Effects of copper contamination on *Hemigrapsus oregonensis*

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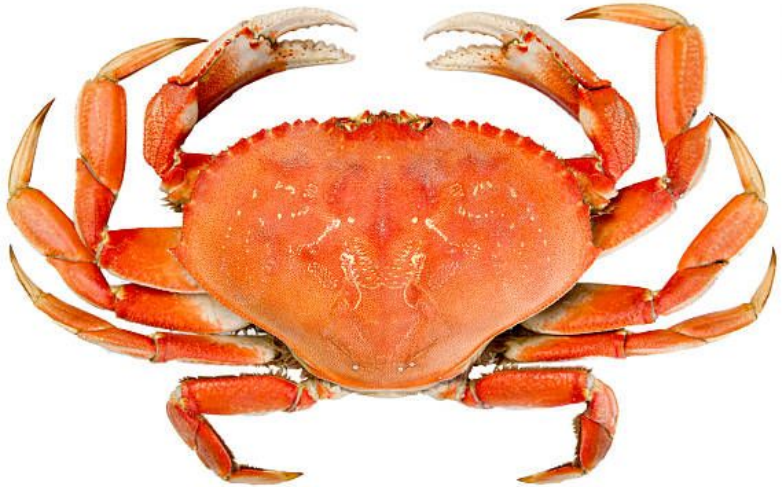
Introduction- Copper Pollution

State of Washington Department of Ecology



Natural Sources: weathering, volcanoes, geological deposits. Coastal water levels low.

Human Sources: Agriculture, industrial processes, antifouling paints. Urban coastal water levels high.

Copper ingested and absorbed by crabs have a wide range of toxic effects: reduced growth, oxidative stress, inhibited senses.

Commercial fishing and human health consequences in the Puget Sound

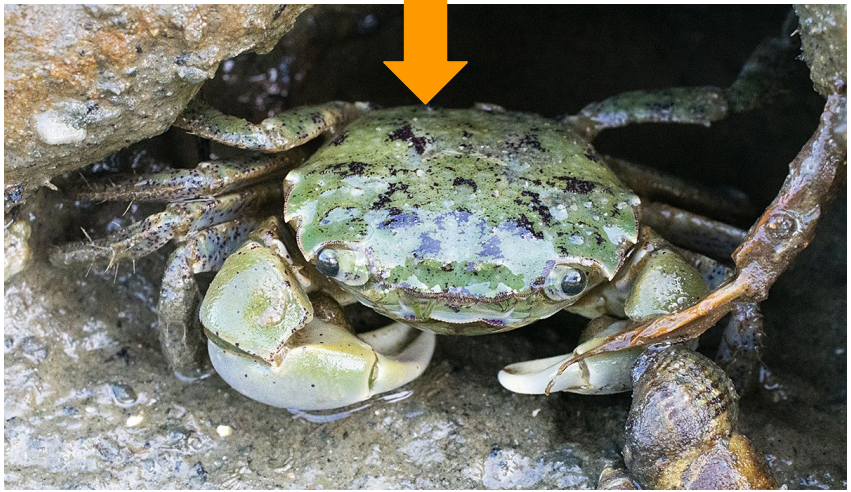
Background- What's known?

1. Ocean copper levels rising, and copper is making its way inside of Puget Sound crabs (Carey et al. 2014, Dagdag et al. 2023)

2. Copper impairs metabolism, behavior, and osmoregulation in crustaceans (Hansen et al. 1992, Krång & Ekerholm 2006; Lara Jacobo et al. 2016)

3. There is a call for research on copper's effects on marine organisms in the Puget Sound to guide legislation (Washington State Department of Ecology 2019, La Colla et al. 2018)

Cu



How will copper pollution affect a model organism crab exactly?

Research Question / Hypotheses

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

Alternative Hypothesis: Copper exposure significantly affects the physiological (metabolic rate) and behavioral (righting time) responses of *Hemigrapsus oregonensis*, with greater copper concentrations leading to more pronounced effects.

Null Hypothesis: Copper exposure has no significant effect on the physiological (metabolic rate) or behavioral (righting time) responses of *Hemigrapsus oregonensis* across different concentration levels.

Experimental Design & Methods

Experimental Design Overview:

- Copper source: Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), dissolved in seawater
- **Treatment groups** (Fig. 1):
  - Control: 0 mg/L Cu
  - Low: 25 mg/L Cu
  - High: 167 mg/L Cu
- **Replicates**:
  - 9 *H. oregonensis* per treatment
  - ≥ 20 individuals in control; total ≥ 38 crabs
- Tanks maintained with constant salinity, temperature, pH, and dissolved oxygen

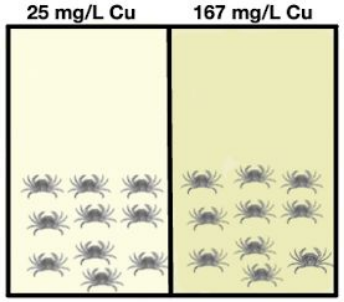


Figure 1. Copper exposure treatments for *H. oregonensis*. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was used to create two concentration groups: low (25 mg/L Cu), and high (167 mg/L Cu). Each treatment group contained 9 crabs, while the control tank contained ≥ 20 or more crabs, totaling ≥ 38 individuals. Tanks were kept under consistent water quality conditions.

Metabolic stress test:

- Respirometry using resazurin dye
- After 7 & 14 days, crabs placed in sealed chambers with 35 mL resazurin (Fig. 2)
- 200 µL samples taken every 30 mins; fluorescence measured
- Values normalized to crab weight; faster color change = higher oxygen consumption

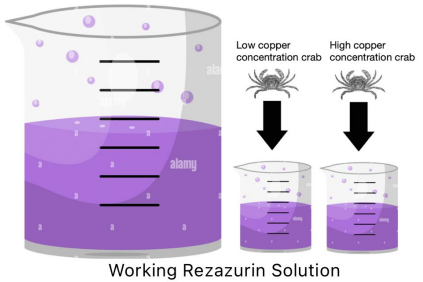


Figure 2. Respirometry using resazurin to measure metabolic rate in *H. oregonensis*. Individual crabs were placed in sealed chambers containing resazurin solution, and the rate of color change from blue (resazurin) to pink (resorufin) was a representation of aerobic metabolic rate Values were normalized to crab weight. Faster color changes indicated higher oxygen consumption and stress.

Behavioral test:

- Righting time measured on days 7 & 14 (Fig. 3)
- Crabs placed upside down; time to self-right recorded
- Conducted in control-condition seawater
- Indicates potential motor or defensive impairment

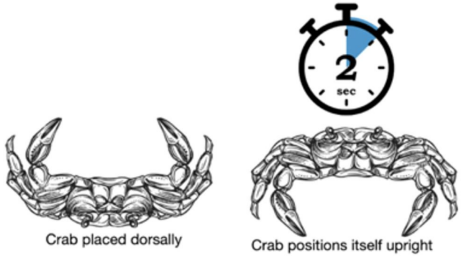


Figure 3. Righting response test for *H. oregonensis*. Crabs were flipped onto their dorsal side, and the time to self-right was recorded. Longer righting times indicated potential behavioral impairment from copper exposure.

Data analyzed using Microsoft Excel and RStudio

Justification

Why This Design?

- Comprehensive: Detect both physiological (metabolic rate) and behavioral (righting time) effects
- Three copper levels (control, low, high) test for dose-dependent responses

Why This Species?

- The yellow shore crab (*Hemigrapsus oregonensis*)
- Found in areas affected by copper pollution: marinas where antifouling paints leach copper into the water (Hobbs et al. 2022)
- Exposed through ingestion and absorption of copper from the environment
- Relevant to ecological risk assessments and conservation of other coastal invertebrates

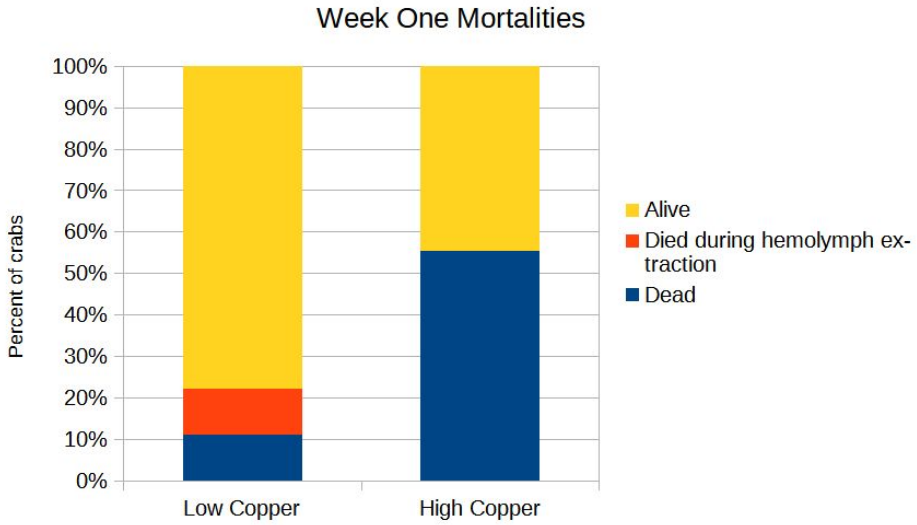
Copper Source & Relevance

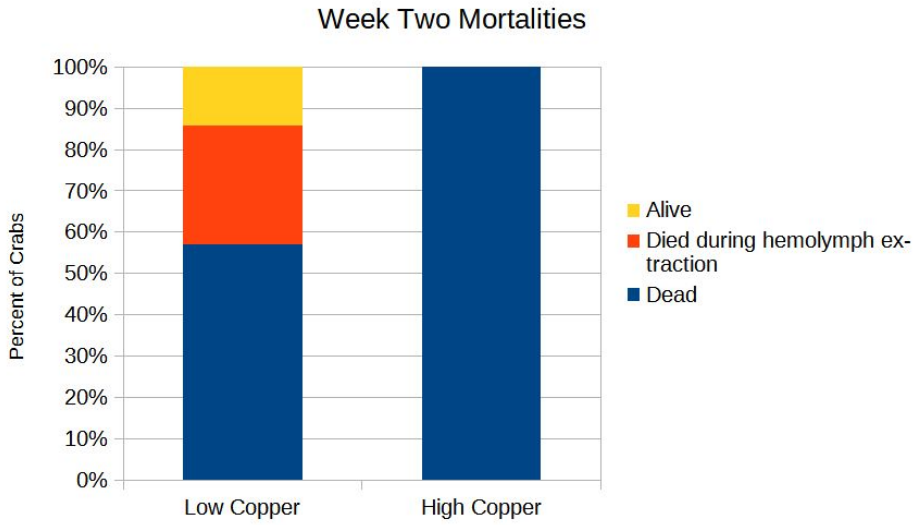
- Compound: Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) dissolved in seawater
- Commonly used in past studies on other crab species (Xu et al. 2025)

Real-world relevance

- Agriculture: fungicide, algaecide (Benhalima et al. 2019)
- Aquaculture: algae/parasite control (Watson & Yanong 2014)
- Wastewater treatment (Burandt et al. 2024)

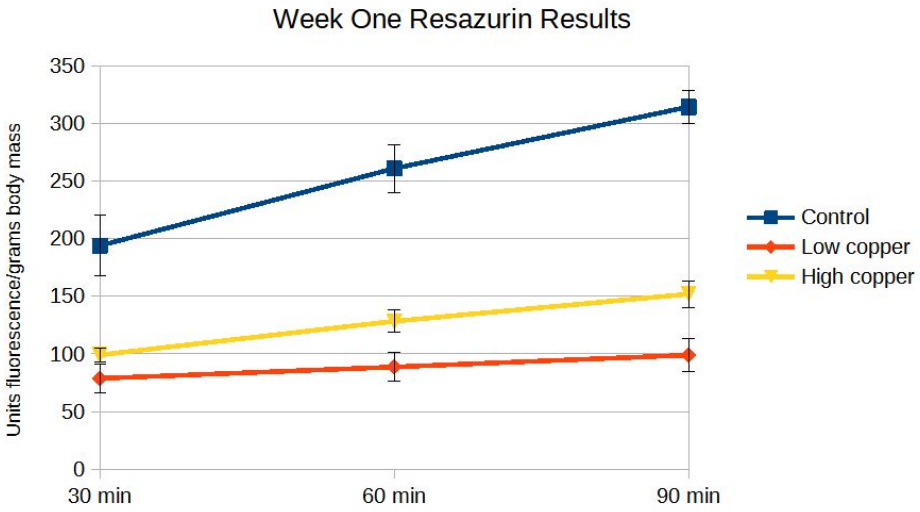
Mortalities

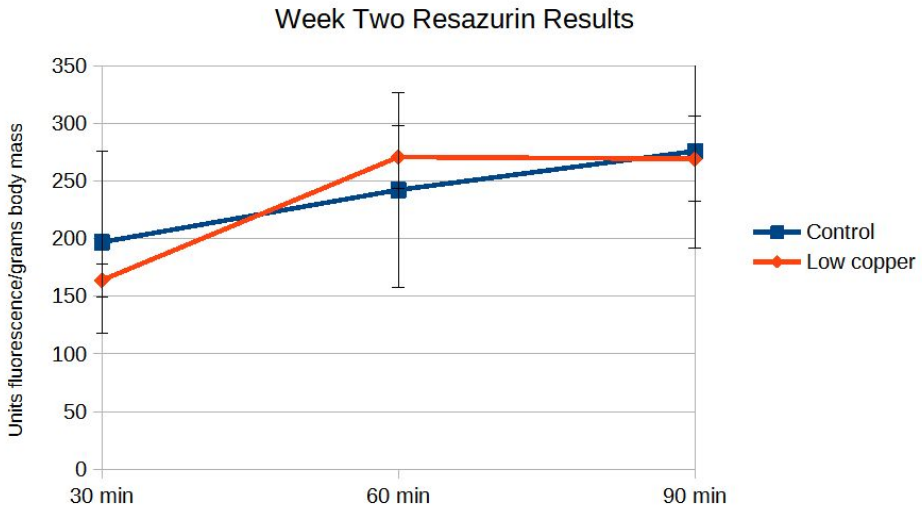




Crabs that died during the resazurin assay are counted as dead due to the treatment

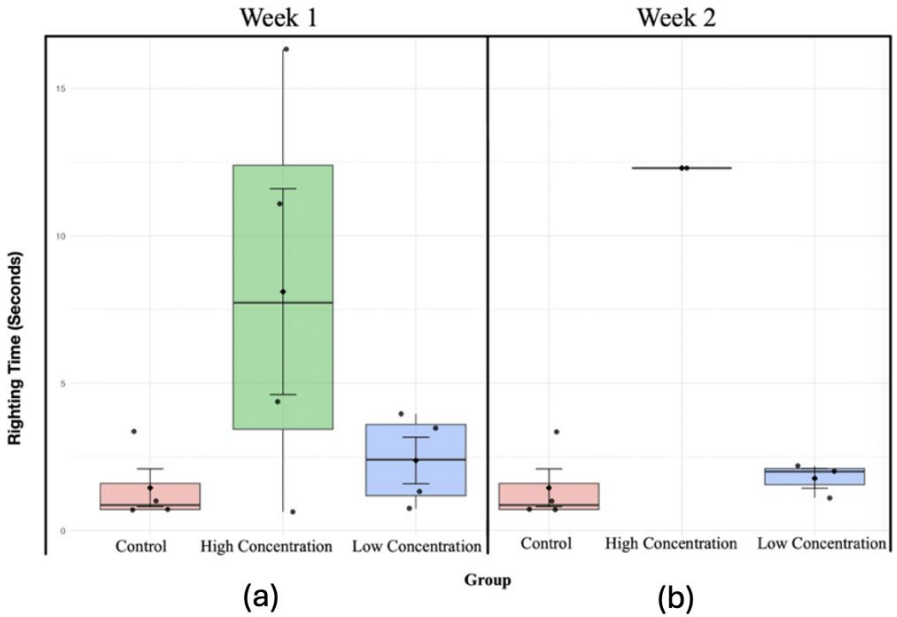
Resazurin





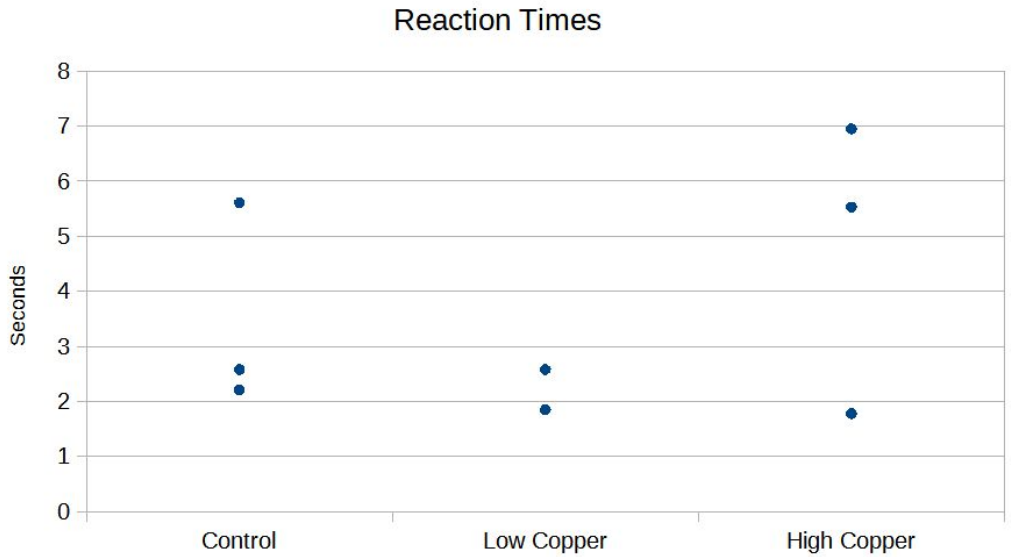
Three crabs per group tested week one with one high copper crab dying during the resazurin assay. Week two control was three crabs, low copper was four crabs, and high copper was one crab, with two low copper crabs and the high copper crab all dying. Data from dead crabs was excluded.

Righting Times



Both weeks are using the same control data. One low copper crab in week two is not included as it was unable to right itself and so does not have a righting time that could be shown.

Threat Response



Reaction times of crabs who fled (three control, two low copper, three high copper). Crabs who froze not shown (one control, two low copper, one high copper)

Conclusions

- Sample size a limiting factor
- Mortalities a clear effect
- Righting times inconclusive
  - Potential neurological effect
- Resazurin also inconclusive
  - Week one clear difference between copper exposure and control
  - Week two no clear difference between groups
- Difficulties with threat response
  - Outside distractions

Future Work

- Larger sample sizes
  - High mortality rates shrunk already small sample size even further, making it challenging to collect useful data
- Lower concentrations
  - Future studies that aim to study non fatal behavioral stressors should ensure copper concentrations in treatment tanks are not fatal
  - A determination of *H. oregonensis* LD50 could help inform future work.
- Different form of copper?
  - Other forms of copper are more representative of copper contamination in the Salish Sea ecosystem
  - Potential use of antifouling paint flakes as copper substrate.
- Modified experimental design for behavior
  - Fewer distractions
  - Potentially a different method for measuring reaction time

Literature

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