PURDUE UNIVERSITY | Extension

FNR-595-W

EXPERT REVIEWED

The NATURE of TEACHING

WILDLIFE

Ecotoxicology and Environmental Health

LESSON PLAN

This unit highlights the effects of environmental contaminants on the health of wildlife and ecosystems.

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OVERVIEW

ESTIMATED TIME

2 lessons, 45-90 minutes each

VOCABULARY

- Contaminant
- Dissolved oxygen
- pH
- Turbidity
- Eutrophication
- Riparian buffer
- Ecotoxicology
- Bioassay
- LC_{50} value
- Dose-response curve
- *Daphnia*
- Correlative relationship

OBJECTIVES

- Describe basic water quality measurements
- Analyze water samples and evaluate how water quality is impacted by land use
- Critically assess the results of a laboratory study and summarize their findings in a lab report
- Apply the scientific method to determine how a common contaminant impacts aquatic organisms
- Evaluate the results of a bioassay by plotting data and extrapolating an LC_{50} value.

REQUIRED MATERIALS

- Outlined on the first page of each lesson (pgs. 7 and 17).

ACKNOWLEDGEMENTS

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ACTIVITY ICONS

Use these icons — located at the top of each lesson plan — to indicate the disciplines to which certain activities belong. These disciplines include:

READING | WRITING | MATH | SCIENCE | STEM (science, technology, engineering, math) | STEAM (science, technology, engineering, art, math)

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LESSON STANDARDS

Lesson One

Next Generation Science Standards

MS-LS2-1
MS-LS2-4
MS-ESS3-3
HS-LS2-6
HS-LS2-7
HS-ESS3-4

Math

CCSS.MATH.CONTENT.HSS.IC.B.6

English Language Arts

CCSS.ELA-LITERACY.W.6.7
CCSS.ELA-LITERACY.W.6.9
CCSS.ELA-LITERACY.W.7.7
CCSS.ELA-LITERACY.W.7.9
CCSS.ELA-LITERACY.W.8.9
CCSS.ELA-LITERACY.W.9-10.9
CCSS.ELA-LITERACY.W.11-12.9

Lesson 2

Next Generation Science Standards

MS-LS2-4
MS-ESS3-3
HS-LS2-6
HS-LS2-7
HS-LS4-6

Math

CCSS.MATH.CONTENT.6.EE.A.2
CCSS.MATH.CONTENT.7.EE.B.3
CCSS.MATH.CONTENT.HSA.SSE.A.1
CCSS.MATH.CONTENT.HSS.ID.A.1
CCSS.MATH.CONTENT.HSS.ID.A.4
CCSS.MATH.CONTENT.HSS.ID.B.5
CCSS.MATH.CONTENT.HSS.ID.C.7
CCSS.MATH.CONTENT.HSS.IC.B.6

Lessons 1and 2

English Language Arts

CCSS.ELA-LITERACY.RI.6.7
CCSS.ELA-LITERACY.RI.6.10
CCSS.ELA-LITERACY.RI.6.4
CCSS.ELA-LITERACY.W.6.1
CCSS.ELA-LITERACY.W.6.10
CCSS.ELA-LITERACY.SL.6.1
CCSS.ELA-LITERACY.RI.7.4
CCSS.ELA-LITERACY.RI.7.10
CCSS.ELA-LITERACY.W.7.1
CCSS.ELA-LITERACY.W.7.10
CCSS.ELA-LITERACY.SL.7.1
CCSS.ELA-LITERACY.RI.8.10
CCSS.ELA-LITERACY.W.8.1
CCSS.ELA-LITERACY.W.8.10
CCSS.ELA-LITERACY.SL.8.1
CCSS.ELA-LITERACY.SL.8.6
CCSS.ELA-LITERACY.RI.9-10.10
CCSS.ELA-LITERACY.W.9-10.1
CCSS.ELA-LITERACY.W.9-10.10
CCSS.ELA-LITERACY.SL.9-10.1
CCSS.ELA-LITERACY.SL.9-10.6
CCSS.ELA-LITERACY.RI.11-12.10
CCSS.ELA-LITERACY.W.11-12.1
CCSS.ELA-LITERACY.W.11-12.10
CCSS.ELA-LITERACY.SL.11-12.1
CCSS.ELA-LITERACY.SL.11-12.6

Ecotoxicology is an interdisciplinary field focused on the study of the effects of toxic chemicals on biological organisms, populations, and ecosystems. **Contaminants** (substances that, when accidentally or deliberately introduced into the environment, have the potential to harm people, wildlife, or plants) of particular interest in ecotoxicology include things such as pesticides, petroleum products, heavy metals, and urban runoff. When these contaminants enter natural ecosystems, they have the potential to cause direct mortality, disrupt food chains, reduce reproductive success, and alter the course of evolutionary change. Given these wide ranging effects on animals, plants, and ecosystems and our increasing usage of chemicals, ecotoxicology is becoming an important field of study.

LESSON 1: THERE'S SOMETHING IN THE WATER! – VOCABULARY

- **Contaminant –** substance that, when accidentally or deliberately introduced into the environment, has the potential to harm people, wildlife, or plants
- **Dissolved oxygen –** how much oxygen is dissolved in the water and available to be used by aquatic organisms
- **pH –** how acidic or alkaline the water is
- **Turbidity –** how clear the water is
- **Eutrophication –** excessive levels of nutrients in a pond, frequently due to runoff from the land, which causes a dense growth of algae and death of other life from lack of oxygen
- **Riparian buffer –** a vegetated area near a stream that helps shade and protect the stream from the impact of adjacent land uses

LESSON 1: THERE'S SOMETHING IN THE WATER! – BACKGROUND

Freshwater is one of Earth's most valuable and important resources. Humans rely on freshwater for drinking, agriculture, industry, sanitation, and recreation. However, freshwater accounts for only 3% of all water on Earth, with only a small fraction (~0.5%) of that water making up the world's freshwater ecosystems. These ecologically and economically important habitats come in the form of rivers, streams, lakes, ponds, and wetlands and are home to a wide variety of organisms, including fish, amphibians, reptiles, mammals, birds, and invertebrates. These ecosystems also provide a variety of economically valuable services (i.e., ecosystem services) such as flood control, water purification, climate change mitigation, and erosion prevention. These ecosystems, however, are often susceptible to contamination and degradation due to human activities.

Common contaminants include fertilizers, sewage, pesticides, and industrial waste. The introduction of contaminants can cause changes in important indicators of the health of an aquatic ecosystem, such as **dissolved oxygen** (how much oxygen is dissolved in the water and available to be used by aquatic organisms), **pH** (how acidic or alkaline the water is), **turbidity** (how clear the water is), and nutrient content. For example, it is common for farm ponds to receive excess nutrients, such as nitrate and phosphate, from surface runoff. This initiates a process known as **eutrophication**. Elevated nutrient levels result in excessive plant and algal growth. These plants and algae eventually die and are decomposed by bacteria. These bacteria use up much of the oxygen in the water, reducing dissolved oxygen levels and resulting in the death of aquatic organisms.

The goal of this exercise is to teach students about common measures of water quality and how the surrounding environment can influence the quality of a water body. In the process, students will also learn the impacts that human activities can have on aquatic ecosystems.

Lesson Preparation
One of the most exciting parts of this lesson for students is learning about the connection between land use and water quality using specific examples from your locality. This will require some preparation and sample collection prior to the activity.

On the morning of or day before the lesson, collect water samples from three ponds. For best results, it is recommended that you pick ponds situated in habitats that differ drastically from one another (a farm pond, a suburban pond, a forest pond, a golf course pond, etc.). To collect a water sample, submerge a container (such as a mason jar, bucket, or Tupperware) in an undisturbed area on the margin of the pond. Fill the container to the brim, place a lid on the container, label with the collection location, and immediately store the sample in a cooler on ice. This will help to maintain the quality of the samples, especially if you collect them the day prior to the lesson. Alternatively, have students collect water samples and bring them into class for homework after discussing best water collection practices. If you have no access to freshwater ecosystems, you can process samples of bottled water, tap water, and well water to compare.

At each site, make sure to record the GPS coordinates of your location. This can be done with most smartphones. On an iPhone or Android phone, open the Google Maps app. Next, touch and hold on the area of the map for coordinates. You will see a red pin appear. Depending on your device, you will find the coordinates in the search box at the top, or in the details under the "dropped pin" tab. Optionally, you may also take pictures at each location of the sampling site and other important land features. You can then provide these pictures to the students to supplement the Google Maps satellite images they will be accessing.

This lesson plan is designed to be used in concert with LaMotte GREEN Program Low-Cost Water Monitoring Kit (http://www.lamotte.com/en/education/water-monitoring/3-5886.html). These kits are relatively easy to use and economical (~$40). Each kit comes with enough supplies for 10 pH samples, 10 dissolved oxygen samples, 10 nitrate samples, 10 phosphate samples, three coliform bacteria samples, and unlimited turbidity samples. *Note that this lesson plan can easily be adapted to work with other water monitoring kits if more precision is desired or other resources are available.*

At the end of the activity, students will be asked to do a write-up of the lab activity. A rubric for grading the write-up, including the homework questions, is included in the lesson. Please provide the rubric to the students before they begin the write-up so that they understand what is expected.

LESSON 2: VOCABULARY

- **Ecotoxicology –** the study of the effects of toxic chemicals on biological organisms
- **Bioassay –** is an experiment in which a living organism is used as a test subject to determine the potency of a chemical substance
- **LC_{50} value –** the lethal concentration of a contaminant required to kill 50% of a test population
- ***Daphnia*** **–** small, aquatic crustaceans commonly used for biological research
- **Dose-response curve –** describes the magnitude of the response of an organism to a chemical
- **Correlative relationship –** degree of relationship between two variables

LESSON 2: BACKGROUND

Because humans release thousands of different chemicals into the environment, it is important to study and understand the effects of these potentially dangerous chemicals on organisms and ecosystems. A **bioassay** is an experiment in which a living organism is used as a test subject to determine the potency of a chemical substance. When a stimulus is applied, the organism's responses are measured. Bioassays provide a means to quantify the toxicity of a chemical. In a typical bioassay, organisms are exposed to a potentially toxic chemical at a range of concentrations. This allows researchers to observe the effects of the chemical on the organism's behavior and survival and determine at what concentration a chemical becomes harmful.

In this lesson, students will be using a bioassay to determine the lethal concentration of a contaminant required to kill 50% of a test population (**LC_{50} value**). For this bioassay, the test animals will be *Daphnia magna*, a small, aquatic planktonic crustacean also known as "water fleas." *Daphnia* are key organisms in ponds and lakes, acting as a primary consumer and serving as a food source for many other organisms. *Daphnia* make excellent experimental organisms because they are easy to keep in the lab, can asexually reproduce, and are easy to observe.

The focal contaminant for this bioassay will be salt (NaCl). Salt is a contaminant of concern in aquatic ecosystems because of its frequent use in road treatments. Ponds, lakes, and wetlands near roads receive runoff containing high salt contamination, especially after the spring melt. Salt is also easy to acquire for the lessons. *If desired, you can easily adapt the lessons found here to use other contaminants, such as ethanol, caffeine, or cleaning products, to compare their effects.*

Another critical component of evaluating the effects of a contaminant on a living organism is creating a **dose-response curve**, which describes the magnitude of the response of an organism to the chemical. Thus, after conducting bioassays on the *Daphnia* and recording data, groups will graph their data to create a dose-response curve. This curve will reveal a correlative relationship between the dose of contaminant administered and the proportion of *Daphnia* surviving at the end of the assay. By creating their own dose-response curve using data they have collected, students will be able to extrapolate an LC_{50} value.

Throughout the activity, students will be asked to answer questions regarding expected outcomes, aspects of experimental design, and the interpretation of the results. This approach will ensure that students are thinking about the scientific process and the implications of their results for real ecosystems.

Daphnia purchasing/care

- Live *Daphnia magna* cultures can be ordered from biological supply companies such as Carolina Biological Supply (https://www.carolina.com). You may choose to order either individual *D. magna* cultures (https://www.carolina.com/Daphnia/Daphnia-magna-living/142330.pr) or a culturing kit (https://www.carolina.com/Daphnia/Daphnia-culture-kits/FAM_142304.pr) to rear and maintain a larger quantity of *D. magna* in your classroom. Each culture is ~$10.00, and a culturing kit is ~$50.00. (As of early 2020.) Each culture contains approximately 30-40 organisms per culture container (a class of 20 would require 3-4 cultures). If you choose to maintain *D. magna* cultures in your classroom for this and future experiments, there are many online resources with instructions for care and rearing.
  - https://www.carolina.com/teacher-resources/Interactive/living-organism-care-guide-Daphnia/tr10492.tr
  - https://www.flinnsci.com/api/library/Download/b049dbe22e12461f83e3da6afab121ca
  - https://www.wikihow.com/Start-Daphnia-Cultures
- Be sure not to use detergents or soaps on any of your experimental units or supplies, as they may be toxic to the *Daphnia*.
- Use spring water or natural well water to rear your *Daphnia*. If only treated tap water is available, be sure to dechlorinate it before use by leaving it to sit for 3-4 days, adding a bubbler to it, or by adding a commercial dichlorination solution.

Solution Preparation

- To prepare a **5% salt (NaCl) solution preparation**, you will need table salt and water. The volume you will need to prepare will depend on your class size. To create a 5% salt solution by mass, you will need to add **5g of salt per 100 mL of water**.

  For example:
  - if you want to create 500 mL of solution, you should first mix **25g** of salt with **400 mL** of water in a graduated cylinder. Once the salt has completely dissolved, add water to bring the final volume of the solution to **500mL**.
- Supply each group with a bottle containing approximately 100mL of the 5% salt solution.

Experiment timing

Although the experiment recommends mortality checks of the *Daphnia* after 1 and 24 hours, we recommend that instructors adjust these time checks to fit best with their class schedules. For example, if your class meets every other day, you may change the checks to after 1 hour and after 48 hours and move forward with the experiment accordingly.

Alternatives to using Daphnia

Although it is encouraged that instructors to use *Daphnia* as the experiment organism for this lesson, some schools may have policies that prevent the use of live animals in this capacity. In this case, an alternative organism to use is **duckweed**, an aquatic plant. Like *Daphnia*, duckweed can be purchased from Carolina Biological Supply (https://www.carolina.com) at an affordable price. Although using duckweed should yield similar results to the *Daphnia* experiment qualitatively, the duration of the experiment may need to be extended and some of the *Daphnia*-specific questions and protocols will need to be adjusted accordingly.

LESSON 1

READING WRITING MATH SCIENCE

LESSON 1: THERE'S SOMETHING IN THE WATER!

This lesson teaches students about important water quality parameters and allows them to explore how land use has a significant effect on water quality.

Estimated time

1-2 class periods

Procedure

1. The day before the lesson assign the students the pre-lab homework questions.
2. Collect water samples from three ponds and one water sample from the tap.
3. Use tape and marker to label water samples 1-4 (3 ponds + 1 tap water)
4. Break the students into at least 4 groups. Each group will process one water sample. Use groups of 3-4 students.
5. Assign each group a lab station with the required materials. Have students work through the **There's Something in the Water! Lab Activity**.
   a. As students process their water samples, have each group record their results on the board along with their sample number so that each group has access to the information.
6. Give students access to the GPS coordinates for each site by writing them on the board and have them copy them into Table 1. These can be written in any of the following formats:
   - Degrees, minutes, and seconds (DMS) (41°24'12.2"N 2°10'26.5"E)
   - Degrees and decimal minutes (DMM) (41 24.2028, 2 10.4418)
   - Decimal degrees (DD) (41.40338, 2.17403)
7. After each group has completed the activity through **Part III** of **There's Something in the Water! Lab Activity**, come together as a class and tally on the board where the students predicted each sample came from. Once a consensus has been reached, ask the students in the class to justify their predictions based on what they observed from the satellite images.
8. Reveal which samples came from what sites.
9. Have the students complete **Part IV**.
10. Have students share what they wrote in their write-up.

Required Materials

- Laptop computer with access to Google Maps (1 per group)
- LaMotte GREEN Program Low Cost Water Monitoring Kit
- 4 containers to collect water samples
- 4 copies of the LaMotte GREEN water sampling kit manual (https://earthforce.org/GMGREEN/wp-content/uploads/2016/03/Lamotte-Water-Monitoring-Kit-Manual.pdf)
- Tape and marker
- Pencil (1 per student)
- Paper (1 per student)
- There's Something in the Water! Worksheet (1 per group)
- Pre-Lab Homework Questions (1 per student)

LESSON 1: PRE-LAB HOMEWORK QUESTIONS

Background

Freshwater is one of Earth's most valuable and important resources. Humans rely on freshwater for drinking, agriculture, industry, sanitation, and recreation. However, freshwater accounts for only 3% of all water on Earth, with only a small fraction (~0.5%) of that water making up the world's freshwater ecosystems. These ecologically and economically important habitats come in the form of rivers, streams, lakes, ponds, and wetlands and are home to a wide variety of organisms including fish, amphibians, reptiles, mammals, birds, and invertebrates. These ecosystems also provide a variety of economically valuable services (i.e., ecosystem services) such as flood control, water purification, climate change mitigation, and erosion prevention. These ecosystems, however, are often susceptible to contamination and degradation due to human activities.

Contaminants are substances that, when accidentally or deliberately introduced into the environment, have the potential to harm people, wildlife, or plants. Common contaminants include fertilizers, sewage, pesticides, and industrial waste. The introduction of contaminants can cause changes in important indicators of the health of an aquatic ecosystem, such as dissolved **oxygen, pH, turbidity,** and **nutrient content**. For example, it is common for farm ponds to receive excess nutrients and sediment input from surface runoff. This results in elevated levels of **nitrate** and **phosphate**, a process known as **eutrophication**. Eutrophication, in turn, results in excessive plant and algae growth. These plants and algae eventually die and are decomposed by bacteria. These bacteria use up much of the oxygen in the water, reducing dissolved oxygen levels and resulting in the death of aquatic organisms.

The goal of this exercise is to teach students about common measures of water quality and how the surrounding environment can influence the quality of a water body. In the process, students will also learn the impacts that certain human activities, such as agriculture, have on aquatic ecosystems. Another goal of this activity is to learn about common measures of water quality and how the surrounding environment can influence the quality of a water body. The following questions will prepare you for the laboratory experiment you will be conducting this week. Please use online resources found through Google searches to answer the questions. Helpful links are provided below the homework questions.

Helpful resources to research answers to the homework questions:

Why monitor water quality?
https://water.usgs.gov/owq/WhyMonitorWaterQuality.pdf

Phosphate in surface water streams lakes:
https://www.water-research.net/index.php/phosphate-in-water

Nitrates and Their Effects on Water Quality:
http://www.wheatleyriver.ca/media/nitrates-and-their-effect-on-water-quality-a-quick-study/

The Science Behind the Need for Riparian Buffer Protection:
https://conservationtools.org/guides/131-the-science-behind-the-need-for-riparian-buffer-protection

Definition of Water Quality Parameters:
http://fosc.org/WQData/WQParameters.htm

Water Quality- Environmental Measurement Systems:
https://www.fondriest.com/environmental-measurements/parameters/water-quality/

Background Questions

1. What are three reasons why scientists monitor water quality?

2. Define pH (i.e., what does it measure?).

3. Define dissolved oxygen (DO).

4. Define turbidity.

5. What are phosphates and nitrates? How do they relate to eutrophication?

6. What is nutrient runoff? Name three common sources of nutrient runoff.

7. Define riparian buffer.

Critical thinking questions (optional)

1. What does a low or high pH indicate about a water body?

2. What does dissolved oxygen tell us about a water body? What is the lower limit of dissolved oxygen necessary to maintain fish populations?

3. What does turbidity tell us about a water body and the surrounding habitat?

4. What are three negative consequences of excess nutrients in a water body?

5. Why are riparian buffers beneficial for a water body? How wide are riparian buffers recommended to be to help protect water quality?

LESSON 1: THERE'S SOMETHING IN THE WATER! LAB ACTIVITY

Today, a state environmental scientist named Beth and her team collected water samples at several local water bodies as part of a long-term water quality monitoring effort. Beth has brought their samples to your lab to be analyzed for several indicators of water quality, such as pH, dissolved oxygen, nitrates, phosphates, turbidity, and coliform bacteria presence. However, upon arrival, Beth realizes that she forgot to bring the datasheet that connects the numbered samples to the sites that they came from. Ever the ambitious scientist, you decide to have some fun with the situation: using only the water quality data obtained from the samples along with satellite images of the sampling sites, you bet Beth that you can predict which sample came from each site. Beth skeptically accepts your bet and heads out to return to her office. With not a second to waste, you quickly get to work analyzing the samples!

Materials

- Clean, tap water sample for reference
- 4 unknown water samples
  - 3 from sites located in different environments
  - 1 tap water sample
- Water monitoring kit
- Sheets of paper for recording answers and observations

Procedure

Part 1: Sample analysis

1. Working in your assigned group, you will be given one water sample to analyze. Before you begin, make sure to follow all lab safety procedures, including wearing safety goggles, tying back hair, wearing gloves, safely handling materials, and properly disposing of waste. Additionally, make sure to read the safety procedures provided with your water monitoring kit as well each water quality card to understand each test before conducting it. Make sure to keep your station clean as you work and clean up your station and equipment afterward.
2. Follow the directions provided with your Lamotte GREEN Program Low-Cost Water Monitoring Kit (or alternative water sampling kit) to conduct water quality testing for the following water qualities:
   a. Turbidity
   b. Dissolved oxygen
   c. Nitrate
   d. Phosphate
   e. pH
3. As you take measurements of your sample, record them in Table 1.

TABLE 1: Water quality data

Group #	Sample #	GPS coordinates	Water appearance	Turbidity (JTU)	Dissolved oxygen (ppm)	Nitrate (ppm)	Phosphate (ppm)	pH

TABLE 2: Water quality reference values

Turbidity (JTU)	Dissolved oxygen (ppm)	Nitrate (ppm)	Phosphate (ppm)	pH
0 – Excellent 0 to 40 – Good 40 to 100 – Fair >100 – Poor	>8 ppm – Excellent 6 to 8 ppm – Good 4 to 6 ppm – Fair <4 ppm – Poor	0 to 5 ppm – Good 5 to 20 ppm – Fair 20 – 40 ppm – Poor >40 ppm – Very poor	0 to 1 ppm – Excellent 1 to 2 ppm – Good 2-4 ppm – Fair >4 ppm –Poor	7 – Excellent 6 or 8 – Good Less than 6 – Poor Greater than 8 – Poor

4. Write your sample measurements from the table on the board.
5. Copy the data from the other groups into your table.

Part II: Sampling Site Assessments

1. Now that all the sample measurements are on the board, it's time to take a look at the sampling sites to record information about pond and habitat characteristics in Table 3. To do this, you'll need computer access. Go to https://maps.google.com/ and then click on satellite view.
2. Type in the coordinates of the first site in the search bar of Google Maps. This should take you close to the pond. If this doesn't work, first make sure you have typed in your coordinates correctly. (If you think you've done everything correctly and are still having trouble, raise your hand and wait for your teacher.)
3. Practice using the "measure" feature in Google Maps a few times. You'll need it to answer questions on your Pond and Habitat Characteristics sheet (Table 3). To use the "measure" feature:
   a. Right-click where you want to start your measurement and choose "measure distance."
   b. Left click where you want to end your measurement. The distance of the measurement will display on the line.
   c. To clear your measurement, right-click and choose "clear measurement."
4. First, zoom in close to the pond. What does the pond look like? Describe the water color and shape of your pond. Does the pond appear to be natural or man-made? Do you see any algae in the pond?
5. Does the pond have a riparian buffer? If so, measure and record its width.
6. Zoom out so that you can see the pond and the surrounding landscape.
   a. Is there a farm nearby? If so, how far is the closest field?
   b. Are there any other possible sources of excess nutrients or pollution nearby (residences, golf courses, factories, mines, drainage ditches, etc.)? If so, how far away are they?

TABLE 3: Pond and habitat characteristics

Pond #	**#1**	**#2**	**#3**
Color			
Shape			
Natural or man-made?			
Estimated % cover by algae			
Riparian buffer width (m)			
Distance to closest farm (m)			
Distance to other source(s) of nutrients/pollution (m)			
Predicted Water Sample			

Part III: Pulling it All Together

1. Based on your observations in Table 3, which pond would you describe as being in the most pristine habitat? Which pond would you describe as being in the most degraded habitat? Please provide reasoning.

2. Compare your measurements in Table 1 to the reference values in Table 2. Which water sample would you describe as being the highest quality? Which water sample would you describe as being the lowest quality? Are there samples that are difficult to distinguish between? Please provide reasoning.

3. Compare your visual pond quality assessments in Table 3 with the water quality measurements in Table 1. Make predictions about where each water sample came from and which water sample came from the tap. Justify each prediction with reasoning based on your background knowledge and the information available.

4. Once all the groups have completed Part III, tally your predictions on the board. If there are disagreements, discuss your predictions as a class and justify your group's decisions with the reasoning you used to answer the questions above.

Part IV: Lab Report

1. In Microsoft Word, write a 1-2 page (12-point font, Times New Roman, 1.5 Spaced) summary of this activity. Please include the following sections:
   a. Sample analysis
      - Describe the physical properties of the sample that you measured and briefly describe the methods you used.
      - Issues you encountered during analysis
      - Limitations of the analysis you conducted (e.g., how accurate the kits were)

   b. Sampling site assessments
      - A description (1-2 sentences) of each sampling site
      - 2 benefits and 2 limitations of using Google Maps to assess habitat quality
      - Other information about the sampling sites that would have helped you match them to the water samples

   c. Pulling it all together
      - Summarize your predictions of where each water sample came from, with brief justifications.
      - Discuss how your predictions matched up with where the samples actually originated. How did/didn't things match up? Why might this be?

LESSON 1: Lab Report grading rubric

	20 pts	15 pts	10 pts	0 pts	Score
Homework	Homework complete with satisfactory answers to all questions	Homework complete with satisfactory answers to most questions	Homework complete, but many incorrect answers	Homework incomplete	
	15 pts	**12 pts**	**8 pts**	**4 pts**	**Score**
Grammar, spelling, & writing style	No misspellings or grammatical errors	Very few (1-3) minor grammatical or spelling errors	Multiple (3-6) spelling or grammatical errors	Many (>6) spelling or grammatical errors	
	5 pts	**4 pts**	**2 pts**	**1 pts**	**Score**
Formatting	12-point font, Times New Roman, 1.5 Spaced, 1-2 pages	All but one formatting requirement met	Two formatting requirements not met	Three formatting requirements not met	
	20 pts	**16 pts**	**12 pts**	**8 pts**	**Score**
Sample analysis	Section addresses all three main points with well-thought-out responses and descriptions	Section addresses all three main points adequately	Section addresses only 2 of 3 main points adequately	Section addresses 1 or fewer main points adequately	
	20 pts	**16 pts**	**12 pts**	**8 pts**	**Score**
Sample site assessments	Section addresses all three main points with well-thought-out responses and descriptions	Section addresses all three main points adequately	Section addresses only 2 of 3 main points adequately	Section addresses 1 or fewer main points adequately	
	20 pts	**16 pts**	**12 pts**	**8 pts**	**Score**
Pulling it together	Section addresses both main points with well-thought-out responses and descriptions	Section addresses both main points adequately	Section addresses only 1 of 3 main points adequately	Section addresses no points adequately	
Points earned: /100 **Comments:**					

PRE-LAB HOMEWORK QUESTIONS – ANSWER KEY

Background Questions

1. What are three reasons why scientists monitor water quality?

   Many plausible reasons. Examples include to detect chemical contaminants, to assess the quality of water for aquatic organisms, to ensure safety for drinking, or to determine long-term trends in water quality.

2. Define pH (i.e. what does it measure?).

   It tells how acidic or alkaline a substance is by measuring the molar concentration of hydrogen ions in the solution.

3. Define dissolved oxygen (DO).

   DO is a measure of how much oxygen is dissolved in the water and available to be used by aquatic organisms.

4. Define turbidity.

   Turbidity is a measurement of water clarity. Turbid water contains a high amount of suspended matter, such as silt and organic matter.

5. What are phosphates and nitrates? How do they relate to eutrophication?

   Phosphate and nitrate are key nutrients required by aquatic plants. High concentrations of phosphates and nitrates can enter water bodies through mechanisms such as agricultural runoff or sewage entry. Excess phosphates and nitrates in the water can result in eutrophication.

6. What is nutrient runoff? Name three common sources of nutrient runoff.

   Nutrient runoff is when nutrients from the surrounding terrestrial environment enter aquatic ecosystems. Manure, septic system, urban runoff, industrial waste, car emissions, sewage treatment plants, artificial fertilizers.

7. Define riparian buffer.

   A vegetated area surrounding a water body.

Critical thinking questions (optional)

1. What does a low or high pH indicate about a water body?

   Low pH indicates an acidic environment, and high pH indicates an alkaline environment.

2. What does dissolved oxygen tell us about a water body? What is the lower limit of dissolved oxygen necessary to maintain fish populations?

   Aquatic organisms need dissolved oxygen to live, so low DO has the potential to cause stress and mortality. Low DO can be indicative of eutrophic conditions. The lower limit to maintain fish populations is between 1-3 mg/L.

3. What does turbidity tell us about a water body and the surrounding habitat?

   Turbid water can be indicative of high amounts of soil erosion, algal blooms, or excess nutrient inputs.

4. What are three negative consequences of excess nutrients in a water body?

   Many plausible answers. Examples include eutrophication, animal die-offs, algal blooms, increases in bacteria levels.

5. Why are riparian buffers beneficial for a water body? How wide are riparian buffers recommended to be to help protect water quality?

   Riparian buffers reduce the amount of runoff that enters a water body and help shade a water body, helping keep temperatures down. Riparian buffers should be wider than 50 feet.

READING WRITING MATH SCIENCE

LESSON 2: INVESTIGATING THE EFFECTS OF SALT CONTAMINATION ON *DAPHNIA*

This lesson gives students the opportunity to conduct a bioassay to explore how a common contaminant can impact aquatic organisms.

Estimated time

1-2 class periods

Procedure

1. To prepare students for this activity, ask them to define what an environmental contaminant is and to name some common contaminants that come to mind.
2. If it hasn't been mentioned already, ask the students if salt could be considered a contaminant. Discuss the different ways that salt can get into the environment.
3. Go through the background material with the students, focusing on what a bioassay is and what *Daphnia* are.
4. Break the students into groups. Assign each group a lab station with the required materials and have them work through the Lesson 2: Lab Activity.
5. Come together as a class to discuss the answers to the questions from the activity.

Required Materials

For each group:

- One *Daphnia magna* culture
- Six 100 mL Petri dishes (plus 1-2 extra)
- Labeling tape for experimental units
- Markers to label tape
- Wide-mouth plastic transfer pipettes
- Timer
- 1 graduated cylinder (at least 50 mL)
- 1 gallon dechlorinated/spring water
- 5% salt (NaCl) solution (100 mL/group)
- Dissecting microscope
- Lesson 2: Investigating the effects of salt contamination on *Daphnia* Lab Activity
- Lesson 2 Discussion Questions

LESSON 2: INVESTIGATING THE EFFECTS OF SALT CONTAMINATION ON *DAPHNIA* LAB ACTIVITY

Background

Ecotoxicology is the study of the effects of toxic chemicals on biological organisms. One of the cornerstones of ecotoxicology is the **bioassay**, an experiment in which a living organism is used as a test subject to determine the toxicity of a contaminant. In a typical bioassay, organisms are exposed to a potentially toxic chemical across a range of concentrations. This allows researchers to observe the effects of the chemical on the organism's behavior and survival and determine at what concentration a chemical becomes harmful.

For this bioassay, the test animals will be *Daphnia magna,* small, aquatic crustaceans also known as "water fleas." *Daphnia* are key organisms in ponds and lakes, acting as a primary consumer and serving as a food source for many other organisms. *Daphnia* make excellent experimental organisms because they are easy to keep in the lab, can asexually reproduce, and are easy to observe. *Daphnia* are also sensitive to new chemicals in their environment.

Here, you will use a bioassay to determine the lethal concentration of a contaminant required to cause mortality to 50% of a test population (LC_{50} value). The particular contaminant that you will use in this lesson is sodium chloride ($NaCl$), also known as salt.

You may wonder why you will be testing the toxicity of salt. You've probably eaten salt today. It's found in most foods! An important concept to keep in mind is that depending on the dose, any substance can be a poison; in other words, the dose makes the poison. Although almost all forms of life require the ions found in salt, too much salt can be bad news. Our *Daphnia* species is adapted to freshwater ecosystems, which are typically 0.05% salt, so too much salt in the environment can cause reduced health and mortality. In contrast, ocean water is approximately 3.5% salt. Salt often makes its way into freshwater aquatic ecosystems because of its frequent use in winter road treatments, making it a relevant contaminant for us to study.

***Daphnia* under a microscope. Photo credit: Turner Deblieux**

LESSON 2 LAB ACTIVITY

Procedure

1. First, take a few minutes to take a closer look at your *Daphnia* and observe their behavior. Within your group, discuss any interesting observations, including:
   - Their movement patterns
   - Where they spend their time in the water column
   - If there is any significant variation in size between individuals
   - Unique aspects of their anatomy (shape, color, body parts, etc.)
   - Approximately how many individuals you have in your container

2. Next, using the provided 5% salt solution, you will need to mix the salt treatment concentrations you will be using for your bioassay using the provided 5% salt solution. The range of values you will be using for the bioassay are filled into the first column of Table 1.

   You'll need to use a bit of math to determine the volume of the salt solution you need to add to each treatment to reach your desired concentration. A simple formula to use is $\mathbf{C_1V_1 = C_2V_2}$ where:

   C_1 is the concentration of the salt solution (5%)

   C_2 is your desired treatment concentration (Table 1)

   V_2 is the volume of the treatment solution (50 mL)

You will need to **solve for V_1**, the volume of the salt solution you need to add to reach your desired concentration. Plug in each of the different treatment salt concentrations from Table 1 as C_2 to calculate V_1 (volume of spring water). See Step 1 below for an example calculation. You should then subtract V_1 from 50 mL to get the volume of water you need for the treatment. See Step 2 below for an example calculation. This example is if you want a treatment of 1% salt:

Equation: $\mathbf{C_1V_1 = C_2V_2}$,

Example step 1:
$(5\%)(V_1) = (1\%)(50mL) \rightarrow V_1 = 10$ mL of salt solution

Example step 2:
(50 mL H_2O) – 10 mL salt solution = *40 mL spring H_2O treatment*

TABLE 1: Experimental salt concentrations for *Daphnia* bioassay treatments. The total volume in each treatment should equal 50 mL

Treatment Salt concentration	Volume of Spring Water (mL)	Volume of Salt Solution (mL)
0%	50	0
0.2%		
0.4%		
0.6%		
0.8%		
1%	40	10

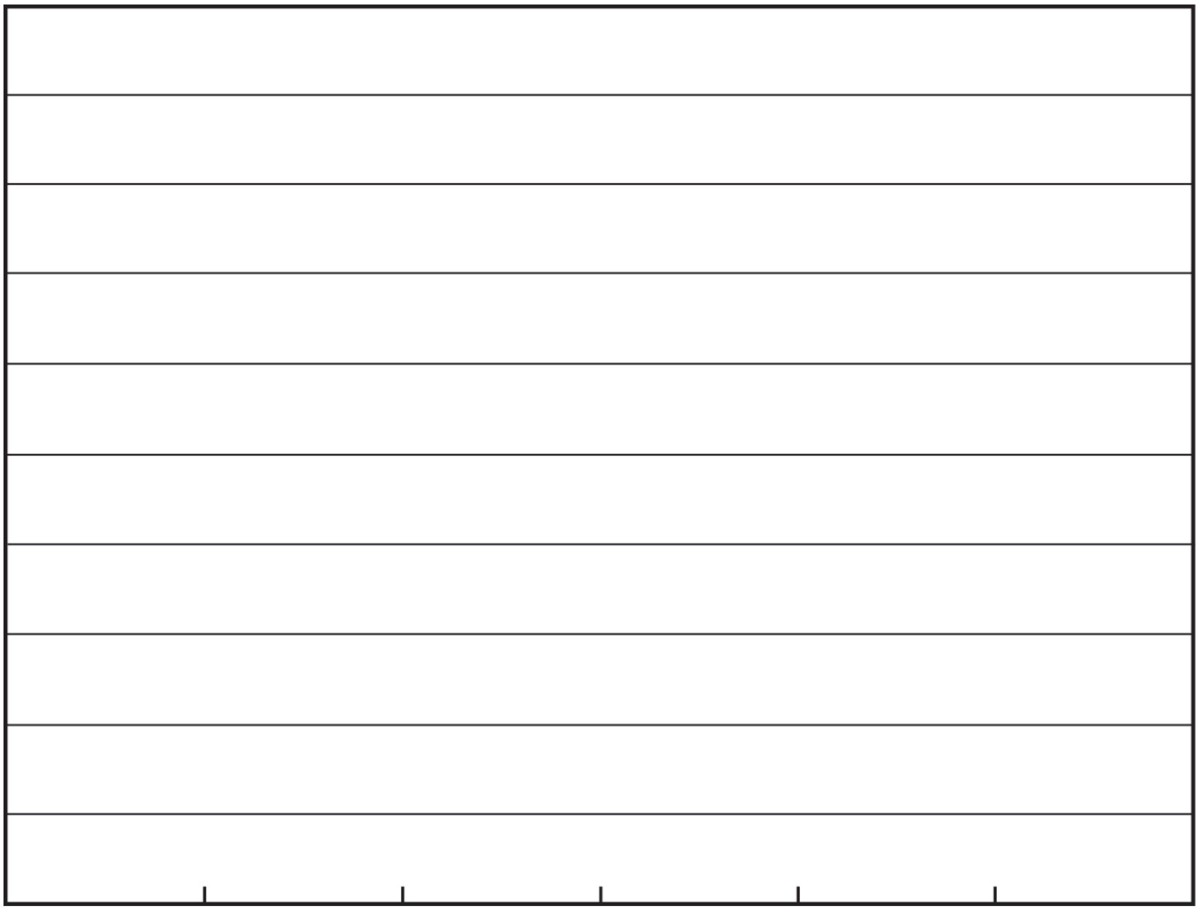
3. Label your 6 experimental units (i.e., Petri dishes) with the 6 standardized salt concentrations that you will be using in your experiment, found in column 1 of Table 1.
4. Using a graduated cylinder, add the volume of spring water calculated in Table 1 to each experimental unit.
5. Using a graduated cylinder, add the volume of salt solution calculated in Table 1 to each experimental unit.
6. Soon, the *Daphnia* will be placed into the experimental units and the bioassay will begin. There are a few things you need to consider first, however.
   - It is important for an experiment to be carefully designed so that the animals in each treatment are exposed to the same conditions outside of the experimental manipulation (salt concentration). This allows us to be more confident that the experimental results are because of the treatments and not because of an outside variable. Make sure that all your experimental units are in the same environmental conditions (e.g., same light levels).
7. The number of *Daphnia* you add to each treatment will depend on how many *Daphnia* you have on hand but will likely range from **5-10 individuals** per treatment. It is critical to add the same number of individuals to each treatment! Record how many individuals you decide to add to each treatment:
   - ___________ individuals/treatment

8. Next, use a pipette to carefully add the individuals to each treatment, keeping in mind the considerations you made above. **Be sure to add as little excess water as possible when adding the *Daphnia*** – excess water could dilute your treatments! Note the time that you start adding the *Daphnia* to the experimental units. *Tip: To limit the amount of excess water you add, first add *Daphnia* to an extra petri dish for easier collection with less water added.
   - Time: ____________________
9. After 1 and 24 hours, count and record the total number of *Daphnia* that have died. Next, compare the general behavior of the surviving *Daphnia* among all six treatments. Record your observations in Table 2 below.
10. Label the blank graph provided in Figure 3 below and make a line graph representing % **mortality after 24 h vs salt treatment concentration** using the data that you recorded in Table 2. Using prior knowledge, decide:
    - Which variable is the dependent variable, which is the independent variable, and why?
    - Which variable belongs on the x-axis and which variable belongs on the y-axis?
    - What is an appropriate title?
11. Determine a 24-hour LC_{50} value for salt exposure in *Daphnia*. This is the salt concentration at which 50% of the *Daphnia* have died after 24 hours.

TABLE 2: The effects of experimental salt concentrations on *Daphnia* survival and behavior

Salt Conc. (%)	# of starting *Daphnia*	# Dead after 1 h	# Dead after 24 h	% mortality after 24 h	Behavioral observations
0%					
0.2%					
0.4%					
0.6%					
0.8%					
1.0%					

FIGURE 3: Percent mortality after 24 h vs salt treatment concentration



LESSON 2: DISCUSSION QUESTIONS

1. Why is it important to include a treatment with no salt in addition to the salt treatments? What is this treatment called?

2. Did all the organisms in the 0% salt treatment survive? If not, what might have caused mortality in this treatment? How does this affect the interpretation of the survival results from the other treatments?

3. List three environmental conditions that you standardized across all of your treatments. Explain how you did so (ex: We controlled for water temperature by taking all water from the same source).

4. The curve that you drew with your data above is called a dose-response curve. Which axis represents the dose? Which axis represents the response?

5. Do you think the 12-hour LC_{50} value would be higher or lower than the 24-hour LC_{50} value? What about the 48-hour LC_{50} value? What would a difference between these LC_{50} values tell you about the toxicity of salt to *Daphnia*?

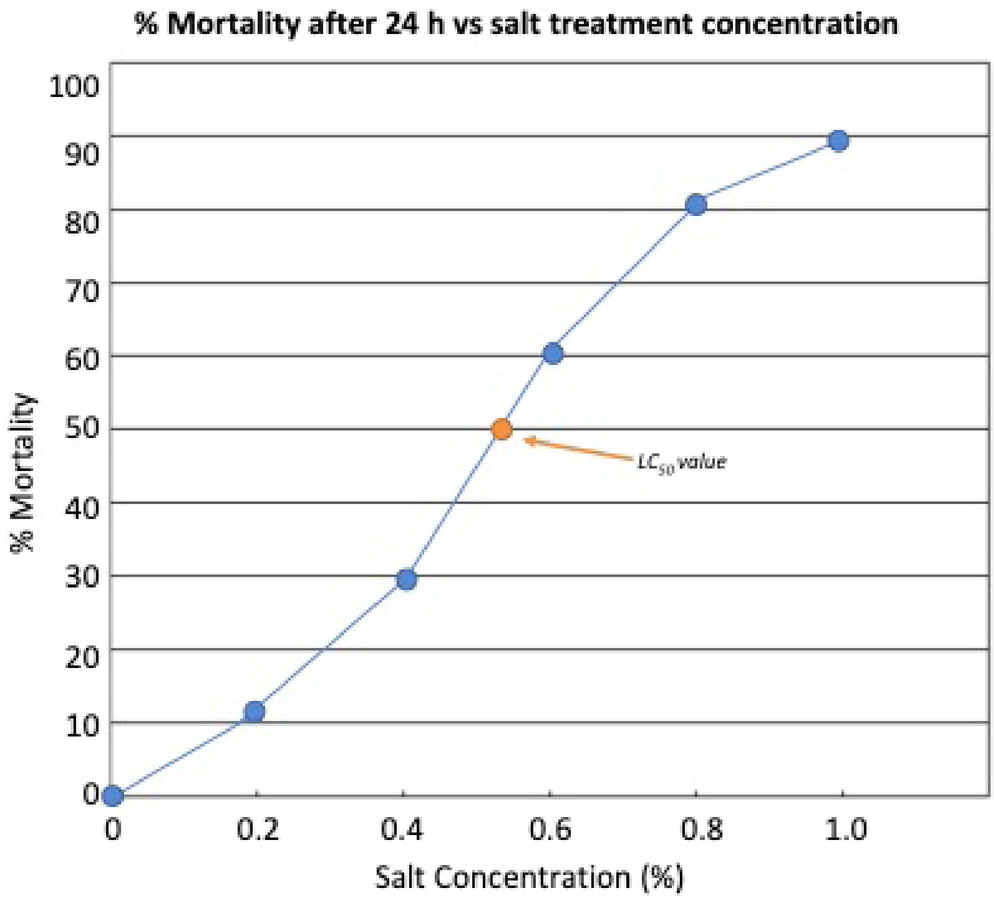
6. An LC_{50} is just one way to assess the toxicity of a contaminant. Based on your experience of conducting an LC_{50}, what are two strengths of this experimental method? What are two weaknesses?

7. Why might a high salt concentration be toxic to *Daphnia* and other freshwater organisms?

8. If you were to conduct this experiment again, what improvements could be made? What would you change?

9. Compare your results and data with the other groups in the class. What conclusions can be drawn and what new questions are raised based on your comparisons? What other information would you need to make road salt use recommendations to your local government?

LESSON 2: LC_{50} PLOT EXAMPLE



LESSON 2: DISCUSSION QUESTIONS – ANSWER KEY

1. Why is it important to include a treatment with no salt in addition to the salt treatments? What is this treatment called?

   This allows us to be more confident that the experimental results are because of the treatments and not because of an outside variable. This is called a control treatment.

2. Did all the organisms in the 0% salt treatment survive? If not, what might have caused mortality in this treatment? How does this affect the interpretation of the survival results from the other treatments?

   Answers will vary. Possible reasons for control deaths include water quality (chlorine present, low oxygen, etc.), starvation, and accidental contamination with salt.

3. List three environmental conditions that you standardized across all of your treatments. Explain how you did so (ex: We controlled for water temperature by taking all water from the same source).

   Many possible answers. Examples include controlling for light levels, table surface, location in the room, and water quality.

4. The curve that you drew with your data above is called a **dose-response curve**. Which axis represents the dose? Which axis represents the response?

   The x-axis represents the dose (dose of salt). The y-axis represents the response (Daphnia mortality).

5. Do you think the 12-hour LC_{50} value would be higher or lower than the 24-hour LC_{50} value? What about the 48-hour LC_{50} value? For both of your responses, explain why. What would a difference between these LC_{50} values tell you about the toxicity of salt to *Daphnia*?

   The 12-hour LC_{50} value should be higher (i.e., higher % of salt needed to cause 50% mortality) than the 24-hour LC_{50} value. The 48-hour LC_{50} value should be lower. This is because at 12 and 48 hours there is less or more time for mortality to occur, respectively, which would, in turn, make the LC_{50} value higher or lower.

6. An LC_{50} is just one way to assess the toxicity of a contaminant. Based on your experience of conducting an LC_{50}, what are two strengths of this experimental method? What are two weaknesses?

   Answers will vary based on student experiences. Possible strengths include: Easy to conduct, low cost, easy to interpret results, relatively fast, easily adaptable to many contaminants/toxins. Possible weaknesses include: can be imprecise (variation between groups), doesn't consider sublethal effects of a contaminant, requires the use of animals, lab conditions don't take into account other stressors (parasites, predators, temperature fluctuations) that wild animals face that could increase the toxicity of a contaminant.

7. Why might a high salt concentration be toxic to *Daphnia* and other freshwater organisms?

   Answers will vary. A main effect is that they can disrupt the osmotic balance in the body of an organism. Can also have indirect effects, such as decreasing the growth rate of food items (such as aquatic plants) and reduce overall food availability.

8. If you were to conduct this experiment again, what improvements could be made? What would you change?

   Answers will vary and depend on student experiences. Answers to this question may be good to discuss as a class.

9. Compare your results and data with the other groups in the class. What conclusions can be drawn and what new questions are raised based on your comparisons? What other information would you need to make road salt use recommendations to your local government?

   Discuss as a class.

SOURCES

Ghazy, Mahassen M. El-deeb, Madlen M. Habashy, Faika I. Kossa, and Eman Y. Mohammady. 2009. "Effects of Salinity on Survival, Growth and Reproduction of the Water Flea, Daphnia Magna." *Nature and Science* 7(11): 28–42.