



FNR-594-W

EXPERT REVIEWED

Disease Ecology

LESSON PLAN

This unit introduces students to basic principles of disease ecology, including the diversity of parasites, how diseases are modeled, and how parasites and hosts interact.

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OVERVIEW

ESTIMATED TIME

3 lessons, 45-90 minutes each

VOCABULARY

- Disease Parasite
- Epidemic
- Endemic
- Susceptible
- Infected

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.

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Recovered

Transmission rate

Recovery rate

Infection rate

Cercariae

Interaction rate

Adaptive behavior

 Macroparasites Generalist

- Microparasites

- Specialist
- Host

.

- Transmission
- Direct transmission .
- Indirect transmission
 - Immune response Behavioral avoidance
 - Vector
- Direct life cycle
 - Indirect life cycle

OBJECTIVES

- Define what a parasite is and explain its effect on a host.
- Summarize scientific literature to identify hypotheses and results.
- Plot and interpret the progression of disease through a hands-on simulation.
- Evaluate the progression and dynamics of a disease using . a common model of disease spread.
- Apply the scientific method to determine how tadpoles behaviorally respond to parasites.
- Predict and assess how the environment can influence anti-parasite behavior.

REQUIRED MATERIALS

. Outlined on the first page of each lesson (pgs. 9, 15, and 26).

ACKNOWLEDGEMENTS

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OVERVIEW

LESSON STANDARDS

Lesson One

Next Generation Science Standards MS-LS2-4 MS-ETS1-1 HS-ETS1-1

English Language Arts

CCSS.ELA-LITERACY.W.6.2 CCSS.ELA-LITERACY.W.6.7 CCSS.ELA-LITERACY.W.6.9 CCSS.ELA-LITERACY.SL.6.4 CCSS.ELA-LITERACY.SL.6.5 CCSS.ELA-LITERACY.SL.6.6 CCSS.ELA-LITERACY.W.7.2 CCSS.ELA-LITERACY.W.7.7 CCSS.ELA-LITERACY.W.7.9 CCSS.ELA-LITERACY.SL.7.4 CCSS.ELA-LITERACY.SL.7.5 CCSS.ELA-LITERACY.W.8.9 CCSS.ELA-LITERACY.SL.8.4 CCSS.ELA-LITERACY.SL.8.5 CCSS.ELA-LITERACY.W.9-10.9 CCSS.ELA-LITERACY.SL.9-10.4 CCSS.ELA-LITERACY.SL.9-10.5 CCSS.ELA-LITERACY.W.11-12.9 CCSS.ELA-LITERACY.SL.11-12.4 CCSS.ELA-LITERACY.SL.11-12.5

Lesson 2

Next Generation Science Standards

MS-LS2-4	HS-LS4-6
MS-ETS1-1	HS-ETS1-1
MS-ETS1-3	HS-ETS1-4

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.3 CCSS.MATH.CONTENT.HSS.ID.C.7 CCSS.MATH.CONTENT.HSS.IC.A.2 CCSS.MATH.CONTENT.HSS.IC.B.3 CCSS.MATH.CONTENT.HSS.IC.B.6

Lesson 3

Next Generation Science Standards MS-LS2-2 MS-LS2-4

Math

CCSS.MATH.CONTENT.7.SP.A.1 CCSS.MATH.CONTENT.7.SP.A.2 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.IC.B.4 CCSS.MATH.CONTENT.HSS.IC.B.5 CCSS.MATH.CONTENT.HSS.IC.B.6

Lessons 1, 2 and 3

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

INFECTIOUS DISEASE ECOLOGY OVERVIEW

Infectious disease ecology is best described as the study of host-parasite interactions within the context of the surrounding *environment* and *evolution* (Kilpatrick and Altizer 2010) and can be visualized in the form of a triangle in which the **host**, **parasite**, and **environment** interact to determine disease outcomes (Figure 1). Disease can occur only if all three components of the triangle are present, and modifying any component of the triangle can change **disease outcomes**.

FIGURE 1: Disease triangle model



In natural systems, the environment is constantly changing, and host and parasite populations are constantly evolving in response to the selection pressures around them, so disease dynamics are often in constant flux. For example, a seasonal increase in ambient temperature (i.e., a change in environment) may modify the reproductive rate of the parasite or the immune function of the host, thus altering overall disease incidence and outcomes in the system.

Disease ecology is, at its core, an integrative field that combines information from the disciplines of parasitology (the study and description of parasites), epidemiology (the statistical patterns of disease for individuals and populations), and medicine (the study of disease cure and prevention). However, the key distinction of disease ecology is that it looks at disease incidence and outcomes as the product of fundamental biological processes rather than from purely statistical terms. In other words, it moves beyond the how of disease and seeks to understand the why of disease. Why does an increase in host diversity decrease disease risk? Why does a parasite prefer one host over another? In this way, disease ecology generates a deeper understanding of the mechanisms driving disease processes, which can help control epidemics and disease outbreaks when they occur. The field of disease ecology has received increasing attention over the past several decades as disease outbreaks in human and wildlife populations have become more common. For example, nearly 1/3 of amphibian species are threatened with extinction, and a fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), has played a significant role in these massive amphibian declines. Additionally, white-nose syndrome, a disease caused by another fungal pathogen, has devastated bat populations in North America, with some species declining by over 90%. Disease ecologists are hard at work trying to find the causes of and possible solutions to these problems through a combination of observational field studies, experimental studies in the lab, and predictive mathematical models.

Although disease ecologists frequently work in wildlife systems, principles from disease ecology have helped solve some of history's biggest human health issues. For example, the principle of herd immunity – where a large proportion of a population becomes immune to a disease, protecting individuals who are not immune by reducing transmission – played a key role in eradicating poliovirus globally. By identifying key characteristics of the virus, such as transmission rate, scientists were able to pinpoint what percentage of a population needed to be vaccinated in order to halt the disease in its tracks. This same principle is used to inform vaccination practices for other deadly diseases and has helped reduce disease transmission and improve health conditions around the world.

At a time when many wildlife populations are imperiled by habitat loss, climate change, and disease, disease ecology is becoming an increasingly important field to teach and understand. As is made clear from the disease triangle (Figure 1), environmental changes as a result of human activity play a key role in determining disease outcomes, and shifts in the environment can be the determining factor in whether a disease becomes an epidemic. Disease ecology provides a more holistic understanding of the three main factors that drive disease (host, parasite, and environment), which will allow wildlife managers, public health specialists, and medical practitioners to better combat these diseases in an efficient and sustainable manner.



LESSON 1: PARASITE DIVERSITY ACTIVITY – VOCABULARY

- **Disease:** any deviation from, or impairment of, the normal structure or function of any part, organ, or system of the living animal or plant body.
- **Parasite:** an organism that lives in or on another organism (its host) and benefits by acquiring nutrients at the host's expense
 - Microparasite: a parasite that is short-lived, microscopic, and reproduces within its host (ex., viruses, bacteria)
 - Macroparasite: a parasite that is relatively long-lived, visible to the naked eye, and reproduces outside the host (ex., ticks, roundworms)
- **Generalist:** a parasite that is able to survive and thrive in a variety of host species
- **Specialist:** a parasite that can survive only on one or a select few host species
- Host: an organism that a parasite lives on or in
- Transmission: passing of parasites from an infected individual to a susceptible individual
 - Direct transmission: occurs when there is physical contact between an infected organism and a susceptible organism (ex., touching, kissing)
 - Indirect transmission: occurs when there is no direct contact between an infectious and susceptible organism (ex., airborne, vector-borne, waterborne)
- Vector: an organism that does not cause disease itself, but which spreads infection by conveying pathogens from one host to another
- **Direct life cycle:** parasites that infect a single species to complete their life cycle
- **Indirect life cycle:** parasites that must infect more than one host species to complete their life cycle
- **Epidemic:** disease that appears at a time or place where it does not normally occur, or with a frequency substantially greater than that expected for the time period
- **Endemic:** disease that occurs in a population at a regular, predictable or expected rate

LESSON 1: PARASITE DIVERSITY ACTIVITY – BACKGROUND

Earth is home to an astonishing variety of life, with an estimated 8.7 million different living species. Even more astonishing, however, is that an estimated 50% of species are **parasites!** Indeed, parasites are much more common than we realize, and are often understudied and misunderstood.

With such a large number of species comes an impressive amount of diversity and variation. For example, certain parasites, called **microparasites** (ex., viruses, bacteria, protists), are too small to see without a powerful microscope. On the other end of the size spectrum are **macroparasites** (ex., trematodes, arthropods, nematodes), which are visible to the naked eye. Some of these parasites are **generalists** (able to survive and thrive on a variety of host species), and others are **specialists** (can survive only on one or a select few host species).

Parasites can be spread from one infected individual to another (**transmission**) in many ways. The broad categories of transmission are **direct transmission** and **indirect transmission**. Direct transmission involves contact between an infected individual and a susceptible individual. For example, sexually transmitted diseases are spread through direct transmission through contact. Indirect transmission occurs when there is no direct contact between an infectious and susceptible organism. For example, coughing can spread the common cold through the air.

Life cycles are also highly variable between parasites. Many parasites, such as measles and fleas, have direct life cycles (infect a single species to complete their life cycle), while others have indirect life cycles (must infect more than one host species to complete their life cycle). One common example of an indirect life cycle is the trematode life cycle. Many trematode species have five life stages and utilize three different hosts! The adult trematode sexually reproduces in the definitive host, which is warm-blooded animal, such as a bird or mammal. The eggs are then released into the water and hatch into free-swimming miracidia, which seek out a snail, their first intermediate host. Once inside the snail, the miracidia eventually form into rediae. Once mature, the rediae will produce free-swimming **cercariae**. The cercariae seek out a tadpole host, and upon finding one, encyst in their kidneys as metacercariae. Once the tadpole is consumed by the definitive host, the life cycle starts over. A simplified version of this life cycle is shown in Figure 2.



TEACHERS' NOTES



FIGURE 2: Simplified trematode life cycle.

Parasite life stages are enlarged for visualization. Modified from photo by unknown author licensed under CC BY-SA, and photo by unknown author licensed under CC BY.

This activity is designed to highlight the variety of parasites types that exist, including host preferences, different ways that they are transmitted, and the differences in their life cycles. Students will break into groups to research a parasite that has been assigned to their group. Each group will present what they have learned about their parasite and write the key features on the board. Once everyone has presented their parasite, break into a larger class discussion about the major differences between the parasites that have been presented and how these differences might influence transmission.

LESSON 2: MODELING DISEASE TRANSMISSION – VOCABULARY

- **Susceptible:** individuals in a population that are not infected with a parasite but can become infected
- **Infected:** individuals in a population that are infected with a parasite and can spread it to susceptible individuals
- **Recovered:** individuals who were previously infected with a parasite, but have cleared infection and are now immune to future infection
- **Transmission rate:** rate of disease spread from infectious to susceptible individuals

- **Recovery rate:** rate at which infected individuals recover from infection
- Interaction rate: rate at which organisms in a population interact
- **Infection rate:** rate at which organisms acquire infections from interactions with individuals in the population

LESSON 2: MODELING DISEASE TRANSMISSION – BACKGROUND

Researchers are often interested in understanding how diseases spread in a population. This activity will introduce students to a basic disease modeling approach: The Susceptible-Infected-Recovered (SIR) model. The SIR model is a simple mathematical model that predicts disease progression in a fixed population. Students can be intimidated by math, so before jumping into manipulating the mathematical model, the class will conduct a simulation of how disease might progress in an SIR model. After the Modeling Disease Transmission: Class Activity, students will break into groups and be asked to manipulate the parameters of an SIR model in Microsoft Excel or Google Sheets to test how varying different parameters influence disease in a population. It is important to emphasize that because it is difficult to conduct experiments on disease transmission in humans, models are useful tools to conduct many "experiments" very rapidly. After each group has completed each activity, come together as a class to critically discuss the findings of the SIR simulations.

LESSON 3: PARASITE AVOIDANCE BEHAVIOR IN TADPOLES – VOCABULARY

- **Immune response:** the body's response caused by its immune system being activated by a foreign substance (i.e., parasites)
- **Behavioral avoidance:** a behavior that reduces exposure and infection risk of the host
- Adaptive behavior: a behavior that increases an animal's chance of survival and reproductive success
- Cercariae: a free-swimming larval stage of trematode parasites



LESSON 3: PARASITE AVOIDANCE BEHAVIOR IN TADPOLES – BACKGROUND

A parasite's goal is to find a host organism and benefit by acquiring nutrients from it. This makes acquiring parasites costly to the host. Parasites not only use up valuable energy resources, but also cause an energetically costly immune response in the host. Moreover, parasites can cause disease, physical damage, malformations, and even mortality to the host! Therefore, it is beneficial for a host to employ strategies to prevent parasitic infection in the first place.

One of the most common methods that animals employ to prevent parasitic infections is **behavioral avoidance**. The goal of behavioral avoidance is to reduce exposure and infection risk of the host, thereby reducing the costs of parasitism by avoiding it altogether. Behavioral avoidance comes in many forms and includes reducing activity, increasing activity, seeking new habitats, and changing feeding behavior.

Behavioral avoidance of parasites is especially important in aquatic habitats, as many aquatic parasites are freeswimming and can seek out hosts in the water column. One common example of this is the cercariae of trematodes, which are a free-swimming life stage that are often highly effective at finding and infecting hosts. Common hosts for trematode cercariae to seek out include snails, tadpoles, and fish. Through this activity, students will learn about host-parasite interactions, anti-parasite behavior, and how the environment can influence anti-parasite behavior by gaining firsthand experience with an aquatic

Students will first expose tadpoles to free-swimming trematode cercariae to study how tadpole behavior changes in the presence of parasites. They should observe that tadpole activity increases when they are exposed to trematode cercariae. This is an adaptive anti-parasite behavior to avoid cercariae and "shake off" cercariae from their bodies.

Students will then expose bullfrog tadpoles that have been held in a cold environment (i.e., refrigerator) to trematode cercariae to study the effect that the environment can have on anti-parasite behavior. Students should find that because amphibians are cold-blooded, being held in a cold environment reduces anti-parasite behavior, as this slows tadpole metabolic rate and overall activity levels.

For this lesson, some planning and preparation are required. However, the tips and details provided below will make this lesson run as smoothly as possible. *Tip: This lesson will be easiest to conduct in the late spring semester (April-June) or early fall semester (September-early November), because that is when snail infections will be most frequent.

SNAIL COLLECTION AND CARE

Site selection and sampling

The first step in preparing this activity is identifying field collection sites that will contain a high density of snails. Snails are very common and are typically found in well-vegetated ponds and wetlands (Figure 3). Before sampling any pond, be sure to get landowner permission.

Once you have identified a pond, you'll need a few basic supplies for sampling: muck boots/waders, a small net, and a plastic container to hold snails. There are several ways that you can sample for snails. One way is to simply pick them off aquatic vegetation and floating debris around the margins of the pond. You can also collect snails by sweeping your net through the aquatic vegetation and across the pond bottom. For this activity, it is best to collect only "ramshorn" snails that

FIGURE 3: Typical ramshorn snail habitat

FIGURE 4: Ramshorn snail of appropriate size for parasite screening



Photo credit: Logan Billet



Photo credit: Logan Billet



host-parasite system.

TEACHERS' NOTES

are the diameter of a dime or larger; larger snails are most likely to contain trematode parasites (Figure 4). It is important to collect as many snails as possible before screening; in the field there is no easy way to tell if a snail is infected, and infection prevalence can sometimes be low.

Screening snails for infection

Once you've finished collecting snails, the next step is to screen them for trematode infection. For this, you will need a rack of conical tubes and a heat lamp (such as a 100 W incandescent light bulb). Fill each conical tube halfway with pond water, spring water, or dechlorinated tap water (if only 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). Next, place one snail into each tube. To stimulate the emergence of cercariae in infected snails, place the rack of tubes approximately 12 inches below the heat lamp and wait one hour. After one hour, check for cercariae. To the naked eye, trematode cercariae will look like small, white specks of dust in the water swimming at a fast pace (video: https://www.youtube.com/watch?v= JeOULMLfmYE). It is recommended that you hold each tube under a magnifying glass or stereo microscope to spot trematode cercariae, as they can be difficult to see with the naked eye. Mark the tubes of infected snails with tape.

There are *many* different species of trematode, so it will be difficult to know exactly what species of trematode you have. Knowing the species is not critical for this activity. In Indiana, it is common to find ramshorn snails infected with cercariae belonging to the family Echinostomatidae (Figure 5). These cercariae are typically constantly swimming, fast-moving, and appear to spin in a figure-8 pattern. If you are interested in more in-depth trematode identification, a good resource is *Handbook of trematodes of North America north of Mexico* (Schell 1985).

Caring for infected snails

To care for infected snails leading up to the activity, place snails individually in 1-L or larger cups filled with unchlorinated water. To feed the snails, place a piece of romaine lettuce in each cup. Water should be changed every few days. While it is recommended that infected snails be kept in a refrigerator for long-term maintenance to reduce trematode shedding, snails can be maintained at room temperature if being held for less than a week.





Photo credit: Logan Billet

TADPOLE PURCHASING AND CARE

Live bullfrog tadpoles can be ordered from biological supply companies such as Carolina Biological Supply (https://www.carolina.com). A pack of 12 medium tadpoles is ~\$20.00. (As of early 2020.)

Along with the directions provided by the tadpole supplier, there are many excellent online resources that detail how to care for tadpoles. Search using phrases such as "tadpole husbandry" and "bullfrog tadpole care."

After you complete the activity, tadpoles can be maintained in a classroom aquarium, donated to another classroom for experiments, taken home by students (with parental permission), or, if necessary, humanely euthanized. The tadpoles should not be released into the wild because they could carry novel parasites and are not from the local area.





LESSON 1: PARASITE DIVERSITY ACTIVITY

This lesson teaches students about the variety of parasites and their characteristics.

Estimated time

One class period

Procedure

- 1. Review the Teacher Information provided on disease ecology.
- 2. Ask the students to write down (on a sheet of paper or notecard) the answers to a few primer questions: how they would define a parasite, what image comes to mind when they hear the word parasite, what proportion of organisms are parasites, and what factors we might need to know about a parasite to understand how it is transmitted.
- 3. Introduce the terms and information from the introductory PowerPoint and supplementary materials. You may have the students take notes or provide the printout of the vocabulary sheet. Walk the students through the questions on the Parasite Diversity worksheet to confirm understanding.
- 4. Guide students through the Types of Disease Activity.
 - a. Break students into groups of 3-4 and assign each group one of the parasites pictured on the Parasite Printout page. Give each group a Parasite Diversity worksheet as well.
 - b. Give the students 30-45 minutes to research their parasites and fill out their Parasite Diversity worksheets. Each group will have very different answers specific to their parasites.
- 5. Have students present their results to the class. This can be done in any way you find appropriate, including writing important details on the board or making short PowerPoint presentations. Give each student the Parasite Presentation Note Sheet so that they can take notes during each presentation.
- 6. As a class, have students discuss the major differences between the different parasites that have been presented and how these differences might influence transmission, which parasites are viewed as the most deadly, why parasites might utilize such different life cycles, etc. It might also be interesting to discuss if parasites are universally "bad" and what would happen if parasites disappeared.

Required Materials

- Introductory PowerPoint (www.purdue.edu/nature)
- Parasite species cut-outs (1 per group)
- Laptop for research
- Parasite Diversity Worksheet (1 copy per group)
- Parasite Presentation Note Sheet (1 per group)
- Paper (1 piece per student)
- Pencil (1 per student)



LESSON 1: PARASITE DIVERSITY WORKSHEET

Group member names:

1. Parasite species vary dramatically in many aspects of their biology. First, find out what class of organisms your parasite belongs to (*virus, bacteria, arthropoda, Protista, fungi, platyhelminth*). Write the class your parasite belongs to here:

Research and briefly describe the following key characteristics of the class your parasite belongs to:

- a. Size class (microparasite/macroparasite)
- b. Mode(s) of transmission
- c. Three physical characteristics that interest you
- 2. As your research from the last question should have highlighted, even within groups of parasites there is a lot of variation. Now that you have a general understanding of the group that your parasite belongs to, it's time to research your specific parasite. Please research and provide answers to the questions below. Be specific and use reliable sources! Google scholar (https://scholar. google.com/) is a good way to find articles.
 - a. What part of the world is your parasite found in? Is it rare or relatively common?
 - b. What hosts does your parasite utilize? Is it a generalist or a specialist?
 - c. Describe the life cycle of your parasite. What are its different developmental stages? Does it have a **direct** or **indirect life cycle**? How does it reproduce? How is it transmitted?
 - d. What disease(s) does your parasite cause? What are the main symptoms of this disease?
- 3. The diseases that parasites cause can occur on regular, predictable intervals (**endemic diseases**), but they can also occur at a time or place where they do not typically occur or with greater intensity than expected (**epidemic diseases**). Additionally, some parasitic diseases are classified as **emerging infectious diseases**. This means that the disease is newly discovered, spreading to new hosts, or expanding in range or severity. Please synthesize the status of your disease by addressing the following questions:
 - a. Would you classify the disease your parasite causes as currently endemic or epidemic? Why?
 - b. Find a current or historical example of an epidemic of the disease your parasite causes.
 - When and where did it occur?





- What were the impacts?
- Are epidemics of your disease a common occurrence or rather rare?
- c. Does your parasite appear to be emerging or contained in a certain region at stable levels? What is the evidence?
- d. Is there a vaccine or treatment for your disease? How effective is it?
- 4. The severity of disease outbreaks is often influenced by the environment ("the disease triangle"). In nature, the environment is constantly changing, and humans sometimes play a role in these changes. Pick one environmental factor you have an interest in (such as temperature, habitat destruction, season, pollution, rain levels, etc.) and use it to address the following:
 - a. Before doing any research, write a specific **hypothesis** and **prediction** about how you think this environmental factor will influence disease levels. A hypothesis is the biological explanation for your prediction, and a prediction explains what you think will happen based on your hypothesis. For example: *I predict that influenza will be more severe in winter because the flu virus is more stable in cold air.* For your hypothesis and prediction, you may use the same framework as the example: "I **predict** ABC will increase/decrease disease, **because** XYZ."

FIGURE 1: Disease triangle model



- b. Scientists often use experiments to test hypotheses and predictions to answer the basic questions of how the environment influences disease. Use scientific literature to research the hypotheses and prediction you've come up with. Google scholar (https://scholar.google.com/) is a good way to find articles. Use keywords from your hypothesis and prediction to search for papers (ex., "influenza," "season" from the example from 4a.) Summarize the results from two papers by describing:
 - What were the hypotheses and predictions of the study?
 - What were the results of the study?
- c. Based on your research, make a conclusion about your hypothesis and prediction. Did your research support or contradict what you presented? Why?



LESSON 1: PARASITE PRINTOUT

RABIES LYSSAVIRUS (Rabies)



This Photo by Unknown Author is licensed under CC BY-NC-ND.

PSEUDOGYMNOASCUS DESTRUCTANS (White-nose syndrome)



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BATRACHOCHYTRIUM DENDROBATIDIS (Chytridiomycosis)



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PLASMODIUM (Malaria)



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SCHISTOSOMA MANSONI (Schistosomiasis)



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TRYPANOSOMA CRUZI (Chagas disease)



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TABLE 1: Parasite presentation Note Sheet

Parasite Species	Macro or microparasite	Mode of transmission	Disease parasite causes	Host species	Life cycle type	Geographic location	One interesting fact

Name _





LESSON 2: MODELING DISEASE TRANSMISSION

This lesson teaches students about modeling the disease dynamics of a parasite.

Estimated time

One class period

Procedure

- 1. Introduce the concept of disease modeling with the information provided in the teachers' note section for lesson 2.
- 2. Break the class into three groups and conduct the Classroom Disease Spread Simulation (directions below).
- 3. After each group has plotted their data and answered the discussion questions, briefly discuss the results as a class.
- 4. Next, guide the students through the SIR Computer Simulation activity in their groups.
- 5. As a class, discuss the strengths and limitations of this simulation, such as:
 - In what ways does the SIR model correctly represent the spread of and recovery from a disease?
 - In what ways is the SIR model inaccurate?
 - What could be added to the SIR model to make it more realistic or better represent different diseases?

Teacher directions for Classroom Disease Spread Simulation:

The goal of the activity is to visually simulate the progression of a disease in the classroom using an SIR model.

- 1. Before the activity, label sets of 4 opaque cups as 1-4. Each group of four students will get 4 cups. Sort 25 yellow, red, and blue bingo chips (75 chips total) into three opaque cups per set. Place all the yellow chips in cup 1, the red chips in cup 2, and the blue chips in cup 3. Cup 4 will be empty.
- 2. Distribute the sets of cups to each group of students. Also provide each group of students with one SIR Disease Spread Graphing Sheet, and one SIR Disease Spread Recording Sheet.
- 3. Inform the students that yellow bingo chips represent "susceptible" individuals, red bingo chips represent "infectious" individuals, and blue bingo chips represent "recovered" individuals.

Required Materials

- SIR Disease Spread Recording Sheet (1 per group)
- SIR modeling Excel Sheets/Google Sheets (electronic resource available at www.purdue.edu/nature)
- SIR Model Simulation Discussion Sheet (1 per group)
- 4 opaque containers (per group of students)
- Yellow, red and blue bingo chips, 25 of each color (Per group of students)
- SIR Disease Spread Graphing Sheet (1 per group)
- SIR model Simulation Worksheet (1 per group)
- Computer (1 per group)
- Microsoft Excel or access to Google Sheets
- SIR Computer Simulation Worksheet (1 per group)
- SIR Computer Simulation Discussion Worksheet (1per group)
- 4. Have each group set up their simulation in cup 4. This will be the cup that represents the first time period on the SIR Disease Spread recording sheet. Assign each group to set up their simulation cups with one of the following chip ratios:
 - Group 1: 20 "susceptible" chips and 5 "infected" chips
 - Group 2: 15 "susceptible" chips and 10 "infected" chips
 - Group 3: 5 "susceptible" chips and 20 "infected" chips,
- 5. Designate a chip drawer, a chip analyst, a data recorder, and a data plotter in each group.
- 6. Have the groups read through the directions of the SIR Disease spread recording worksheet and complete the activity.



LESSON 2: SIR DISEASE SPREAD RECORDING SHEET

Group number _____

Introductory information

The goal of this activity is to simulate the spread of disease with a common model, the Susceptible-Infectious-Recovered (SIR) model. For this activity, bingo chips represent individuals in a population. Each color bingo chip corresponds with a disease status. **Yellow chips** represent susceptible individuals capable of becoming infected. **Red chips** represent infectious individuals who are infected with a disease and capable of spreading it to susceptible individuals. **Blue chips** represent recovered individuals who have cleared an infection and are now immune to the disease. To model the progression of disease through time, you will draw 2 bingo chips from your simulation cup (cup 4) at random.

The two chips you pull represent an interaction between two individuals in the population, and the results of this interaction will depend on the infection status of the two individuals. In this simulation, disease spread occurs ONLY when a "susceptible" chip comes in contact with an "infected" chip, much like disease spread occurs when an infected individual encounters a susceptible individual. No disease spread occurs when 2 "susceptible" chips or 2 "recovered" chips come in contact, as no individuals are infected. Additionally, no disease spread occurs when ANY chip comes in contact with a "recovered" chip because recovered individuals are immune and thus unable to contract the infection. Recovery occurs when an "infected" chip comes into contact with another "infected" chip, or when an "infected" chip comes into contact with a "recovered" chip. This process represents the natural recovery from infection through time.

To begin, designate a chip drawer, a chip analyst, a data recorder, and a data plotter. Then follow the activity directions below!

Directions

- Have the *chip recorder* record the starting number of susceptible, infected, and recovered individuals in period 1 in the table on page 17.
- 2. Have the *chip drawer* randomly draw two chips from the simulation cup (cup 4).

- 3. Have the *chip analyst* assess the interaction between the two selected chips:
 - 2 "susceptible" chips → put both chips back into cup 4 (no new infections)
 - 2 "infected" chips → Replace one "infected" chip in cup 4 with a "recovered" chip from cup 3 and put the other "infected" chip back in cup 4 (one infected individual clears infection)
 - 2 "recovered" chips
 put both chips back in cup 4 (No new infections)
 - 1 "susceptible" chip and 1 "infected" chip → put the "infected" chip back in cup 4 and replace the "susceptible" chip from cup 4 with an "infected" chip from cup 2 (one new infection)
 - 1 "recovered" chip and 1 "infected" chip → Replace the "infected" chip from cup 4 with a "recovered" chip from cup 3 and put the other "recovered" chip back in cup 4 (infected individual clears infection)
 - 1 "recovered" chip and 1 "susceptible" chip → put both chips back in cup 4 (no new infections)
- 4. Have the *data recorder* count the number of susceptible, infected, and recovered individuals in the simulation cup after each period and write it down on the *SIR Disease Spread* recording sheet.
- 5. Have the *data plotter* plot the number of susceptible, infected, and recovered individuals that the data recorder wrote down at the correct time point on the *SIR Disease Spread* graphing sheet. For each line, use a marker color that matches its corresponding bingo chip color.
- 6. Stir the simulation cup and repeat steps 1-4 for a total of 20 trials.



SIR Disease spread recording sheet					
Time Period	Number Susceptible	Number Infected	Number Recovered		
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					





LESSON 2: SIR DISEASE SPREAD GRAPHING SHEET





LESSON 2: SIR DISEASE SPREAD DISCUSSION SHEET

- 1. How well do the different chip interactions represent disease transmission in real life?
- 2. In what ways was this simulation an unrealistic demonstration of disease spread?
- 3. Was there a general trend for how each status (S, I, and R) changed over time?
- 4. Compare your graph with the other groups. How are they similar? How are they different? What could account for these different outcomes?
- 5. What would happen to the rate of disease spread if, on each turn, you pulled three chips instead of two?
- 6. What are the limitations of this activity for modeling disease in larger populations? How could this activity be improved to better represent disease spread?



LESSON 2: SIR DISEASE SPREAD DISCUSSION SHEET – ANSWER KEY

1. How well do the different chip interactions represent disease transmission in real life?

Disease spread occurs ONLY when a "susceptible" chip comes in contact with an "infected" chip, much like disease spread occurs when an infected individual comes in contact with a susceptible individual. No disease spread occurs when 2 "susceptible" chips or 2 "recovered" chips come in contact, as no individuals are infected. Additionally, no disease spread occurs when ANY chip comes in contact with a "recovered" chip because recovered individuals are immune and thus unable to contract the infection.

2. In what ways was this simulation an unrealistic demonstration of disease spread?

There can be a variety of answers to this, and responses should be evaluated on logic and reasoning. Examples of appropriate answers include:

- Only two individuals come into contact at any one time, when in reality there can be interactions of many individuals at once (e.g., in a confined or crowded place).
- Recovered individuals stay immune forever, when this is not the case for many diseases.
- Infected individuals become immune following an interaction with another infected individual, which is not how disease recovery actually occurs.
- 3. Was there a general trend for how each status (S, I, and R) changed over time?

Responses will depend on the starting conditions. Below are examples of how the plots might look based on the three suggested starting conditions.

4. Compare your graph with the other groups. How are they similar? How are they different? What could account for these different outcomes?

Answers will vary. Common similarities include the same trends for setup 1 and 2 but with different line slopes Common differences include a decrease in infection in setup 3 that does not occur in setup 1 or 2, as well as a more drastic increase in recovered individuals in setup 3. The different starting conditions account for these differences. 5. What would happen to the rate of disease spread if, on each turn, you pulled three chips instead of two?

The rate of disease spread would increase, as there would be a higher likelihood of infected individuals interacting with susceptible individuals on any one draw.

6. What are the limitations of this activity for modeling disease in larger populations? How could it be improved to better represent disease spread?

Answers will vary. Acceptable answers include:

- It would be difficult to conduct such an activity with a large population because it would take an extremely long time.
- Human interactions are much more complicated than the chip interactions can represent.
- Humans typically interact in groups larger than two.











SIR COMPUTER SIMULATION WORKSHEET

Researchers are often interested in understanding how diseases spread in a human or wildlife population. One of the most useful ways to do this is through mathematical models, which describe a real-life scenario using math. Though mathematical models can be very complicated, even simple models are useful for understanding how diseases spread.

The goal of this activity is to introduce a simple computer model of disease spread, the **Susceptible-Infectious-Recovered** (SIR) model. In the SIR model, every individual in a population is in one of three classes:

- Susceptible (S) capable of becoming infected
- Infectious (I) infected with the disease and capable of spreading it to others
- **Recovered (R)** infection has been cleared and individuals gain immunity

FIGURE 1: Visual representation of the SIR model



Models are often used to simplify complicated real-world problems, such as disease spread. This makes the problem easier to understand. However, this requires scientists to make assumptions that may not be entirely realistic but help make understanding a problem more manageable. For example, some of the assumptions of the SIR model are:

- The population is fixed (no births or deaths).
- The only way an individual can leave the **susceptible** group is to become **infected**.
- The only way an individual can leave the **infectious** group is to **recover** from the disease.
- **Recovered** individuals become immune to infection forever.
- All individuals are the same (age, sex, size, etc., not considered).
- The disease is **directly transmitted** from individual to individual.

Models also require the use of **parameters**. Parameters are variables that are used to build the mathematical model and

ultimately determine the rate at which individuals move between classes. In the case of the SIR model that we will be using, the most important parameters are:

- **Population size (N)**: total number of individuals in the population
- Transmission rate (beta β): how fast the disease spreads from infectious to susceptible individuals. Transmission rate is a product of:
 - Interaction rate (lambda λ): rate at which organisms in a population interact
 - Infection rate (rho p): rate at which organisms acquire infections from interactions with individuals in the population
- Initial Infected (I): number of individuals infected to start
- Time increment (t): the timescale (1 is default)
- **Recovery Rate (gamma γ)**: rate at which infected individuals recover from infection

The value that is assigned to each parameter depends on the characteristics of the parasite and the host population. For example, population size depends on how many animals are in the population, and infection rate depends on how easily the disease spreads. The above parameters are then used to create equations that are then used to calculate the number of Susceptible, Infectious, and Recovered individuals:

$$S_{t+1} = (S_t) - (S_t * \beta * I_t)$$
 (1)

$$I_{t+1} = (I_t) + (S_t * \beta * I_t) - (\gamma * I_t)$$
(2)

$$R_{t+1} = (R_t) + (\gamma * I_t)$$
(3)

If you aren't familiar with the notation here, don't worry! It's easier than it looks. Let's use the first equation as an example.

- First, think of S_{t+1} as the amount of people susceptible tomorrow, since we are adding 1 to the current time (subscript t+1).
- Next, think of S_t as the number of susceptible individuals **today** and the product of S_t * β * I_t as the number of individuals that became infected today.
- Finally, to calculate the number of susceptible individuals **tomorrow** S_{t+1} , we must subtract the number of new infections **today** $(S_t * \beta * I_t)$ from the number of susceptible individuals today S_t since infected individuals are no longer susceptible.

From this, we can verbalize the equation as: The number of susceptible individuals tomorrow is equal to the number of susceptible individuals today minus the number of new infections today.



Q: Using the example provided above as a guide, describe what equations 2 and 3 mean in words. Make sure to address (1) what each set of bracketed variables refers to and (2) why some things are being added and other things are being subtracted. (It might be helpful to refer to the graphical representation of the SIR model provided in figure Figure 1.)

Now that you understand how an SIR model works, open up the provided Microsoft Excel file or Google Sheet containing the SIR model. Columns A-D hold values for **Time**, **Susceptible, Infected**, and **Recovered**, respectively. The values in these columns are generated from equations using the parameters in column G. Below the parameters there is a graph generated from the values in columns A-D. We will use this graph to explore the model and make observations about how altering parameter values change disease outcomes.

As you learned above, **transmission rate** is the product of the **interaction rate** (default= 0.0015) and **infection rate** (default=0.2) in this model. Adjust infection rate up and down by increasing or decreasing infection rate in intervals of .05 (ex., 0.2, 0.25, 0.3, 0.35). How does the shape of each graph line change as you increase/decrease infection rate? Observe and record what happens to:

- The magnitude of the epidemic (maximum height of the "infected" curve)
- The duration of the epidemic (width of the "infected" curve)
- The number of immune individuals (where the "recovered" curve ends at time 100)

Now, reset infection rate back to 0.2 and interaction rate back to 0.0015. What happens to the epidemic when you increase/ decrease the recovery rate (default=0.1)? Observe and record what happens to:

- The magnitude of the epidemic
- The duration of the epidemic
- The number of immune individuals

Reset recovery rate back to 0.1. What happens to the epidemic when you increase/decrease the population size? Again, observe and record what happens to:

- The magnitude of the epidemic
- · The duration of the epidemic
- The number of immune individuals



SIR COMPUTER SIMULATION DISCUSSION WORKSHEET

- 1. List and explain two advantages of the mathematical model over the chip modeling activity.
- 2. Look back at your observations about how the magnitude of the epidemic, the duration of the epidemic, and the number of immune individuals change when adjusting different parameters. While most of the observations make sense, some of them may seem counterintuitive.
 - a. Why might increasing the infection rate decrease the duration of an epidemic?
 - b. Why might increasing the recovery rate decrease the number of immune individuals?
 - c. Why might increasing the population size increase the relative magnitude of an epidemic?
- 3. In this SIR model, why does the number of susceptible individuals always decrease through time, the number of recovered individuals always increases through time, and the number of infected individuals increase and then decrease through time? Relate your explanation back to Figure 1.
- 4. Some diseases are better represented by SIR models than others. What is one disease that might be well represented by the SIR model? What is one disease that might not be well represented by this SIR model? Explain. (see Lesson 1: Parasite Printout, for examples.)
- 5. As you may know, vaccination is one of the most important ways of stopping the spread of infectious diseases. Relate the impact of vaccination back to the SIR model. How does it influence the number of individuals initially in each class (S, I and R)? How might this decrease disease spread overall?



SIR COMPUTER SIMULATION WORKSHEET, IN-ACTIVITY QUESTIONS: ANSWER KEY

Q: Using the example provided above as a guide, describe what equations 2 and 3 mean in words. Make sure to address (1) what each set of bracketed variables refers to and (2) why some things are being added and other things are being subtracted. (It might be helpful to refer to the graphical representation of an SIR model provided above.)

$$I_{t+1} = (I_t) + (S_t^*\beta * I_t) - (\gamma * I_t)$$

The number of infected individuals tomorrow is equal to the number of infected individuals today, PLUS the number of new infections today, MINUS the number of individuals that recover today.

$$\mathsf{R}_{t+1} = (\mathsf{R}_t) + (\gamma * \mathsf{I}_t)$$

The number of recovered individuals tomorrow is equal to the number of recovered individuals today PLUS the number of individuals that recover today.

SIR COMPUTER SIMULATION DISCUSSION WORKSHEET: ANSWER KEY

1. List and explain two advantages of the mathematical model over the chip modeling activity.

Advantages: Much faster, can model a much larger population, can account for more complex interactions than just two individuals at a time, is much more flexible

- 2. Look back at your observations about how the magnitude of the epidemic, the duration of the epidemic, and the number of immune individuals change when adjusting different parameters. While most of the observations make sense, some of them may seem counterintuitive.
 - a. Why might increasing the infection rate decrease the duration of an epidemic?

Individuals become infected at a more rapid rate, speeding up the pace and intensity of the epidemic and reducing its length.

b. Why might increasing the recovery rate decrease the number of immune individuals?

If infected individuals recover faster, this means that they are less likely to pass their disease on to others. The only way for an individual to become immune is to recover. Thus, fewer total infections lead to a lower number of immune individuals. c. Why might increasing the population size increase the relative magnitude of an epidemic?

More total individuals come into contact with each infected individual since the interaction rate is the same. This effect compounds at each time, leading to a higher number of total infected individuals in a shorter span of time.

3. In this SIR model, why does the number of susceptible individuals always decrease through time, the number of recovered individuals always increases through time, and the number of infected individuals increase and then decrease through time? Relate your explanation back to Figure 1.

The arrows of the SIR model go in only one direction. Once a susceptible individual becomes infected, the only place this model allows an individual to go afterward is to the recovered class.

6. Some diseases are better represented by SIR models than others. What is one disease that might be well represented by the SIR model? What is one disease that might not be well represented by this SIR model? Explain. (see Lesson 1: Parasite Printout, for examples.)

Answers will vary. Diseases that are well represented should be directly transmitted microparasites such as rabies, poorly represented diseases might be things that are vector-borne or not directly transmitted.

4. As you may know, vaccination is one of the most important ways of stopping the spread of infectious diseases. Relate the impact of vaccination back to the SIR model. How might vaccination influence the number of individuals initially in each class (S, I and R)? How might this decrease disease spread overall?

It would reduce the number of susceptible individuals and increase the number of recovered individuals off the bat. This would, in turn, decrease disease transmission and epidemic magnitude.





LESSON 3: PARASITE AVOIDANCE BEHAVIOR IN TADPOLES

Students will learn about anti-parasite behavior and how the environment can influence anti-parasite behavior by gaining firsthand experience with an aquatic host-parasite system.

Estimated time

1 class period

Procedure

- 1 hour before the class is set to begin, put an individual infected snail in each 50 mL tube filled with 30 ml of water. Place the tubes under the heat lamp to begin shedding. Additionally, place half of the bullfrog tadpoles in a refrigerator set at approximately 4° C.
- 2. Set up 3-5 lab stations with the required equipment. Remove the snails from each tube after shedding is complete and place one tube of cercariae at each lab station.
- 3. To prepare students for this activity, ask them to describe how host organisms typically fight off a parasitic infection. Students will likely describe **immune responses**.
- 4. If it hasn't been mentioned already, ask the students if they can think of any behaviors that can help prevent a parasitic infection (ex., How does human behavior change around a sick person?)
- 5. Go through the background material with the students, focusing on what **behavioral avoidance** is and how behavioral avoidance might be an **adaptation** by the host to reduce infection and disease risk.
- 6. Break the students into groups. Assign each group a lab station with the required materials and have them work through the Lesson 3 activity.
- 7. Come together as a class to discuss the answers to the questions from the activity.

Required Materials

Teacher preparation

- 50 mL tubes for shedding snails
- Rack for 50 mL tubes
- Heat lamps (100 W bulbs)
- Aquarium nets

Per group

- Petri dishes (4)
- Tape and marker for labeling petri dishes
- Stereo microscope for viewing parasites
- Aquarium net
- 50 mL tube containing snail and shed cercariae
- Bullfrog tadpoles (4)
- Trematode-infected snail (1)
- 1-L clear container (4)
- Dechlorinated/spring water
- Graduated pipets (2)
- Stopwatch (2)
- 100 mL Graduated Cylinder
- Lesson 3 Discussion Worksheet
- Lesson 3: Parasite Avoidance Behavior in Tadpoles Lab
 Activity Worksheet



LESSON 3: PARASITE AVOIDANCE BEHAVIOR IN TADPOLES LAB ACTIVITY WORKSHEET

Background

A parasite's goal is to find a host organism and benefit from it by acquiring nutrients at the host's expense. Because parasites are gaining nutrients at their host's expense, parasitic infection is costly to the host. Parasites not only use up valuable energy resources, but infection can also elicit an energetically costly **immune response** in the host to fight off the infection. Moreover, parasites can cause disease, physical damage, malformations, and even mortality to the host! Because of this, it is beneficial for a host to employ strategies to prevent parasitic infection in the first place.

One of the most common methods that animals employ as a first response to prevent parasitic infections is **behavioral avoidance**. Behavioral avoidance of parasites is an **adaptive behavior** (i.e., increases an animal's chance of survival and reproductive success) that reduces exposure and infection risk of the host, thereby reducing the costs of parasitism by avoiding it altogether. Behavioral avoidance can come in many forms, depending on the parasite, and includes reducing activity, increasing activity, seeking new habitat, and changing feeding behavior.

Behavioral avoidance of parasites is especially important in aquatic habitats, as many aquatic parasite taxa are freeswimming and can seek out hosts in the water column. One common example of this is the **cercariae** of trematodes, which are a free-swimming life stage that are often highly effective at finding and infecting hosts. Common hosts for trematode cercariae include snails, fish, and tadpoles.

In today's activity, by gaining firsthand experience with an aquatic host-parasite system, you will learn about hostparasite interactions, adaptive anti-parasite behavior, and how the environment can influence anti-parasite behavior. First, you will expose bullfrog tadpoles to free-swimming trematode cercariae to study how tadpole behavior changes in the presence of parasites. Then, you will expose bullfrog tadpoles that have been held in a cold environment (i.e., refrigerated) to trematode cercariae to study the effect that the environment can have on anti-parasite behavior.

Procedure

- 1. First, take a few minutes to take a closer look at your trematode cercariae and observe their behavior under the stereomicroscope. 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.)
- 2. Next, you'll need to approximate how many cercariae are in your beaker. You can do this by taking out several 1 mL samples of water to calculate the density of cercariae per mL.
 - Step 1: First, use a graduated pipet to gently mix your beaker of cercariae so that they are randomly distributed in the water column.
 - Step 2: Next, use your graduated pipet to pull up 1 mL of water from the center of the beaker. Gently distribute the water from the pipet into a small Petri dish.
 - Step 3: Place the Petri dish under the stereomicroscope and count the number of cercariae swimming around. Record this number in Table 1.
 - Step 4: Repeat steps 1-3 three times.
 - Step 5: Finally, calculate the average number of cercariae/mL by adding up the totals from samples 1-3 and dividing them by 3. Record this value in Table 1.

TABLE 1: Cercariae density calculation

Aliquot #	Number of cercariae/mL
1	
2	
3	
Average	

Average = Number of cercariae in samples (1+2+3)/3 (number of samples)

3. You will be adding approximately 15 cercariae to each of your trematode exposure treatments. Calculate how many mL of water you'll need to add from your beaker to add approximately 15 cercariae using the following formula:

 $\underline{1 \text{ mL}} \times 15 \text{ cercariae} = x \text{ mL}$ (your calculated average number of cercariae/mL)

x = _____mL of water from cercariae beaker



- 4. Use tape and a marker to label your 4 experimental units (i.e., plastic dishes) with the 4 following treatment combinations that you will be using in your experiment.
 - Room temperature tadpole, no trematode (label R-NT)
 - Room temperature tadpole, trematode exposure (label R-T)
 - Cold temperature tadpole, no trematode exposure (label C-NT)
 - Cold temperature tadpole, trematode exposure (label C-T)
- 5. Using a graduated cylinder, add 100 mL of spring water to each experimental unit.

Experiment 1: Effect of trematodes on tadpole activity

You will first conduct the experiment with only the room-temperature tadpoles.

- Add the two bullfrog tadpoles that have been held at room temperature into their experimental units labeled R-T and R-NT.
- 2. Designate two members of your group to be timekeepers – one for the trematode treatment (R-T) and one for the no-trematode treatment (R-NT).
 - Once the trial starts, the role of the timekeeper is to start the stopwatch when the tadpole in their cup is swimming and stop the stopwatch when the tadpole in their cup stops swimming.
- 3. Before the experiment gets started, form a hypothesis about how tadpole behavior will change in the presence of trematode cercariae.
 - I hypothesize that tadpole activity will (increase/decrease) in the presence of trematode cercariae because:
- 4. Use a graduated pipet to gently mix your beaker of cercariae so that they are randomly distributed in the water column.
- 5. Next, based on your calculations in Table 1, use a graduated pipet to add the amount of water necessary to add approximately 15 cercariae to the trematode treatment experimental unit. Add an equivalent amount of clean spring water to the no-trematode treatment as a control.

- 6. Wait for approximately two 2 minutes.
- 7. The trial will run for five 5 minutes. Record your starting time here: _____
 - Begin the trial at your chosen start time. For five 5 minutes, have the timekeepers run their stopwatches only when the tadpole is swimming.
- 8. When five 5 minutes is up, alert the timekeepers to stop recording.
- 9. Record the time spent swimming in each treatment in Table 2.
- 10. After five 5 more minutes, replace the water in both experimental units with clean spring water.

Experiment 2: Effect of temperature on behavioral avoidance

You will now conduct the experiment with the refrigerated (cold) tadpoles.

- 1. Remove two bullfrog tadpoles from the refrigerator and bring them to your lab station.
- 2. Add the two refrigerated bullfrog tadpoles into their experimental units labeled C-T and C-NT.
- 3. Designate two new members of your group to be timekeepers one for the trematode treatment (C-T), and one for the no-trematode treatment (C-NT).
 - Once the trial starts, the role of the timekeeper is to start the stopwatch when the tadpole in their cup is swimming and stop the stopwatch when the tadpole in their cup stops swimming.
- 4. Before the experiment gets started, form a hypothesis about how being held in a cold environment will change tadpole anti-parasite behavior *relative* to the tadpole held at room temperature.
 - I hypothesize that the tadpoles held in the cold environment will display (increased/decreased) anti-parasite behavior relative to the tadpole held at room temperature because:

5. Use a graduated pipet to gently mix your beaker of cercariae so that they are randomly distributed in the water column.



- 6. Next, based on your calculations in Table 1, use a graduated pipet to add the amount of water necessary to add approximately 15 cercariae to the trematode treatment experimental unit. Add an equivalent amount of clean spring water to the no-trematode treatment as a control.
- 7. Wait for approximately 2 minutes.
- 8. The trial will run for 5 minutes. Record your starting time here: _____
 - Begin the trial at your chosen start time. For 5 minutes, have the timekeepers run their stopwatches only when the tadpole is swimming.
- 9. When 5 minutes is up, alert the timekeepers to stop recording.
- 10. Record the time spent swimming in each treatment in Table 2.
- 11. After 5 more minutes, replace the water in both experimental units with clean spring water.

Plotting the data

Now that you have Table 2 filled in with the time spent swimming, you'll need to visualize your data.

- 1. First, calculate the proportion of time the tadpole in each treatment spent swimming by dividing the seconds spent swimming by the total seconds in the trial (300 s).
- 2. Next, plot the data by creating a bar graph in Figure 1, with one bar for each treatment.

TABLE 2: Effects of temperature on anti-parasite behavior and trematode infection

Treatment	Time spent swimming (sec.)	Proportion of time swimming (%) (Time spent swimming/ 300 seconds) x 100)
Room temperature, no trematodes		
Room temperature, trematode exposure		
Cold temperature tadpoles, no trematodes		
Cold temperature tadpoles, trematode exposure		







FIGURE 1: Tadpole activity levels in the presence or absence of parasites at two different temperatures



LESSON 3: PARASITE AVOIDANCE BEHAVIOR IN TADPOLES DISCUSSION WORKSHEET

- 1. Describe how the presence of trematode cercariae changed tadpole behavior relative to the tadpole that was not exposed to trematode cercariae. Does this align with the hypothesis you made regarding how tadpole behavior will change in the presence of trematode cercariae?
- 2. Describe how being held in a cold environment altered tadpole anti-parasite behavior relative to the trematode-exposed tadpole that was held at room temperature. Does this align with the hypothesis you made regarding how being held in a cold environment will change tadpole anti-parasite behavior?
- 3. Compare your bar graph in Figure 1 with another group. What is similar? What is different? What conclusions can be drawn and what new questions are raised based on your comparisons?
- 4. Behavioral avoidance of parasites is considered an **adaptative behavior** (increases an animal's chance of survival and reproductive success).
 - a. How might the parasite avoidance behavior you observed be adaptive?
 - b. If parasite avoidance behavior is adaptive and effective at preventing parasitic infection, would you expect the tadpoles held at a cold temperature (with reduced activity) to have higher or low infection levels?
- 5. If you were to conduct this experiment again, what improvements could be made? What would you change?



LESSON 3: PARASITE AVOIDANCE BEHAVIOR IN TADPOLES ANSWER KEY



LESSON 3: Tadpole activity bar graph example



 Describe how the presence of trematode cercariae changed tadpole behavior relative to the tadpole that was not exposed to trematode cercariae. Does this align with the hypothesis you made regarding how tadpole behavior will change in the presence of trematode cercariae?

Answers will depend on the initial hypothesis. Students should find that tadpole activity increased when exposed to cercariae in order to avoid and "shake off" cercariae.

2. Describe how being held in a cold environment altered tadpole anti-parasite behavior relative to the trematode-exposed tadpole that was held at room temperature. Does this align with the hypothesis you made regarding how being held in a cold environment will change tadpole anti-parasite behavior?

Answers will depend on the initial hypothesis. Students should find that overall tadpole activity was reduced by being held in a cold environment, and as a result, they demonstrate less anti-parasite behavior relative to the room-temperature tadpoles.

3. Compare your bar graph in Figure 1 with another group. What is similar? What is different? What conclusions can be drawn and what new questions are raised based on your comparisons?

Answers will depend on individual group results, but groups should find that they have the same general trends as displayed in the example graph provided. However, the percent time moving may vary between groups, which could be the result of individual differences between tadpoles, slight variations in methodologies between groups, or unintentional disturbance of tadpoles that altered their activity.

- 4. Behavioral avoidance of parasites is considered an adaptative behavior (increases an animal's chance of survival and reproductive success).
 - a. How might the parasite avoidance behavior you observed be adaptive?

Based on the observation that tadpoles increase movements, students will likely hypothesize that increased movement helps to prevent infection by avoiding parasites. b. If parasite avoidance behavior is adaptive and effective at preventing parasitic infection, would you expect the tadpoles held at a cold temperature (with reduced activity) to have higher or low infection levels?

The tadpoles held at a cold temperature should have higher infection levels because they did not display parasite avoidance behavior, making them more likely to get infected.

5. If you were to conduct this experiment again, what improvements could be made? What would you change? Answers will vary. Possible responses can relate to mistakes that the group made during the lesson, additional treatments, or changes in the protocol.

SOURCES

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