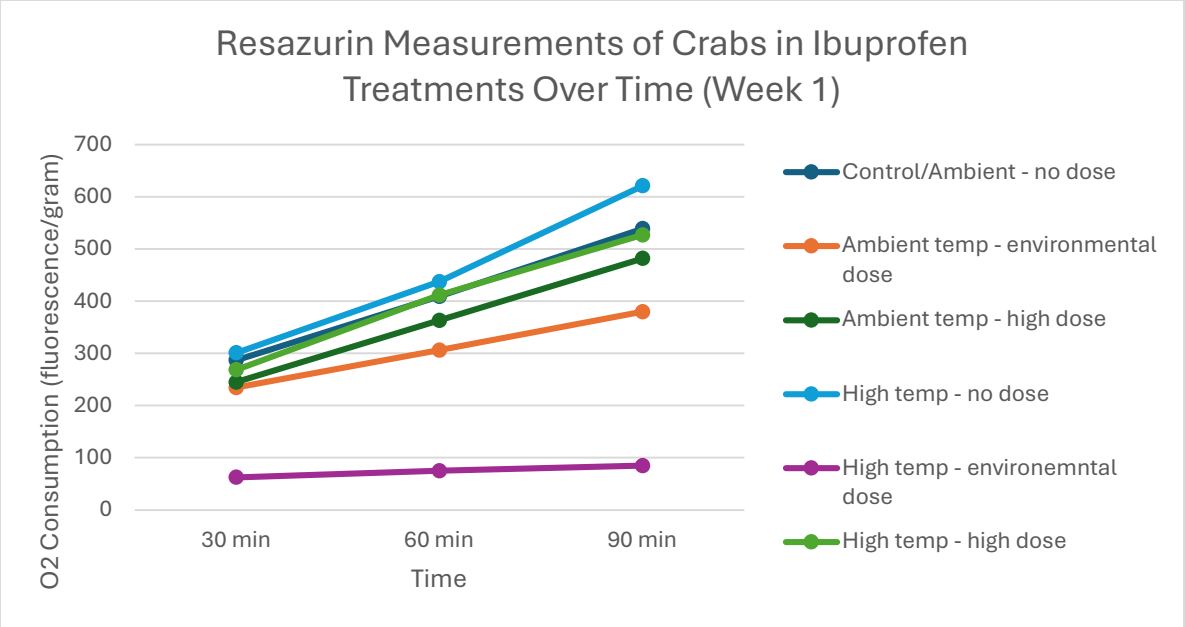

Figure 1a: Oxygen consumption rates across experimental treatments, week 1.

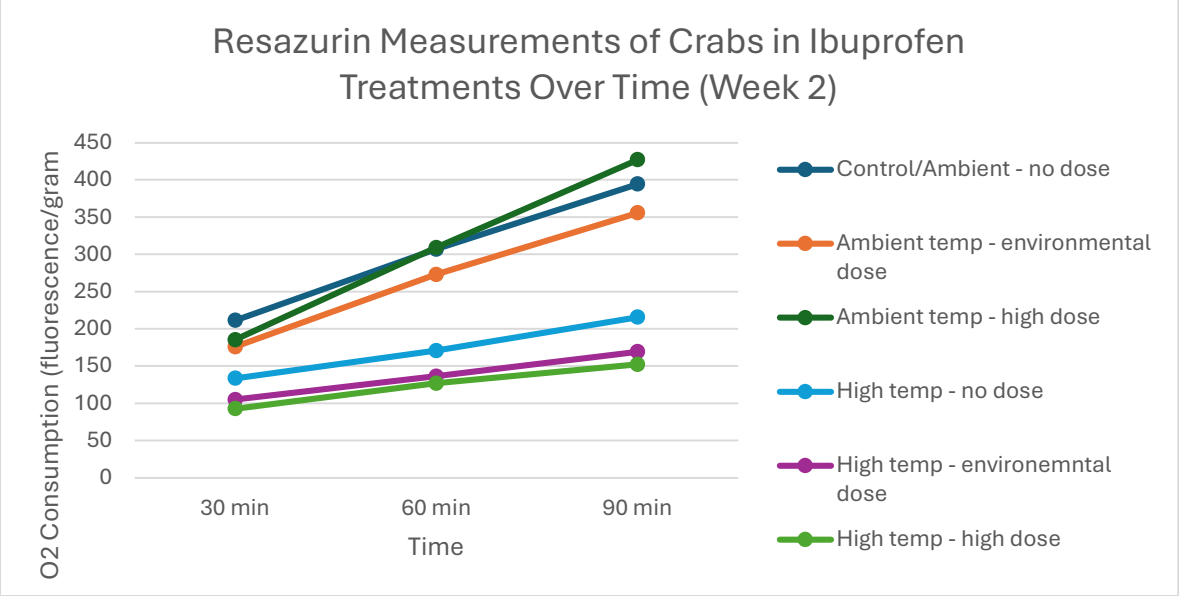

Figure 1b: Oxygen consumption rates across experimental treatments, week 2.

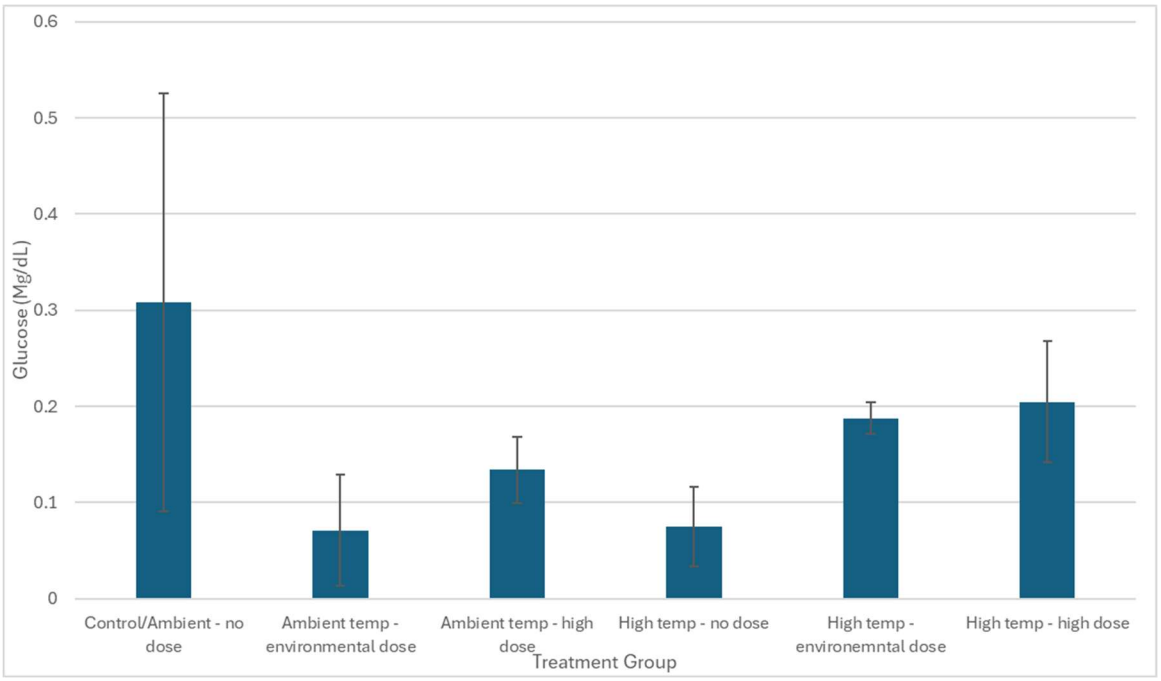

Figure 2: Hemolymph glucose concentrations across experimental treatments.

Discussion

The findings from this study demonstrate that both temperature and ibuprofen exposure independently and interactively influence physiological responses in *H.oregonensis*. Oxygen consumption and glucose concentration patterns suggest that elevated temperature generally increased physiological demand, though the lower glucose in ambient temperatures couldn't be explained by literature.

Increased oxygen consumption during week one under high temperature and no ibuprofen conditions suggests heightened metabolic activity, a response typical of thermally stressed ectotherms (Rodrigues et al., 2015). By week two, all high-temperature groups displayed reduced oxygen consumption, potentially indicating metabolic suppression or fatigue under prolonged heat exposure. This pattern mirrors other research in crustaceans experiencing chronic stress (Yang et al. 2020).

The hemolymph glucose results showed an important factor. Since all crabs were fasted, lower glucose levels are best interpreted as depletion of internal energy stores, signaling more severe stress. The high temperature and no ibuprofen group had the lowest glucose concentrations, showing the elevated stress. In contrast, the control group maintained the highest glucose levels, suggesting the least stress, which makes sense. However, a limitation was that the control group had a very high variance which suggests maybe more replicates should have been used.

Yang et al. 2020 found that fish exposed to NSAIDS and thermal stress reduced oxygen consumption due to fatigue and energy depletion which is congruent will our results. Rodrigues et al. 2015 showed European green crab (*Carcinus maenus*) that had short term heat exposure had elevated O2 consumption, but long-term heat exposure suppressed O2 consumption. We see a similar pattern week over week with our higher temperature treatments respirating less than ambient treatments. Trombini et al. 2021 showed depleted glucose levels in crayfish (Procambarus clarkii) exposed to NSAIDs. All Ibuprofen treatments in our experiment had substantially lower glucose then the control.

This data provides preliminary evidence that *H. oregonensis* is sensitive to both rising temperatures and pharmaceutical contaminants, with interactive effects that complicate stress responses. Future work should extend exposure duration to track chronic responses and study a wider range of biomarkers of stress. Given ongoing warming and pharmaceutical runoff in coastal environments, these findings have implications for estuarine species management and policy reform related to wastewater regulation.

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