Toxicology and Basic Science Outreach

Communicating scientific results to the general public is an essential step in spreading awareness of academic findings outside of the ‘Ivory Tower’. Science communication and outreach allow scientists to engage with the public in a meaningful way that otherwise would not take place. The National Science Foundation (NSF), one of the largest granting agencies in the country for STEM fields, has recently set a precedent to further science outreach to the general public. For instance, the broader impacts portion of NSF grant proposals directly target such outreach initiatives, especially as they pertain to minorities, women, and younger-aged students.

Toxicologists in particular may benefit from such public outreach efforts, as toxicants may affect the public without their knowledge on a day-to-day basis. A more scientifically literate public, along with public engagement with science, could ultimately help to resolve issues related to toxicology as they pertain to people’s day-to-day lives, or the natural resources that they may take advantage of. Ultimately, educating the public on toxicological issues via public outreach efforts, has the potential to lead to a more scientifically literate society. For instance, by conducting science outreach at the high school level, scientists will be better able to engage with the next generation of workers, college students, and professionals.

With such outreach initiatives, scientists can partner with school teachers to communicate to further generations by introducing them at an early age to basic scientific concepts, and helping to improve on skills such as critical thinking and writing. By using scientists as a resource for their students, teachers will be better able to promote careers within scientific fields to their students as well.

Towards these goals of scientific outreach to students, we have arranged a lesson plan for toxicologists to further promote scientific thought and career opportunities within the field of toxicology. This lesson plan may be employed in conjunction with scientists, or can be presented independently and tailored to the specific needs of the class. Ultimately, this lesson plan meets the objectives of the HS-LS:1-6 learning objectives of the Next Generation High School science standards. This is of particular importance, as it is critical for students that are college-bound to be exposed to critical problem solving experiences before the start of their collegiate career.

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<u>Lesson Plan: How Scientists Conduct Bioassays for Ecotoxic Substances</u>

Introduction:

Many human activities require inputs of a large number of different chemicals. Natural Systems with a proximity to these activities receive inputs of these chemicals from runoff, direct application, or both. One natural system that has been shown to be of particular concern with regards to impacts from anthropogenic chemicals are wetlands, ponds and lakes. These systems perform essential ecosystem services for human societies. Thus, understanding the impacts that our chemical intensive lifestyles have on these systems are important.

Scientists perform LC_{50} experiments (lethal concentration, 50%) in order to determine the concentration of a chemical that will cause mortality in 50% of the target population. This is important because loss of certain organisms is linked to altered functioning of these systems. In this lesson we will perform an LC_{50} experiment with NaCl, a common road deicer on the common water flea (*Daphnia pulex*).

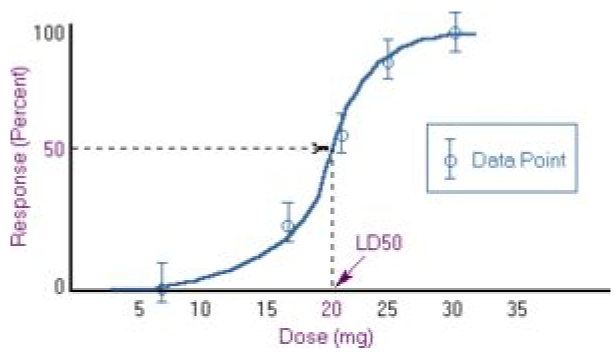
Integrating Ecology and Toxicology:

Why do scientist use D. Pulex as a model organism?

Scientists use *D. Pulex* as a model organism for several reasons. First it is widespread, meaning that it is found in subtropical and northern regions. Therefore findings from tests on *D. Pulex* can be generalizable enough to apply to the entire United States. Next, it is considered the most susceptible to many contaminants because of its relatively unsophisticated metabolism. Lastly, it occupies an important niche or ecological role in aquatic ecosystems. It controls the standing crop of phytoplankton that in turn alters the amount of light reaching submerged plants and algae. It provides an essential linkage in the food web from primary production to higher trophic levels.

What is an LC_{50}?

As noted above, LC_{50} stands for lethal concentration, 50%. This is the concentration of the contaminant that causes 50% mortality in the model organism replicates. This has been used by the EPA, the government agency charged with protecting the environment from chemical contamination, to determine the toxicity of chemicals to a host of organisms. This may be a good indicator for forming



quick assumptions about chemical toxicity. However, it is not necessarily demonstrative of its actual toxicity in real world settings.

Why Salt?

Sodium chloride is ubiquitous within wetland systems. It most commonly enters these systems due to road deicing, with concentrations associated with this mode of transport being as high as 1000 mg l^{-1} within the northeastern United States, and 2700 mg l^{-1} in the midwest. Further, NaCl can also become present in wetlands through the clearing of natural vegetation for agricultural farming, as well as through agricultural irrigation systems. Salinity concentrations due to these agricultural practices can be as high as 46,000 mg 1^{-1} in groundwater, which can ultimately leach or runoff into nearby bodies of water, which has implications for human and wildlife health.

What does ‘mode of action’ mean?

Understanding how chemicals produce ill effects on organisms is important to understanding their eco-toxicity. For example let's look at the common insecticide malathion. It is a cholinesterase inhibitor, meaning it blocks the transduction of signals along the nerve synapses. This leads to death because essential signals cannot be transmitted.

Salt has a different mode of action. It does not bind to important enzymes in the body, causes an imbalance of ions in the body compared to the aqueous environment that surrounds it. This leads to a loss of osmoregulation in organisms, leading to potential mortality.

Purpose: Formulating the Question

Inclass activity 1: (The importance of Questions)

We seek to understand the concentration of NaCl that causes *D. pulex* to lose its ability to effectively osmoregulate. Have the class formulate the question:

Something like: At what concentration of NaCl does 50% of *D. pulex* individuals lose its ability to osmoregulate?

Formulating the Hypothesis: Integrating Research in the Lab and Library

Inclass activity 2: (What is the Literature and How to find it)

We want to know a few things 1) What concentrations of ions are observed in freshwater systems? 2) What is the highest amount of NaCl that *D. pulex* have been observed to survive in? 3) What concentrations should we use in our experiment?

In order to answer these questions we need to get into the literature. Scientists do this by using search engines and databases. One popular search engine is google scholar, or use your school library's journal database.

HW 1: Finding sources: Have students find answers to the questions above. And bring them to the next class period.

Have the students formulate their hypothesis using an if-then statement.

"If *D. pulex* are subject to XX mg/L of NaCl, than 50% of the individuals will die."

Day 1: Preparing the solutions

In this section, students will make solutions of the NaCl at concentrations for the experiment.

Students will prepare solutions of 0 g L^{-1}, 0.3 g L^{-1}, 0.6 g L^{-1}, and 1 g L^{-1} concentrations.

Materials Needed: Rock salt, well water, balance, weighing boats, jars with tops, graduated cylinders.

1) Using the graduated cylinder, fill three mason jars with 800 mL of well water.
2) Determine the amount of salt (in grams) needed to make the 3 concentrations above
    a) Notice that our concentrations are in g L^{-1}, while our mason jars are filled with 800 mL of water.
    b) For our first concentration: (0.3 g L^{-1})(0.80) = 0.24 grams ; second: 0.6 g L^{-1} (0.80) = 0.48 grams; third: (1 g L^{-1}) (0.80) = 0.80 grams - **have the students work the math out on their own**
3) Using the weight boats and balance, weighing out the three quantities of salt
4) Once students have weighed out salt for each jar, they can then add salt to each: be sure to label jars with their respective concentrations.
5) Put lids back onto the jars and shake the concentrations for 10-15 seconds.

Day 1: Exposing *D. pulex* to salinity

In this section, students will expose *D. pulex* to each of the four salt concentrations

Materials Needed: four jars per student/group, plastic pipette for each student, labelling tape and marker, *D. pulex* from a local pond

1) Have students label jars (experimental units) with labelling tape and marker
2) Students will fill each jar halfway with salt solution for each respective unit - one potential issue is that student may accidentally put the wrong concentration into an experimental unit, stress that this may be an issue!
3) Using the plastic pipette, have students place a single *D. pulex* into each experimental unit, going from low concentration to high (0 g L^{-1} through 1 g L^{-1}).

    a) Students should double-check each jar to be sure that each has a single *D. pulex*
4) After all D. pulex have been added, have students determine whether or not *D. pulex* are still swimming within the solution. Students should make notes on their observations between treatments during this time.

Day 2: Checking on experimental units and quantifying data

In this section, students will determine mortality of *D. pulex* between their salt treatments

Materials Needed: one dissecting scope, plastic pipette per student/group, projector

1) Using a computer and projector, project a video of an immobilized *D. pulex* - students may easily misidentify immobilized individuals as being dead.
2) Have students check each of their units and determine the state (mobile, immobile, or deceased) of each *D. pulex*
3) Have students/groups report mortality of each treatment and place results on the board
    a) Discuss the results with students - homework can be assigned for the class-wide results, such as graphing of the data.

Using Data to Make Predictions: Considering the Community and Forming Conclusions

Once ecotoxicologists have data from LC_{50} tests, they can attempt to make predictions on how substances will alter natural communities. To produce the most accurate predictions of a substance's effect on the community, the ecotoxicologist may need to perform LC_{50} tests on several different taxa. Other taxa that are common to wetland communities include:

<u>Amphibians</u>
- Spring Peeper
- Wood Frog
- Spotted Salamanders
- Leopard frog
- American Toads

<u>Odonates:</u>

- Green darter
- Damselflies
- Pond skimmer

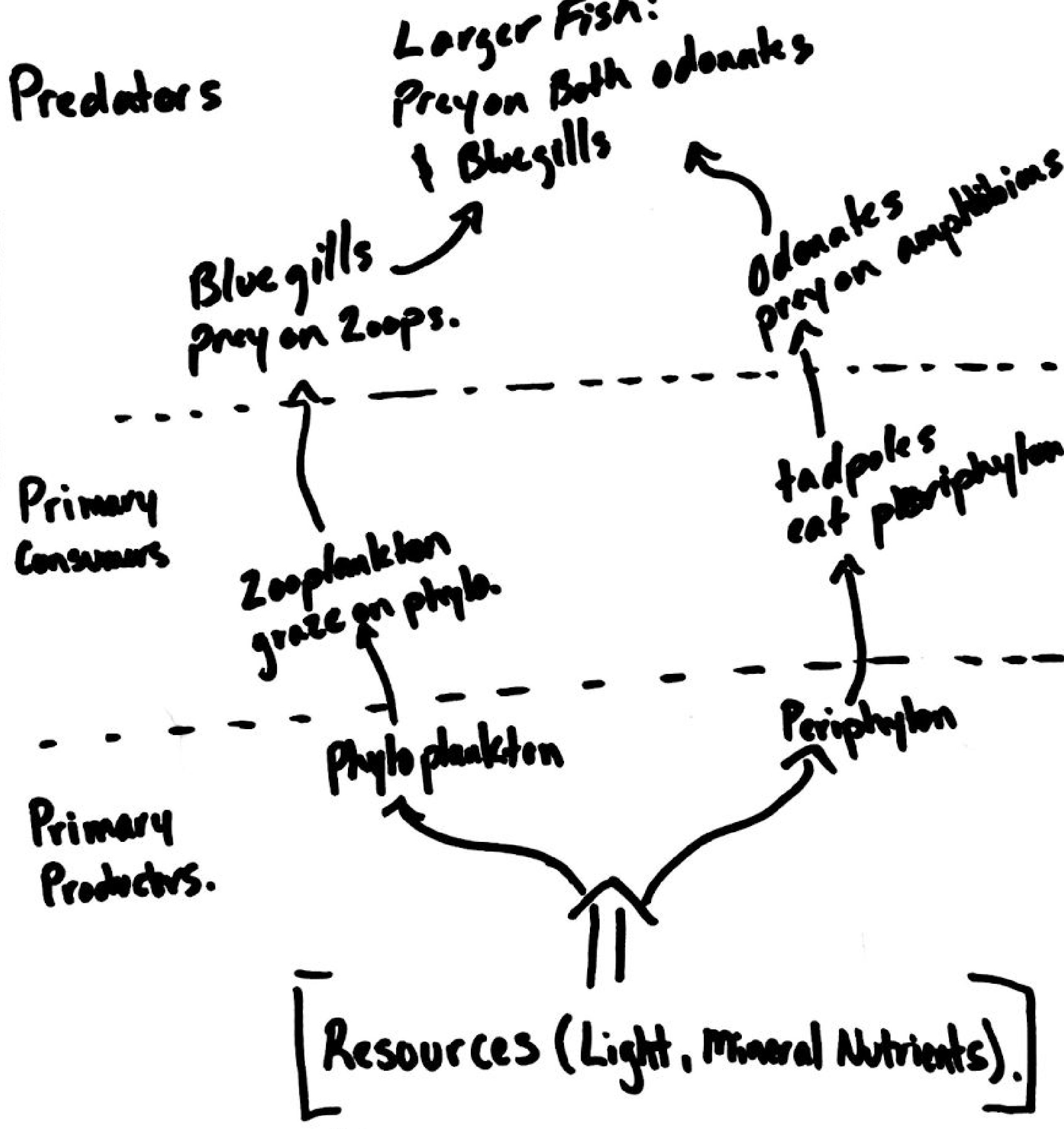
<u>Fish</u>
- Bluegill
- Pumpkinseed

All of the organisms above, along with *D. pulex*, exist as part of an ecological community within wetlands and pond ecosystems. This means that they interact both directly and indirectly with one another. Direct interactions consist of relationships where individuals have direct effects on one another, such as predator-prey relationship. Indirect interactions consist of relationships in which individuals have an effect on one another, but through indirect means. For instance, *D. pulex* helps in maintaining water clarity, which has subsequent effects on epiphytic algae abundance, which is a food source for larval amphibians.

In Class Group Activity:

Have the students construct a sample community consisting of resources (light and mineral nutrients), primary producers (phytoplankton and algae), primary consumers (tadpoles and zooplankton), and predators (fish and odonates). Explain that resources start at the bottom and then the levels build on top of this.

Sample In Class Group Activity.



Using this model community, explain how chemical contamination of systems may disrupt community dynamics.

Use the data from the LC_{50} to demonstrate that in natural systems, zooplankton may be impacted by concentrations that can cause mortality in nature. These can set off what is called a bottom up trophic cascade, this impacts all the levels of the community we constructed by changing the availability of food resources. Lowering the abundance of zooplankton in the systems will have two direct effects: firstly it will release phytoplankton from top-down control on abundance, next it will limit the availability of food to zooplanktivorous fish.

1) Phytoplankton released from top down control will blume or rapidly increase in abundance. This will shade out epiphytic algae attached to surfaces underwater. This will result in a reduction in resources available to larval amphibians, causing their abundance to decline. Odonates will experience a reduction in abundance also resulting in decreased abundance.
2) Reduction in small fish and odonates will result in reduced number of larger fish.
3) These effects can be magnified by the impacts that increased salinity is having on these organisms.

How do Scientists test hypotheses derived from experimental data?

They conduct more experiments to test the impacts on the community ecotoxicologists may perform experiments on constructed communities in order to determine if their predictions are correct. Sometimes they find effects that they did not predict based on the single species toxicity assays or LC_{50} tests.

Final Activity: Student lab report (see Scientific Writing Guide)

Scientific Writing Guide

General Guidelines

- MLA or APA are common choices. Make sure you have:
- 1 inch margins
- Size 12 font (Times, Cambria, Arial, Calibri okay)
- Double spaced with line numbers
- Minimum of 5 scientific article citations
- Refer to Searle et al., 2016 of what the following sections should contain

Internal Citations should follow this format:

- (Author Last Name, Year Published)
- Examples: 1 Author – (Smith, 2015), 2 Authors – (Smith and Park, 2015), 3 or more authors – (Smith et al., 2015)
- See below for Literature Cited

<u>Title</u>

Same size and font, centered and underlined, may be bolded
Be clear, concise and don't be afraid to spoil the ending

Introduction

Frame your work with a general perspective. Provide relevant background that the reader will need to understand your approach, hypotheses etc. Significance should tell the reader why it is important to do this research, and what applications or benefits may come of it. *This is a good place to use the literature you researched.* Going from broad to specific throughout the paragraph, towards the end should be your <u>working hypothesis</u>.

Methods

This is where you tell the reader what you did (in past tense, as you already did it). Be detailed enough so that other biologists could repeat your experiment just from reading it, but don't encumber the reader with extraneous details. I should see in this section a sound research plan, that controlled for confounders and truly tested the proposed hypothesis. Mention any statistical analyses and *include your null and alternative hypotheses*.

Results

Keep this section short and sweet. What were your results in a *statistically* phrased explanation? I.e. Was there a statistical difference between your categories at the given p value? Include relevant figures where they will *inform the reader*. These should contain *summarized data*, where multiple trials are averaged, or combined in a way relevant to the data you collected. Not just points on a graph for each pollinator. Label your axes, and provide a *numbered* and *descriptive* title (eg. **<u>Figure 1</u>**<u>: Average gender composition across sections of introductory biology sections</u>). All figures and tables go at the end of the document, having a separate page for each.

Discussion

This is the place to discuss your statistical results in a biological manner. It is essential that you do not make assertions that extrapolate beyond what your statistics told you about your data. This is a good section to compare your study with other similar studies, and telling what they found. Provide take-home messages during the end of this section and provide reasons as to why your study was of significance. End with potential further studies.

Literature Cited

Here you can abide by the format of your choice (MLA, APA; use “Purdue OWL” for guidance). Don’t forget the hanging indent for the *second line for each citation.* Remember, <u>you need five references from scientific journals</u>. Use google scholar, web of science, or another search engine to find articles. You may also cite your textbook, your lectures or other relevant material. Do not use web encyclopedias (e.g. Wikipedia) or authorless/dateless entries on websites.