Short communication:

Toxic effects of copper sulfate on the purple Sea Urchin, *Arbacia punctulata*

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Received: July 2024 Accepted: October 2024

Abstract
Copper sulfate is commonly used in marine life support systems (aquaria) for treatment of ectoparasites in fishes. Marine invertebrates are known to be sensitive to copper but the number of quantitative studies defining copper toxicity in specific taxa are limited. In this controlled study utilizing a common species of echinoderm, the purple sea urchin (*Arbacia punctulata*), we determined that there is dose-related mortality in purple sea urchins when exposed to therapeutic levels of copper sulfate (0.10-0.20 mg/L) in the water and that sea urchins, and possibly all echinoderms, should not be exposed to any level of copper sulfate immersion.

Keywords: Copper sulfate, toxicity, echinoderm, *Arbacia punctulata*, sea urchin

[DOI: 10.22034/INJBIR.3.1.155]

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Introduction

Copper is a heavy metal present in various forms within the natural world. The cupric ion with the 2+ charge is the most effective and common form of copper in aquatic medicine, and it can be released from copper sulfate ($CuSO_4$) when applied to water. For this reason, copper sulfate is an effective and prevalent treatment for algae and parasite control in teleost fishes. The most common diseases in captive marine fishes that copper sulfate is used to treat are "white spot disease" (*Cryptocaryon irritans*) and "velvet disease" (*Amyloodinium ocellatus*) (Yanong and Lewbart, 2024). The target concentration of copper sulfate for the aforementioned diseases ranges from 0.15 to 0.20 mg/L Cu^{2+} (Yanong, 2013). Treating with copper sulfate must be done gradually and with close monitoring due to a low therapeutic index. Copper has been shown to be toxic to gill tissue in various teleosts as well as fatally toxic to invertebrates and elasmobranchs (Hadfield and Clayton, 2011; Yanong and Lewbart, 2024).

Copper concentrations are difficult to maintain due to numerous binding capabilities, which can be affected by the water quality such as concentration, salinity and pH. Depending on the water quality that copper is dissolved in, there can be various compounds or living organisms that bind or absorb copper and remove it as an available ion. Salinity can decrease the binding of copper to surfaces due to increased competition with sodium ions, allowing for copper to remain in higher concentrations in the water. Similarly, if the water pH becomes more acidic, copper is released from previously bound chloride molecules which increases the concentrations of copper as well (Yanong, 2013). When prescribing copper sulfate as a treatment, clinicians must be cautious and monitor water quality parameters, copper concentration levels, and may have to separate the treated species from other cohabitant organisms such as invertebrates.

The pathophysiology of copper toxicity is yet to be completely understood but has been suspected to be caused by oxidative stress, DNA damage, impaired ion regulation, and metabolic derangements due to impaired oxygen uptake (LaDouceur et al., 2016; Smriti et al., 2023; Cui et al., 2024). Sea urchins have been shown to be severely affected over short periods and exhibit generalized tissue necrosis with the toxicity occurring at concentrations of 15 – 50 ug/L (Wynne *et al*., 2015). Sea urchins are frequently used in marine invertebrate studies due to abundance in their environment, simplicity to catch, and presence in shallow waters (Hill and Lawrence, 2003). The purple sea urchin, *Arbacia punctulata*, belongs to the aquatic invertebrate taxonomic group of echinoderms (sea stars, sand dollars, sea cucumbers, etc.). This species of sea urchin is prevalent and unchallenging to catch due to it being found in shallow waters of rocky ocean bottoms on the east coast of North and South America. They are easy to maintain in captivity with an omnivorous diet mainly

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consisting of kelp, algae, zooplankton, and sea sponges.

Toxicology studies of the phylum Echinodermata have previously shown the fatal consequences of copper sulfate treatments. Though sea urchins have been seen to be susceptible to copper levels in vivo and their natural habitats, determining the level of tolerability of copper sulfate has not been closely studied. It may be beneficial to determine if there are tolerable copper sulfate levels in marine invertebrates as this can create more opportunities for aquarists in how they treat co-inhabited tanks. The purpose of this study was to determine the sensitivity of the purple sea urchin to copper sulfate exposure at different concentrations.

Materials and methods

Tank and Water Quality

Four 75 L tanks were assembled with only a sponge filter and sea water made with Instant Ocean® sea salt (Spectrum Brands, Blacksburg, Virginia, USA). Water quality parameters were measured daily at the same time and included: salinity (hydrometer), temperature (floating thermometer), ammonia, nitrite, nitrate, and pH (Hach® colorimetric tests, Hach Company, Loveland, Colorado, USA).

Specimen Quarantine and Husbandry

Urchins were collected off the coast of North Carolina by free divers. The urchins were acclimated to the tanks and water quality for two months prior to the study. Nine sea urchins were placed in each tank for a total of 36 specimens. The diet consisted of kelp and shrimp which were measured and recorded daily. The urchins were determined to be healthy by a veterinarian when the study began. Characteristics that determined health status were spine appearance, appetite, mobility, and ability to grip substrates.

Copper Concentration and Measurements

Each tank contained different levels of copper with tank one being the control – 0.00 mg/L, tank two – 0.10 mg/L, tank three – 0.15 mg/L, tank four – 0.2 mg/L. Copper levels and water quality parameters were tested with a Hach 2010 spectrophotometer daily. A solution of 1.0 mg/mL copper sulfate ($CuSO_4$-$5H_2O$) was prepared and used throughout the study. All tanks were confirmed to have a 0.00 mg/L copper level prior to the first copper administration. Copper levels were measured daily for 18 days and then at least every other day for 30 days.

Results

Mortalities were the quickest and highest in tank four. Tank four (0.2 mg/L copper) experienced the first two mortalities 5 days after the first copper sulfate treatment. Mortalities followed with three sea urchins found dead on day 6 in tank four and two mortalities in tank three (0.15 mg/L copper) the same day. On day 7 two more urchins died in tank four and five in tank three. By day 10, all sea urchins were dead from tank four. By day 15, all urchins in tank three were dead. Only one mortality occurred in

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tank two (0.10 mg/L copper) during the copper testing. All urchins were placed back in water lacking copper after the study, but those from tank two died within 70 days following the study. There were no mortalities from tank one, the control aquarium, during or after the study (70-plus days).

Discussion

Results of this study revealed all copper sulfate concentrations, 0.01 mg/L to 0.2 mg/L, were associated with mortality in the purple sea urchin, *A. punctulata*. Due to copper sulfate being a commonly used antiparasitic in teleosts in these dose ranges, these results preclude medicating mixed populations of teleosts and invertebrates, specifically echinoid echinoderms.

Various marine invertebrates, such as mollusca, cnidaria, and echinoderms have been shown to concentrate heavy metals from 10 – 20,000+ times the environmental level making them susceptible to copper toxicosis at lower therapeutic concentrations than those used to treat parasites in teleosts (LaDouceur et al., 2016). Due to copper not causing discoloration of the water and success as an antiparasitic, it is commonly used in display aquarium facilities (Yanong, 2013). One study showed that 60% of surveyed AZA institutions use copper sulfate as a prophylactic parasitic treatment during the quarantine process of their marine teleosts (Hadfield and Clayton, 2011).

The results of this study are consistent with previously conducted copper sulfate toxicity studies in numerous invertebrates such as sea anemones (Main et al., 2010), apple snails (Rogevich *et al*., 2009), spiny lobsters (Maharajan *et al*., 2012), sea hares, sea stars, shore crabs and some fish species (LaDouceur et al., 2016). The pathogenesis of copper sulfate toxicity has various hypotheses in aquatic invertebrate species, but further research is indicated to determine the specific disease process. In addition, diagnostic work up such as cytology and histology of affected tissues are not well documented with multiple inconsistencies reported. Advancing the understanding of the pathophysiology of copper sulfate toxicity in aquatic invertebrate species can lead to improvement of our knowledge of the physiologic mechanisms of each species and the organization of safe treatment protocols in aquatic facilities.

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[DOI: 10.22034/INJBIR.3.1.155]