Effects of copper contamination on *Hemigrapsus oregonensis*

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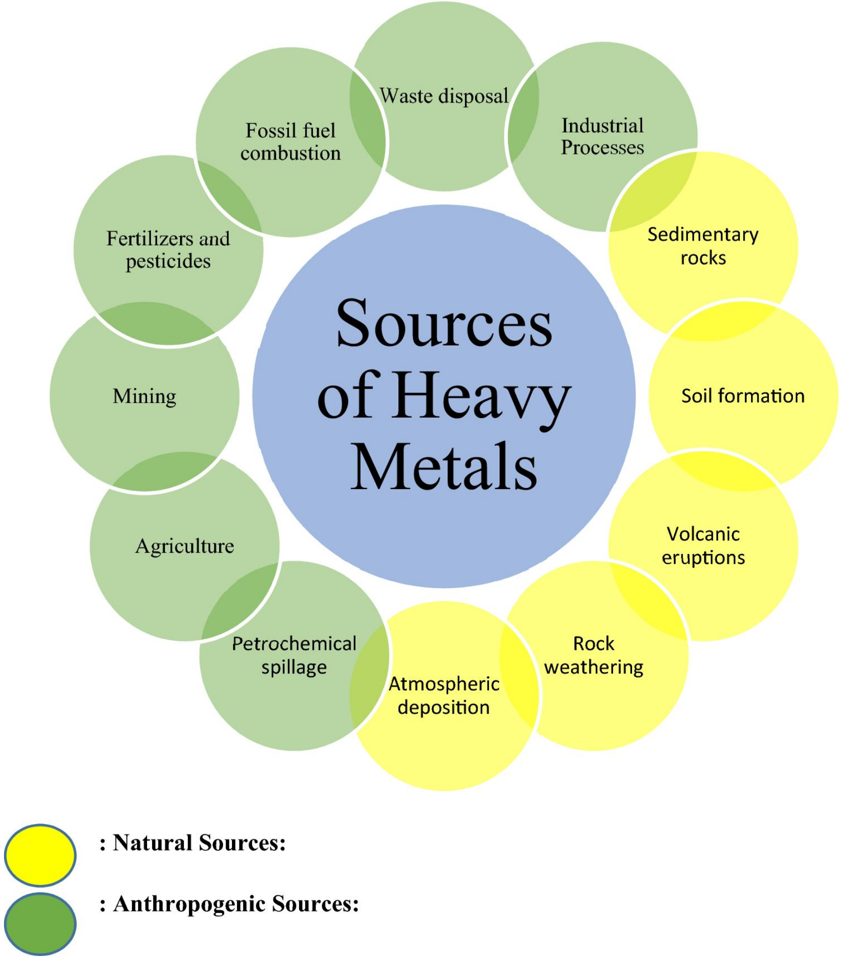
Heavy metal pollution

- Heavy metals are introduced to the environment by human activity
  - Arsenic, Copper, Lead, Mercury, etc.
- Heavy metals are found in water, and are ingested by organisms
- Higher concentrations frequently associated with negative effects on exposed wildlife
  - Even at low concentrations, can pose an issue due to bioaccumulation



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Hemigrapsus oregonensis

- The yellow shore crab (*Hemigrapsus oregonensis*) lives along the West Coast of the US and Canada
- Typically intertidal, eats primarily diatoms and green algae
- Habitat and range overlap with other crabs such as Dungeness crabs and invasive European green crabs
    - Potential use of *Hemigrapsus oregonensis* as model organism

https://wsg.washington.edu/hemigrapsus-oregonensis-jaws/

Prior studies on copper pollution

- Behavioral impairment
  - Male *C. maenas* exposed to copper contamination experienced impairment to their ability to find a mate and to perform certain mating behaviors (Krång and Ekerholm, 2006)
- Inhibited ovarian growth
  - Female C. granulata experienced inhibited ovarian growth (Medesani et al. 2004) and female C. maenas showed signs of impaired ovarian activity (Elumalai et al., 2005)
- Impaired osmoregulation
  - *C. antennarius* injected with copper sulfate had their osmoregulatory capacity adversely affected (Jacobo et al., 2016)

A male *C. maenas* found in San Francisco Bay
Photo by Andrew N. Cohen
www.exoticsguide.org

Research Question

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

Hypothesis

Null Hypothesis: ***Hemigrapsus oregonensis*** **will show no statistically significant change in stress response from exposure to copper**

Alternative Hypothesis: ***Hemigrapsus oregonensis will show a statistically significant change in measurable by respirometry, osmolarity, or behavior***

Experimental Design

Copper Source: copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) dissolved in seawater.

Justification:

Another study: Toxic effects of copper sulfate pentahydrate on antioxidant enzyme activities and lipid peroxidation of freshwater fish *capoeta Umbla* tissues

- Agriculture (as a fungicide or algaecide),
- Aquaculture (to control algae or parasites),
- Industrial processes (mining, electroplating),
- Wastewater treatment (Kubitza 2023).

Experimental Design

Copper exposure setup - Examine the effects of copper on Hemigrapsus oregonensis under controlled conditions.

- Treatment Tanks:
  - Control: 0 mg/L Cu (no copper added)
  - Low: 0.1 mg/L Cu
  - High: 0.5 mg/L Cu
- Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) dissolved in seawater.
- Monitor and maintain water salinity, temperature, pH, and dissolved oxygen throughout the experiment.
- 20 crabs total, 10 in each tank (control has separate crabs)

Respirometry Using Resazurin - To measure metabolic rate as a proxy for physiological stress.

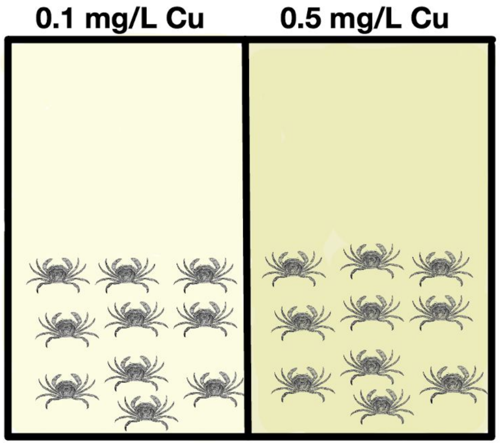
- Prepare the resazurin solution
- Incubate individual crabs in chambers containing the solution
- Measures aerobic metabolism via oxygen consumption
- Blue (resazurin) → Pink (resorufin) indicates metabolic rate

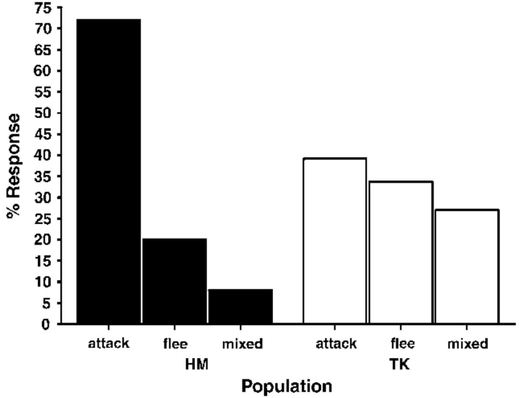
Osmolarity Assays - To assess osmoregulatory response under copper stress.

- Normal osmolarity = crab is healthy
- Lower osmolarity = possible ion loss or gill damage
- Higher osmolarity = stress

Behavioral Observations - To assess behavioral changes (avoidance, defense) under copper stress.

- Measure righting time (time it takes for crab on its back to turn itself upright)
- Stimulate the crab with a probe (wood skewer) and record latency to respond (claw at pencil or attempt to escape)
- Score defense intensity on a scale (0 = no response, 1 = minor movement, 2 = full claw display or escape).
- Replication: 10 crab in low copper concentration, 10 crab in high concentration





Reichmuth J.M., MacDonald J., Ramirez J. & Weis J.S. 2011. Fight or flight: an investigation of aggressive behavior and predator avoidance in two populations of blue crabs (*Callinectes sapidus Rathbun*) in New Jersey. *Hydrobiologia 658*, 173–182, doi: 10.1007/s10750-010-0460-z.

Justification

Why this design?

- Multi-assay approach captures both physiological and behavioral effects of copper exposure.
- Using three different copper levels (control, low, high) allows us to detect dose-dependent effects.
- Combines quantitative (respirometry, osmolarity) and qualitative (behavioral scoring) data for a more complete picture of stress response.

Why is it appropriate for your research question or hypothesis?

- Our question is about how copper affects crab physiology and behavior.
- This design directly tests that by measuring:
  - Metabolic rate (respirometry)
  - Osmoregulation (osmolarity assay)
  - Behavioral changes (avoidance, defense)
- These responses are known to be affected by heavy metals and are good indicators of sublethal stress.

Why are your controls and variables set up this way?

- Control group (0 mg/L Cu) provides a baseline for healthy crab function.
- Low and high copper treatments reflect possible environmental contamination levels.
- Using the same crab numbers across groups ensures comparability and reduces bias.

Why is this species or system a good model?

- Common intertidal species on the West Coast—ecologically relevant and easy to collect.
- Insights gained from this study may also be applied to other larger crab species, helping to predict their physiological and behavioral responses to metal pollution and improve management strategies for broader ecological contexts.
- Understanding the effects of heavy metal pollution could inform conservation efforts including habitat restoration measures.

Relevance to real-world ecological or management outcomes

- Natural concentrations of copper are low in coastal environments, but increase from anthropogenic activity:

- In the Puget Sound, the largest source of anthropogenic copper is from antifouling paints used on hulls of ships in marinas
  - Antifouling Paints Law - postponed to study scientific literature further
  - State of Washington Department of Ecology study of copper on Puget Sound biota found higher levels of copper in marinas vs outside marinas

- Knowing the effects copper has on the hairy shore crab could provide insights into how similar species will respond, and help guide policy and urban planning

State of Washington Department of Ecology

Timeline and Milestones

Lab 4: Set up experiment and begin the exposure of crabs to copper. Collect stress and behavioral data on control group of crabs.

Lab 5: Collect stress and behavioral data from experimental group after first 7 days of copper exposure. Record results and methodology used.

Lab 6: Collect stress and behavioral data from experimental group after 14 days of copper exposure. Record results and methodology used.

Lab 7: Present results and initial hypothesis.

Lab 8: Write up paper with background, methods, results, and discussion.

Lab 9: Finish paper and share findings

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