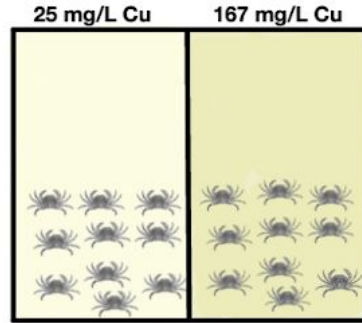
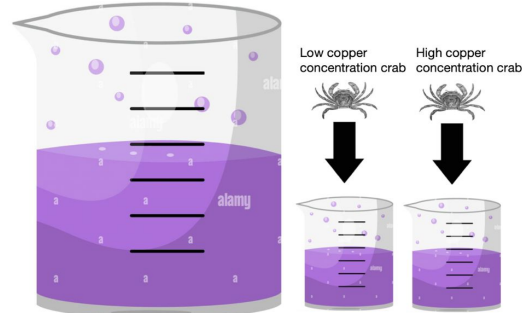


# Effects of copper contamination on *Hemigrapsus oregonensis*: Results

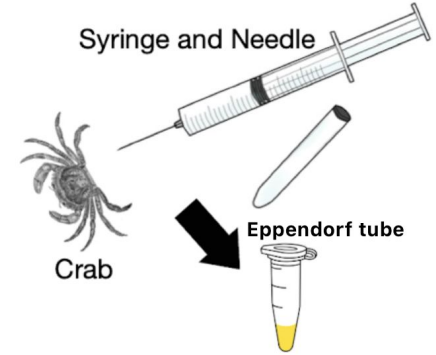
Research question: What are the physiological effects of copper exposure on *Hemigrapsus oregonensis*, assessed through metabolic, osmotic, and behavioral indicators?



**Copper exposure setup**



**Resazurin Assay**



**Haemolymph Extractions**

## Treatment Tanks:

- Control: 0 mg/L Cu (no copper added)
- Low: 25 mg/L Cu
- High: 167 mg/L Cu

Maintain water salinity, temperature, pH, and dissolved oxygen throughout experiment.

Copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) dissolved in seawater.

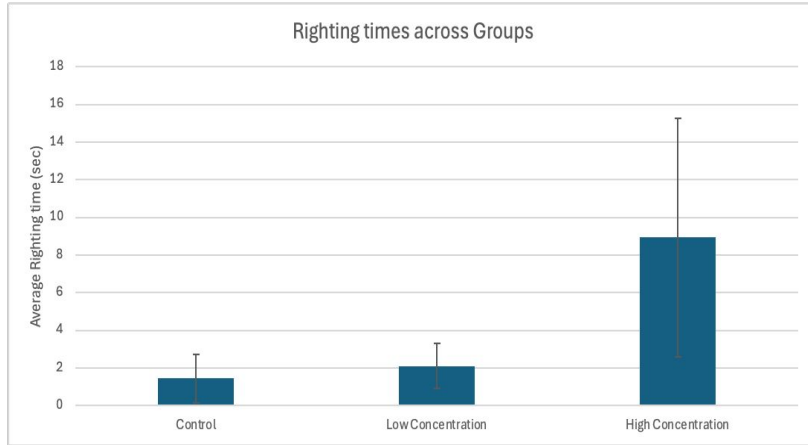
18 crabs total, 9 in each tank (control has separate crabs).

- Prepare resazurin stock solution (0.5 g resazurin, 10 mL DI water, 10  $\mu\text{L}$  DMSO); store cold and dark
- Dilute stock into a working solution using seawater, DMSO, antibiotics; store at 4°C in the dark
- Load 35 mL of working solution into crab chambers
- Weigh each crab and place into individual chambers
- Sample 200  $\mu\text{L}$  every 30 minutes into 96-well plates
- Measure fluorescence (Ex 530 nm, Em 590 nm) using a plate reader

- 7 days after exposure: Using Plastipak syringe and needle, hemolymph was extracted from 3 crabs from each treatment tank.
- 14 days after exposure: hemolymph extraction taken from 3 low exposure crabs, 1 high exposure crabs.
- Stored in eppendorf tubes to be analyzed

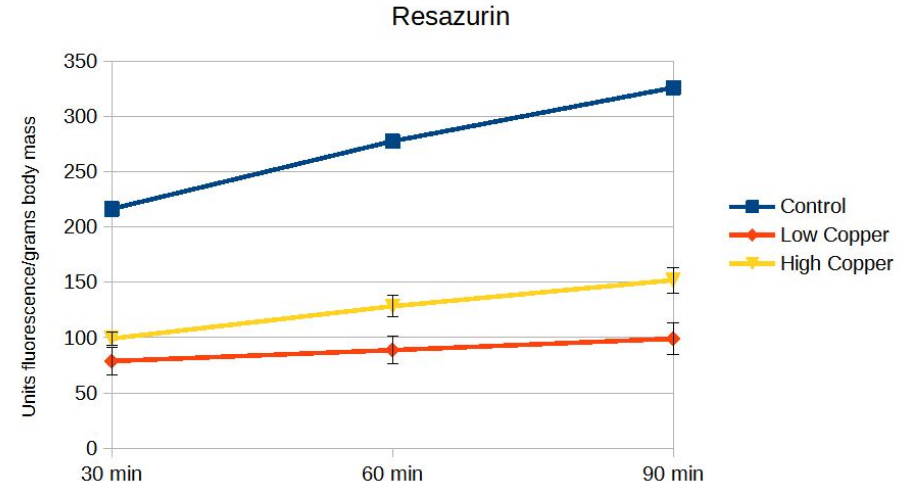
# Results`

## Righting Time Results



Used Excel to measure correlation between copper concentration and righting time. Graphing the averages to observe differences between treatment groups and the control group. Standard deviation was measured and incorporated on the bar graph.

## Resazurin Results



Used Excel to normalize each fluorescence by body weight of the individual. Measurements were taken for three crabs from each treatment group and one control crab at 30 minutes, 60 minutes, and 90 minutes. Measurements were averaged for the two treatment groups, and standard deviations are also given.