Impacts of Antifouling paint on *Hemigrapsus oregonensis* and their stress physiology response

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Introduction

Every day, toxic chemicals leak into coastal habitats and cause irreversible damage to marine organisms and the ecosystems they inhabit. These toxic chemicals are a result of antifouling paint. Antifouling paint is a product that is typically applied to marine substrates such as boats and buoys to prevent biofouling, the accumulation of algae and small marine organisms on surfaces. Antifouling paint is used to prevent the organisms from latching onto surfaces and is known to contain toxic amounts of metals such as copper, lead, and zinc (Rees et al., 2014). When trace metals like copper, zinc, and lead enter the marine environment, they tend to dissolve after a period of time. When this happens, marine species can absorb these trace metals through their gills and skin and from consuming sediment. Although trace metals are important nutrients, (copper is used as a cofactor for enzymes involved in respiration and other metabolic processes (Turner, 2010)), when they are over a certain concentration, they can become toxic and can impact growth, regulation, and other processes (Katranitsas et al., 2003). These toxic chemicals impact many species in the coastal habitats they leak into, such as hairy shore crabs (*Hemigrapsus oregonensis*). Hairy shore crabs are a ubiquitous native species in the Puget Sound, serving as model organisms due to ease of accessibility (Rudy Jr et al., 2013). Given the information about the toxicity of antifouling paint, we were curious to determine its impacts on the stress physiology of hairy shore crabs. Our experiment took inspiration from a model experiment about the impacts of antifouling paint on *Artemia* brine shrimp. When *Artemia* were exposed to a 100 mm2 surface panel covered with antifouling paint in 8 mL of seawater, there was a reported 90% mortality after 48 hours (Persoone & Castritsi-Catharios, 1989). In other studies, copper was found to be toxic through oxidative stress. The osmotic and ionic processes cease when copper increases (Cima & Varello, 2023), and this will prevent the organism from maintaining a homeostatic state and increase the amount of stress on the individual. When considering the size of boats and buoys, they are covered by much more antifouling paint than the test panel in the Artemia study, indicating that they are likely much more toxic. Given the variation in scale of antifouling paint applications in the real world, determining baseline toxicity impacts of antifouling paints from experiments can provide important information about how hairy shore crabs are impacted by the antifouling paints in the coastal ecosystems. As a proxy for stress physiology, lactate, triglycerides, and righting time (time it takes for a crab to right itself) data can ascertain the influence of antifouling paints on hairy shore crab function. Lactate is a byproduct of anaerobic respiration and elevated levels typically indicate shock/lack of oxygen, and triglycerides are a form of energy storage that can indicate if environmental stressors are

depleting energy, and with changes in antifouling paint concentrations there are changes in the concentrations of these molecules. With an increase in concentration of antifouling paint, there will be a correlating increase in righting time and lactate and triglyceride use.

Methods

To conduct this experiment, 3 tanks were filled with 1 L 15 ℃ water and 5 crabs were put in each tank, shown in Figure 1 below. A piece of aluminum foil of varying size covered in Trilux 33 antifouling paint was placed into each tank at the beginning of the experiment to simulate chemical leaking. One tank had a piece of 2 cm x 2 cm foil, another had a 4 cm x 4 cm piece, and the third had an 8 cm x 8 cm piece.

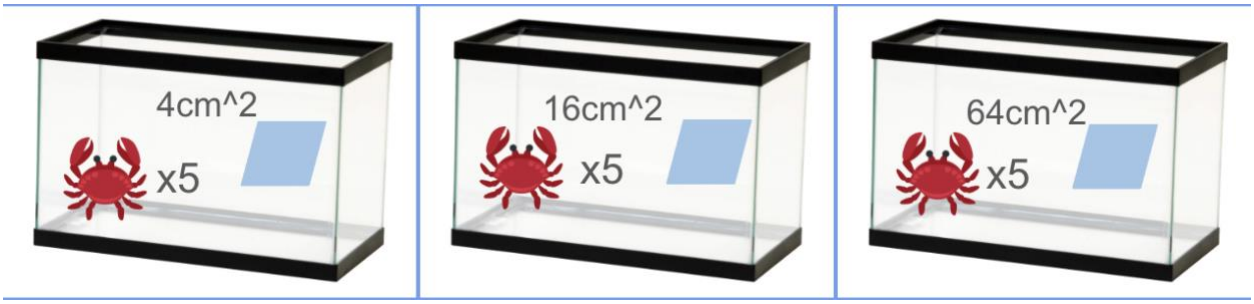

Figure 1 – Depiction of antifouling paint experiment, consisting of 3 tanks each containing 5 crabs and a piece of antifouling paint.

To determine the time it took for crabs to right themselves, each crab was placed on its back and a stopwatch was started and stopped when the crab was back on its legs, and this was repeated for each crab. To assess lactate & triglyceride concentrations in hairy shore crabs, hemolymph samples were collected from the 15 individuals. Extractions were taken from the base of the walking legs of the crabs using sterile 1 mL needles. Physiological assays were conducted following Cayman Chemical L-Lactate Assay & Triglyceride Colorimetric Assay manufacturer instructions.

Results

Righting time data and lactate and triglyceride data was to be used as a proxy for the stress response of *Hemigrapsis oregonensis* in response to the presence of antifouling paint. At the end of the experiment, there were two non-samples (one mortality, one pregnancy). Hemolymph samples were not sufficient for lactate and triglyceride data, so only lactate data was analyzed, depicted below in Figure 2.

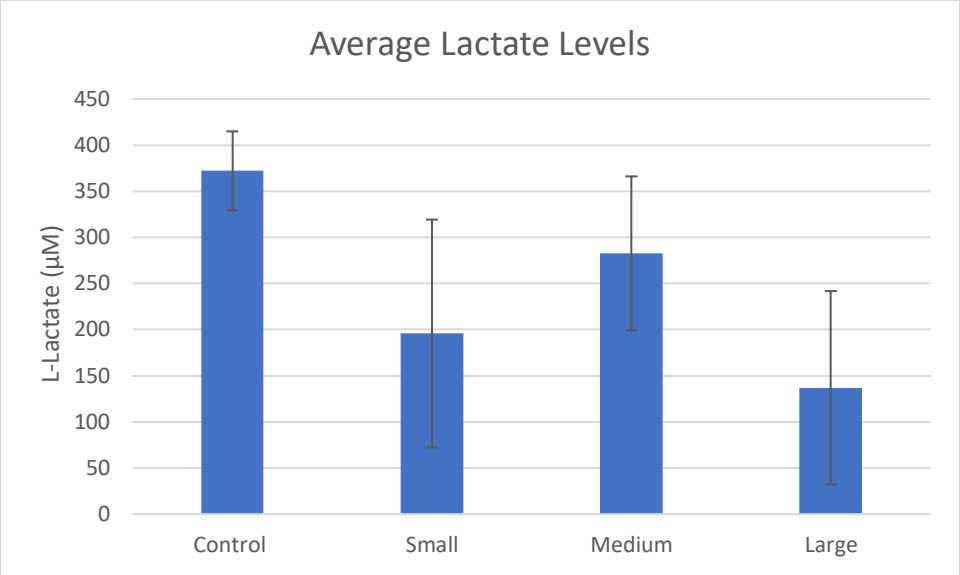

Figure 2 – Average Lactate concentrations per group, with small, medium, and large indicating antifouling paint amout.

Lactate levels were lower in the small 4cm^2 group (SD 123.5±), larger in the medium 16cm^2 group (SD 83.5±), and lowest in the 64cm^2 group (SD 104.8 ±). There were similar changes in righting time, depicted below in Figure 3.

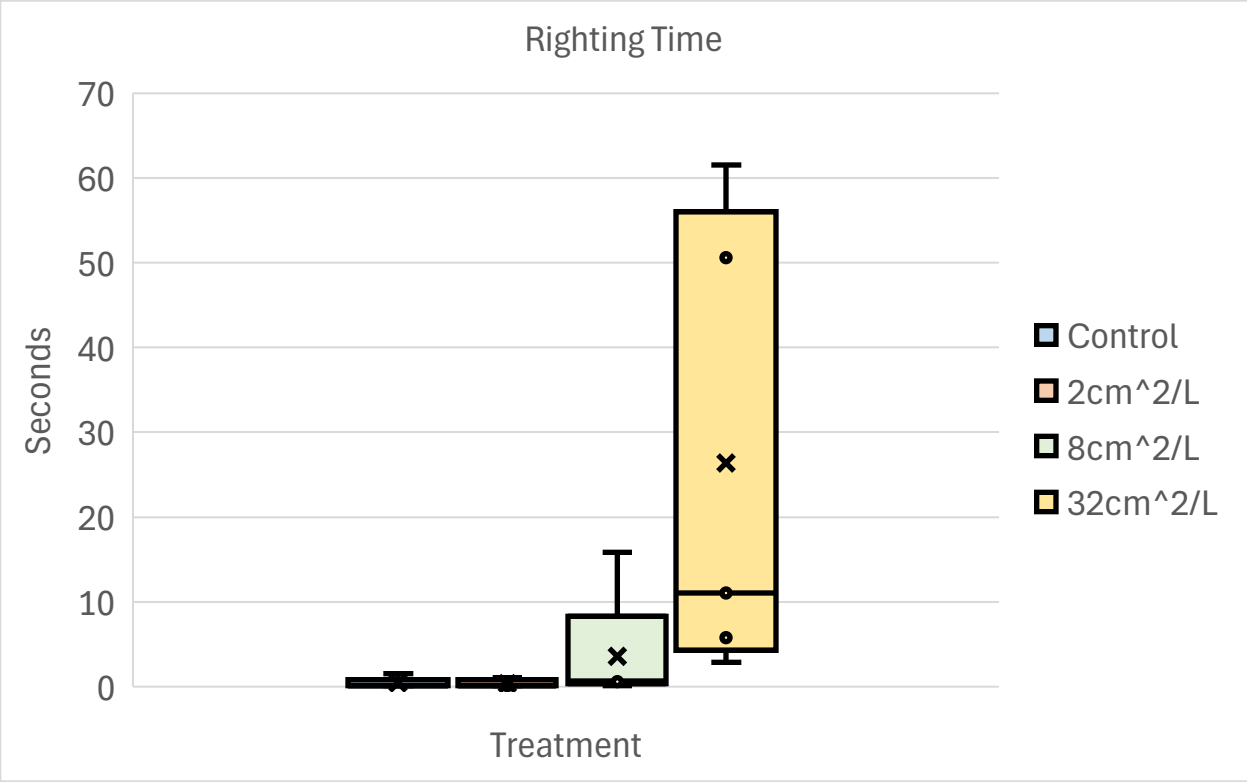

Figure 3 – A box and whisker plot showing the change in righting time for each size of antifouling paint, with x marking median data and dots indicating upper and lower quartile ranges.

There was little to no change between the control and small antifouling paint group, but there was a noticeable increase from the small to medium group, and a significant increase in the large antifouling paint group. The p-values suggested some statistical significance between the control and large antifouling paint group ($p < 0.05$) but not for the small and medium groups ($p = 0.44$ and $p=0.17$, respectively.

Discussion

The aim of this study was to determine baseline toxicity impacts of antifouling paints from experiments that could provide important information about how hairy shore crabs are impacted by the antifouling paints in the coastal ecosystems. We hypothesized that with an increase in antifouling paint concentration, we would see an increase in lactate concentrations, triglyceride energy storage use, and righting time, all signs of a stress response. Our experiment yielded results that were mostly consistent with our hypothesis with some slight deviations. Righting time increased with increased antifouling paint concentration as expected, although the results were not statistically significant (excluding the large group). This is likely due to sample size of only $n = 5$ crabs, and variations in how the crabs were placed on their backs, as some crabs slipped out of our hands from higher up, which could contribute to increased righting time as they may have been dazed. With consistent placement of crabs, more accurate stopwatch information such as through laser timing, and a much larger sample size, data might paint a more accurate picture and be more statistically significant. Lactate concentrations were unexpected as the large antifouling paint concentration group showed the least amount of lactate present, when it was expected that they would be highest. This may have been due to extra oxygen being supplied to the tank, making anaerobic respiration not necessary. Regardless of these experimental errors, the data clearly shows that antifouling paint has a noticeable impact on crab physiology and stress response in experimental conditions with small concentrations of antifouling paint, indicating the in-situ impacts could be significantly worse. In comparison with prior studies about the toxicity of antifouling paint (Persoone & Castritsi-Catharios, 1989), crabs did not suffer majority mortality, which could indicate that different antifouling paints are more toxic than others, or some take more time to degrade and become harmful, as our study only ran for 1 week. This could be another area of study, determining if there is a less destructive type of paint. When we consider the sheer scale of antifouling paint that is present in marine environments where *Hemigrapsis oregonensis* is also present, there is a clear need to further understand the impacts of antifouling paint on the physiology of hairy shore crabs to not only understand how they will be impacted, but how other species could in turn be affected as well.

References

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