Impacts of Antifouling Paint on *Hemigrapsus Oregonensis*

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Background

What is antifouling paint?

- Material applied to boats, buoys, mile markers, and other marine infrastructure to prevent biofouling - accumulation of microorganisms, plants, algae, and small animals on unwanted surfaces.

Why should we study antifouling paint?

- Antifouling paints contain noticeable amounts of trace metals, which are known to impair growth and reproduction in marine organisms and disrupt ion regulation.

How Strong is Our Paint ?

5086
Aluminum

5086
Aluminum &
Trilux ® 33

Courtesy of Washington Wave RSO

Previous Research on Antifouling Paint

A simple bioassay with *Artemia* larvae to determine the acute toxicity of antifouling paints

Persoone, G. & Castritsi-Catharios, J.

- Created different surface area-to-volume ratios of antifouling paint to expose larval brine shrimp
- Resulted in an 80-95% mortality rate with high exposure

Effects of waterborne copper delivered under two different exposure and salinity regimes on osmotic and ionic regulation in the mudflat fiddler crab, Minuca rapax (Ocypodidae, Brachyura)

Capparelli et al.

- Copper is an osmoregulatory toxicant, especially when above 250 µg Cu/L
- The osmotic and ionic processes cease when copper increases

Research Question

How does antifouling paint impact *Hemigrapsus oregonensis* crab ecophysiology?

Experiment Hypothesis

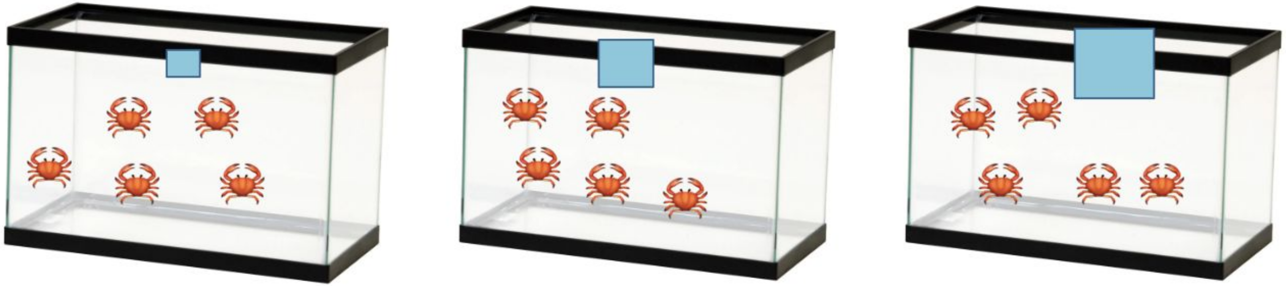
Null: There will be no changes to crab ecophysiology in response to antifouling paint

Alternative: There will be an increase in the righting time and lactate levels with crabs exposed to increasing amounts of antifouling paint

Experimental Setup

- 15 °C and 33 ppt salinity
- 5 crabs per 2 liter tank
- One piece of tinfoil sprayed with antifouling paint per tank
  - Ratios: 2cm^2/L, 8cm^2/L, 32cm^2/L

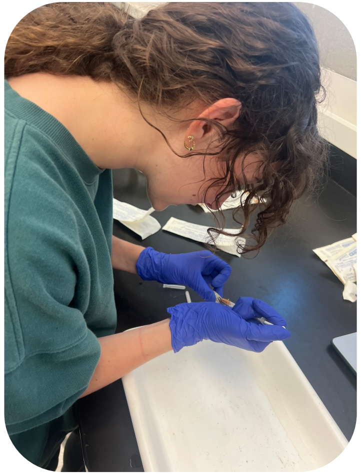
<u>Active ingredient:</u>
Cuprous Thiocyanate biocide
<u>Chemical Formula:</u>
$CuSCN$



Surface Area of ■ : 4 cm^2 16 cm^2 64 cm^2

Stress Response Methods

- **Righting Time** - how long it takes a crab to flip back over after being placed on its back
  - Immediate flips counted as 0.1 seconds to account for human delay in stopping timer
- **Lactate Levels -** hemolymph extracted and assayed using Cayman L-Lactate Assay



Circle of Life

- 1 crab died
- 1 crab became gravid -> righting time taken but no hemolymph
- Both in $2cm^2/L$

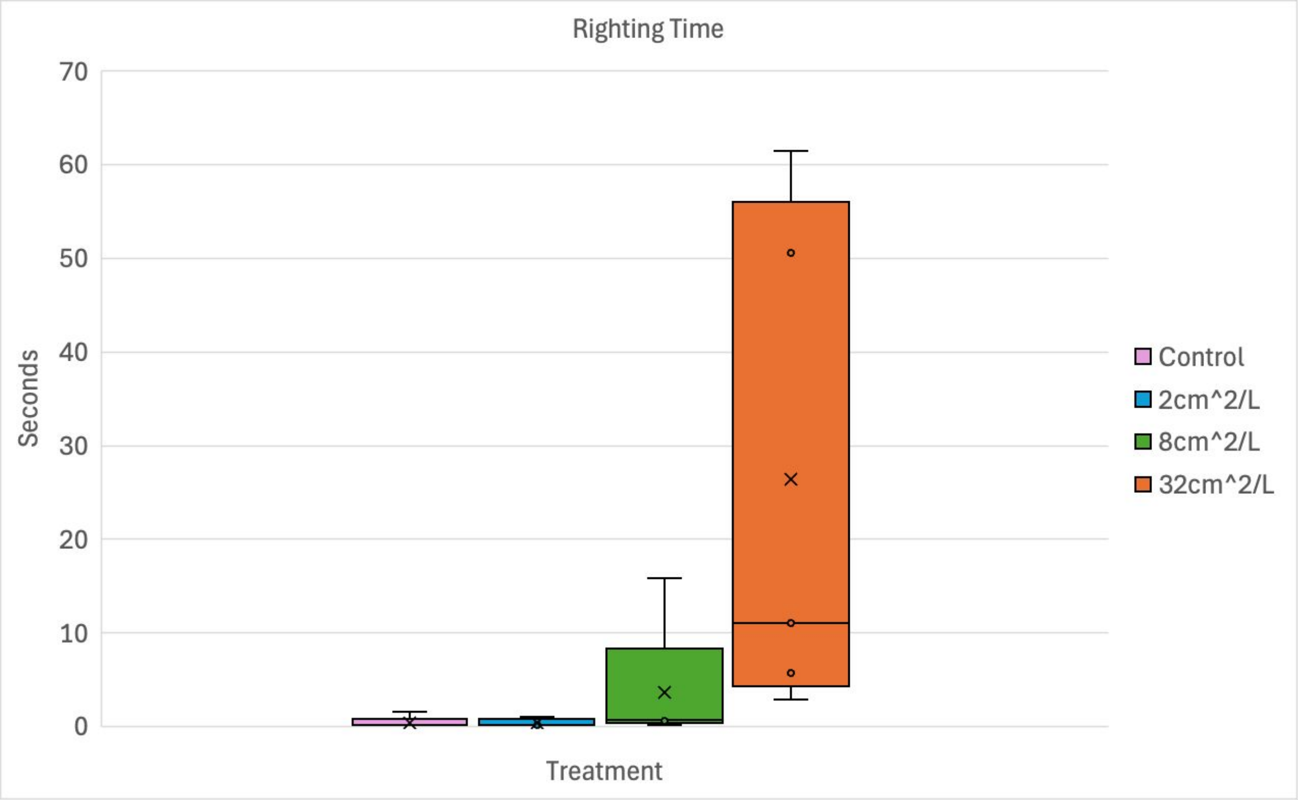


Figure 1. Box and whisker plot of the righting time of *H. oregonensis* within each treatment group. Average times are denoted with an X and each data point is denoted with an o as well as the whiskers being points.

t-Test Comparison	Control $2cm^2/L$	Control $8cm^2/L$	Control $32cm^2/L$	$2cm^2/L$ $32cm^2/L$	$2cm^2/L$ $8cm^2/L$	$8cm^2/L$ $32cm^2/L$
p-Value	0.89	0.36	0.10	0.10	0.35	0.15

Table 1. Results of two sample t-tests assuming unequal variances for righting time.

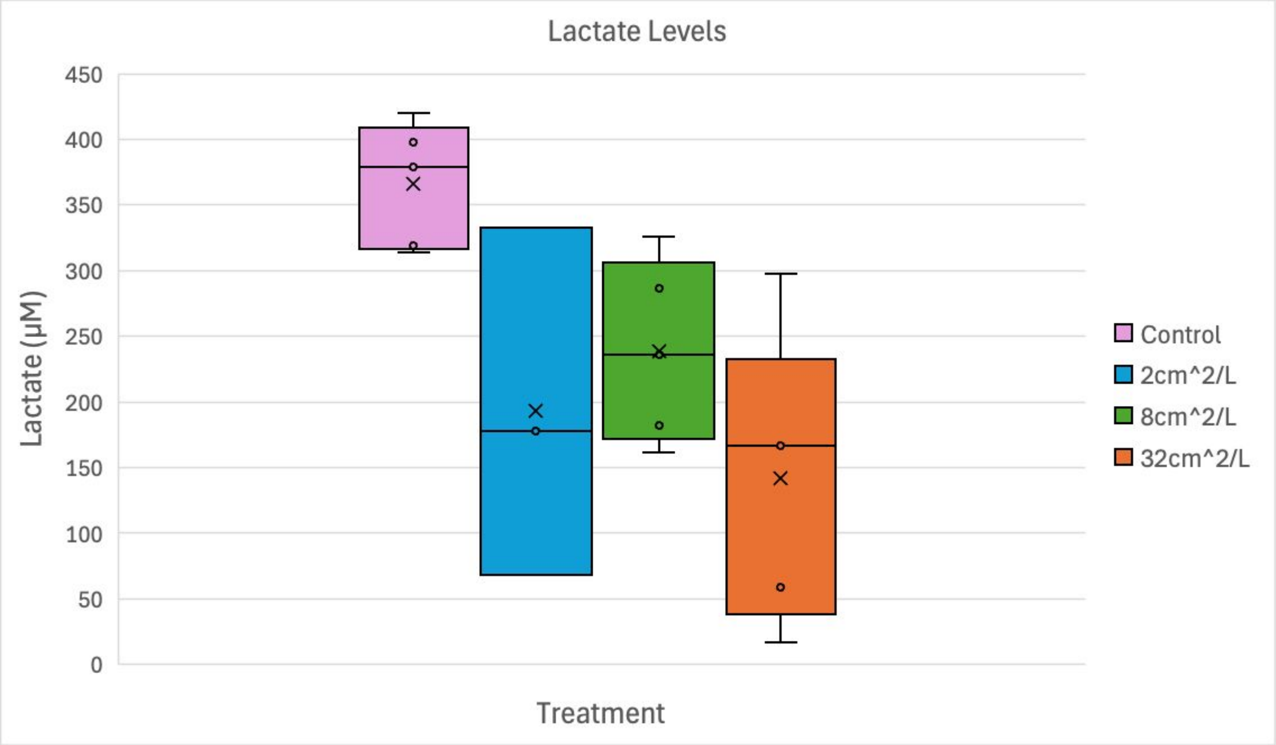


Figure 2. Box and whisker plot of the lactate levels µM of *H. oregonensis* within each treatment group. Average times are denoted with an X and each data point is denoted with an o as well as the whiskers being points. The $2cm^2/L$ treatment had one crab die and another had eggs underneath her carapace, so hemolymph was not extracted, thus a smaller sample size

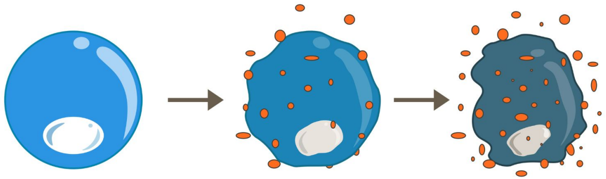
* one mortality $2cm^2/L$
* one pregnancy $2cm^2/L$

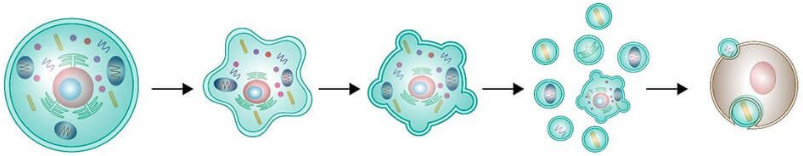
t-Test Comparison	Control $2cm^2/L$	Control $8cm^2/L$	Control $32cm^2/L$	$2cm^2/L$ $32cm^2/L$	$2cm^2/L$ $8cm^2/L$	$8cm^2/L$ $32cm^2/L$
p-Value	0.16	0.01	0.008	0.60	0.62	0.14

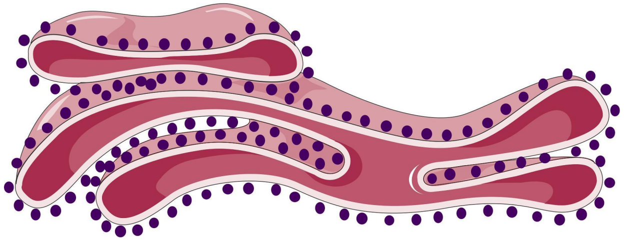
Table 2. Results of two sample t-tests assuming unequal variances for lactate levels.

Potential Explanation of Righting time Increases

- **Damage to statocysts** - seen with rock lobsters exposed to intense sound waves
- **Oxidative Stress -** leads to DNA, lipid and protein damage
- **Apoptosis -** Upregulated genes seen within the hepatopancreas, gills, and muscles of Chinese Mitten crab
- **Endoplasmic Reticulum Stress -** disrupts protein folding, assembly, and transport







(Day et al., 2019)
(Feng et al., 2022)

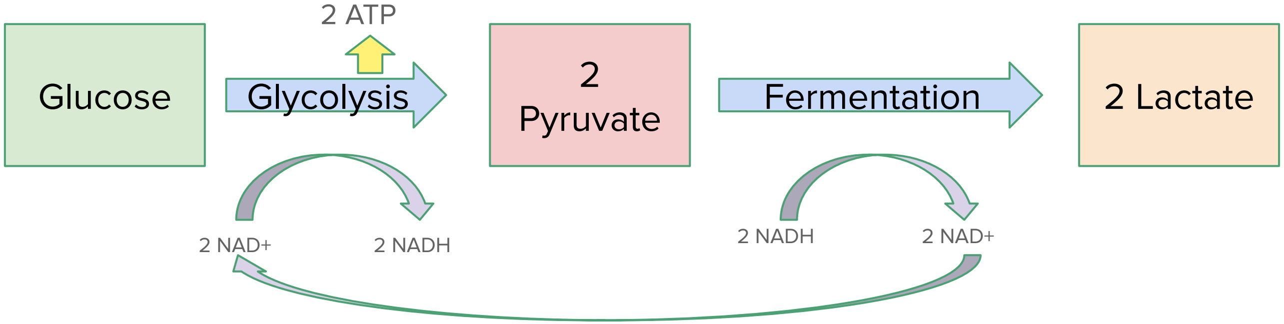
Potential Explanation of Displayed Lactate Levels

Other Studies

- ↑ lactate in ↑ concentration -> Indicative of switch to anaerobic respiration
- Copper causes damage to gills

What We Found

- Opposite Pattern -> ↓ lactate with ↑ concentrations
- Had plenty of oxygen -> no switch to anaerobic respiration



(Asih et al., 2013)

Implications and Future Experiments

Implications

- Anti-fouling paint results in decreased alertness in hairy shore crabs.
- Increase stress can reduce the ability to fight infection or diseases, lowering the overall fitness.
- Act as a comorbidity, increase chance of death

Future Experiments

- Increasing the sample size and creating true replication.
- Specific impacts of cuprous thiocyanate on anaerobic respiration.
- The specific amount of copper being released into the water, based on the surface area-to-volume ratio.

Sources

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