The effects of salinity and dissolved organic matter on lethal and sub-lethal copper toxicity endpoints using a hydroid (*Eudendrium carnuem*)

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Introduction

Estuaries are brackish (salinities of 1-35ppt) bodies of water that connect freshwater lakes and rivers with oceans. Hall and Anderson (1995) found that the toxicity of some metals (e.g. cadmium, zinc, nickel, and copper) to estuarine organisms increased with decreasing salinity. This relationship was based off of a relatively small estuarine dataset of a limited number of both species and families so the consistency of this trend among estuarine organisms remains uncertain.

In order to investigate this trend further, the effects of salinity on copper (Cu) toxicity will be studied using the euryhaline hydroid *Eudendrium carneum* (Figure 1). Cu enters estuaries through both natural and anthropogenic sources (e.g. anti-fouling paints, and mine tailings) and there is a lack of information on Cu toxicity in estuarine environments. *E. carneum* is part of an understudied family, and can tolerate salinities from 10-35ppt which makes this hydroid an excellent candidate for studying the effects of salinity on Cu toxicity.

Figure 1. Typical appearance of *E. carneum* colonies used.

Estuaries are sites that often have dissolved organic matter (DOM) present. DOM sequesters metals and can reduce the presence of the free-metal ion, Cu^{2+}, and reduce the toxicity of Cu to estuarine organisms. The effect of DOM on Cu toxicity will also be determined to better understand toxicity-modifying factors on Cu toxicity in estuaries.

Objectives

The objectives of this study were to:
1) Determine the toxicity of Cu to this hydroid at different salinities, and
2) Assess how Cu toxicity is altered using DOM, a toxicity-modifying factor

Materials and Methods

- Hydroids were cultured at 21°C and at salinities of 15, 25, and 30ppt. Salt water for both culture and testing was made by mixing de-ionized water with Kent Sea Salt. Cultures had 20% of the total water volume changed daily and were fed *artemia* nauplii once every 1-2 days.
- For each test concentration, hydroids on microscope slides were placed in 50mL falcon tubes with 50mL of medium in duplicate.
- Cu solutions were prepared by dilution of salt water solutions spiked with 1g/L atomic absorption standard. Samples were taken for dissolved and total concentrations and measured using furnace atomic absorption spectrophotometry (Varian SpectrAA-880).
- Hydroids were fed 24 hours before the test and observed using a light microscope (EVOS™) at x2-4 magnification at 24, and 48 hours.
- Response was measured using a hydranth scoring system (Figure 2).
- All EC_{50} and LC_{50} values were calculated using the program CETIS (Comprehensive Environmental Toxicity Information System); EC_{50} and LC_{50} values and 95% confidence limits were determined using the Spearman-Karber method and a significance value of p=0.05 was used.
- The source of dissolved organic matter (DOM) was Kouchibouguac National Park, which was diluted to a nominal concentration of 5mg/L of DOM from a stock solution of 349mg/L for use in toxicity testing.

Results

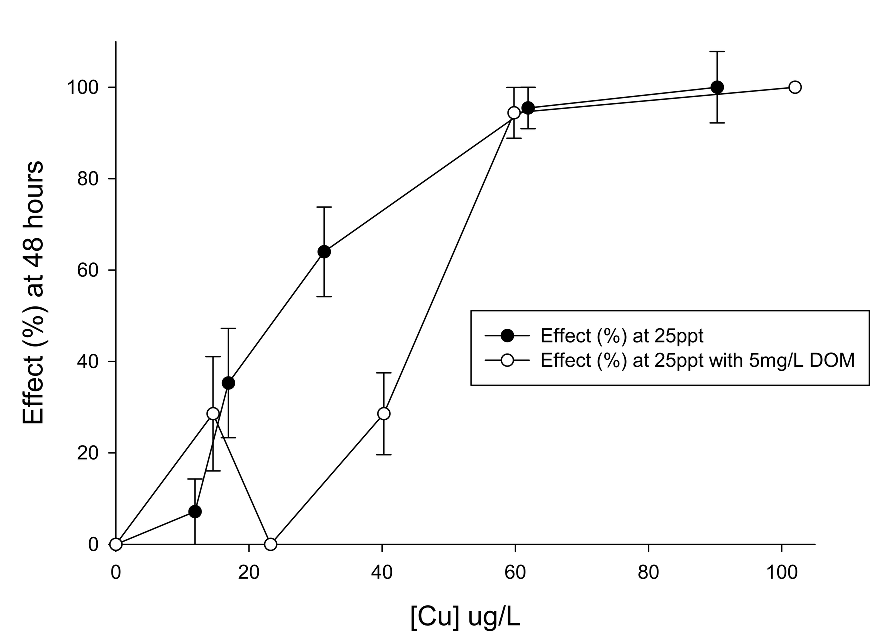

Figure 3. A measure of the percent of hydranths that showed a response of a score of 3 or less at 25ppt and at 25ppt with 5mg/L DOM.

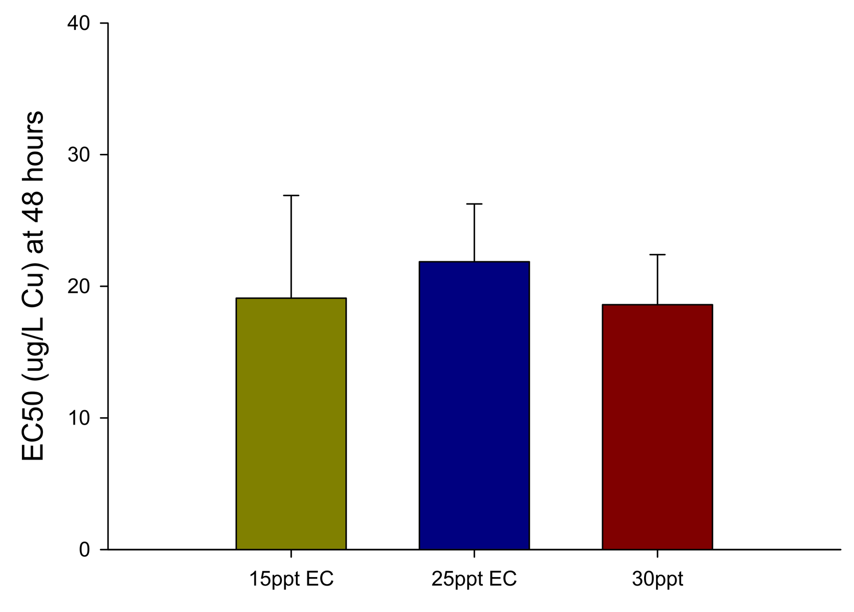

Figure 4. Comparing the EC_{50} values at salinities of 15, 25, and 30ppt with 95% confidence intervals. There were no significant differences from the EC_{50} at 25ppt.

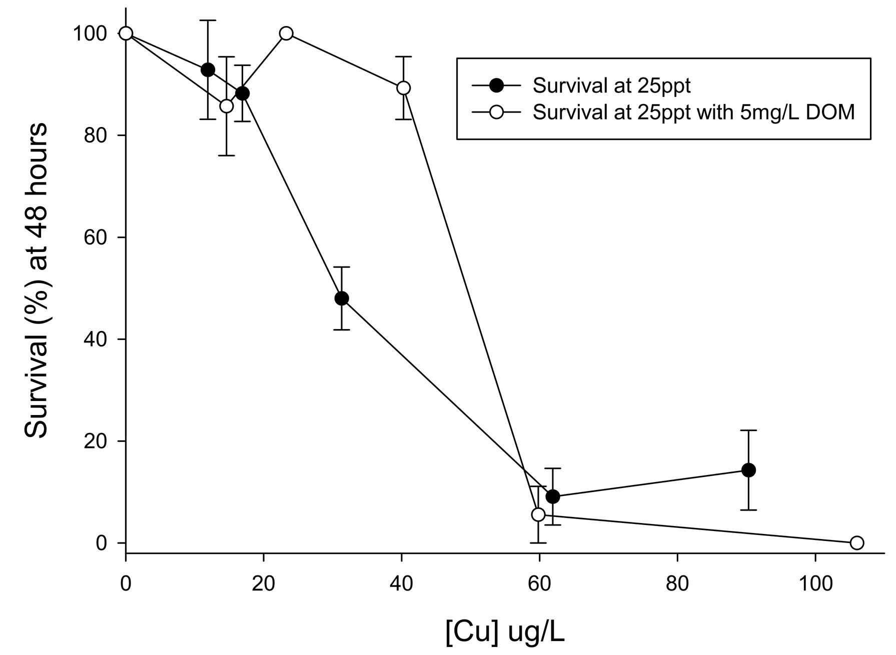

Figure 5. Survival (%) of hydranths of *E. carneum* with and without 5mg/L DOM at 25ppt with standard error bars.

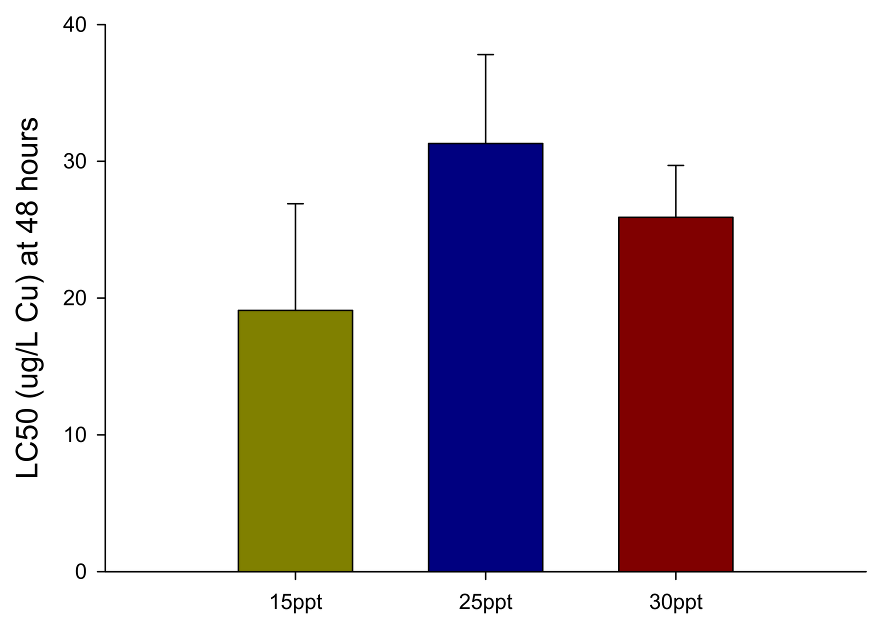

Figure 6. A comparison of LC_{50} values at salinities of 15, 25, and 30ppt with 95% confidence intervals. There were no significant differences from the LC_{50} at 25ppt.

Methods: The Hydranth Scoring System

Figure 2. Representatives of each of the stages of the hydranth scoring system in *E. carneum*. From left to right (A-E), the score values for each stage were: 4-Normal, 3-Clubbed tentacles, 2-Shortened tentacles, 1-Tulip phase, and 0-Disintegrated. The sub-lethal endpoint used in this research was clubbed tentacles (figure 2, B), which was used to determine EC_{50} values and the lethal endpoint was the tulip-phase (figure 2, D) which was used to calculate LC_{50} values. For panel A, phase-contrast microscopy was used and for panels B-E bright-field microscopy was used.

Discussion

- The scoring system that was adapted from Trottier and Blaise (1996) described the responses of *E. carneum* to Cu toxicity (Figure 2, A-E).
- There was no significant difference in EC_{50} values between 15, 25, and 30ppt (figure 4).
- There was a difference in the LC_{50} values between salinities of 15 and 25ppt; whereas there was little difference between LC_{50} values at 30 and 25ppt (figure 6). The decrease in LC_{50} at 30ppt may be the result of the pairing of Cu toxicity with non-optimal salinity and osmotic stress (Hall and Anderson, 1995).
- There was a significant difference between EC_{50} values with DOM at 25ppt and the EC_{50} at 25ppt (21.9µg/L (CL 18.18, 26.28), and 43.2µg/L (CL 38.9, 48.0) (Figure 3). The presence of DOM showed a significant increase in the LC_{50} value when compared to the LC_{50} at 25ppt with no added DOM (46.3 (CL 41.6, 51.6), and 31.3µg/L (CL 25.8, 37.8) respectively) (Figure 5).
- The trends in LC_{50} values suggest that, while not significant, there appears to be a slight trend of increasing response to Cu toxicity with decreasing salinity. This trend is not evident in EC_{50} values.

Conclusions

- The lethal endpoint is more sensitive to changing salinity than the sub-lethal endpoint.
- Decreasing salinity had no significant effect on Cu toxicity.
- DOM provided a significant protective effect to Cu toxicity.
- *E. carneum* is an ideal species to add to estuarine datasets.

Future Directions

- Determine the validity of this test method
- Investigate the effects of other metals along salinity gradients to this hydroid (e.g. Zn^{2+}, Cd^{2+}, and Ni^{2+}).
- Examine colony-wide responses (e.g. colonial growth rate) to metal toxicity along salinity gradients.

References

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