Professional Communication in Biology
BIOL 230
Spring 2025

Environmental Toxicology: An Experiment with *Daphnia* and Copper Sulfate

OBJECTIVES:

- Conduct an experiment to test the dose-response effect of a potential environmental toxicant.
- Analyze your data and assess its statistical significance using standard statistical tests.
- Obtain and read relevant, peer-reviewed primary literature, evaluating your own results in its context.
- Write a formal scientific research paper ("lab report") on your study and results, and peer review others.'

INTRODUCTION:

Intentionally or not, human activities have introduced a variety of chemical substances into the environment. These substances can have unintended ecological and biological consequences, including for humans. Organisms don't necessarily need to be exposed to high concentrations of a substance for serious effects to occur; sometimes exposures only need to be in the µg/L (microgram per liter, or parts per billion) or even ng/L (nanogram per liter, or parts per trillion) range. Minute concentrations of 1 µg/L and 1 ng/L are difficult to conceptualize—so for context these are equivalent to 3 seconds out of a century and 3 seconds out of one hundred thousand years, respectively! Some substances are poisonous, with acute (immediate) or chronic (long-term) toxicity; others 'bioaccumulate' in the bodies of organisms over time from continuous exposure, potentially reaching concentrations that are hundreds or thousands of times greater in the tissues than what is found in the external environment; others are endocrine disruptors that inhibit or enhance the effect of natural hormones, affecting organism growth, development, and reproduction; countless others may have effects that are as of yet undocumented. **Toxicology** is a field that combines biology and chemistry to study the adverse effects of substances on living organisms, including the specific mechanism of action and the exposure levels (dosage and time) that are required to induce the adverse effect. When a substance has an unknown effect, toxicologists will often first conduct a **LC_{50} bioassay** whereby laboratory test organisms are exposed to range of concentrations of the putative toxicant for a short period of time (hours to a few days), and the "lethal concentration" that kills at least 50% of the test organisms (the LC_{50}) is determined from the resulting **dose-response relationship.**

An example of a substance that humans apply to many freshwater ecosystems (lakes, ponds, and even home aquariums) is copper sulfate, an algaecide used to kill nuisance algae. Although a healthy aquatic ecosystem requires algae and plant growth to energetically support the herbivores and carnivores 'higher' in the food chain, lakes that have become polluted with excess amounts of nutrients (like phosphorus) from agricultural and residential applications of fertilizers will have explosive blooms of certain species of algae and weedy plants that foul the water for human use and cause serious ecological deterioration. To combat this problem, many states authorize the chemical treatment of impaired lakes with copper sulfate; you can also readily purchase similar products for treating backyard ponds and home aquaria. The copper binds to and denatures proteins in algae cells, causing cell damage and death. However, copper is known to be toxic to other organisms in the right quantities, and some studies have found adverse effects of copper sulfate on non-target aquatic organisms from invertebrates to fishes. Your objective in this class experiment is to conduct a LC_{50} bioassay to assess the dose response relationship for copper sulfate on a common freshwater animal, *Daphnia.*

Daphnia are small (~1.5-3 mm) crustaceans and members of the freshwater zooplankton community in lakes, living in the open water zone. Known as "water fleas" because of their fluttering swimming behaviors, they are filter-feeders that consume phytoplankton (suspended microalgae), heterotrophic protists, and bacteria. Movement of their thoracic feeding appendages creates a current that sweeps food particles to their mouth. Reproduction in *Daphnia* is primarily asexual by parthenogenesis, enabling short generation times and high reproductive output. Like other crustaceans, *Daphnia* possess a chitinous exoskeleton (called a carapace) that is molted with growth. In the case of *Daphnia*, the carapace is translucent, enabling observation of internal organs including the intestine, brood pouch, and heart (Figure 1). This makes *Daphnia* an ideal organism to test the biological effects of various environmental factors. For these and other reasons, *Daphnia* are considered "**model organisms**" in biology and have been extensively employed in research on ecology, evolution, physiology, and, as you will explore in this experiment, toxicology.

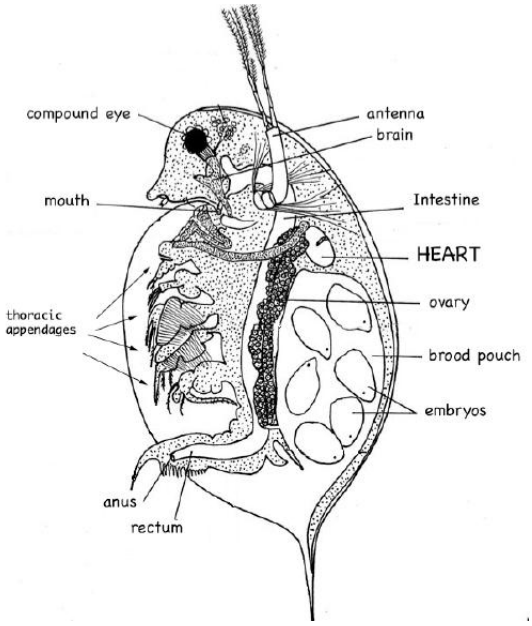

Figure 1. Anatomy of *Daphnia*.

BEFORE CLASS:

Carefully read through this entire handout, including the Procedure section. Look up any terms or concepts you are not familiar with. Then, (1) phrase the **research question** you will be addressing in your own words, (2) propose a **testable hypothesis** for that question, and (3) advance at least one specific **prediction**. Write these down in your notebook so that you will have them for later. Be thinking about the scientific relevance of your question, and how addressing it will increase current understanding of the topic.

Also, be thinking about the different components of the experimental procedure, and their purpose. You should write brief notes in response to the following points in your notebook:

- Identify the specific **independent** (manipulated)**, dependent** (measured), and **controlled** (constant) **variables**.
- What are the respective purposes of the **control group** and the different **treatment groups?**
- What is the **sample size** for the experiment?

MATERIALS:

- *Daphnia magna* culture
- *Ankistrodesmus* culture
- Disposable transfer pipettes, tips cut off
- Spring water (gallon jugs and squirt bottles)
- Copper sulfate pentahydrate stock (500 µg/L Cu)
- Conical tubes, 50 mL
- Tube racks
- Micropipettes and tips
- Gloves
- Volumetric flasks with stoppers
- Beakers (100, 250 mL)
- Graduated cylinders (10, 25, 50 mL)
- Fine-tip sharpies
- Labeling tape
- Parafilm
- Thermometers

PROCEDURE:

Since the class is online this semester, we will not actually perform this experiment ourselves. Instead, data from a previous semester will be provided in a shared class dataset. The protocol describing the experimental set up and how the data were collected is below. Please read it carefully and ask any clarifying questions you may have. You will eventually still need to describe the materials and methods from this experiment in your research paper.

Please remember that although *Daphnia* are invertebrate animals and experimentation on them is therefore exempt from IACUC regulation, they are living animals and should be extended their due respect. Avoid unnecessary manipulations and handling that could cause them additional stress. *Daphnia* are cultured at 21°C in 1L bottles containing spring water, with a 12hr:12:hr light:dark cycle, and fed satiating quantities of *Ankistrodesmus.*

1. While wearing gloves, prepare the following six solutions from the provided 500 µg/L copper sulfate stock solution. Test solutions are made by diluting the 500 µg/L stock with spring water—not deionized water or tap water—in the given ratios (Table 1).
    a. Prepare solutions in a 250 mL volumetric flask by first filling the flask about hallway with spring water, then adding the appropriate volume of 500 µg/L stock with a graduated cylinder or micropipette.
    b. Add 5 mL of *Ankistrodesmus* culture (this is food for the *Daphnia;* final food concentration = 2%).
    c. Fill the remainder of the volumetric flask to the etched 250 mL calibration line with spring water. Careful! The neck of the volumetric flask fills very quickly—once the volumetric flask is filled to the base of the neck, it is easier to use a squirt bottle of spring water to fill to the calibration line. Be sure the meniscus lies on the calibration line. Cap tightly with Parafilm and invert the flask several times to mix.
    d. The 500 µg/L solution is made with just 5 mL *Ankistrodesmus*, filled to the calibration line with undiluted stock (no spring water added).
    e. The 0 µg/L solution is made with just 5 mL *Ankistrodesmus*, filled to the calibration line with spring water (no stock solution added); this will serve as the control.

Table 1. Preparation of the six test solutions of copper sulfate. Combine the given volumes of copper sulfate stock solution, *Ankistrodesmus* culture, and spring water in a volumetric flask. All solutions have a total volume of 250 mL.

Test solution Concentration (µg/L Cu)	Volume 500 µg/L stock (mL)	Volume *Ankistrodesmus* (mL)	Total volume of test solution (mL)—any remainder is spring water
500	245.0	5.0	250
100	50.0	5.0	250
50	25.0	5.0	250
25	12.5	5.0	250
10	5.0	5.0	250
0	0.0	5.0	250

2. Pre-label a set of six 50 mL conical tubes (one for each test solution, including the control), and your initials.

3. Once all solutions are made, pour 30 mL of each test solution into their respective conical tubes.

4. Use a wide-bore transfer pipette to transfer 6 healthy *Daphnia* into each conical tube, including the control. Minimize the amount of spring water that is transferred along with the *Daphnia*: while holding the pipette vertically with the tip pointing down, either let the *Daphnia* swim down to the tip on their own or expel the excess water until the *Daphnia* are forced down to the tip so that each *Daphnia* is transferred in only one small drop of water. To the fullest extent possible, select animals that are approximately the same size.

5. Place each conical tube, with the caps loose, into a tube rack. Note the time; the experiment is now underway. Make note of any other relevant environmental conditions of the experiment (e.g., room and/or test solution temperature, etc.), or observations of the individual test *Daphnia.*

6. The tubes will be incubated at ambient temperature until the following class period, at which point you will count the number of live *Daphnia* in each conical tube and pool data with the class (if you wish to check the progress of the experiment during the time between class meetings, notify the instructor and room access will be arranged). We will discuss determination of the LC_{50} and how to perform appropriate statistical analyses on the data. Once analyzed, you will produce a formal **scientific research paper** ("lab report") on the results over the coming weeks, and participate in the **peer review** process by providing constructive feedback on a peer's first drafts. We will discuss all the elements of scientific writing in great detail during upcoming classes.