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Acute Copper (Cu) Toxicity in Dragonfly Naiads (Insecta: Odonata)

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Abstract

The objective of this study was to examine the effects Copper (Cu) on mortality within a pair of test groups of naiads in the order Odonata, and to replicate previous observations regarding their potential uses as indicators for water quality. Few past studies have examined toxicity within relation to the odonates. It has been suggested that this order may contain valuable model organisms (Tollett et al., 2008). Very little focus has been given to odonates as bioindicators, with the few studies done being performed on species that would typically inhabit lentic environments (Trevino, 1999). Similarly, the test subjects for this study belong to a species of skimmer (Anisoptera: Libellulidae), which were observed over the course of a pair of 96-hour, non-renewal acute toxicity assays. Findings indicate a high resistance to copper toxicity. Although examinations on the effects of Cu on fitness were not feasible during the course of study, trends in mortality indicate stress in concentrations below 10 parts per million. This research suggests that skimmers may be useful as laboratory model organisms in the field of environmental toxicology.

Introduction

Studies have long shown that heavy metal pollution within aquatic systems has the tendency to reduce the abundance and species richness of the macro-invertebrate community (Resh et al., 1984). Odonata represents an understudied, potentially valuable general bioindicator of habitat quality. While typically acting as the top invertebrate predators in their respective habitats, odonate larvae serve an important role as a source of food for fish, birds, and to some lesser extent amphibians (Corbet, 1999). Little research has been conducted in laboratory settings to examine the effects of environmental toxicity among the odonates.

Tollett et al. (2008) were able to conduct a range of heavy metal toxicity testing on a group of skimmer naiads (Anisoptera: Libellulidae) and observed that only exposure to Cu demonstrated any effect on mortality at concentrations above 150 µg Cu/L (0.15 ppm). Environmental protection agency limits for the protection of aquatic life set concentrations of Cu at 9.00 µg/L (0.009 mg/L) (Tollett et. al., 2008). Of all the metals observed, copper (Cu) appeared to have the greatest negative impact on naiads. This study attempts to further examine the effects of Cu up to 10 parts per million (ppm), as it relates to mortality in *Tramea carolina* (Anisoptera: Libellulidae).

Outside of an environmental disaster scenario, 10 ppm Cu is unlikely to be encountered in lotic environments. This high level was chosen in order to establish potential uses as laboratory model organisms. It was important to ensure that the stock used to examine potential mortality would be toxic. It has been observed that Cu levels above 10 ppm result in mortality or sublethal complications among several species of macroinvertebrates (Joachim et al., 2017), (Australian Government Initiative, 2000).

Materials and Methods

Two tests were conducted, each test consisting of 24 individual organisms, randomly selected, and placed into a randomized board of varying solutions ranging from non-toxic to toxic. Mortality was recorded over 24-hour periods of each test, for a duration of 96 hours to observe potential effects of acute toxicity. Upon termination of both tests, organisms were collected, and identified utilizing a dissecting scope and dichotomous keys (Merritt &Cummins, 1996; Needham et al., 2000).

Toxicant: Thirty grams of 150 mesh laboratory grade copper (Cu) powder was obtained from Home Science Tools. In order to attain a solution measuring 10 parts per million (ppm) Cu, 0.00400 grams were measured and combined with 4 liters of moderately hard deionized laboratory water, commonly used as a control solution for whole effluent toxicity testing. Sixty liters of super deionized moderately-hard control water was made and continuously aerated via a six-inch air-stone on December the 28, 2022 at 3:00 pm MST. The control was analyzed on December 31, 2022, for hardness, pH, alkalinity, dissolved oxygen (D.O), and conductivity (Table 1).

Table 1: Moderately hard control water bench chemistry analysis.

Hardness (mg/L)	pH (SU)	Alkalinity (mg/L)	Dissolved Oxygen (mg/L)	Conductivity(µS)
102	8.2	70	7.3	290

The test toxicant was made approximately 24 hours before initiation of both tests on January 4, 2023. After the addition of the Cu, the toxicant stock was set on a stir plate and agitated for 30 minutes. Following agitation, the stock was refrigerated at 4 ℃. Prior to test initiation, toxicant was placed in a hot water bath set at 44.5 ℃ and brought up to 20 ℃. Bench chemistry analysis was performed on January 5, 2023, at 12:35 pm. Parameters examined were hardness, pH, alkalinity, D.O, conductivity, total residual chlorine (TRC), ammonia, and total dissolved solids (TDS) (Table 2).

Hardness (mg/L)	pH (SU)	Alkalinity (mg/L)	Dissolved Oxygen (mg/L)

Table 2: Cu toxicant initial bench solution chemistry.

104	7.7	70	7.5

Conductivity(µS)	TRC (mg/L)	Ammonia (mg/L)	Total Dissolved Solids(mg/L)
311	<0.02	0.0059	153

Organisms: Odonate naiads were obtained from Carolina Biological Supply (item #: 143526). While organisms were advertised as belonging to either genus *Pantala* or *Libellula,* all specimens obtained were identified as *Tramea carolina* (Figure 1), a species of skimmer in the family *Libellulidae* commonly referred to as the Carolina saddlebags. (Needham et al., 2000; Merritt & Cummins, 1996).

Figure 1: *Tramea carolina*

Like most of the skimmers*, T. carolina* is a sprawler, lying in wait to ambush unsuspecting prey or stalking the benthic region of their habitat (Bay, 1974). Twenty-four naiads (undetermined instar) were utilized for each study.

Utilities: Trials were conducted within a temperature-controlled incubator set to 20.0 ℃. Two hundred milliliter plastic containers were utilized to house individual specimens during the 96-hour duration of the study. A 96-hour testing period was chosen in accordance with EPA guidelines regarding acute toxicity testing, which allow for 24, 48, and 96-hour periods to allow for permutations such as temperature, and frequency of effluent renewal. Test boards would be examined to record mortality over time at 24-hour intervals (Environmental Protection Agency, 2020). Containers were stored on a plastic frame topped with a plastic sheet with large diameter holes drilled out to secure each housing unit (Figure 2).

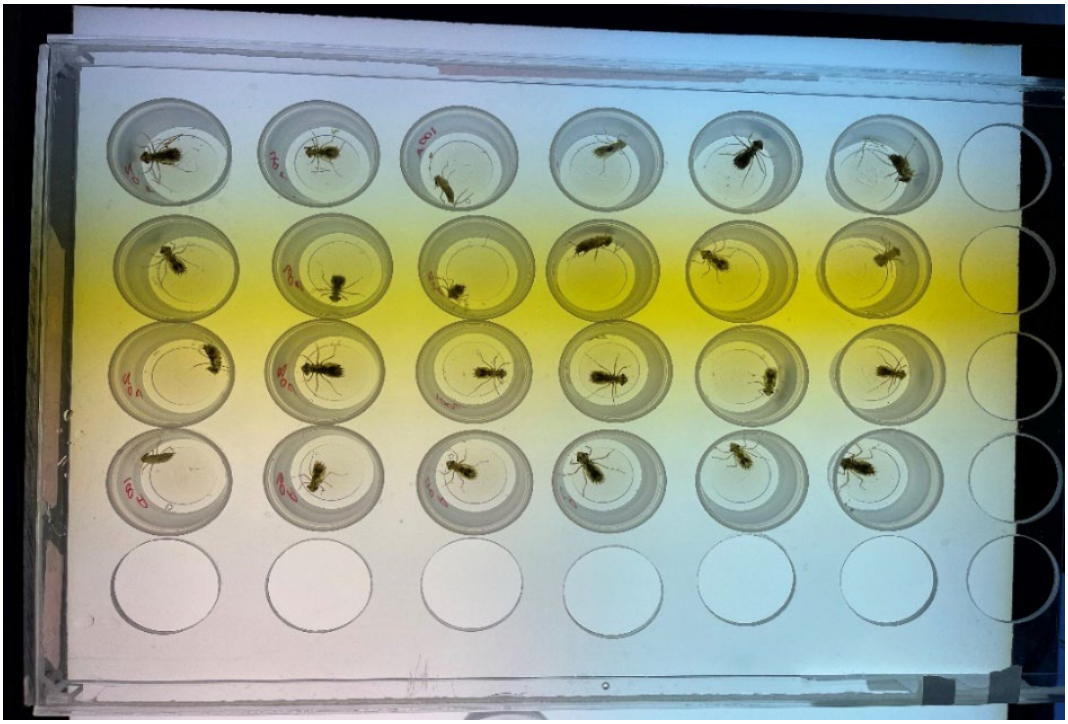

Figure 2: Test board # 1

Initiation: Utilizing a combination of moderately hard laboratory water, and the prepared toxicant stock, dilutions were made with a graduated cylinder, and stored within labeled plastic containers. Dilutions ranged from a 0% control, to a 20, 40, 60, 80, and 100% stock concentration, with percentages representing the ratio of 100% toxicant stock diluted with moderately hard control water. In total, 50 mL of each solution were added to 4 containers per toxicant concentration and placed in the board with the use of a randomizing template. A fine mesh screen, periodically rinsed with deionized water between contact with toxicant, was used to transport each *T. carolina* into its randomized test chamber from its holding tray. Each test consisted of six dilution sets, with each possessing four replicate organisms per set.

Both tests were initiated on January 5, 2023. Test #1 was initiated at 9:50 am, and test #2 was initiated at 10:20 am. Both tests were conducted as acute 96-hour non-renewal tests, with

observations for mortality events performed every 24 hours from the time of initiation to 96 hours. This is in accordance with the Environmental Protection Agencies standard operating procedure regarding acute toxicity to freshwater organisms when conducting whole effluent toxicity testing (EPA, 2020). Deceased organisms were removed from their containers and stored in a solution of 95% denatured alcohol. Upon test completion both groups of remaining naiads were euthanized, first with a 5% ethanol immersion to induce anesthesia, followed by euthanasia via a 95% ethanol immersion (Wyatt & Gilbertson, 2016). Bench chemistry was then utilized to analyze each dilution for D.O., conductivity, pH, and temperature.

Data analysis: Within the field of pharmacology, toxicant potential is measured with an EC_{50} or effective concentration estimate. The lower an estimate, the lower concentration of a substance is necessary to yield a 50% toxicological effect. An LC_{50} (lethal concentration) estimate is a unique case for an EC_{50}, in which the effect recorded is death. This can be summarized as the point on a trend line where the factors examined are mortality over a set period of time; or the intersection between the 50% point, and the point of time during the duration of the test. The LC_{50} is a statistical estimate of a concentration of a toxicant, necessary to yield a desired effect in 50% of the observed population.

For this study, Comprehensive Environmental Toxicity Information System (CETIS) was utilized to generate an LC_{50} rating for the consolidated test results (Tidepool Scientific Software, 2005). CETIS was used to perform a Trimmed Spearman-Karber statistical analysis in order to produce an LC_{50} score.

Results

The findings of this study indicate a high tolerance regarding odonate mortality and heavy Cu concentrations. Both tests exhibited near total die-off in the highest toxicant concentration, as well as scattered mortality from 4-ppm and above. Test 1 (Figure 3) exhibited no mortality for the control and 20% concentration, exhibited 25% mortality in the 40% group after 72 hours, and in the 60% group after 96 hours, and exhibited 0% mortality in the 80% group. The 100% toxicant stock group saw 25% mortality after 24 hours, and 75% mortality after 72 hours.

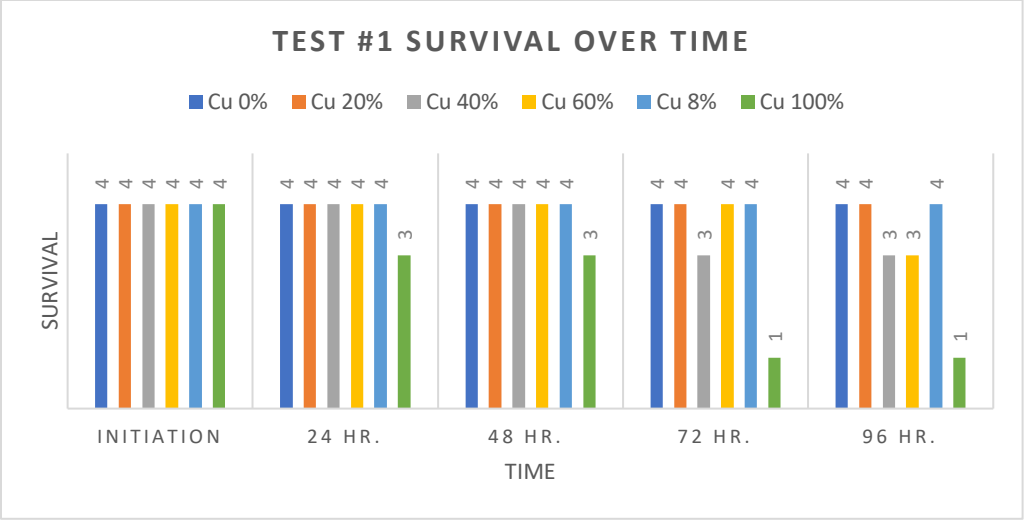

Figure 3: Test 1 Survival rates over time.

Test 2 (Figure 4) also exhibited no mortality in the control, and 20% groups. After 24 hours 25% mortality was observed in the 40% group, which would reach 50% by 96 hours. The 60% group saw a 25% reduction after 24 hours and did not fall further. At 24 hours the 80% saw 50% mortality, and the 100 % toxicant group reached 25% mortality at 24 hours, 75% after 48 hours, and reached 100% mortality after 96 hours.

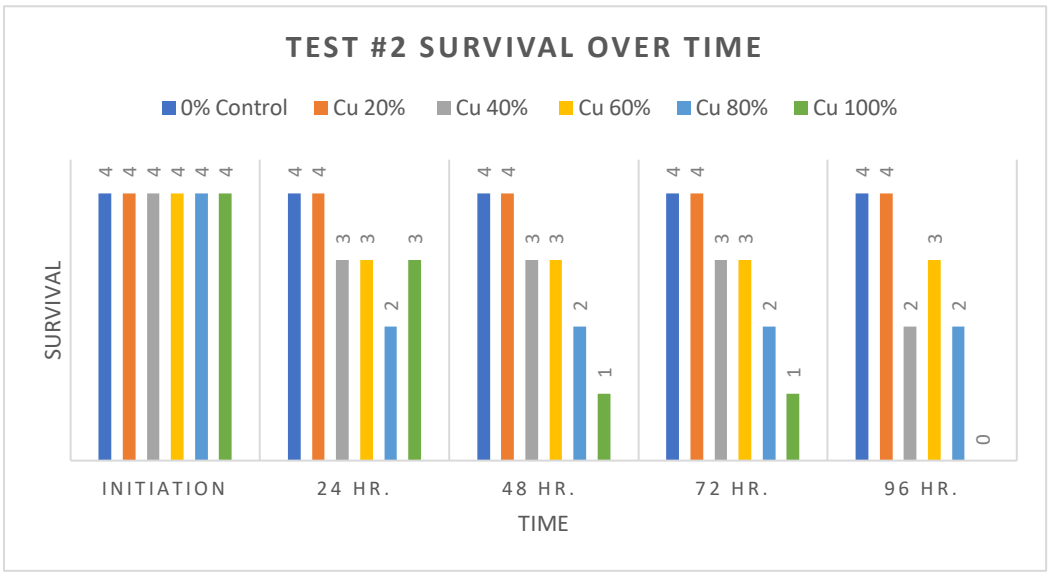

Figure 4: Test 2 survival over time.

The results of the statistical analyses calculating an LC_{50} indicate an LC_{50} of 71.58 mg/L (71.58 ppm), with a lower confidence limit of 54.08 mg/L (54.08 ppm), and an upper confidence limit of 94.73 mg/L (94.73 ppm).

Discussion

The findings of these tests appear to indicate that *T. carolina* exhibits a low susceptibility to Cu exposure. Tollett et al. (2008) postulated that Odonata may be a useful source of model organisms in the field of environmental toxicity testing. It is commonly held in the field of aquatic ecology that Cu is detrimental to most invertebrates (Yanong, 2019), however these findings would suggest that *T. carolina* instead follows the trend observed by Tollett et al. (2008) whose work also focused on the family Libellulidae.

Unfortunately, due to the lack of a single cohort, measurements of growth and biomass were not contributing factors in developing conclusions. The presence of multiple instars made the collection of biomass in order to observe physical incongruities following test termination inconsequential. While there is a trend indicating effects on mortality, sublethal effects such as negative impacts upon fitness and hindrances to morphological development were not able to be quantified.

While more genera should be examined to validate this trend, future areas of focus should be expanded in order to identify if this trend is widely shared, and if it extends to the other transition metals, and toxicants. This experiment was unique in being perhaps one of the first studies to calculate LC_{50} results regarding odonate environmental toxicity. The LC_{50} results indicate high levels of Cu required in the 100% toxicant stock to produce mortality in half of each test's population, as estimates far surpass the 4 mg/L utilized in this study, which represents just 5-6% of the 71.58 mg/L estimate. The EPA considers the action level for Cu to be 0.009 mg/L or 0.009 ppm (Tollett et. al., 2008). Considering the low amount of Cu used to prepare toxicant stock, these estimates appear quite high, and far beyond what might be encountered in nature without the introduction of contamination through disaster or accident.

An issue with this study relates to the inconsistency regarding life stages, or instars, of the model groups. Raising odonates in a laboratory setting is a potentially expensive and time-consuming endeavor. Naiads can take multiple years to mature (Corbett, 1999). The lack of a single identifiable cohort made the measuring of biomass irrelevant to this study. This resulted in lost data regarding effects and potential abnormalities related to fitness in the sublethal concentrations.

Similar to previous studies, these tests observed species that inhabit lentic environments. The means by which toxicity is introduced and sustained between lentic and lotic environments may differ substantially. The main area of focus in this area has been on the libellulids. If rearing technologies can be advanced to the point of practicality, examining Cu toxicity in further understudied odonate groups should be considered.

Focus moving forward should be directed at expanding the responses to varied levels of Cu to narrow observed confidence intervals, as well as other toxicants, preferably beginning with the other transition metals that easily bind with oxygen (manganese, tungsten, molybdenum, etc.). Methods for laboratory rearing should also be examined, both for the purposes of

endangered species protection, as well as a potential source of test organisms for toxicity laboratories adding to the range of research organisms currently in use. While this field has been grossly under examined, these findings indicate potential uses for Libellulidae as a useful bioindicator as well as a potential model laboratory organism within the field of whole effluent toxicity testing.

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