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Physiological Effects of Carbon Contamination on *Hemigrapsus oregonensis*

Abstract

Urbanization leads to heavy metal pollution from sources such as stormwater runoff, marinas, infrastructure, and industrial processes. This has been seen especially in the Puget Sound in Washington State, where highly urbanized areas with high boat traffic lead to increased copper being transported into the ocean. It is of special importance to monitor the effects copper has on marine organisms, especially those of great commercial importance. In the Puget Sound, this includes several species of crab used in commercial fishing. In this study, we tested the physiological effects of copper contamination on a model crab species, the Yellow shore crab (*Hemigrapsus oregonensis)*, in a laboratory setting. We exposed nine individuals to copper sulfate pentahydrate to a concentration of 25 mg/L Cu and nine individuals to a concentration of 167 mg/L Cu. Respiration data were collected using resazurin assays, and righting times were collected for each treatment and control group. It was found that crabs exposed to copper showed significantly lower levels of respiration than crabs not exposed to copper. We also found that copper-exposed crabs generally had longer righting times than control crabs. Copper-contaminated crabs also had a higher mortality rate than control crabs, with the highest polluted group having the highest levels of mortality. These results suggest that copper contamination affects respiration and cognitive function in Yellow shore crabs to a lethal level. This study shows the potential dangers of copper on commercially significant species, and actions need to be taken in order to control copper pollution in order to conserve species of great importance.

Introduction

While natural concentrations of dissolved copper (II) in coastal waters are low, anthropogenic sources are causing increases of copper pollution near urbanized areas (Gledhill et al. 1997). Anthropogenic sources include surface runoff, groundwater, wastewater treatment, and atmospheric deposition from particles of industrial processes (Norton et al. 2011). Even though copper is an essential micronutrient that is necessary for many metabolic processes, elevated levels of copper become toxic for marine organisms (Flemming and Trevors 1989). In the Puget Sound area of Washington State, special interest has been taken in monitoring and evaluating the effects of copper contamination on marine organisms of commercial importance. These species include the Dungeness crab (*Metacarcinus magister*) and the Red rock crab (*Cancer productus*). The fisheries of these species are valued at over $88.2 million in recent years (Washington Department of Fish and Wildlife 2025). An anthropogenic source of copper of interest to state managers is antifouling paints used on marine vessels to prevent biota from growing on hulls and to reduce the costs of maintenance (Goetz 2018). These antifouling paints are copper-based and work by constantly leaching small amounts of copper into the water to prevent attachment and growth of marine biota (Goetz 2018). Legislation to ban the use of copper-based antifouling paints has been under consideration in Washington since 2011, but has been delayed due to further studies needed to assess the risk posed by copper contamination compared to other heavy metal-based paints (Goetz 2018).

Previous studies on Cu contamination in crabs have found that increased concentrations of copper led to increased heart rates, which was thought to correlate with an impairment of respiratory function (Bamber and Deepledge 1997). It has also been seen to inhibit mating responses by blocking pheromone stimulus receptors, resulting in longer mating initiation times and an increase in non-mating behavior (Krång and Ekerholm 2006). Further studies into the respiratory and behavioral effects of copper contamination are needed to gain better insights into the different pathways copper inhibits physiological processes in crabs.

In this study, we examine the respiratory and behavioral impacts that copper contamination has on the Yellow shore crab (*Hemigrapsus oregonensis*). By exposing shore crabs to different concentrations of copper, using resazurin assays, and measuring righting times, we are able to examine how respiratory and behavioral functions are impacted by copper contamination.

Methods

The Yellow shore crab was used as a model organism due to its presence in the Puget Sound and its phylogenetic proximity to Dungeness and red rock crabs. Nine crabs were placed in a tank of 167 mg/L Cu, and nine crabs were placed in a tank of 25 mg/L Cu. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was used due to its availability and solubility. Temperature was kept at 15°C. Salinity, pH, and oxygen levels were kept the same across treatment and control tanks. Crabs were collected from Lion's Park boat launch between 11:30 and 13:34 on a -1.68 tide in mixed substrate.

To gain respiration data, resazurin assays were performed. After one week of exposure to copper, three low-concentration and three high-concentration crabs underwent rezaruin assays. The assays involved placing the crabs in a container containing 149 mL seawater, 133 µL resazurin stock, 150 µL DMSO, and 1.5 mL antibiotic solution. Every 30 minutes, 200 µL was drawn from each container and placed in wells for 90 minutes total. The solutions were run in a plate reader to obtain fluorescence values. This process was repeated after two weeks of exposure; however, due to mortality in the high concentration group, only one individual was tested. Four low-concentration crabs were measured in the second week as well. Fluorescent levels were normalized for crab body weight. Average fluorescence normalized by body weight was calculated by combining results from both week 1 and week 2. Both weeks were combined due to mortality limits, with week 2 not having enough individuals left for meaningful results to be shown separately.

To gain behavioral data, righting times were measured. Four low-concentration crabs and four high-concentration crabs were placed on their backs in an empty tank filled with seawater by themselves. They were held on their backs, and we timed how long it took them to right themselves up after being let go. This was repeated after two weeks of exposure; however, only three low-concentration crabs were measured, and one high-concentration crab was measured due to mortality.

Results

Five crabs from the high concentration tank died after one week of exposure, with three more dying after two weeks of exposure. One crab from the low concentration tank died after one week of exposure, and three more died after two weeks of exposure. This left us with a mortality rate of 75% in the high concentration tank and 43% in the low concentration tank. A caveat to these results is that we had several crabs die during the resazurin and hemolymph measurements. Hemolymph ended up not being included in this study due to low amounts being able to be extracted and inconclusive results from that. In total, two high-concentration and one low-concentration crab died during resazurin testing.

The resazurin assays revealed that copper exposure led to inhibited respiration (Figure 1). After one week average fluorescence/body mass measured for the control group were: 194

RFU/g 30 minutes, 261 RFU/g 60 minutes, and 314 RFU/g 90 minutes. In the low concentration group: 161 RFU/g 30 minutes, 178 RFU/g 60 minutes, and 178 RFU/g 90 minutes. In the high concentration: 104 RFU/g 30 minutes, 140 RFU/g 60 minutes, and 153 RFU/g 90 minutes. The decrease in RFU/g shows inhibited respiration and metabolic activity in crabs exposed to copper.

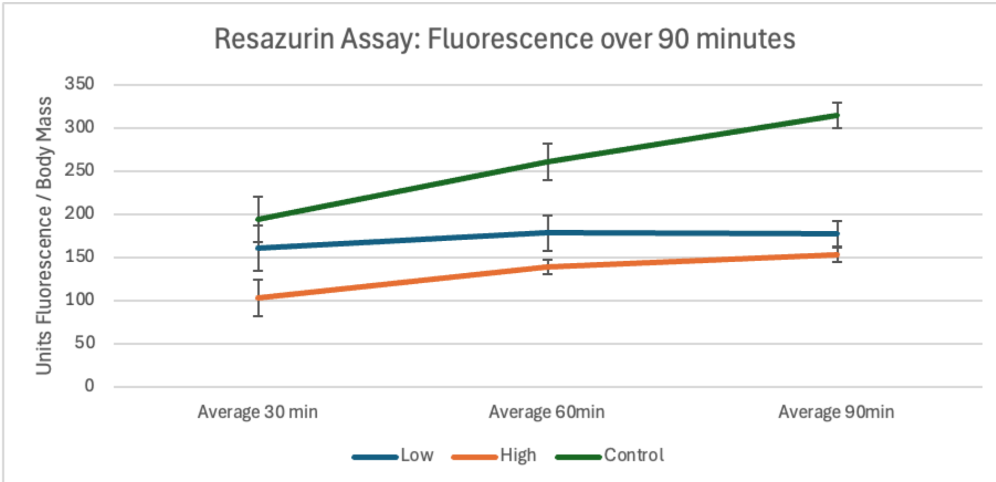

Figure 1: Average units of fluorescence/body mass over 90 minutes in the control, high concentration treatment, and low concentration treatment groups. This graph combines all resazurin assay results from exposure in both week 1 and week 2. Each group shows increased fluorescence levels across the 90 minute period, consistent with the crabs respiring. However, in the treatment groups, we see a significant drop in respiration, with the lowest levels being in the highest concentration group.

We saw an increase in average righting times in the copper-exposed group, but with large standard deviations (Figure 2). While all three groups had at least one individual able to right itself in under one second, the high concentration group was the only group that had individuals take over 5 seconds to right themselves. With the highest taking 16.33 seconds, and one individual not able to right itself altogether, even after 30 seconds. The only high-concentration individual that was able to be tested after two weeks of exposure took 12.33 seconds to right itself. Most individuals in the control group took under 1 second to right themselves, however, one took 3.36 seconds. Most individuals in the low concentration group took between 1-4 seconds to right themselves. The three individuals tested after two weeks of exposure took 2 seconds, 1.11 seconds, and 2.2 seconds to right themselves.

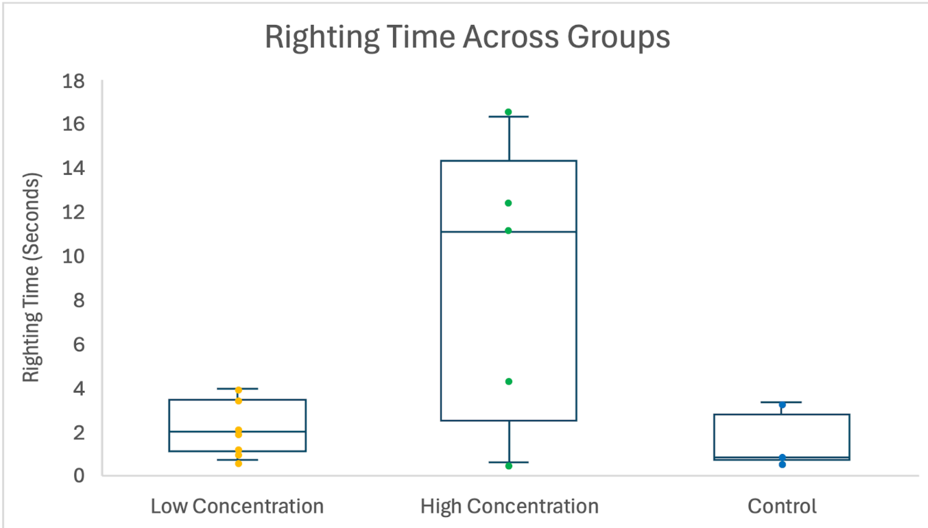

Figure 2: Bar and whisker plot showing average righting times in seconds across treatment and control groups. There were large standard deviations in each group, however, the high concentration group was the only group with significantly long righting times over 5 seconds. The low concentration group had a longer average righting time than the control group, with most falling between 1-4 seconds. These results show a definite trend in copper inhibiting righting function in crabs, however, a few copper-exposed crabs were less affected than others.

<u>Discussion</u>

These results show that copper contamination has lethal, direct negative effects on shore crabs' respiration and righting processes. The inhibition of respiration in crabs could be due to copper's influence on enzymes and metabolites involved in metabolic processes, as seen in other studies (Hansen et al. 1992). The enzymes involved in glycolysis, the Krebs cycle, and mitochondrial electron transport chain have been shown to be inhibited by copper contamination, including hexokinase, phosphofructokinase, pyruvate kinase, and citrate synthase (Lauer et al. 2012). It is possible that the decrease in metabolic enzymes led to inhibited metabolism in the treatment groups, which would explain the lower respiration rates. Respiratory stress may have been the reason behind the mortality in the copper-exposed groups.

Delays in reaction times to mating pheromones have been seen in male shore crabs, however, there are few studies on their effect on righting times (Krång and Ekerholm 2006). The exact mechanisms that cause this delay in righting time are difficult to pinpoint, as copper has been shown to affect several physiological processes, including stimulation of receptor sites, gill cell damage, metabolic rates, and hemolymph osmolarity (Hebel et al. 1997). The delayed righting times seen in this study could be explained by copper's inhibition of stimuli, or possibly the reduced metabolic processes that may have led to the crabs not having the energy to right themselves quickly.

This study highlights just a few ways copper affects the physiological processes of Yellow shore crabs. The results laid out in our study could prove significant to the current legislation considerations of copper-based antifouling paints in the Puget Sound, especially in consideration of similar commercially important species like Dungeness and Red rock crabs. Copper pollution should continue to be monitored, and other antifouling methods should be considered for vessels in the Puget Sound in order to preserve important and sensitive marine organisms.

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