Big, Bigger, Biggest Size and Scale Comparison Scale Models of Viruses, Bacteria and Protozoa

Carl Zimmer writes in A Planet of Viruses:

- 10 skin cells could line up along the side of a grain of salt.
- 100 bacteria would fit along that same salt grain.
- It would take 1,000 viruses to fill that same space!

Create models of a virus, a bacterium and a protozoan to illustrate the size and scale comparison. (Assume that the average protozoan is approximately the same size as a skin cell.) Topics covered include microorganisms, pathogens, characteristics of life, prokaryotes and eukaryotes, the nature of a cell, and cellular organelles.

Salt Crystal Cube:



Tabletop Display

Create a six-sided paper or cardboard salt crystal (cube) with information about pathogens. Cut out six 20 cm x 20 cm squares, glue them together into a cube, and assign two sides to each pathogen. One could be a description of the pathogen with a labeled drawing and the other a size comparison between the three pathogens.



Make a tabletop display (trifold board or diorama) with drawings or 2D models of each pathogen. Include descriptions and size comparisons. Show measurements and appropriate ratios. Use standard art supplies and any additional materials.

Classroom Models

Construct 3D models of each pathogen using art supplies, toys or common household objects. Show correct size comparison ratios. Hang these in the classroom.





	Met or Exceeded	Met Objectives	Did Not Meet
	Objectives		Objectives
	Models are to scale.	Models are close	Models are not to
Size and Scale	The 1:10:100 ratio for	to scale. The	scale. The
	virus, bacterium and	1:10:100 ratio for	1:10:100 ratio for
	protozoan is	virus, bacterium	virus, bacterium and
	accurate.	and protozoan is	protozoan is
		close to accurate.	inaccurate.
	Units used to	Units used to	Units used to
Units of Measure	measure models	measure models	measure models
	were appropriate.	were close to	were inappropriate.
	Scale comparison was	appropriate.	Scale comparison
	accurate.	Scale comparison	was inaccurate.
		was close to	
		accurate.	
	All structures are	Most structures	Few or no structures
Structures	present.	are present.	are present.
	Structure labels are	Structure labels	Structure labels are
Labels	clear and	are somewhat	unclear and
	appropriate.	clear and close to	inappropriate or
		appropriate.	missing.
	Structures are shown	Structures are	Structures are not
Creativity	very creatively.	shown clearly but	shown clearly or are
		basically.	missing.

Suggested Scoring Rubric



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Samples of Student Work







Climate Change and Vector-Borne Disease



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Bacteria and Viruses

The human body is made of about 100 trillion cells. These cells are quite complex. Most have a nucleus and many special parts.

Bacteria are much simpler. A bacterium is made of only one cell but has no nucleus. Bacteria are small; each is about 1/100th the size of a human cell.

Bacteria are like fish swimming in the ocean of your body. As they swim around, they eat and reproduce rapidly. One bacterium can become millions of bacterium in just a few hours.

Viruses are completely different. A virus is a particle of DNA or RNA with a special cover over it. When a virus comes in contact with a living cell, it attaches to it. The virus injects its DNA or RNA into the cell. The virus DNA or RNA takes over and uses the cell to make more viruses.

Eventually the cell dies and bursts open spewing millions of new viruses into the body of its victim. Each new virus particle can infect another cell.



Allonweiner, Wikimedia Commons

This bacterium is only one cell, but it can grow and reproduce.



Cynthia Goldsmith, CDC/Wikimedia Commons

This is the West Nile virus. Viruses have no living parts and must take over living cells to grow. Name

Date _____ Class _____

Scale of the Universe: An Out-of-this-World Website

A virus is an infectious agent of small size and simple composition that can multiply only in living cells of animals, plants, or bacteria. Viruses are microscopic; they range in size from about 20 to 400 nm (nanometers) in diameter (1 nanometer = 10⁻⁹ meters). By contrast, the **smallest** bacteria are about 400 nm. To get a frame of reference for just how small viruses and bacteria are, we will use a website to compare them to other familiar items.

Google "Scale of the Universe 2012" or go to http://htwins.net/scale2/. Click English and Start. The scroll bar allows you to zoom in and out. Click on any image for more information, such as size in meters. This website was designed by twins Cary and Michael Huang when they were seventeen years old.

Items (size in meters)	List items in order from smallest to largest:
Largest virus	
Largest bacterium	
<i>E. coli</i> bacterium	
Red blood cell	
Paramecium	
Width of human hair	
Water molecule	
Human skin cell	
HIV (Human Immunodeficiency Virus)	

Write some fun facts you learned from this website.



Name	Date	Class
A Plan "A Contagiou	<i>et of Viruses</i> by Carl Zimr Guided Reading Introduction (pages 1–6) Is Living Fluid": Tobacco Mos	ner saic Virus
Cave of Crystals (paragraphs 1–2) translucent: clear or transparent geologist: a person who studies th subterranean: below the surface of surreal: dreamlike	e origin, history and structure of I of the earth	Earth
Number these statements in the orde Crystals:	r that these events occurred in the	e formation of the Cave of

____ Underground chambers filled with hot, mineral-laced water.

Volcanoes formed mountains 26 million years ago.

_____ Water stayed at 136 °F for hundreds of thousands of years and the crystals grew very large.

_____ Volcanic magma held water temperature at 136 °F, minerals settled out and formed crystals.

Viruses in the Cave of Crystals (paragraphs 3–4)

Why was Dr. Curtis Suttle surprised to find viruses in the Cave of Crystals?

"Each drop of cave water may hold two hundred million viruses." Which number represents that measurement?

a. 200,000b. 2,000,000

c. 20,000,000 d. 200,000,000 e. 2,000,000,000

Human Viruses (paragraph 5):

sputum: a mixture of saliva and mucus, expelled from the lungs cystic fibrosis: a hereditary disease in which thick mucus collects in the lungs, leading to infection sterile: free from living microorganisms (bacteria, viruses, protozoans) menagerie: a collection of unusual wild animals Scientists find viruses practically everywhere that they look for them.

- A. TRUE
- B. FALSE: Viruses are rare and hard to find.
- C. FALSE: Viruses are so hard to see that scientists rarely find them.
- D. FALSE: Viruses only infect humans.

Describe the experimental design used by Dana Willner, biologist, San Diego State University.

What was the scientific question in this experiment?

How many test subjects took part in the experiment and what groups were they in?

What was the procedure? ______

What might have been the hypothesis tested by Dr. Willner using the experimental groups listed above?

What were the **results** of the experiment?

Most of the viruses found in the lungs of these patients had viruses that were already well known.

- a. TRUE
- b. FALSE: None of the viruses were already well known.
- c. FALSE: Very few of the viruses were already well known.
- d. FALSE: About half of the viruses were already well known.

Science of Virology (paragraphs 6–7)

contagious: capable of being transmitted by bodily contact with an infected person or object **discharge:** a fluid that is released or emitted **impregnate:** to saturate, soak or infuse **mosaic:** a picture made of small pieces, often inlaid stone or glass

Scientists have only been able to see and identify viruses for a short time, but people have known about viruses for thousands of years. How? Give evidence to support your answer.

Tobacco Mosaic Diseas	e and Adolph Mayer (pa	aragraphs 8–9)
scourge: a cause of	disease or misfortune	
parasite: an organis and is ofte	sm living on another orga en harmed	anism (host) for nourishment; the host does not benefit,
extract: to pull or d	raw out	
pathogen: any dise incubate: to mainta	ase-producing organism, in at a favorable tempera	especially a virus, bacterium or other microorganism ature and other conditions to promote development
What happened in The	Netherlands in the late 1	1800s that led to Adolph Mayer's experiments?
Summarize Adolph Ma	yer's experiments.	
What three things did A	Adolph Mayer determine	were NOT causing TMD in plants?
1	2	3
Tobacco Mosaic Diseas	e and Martinus Beijering	ck (paragraphs 10–12)
inconceivably: unb	, duplicate or reproduce elievably, incredibly	
infectious: spreadir	ng from one person to and	other or from one part of the body to another
What did Martinus Beij	erinck discover from his e	experiments?

Size and Scale of Viruses	(paragraphs 13–15)
---------------------------	--------------------

dissect: to cut apart to examine structure **paltry:** ridiculously or insultingly small, utterly worthless

List what Beijerinck described that a "virus" is NOT.

1		3		
2		4		
What tool allowed scie	ntists to see viruses?	?		
Describe the <u>size comp</u>	<u>arison</u> between a gra	ain of salt and hum	nan skin cells, bacteri	a and viruses. Use the
Grain of Salt = _	Skin Cells			
Grain of Salt =	Bacteria			
Grain of Salt=	Viruses			
evade: to elude, ese evolve: to develop diversity: change, d human genome: all maintain a human o	agraphs 16–17) cape or avoid gradually lifference, variation, the genes in a huma organism	dissimilarity an; all the biologica	al information requir	ed to build and
Based on what you readyou were a scientist, where a scientist, where a scientist and the scientist and the science of the scie	d, what else do you t hat questions would	think scientists sho you still have abou	ould investigate or fin ut viruses? List at lea	d out about viruses? If st three ideas.
2				
3				

Further Research

Describe the Germ Theory of Disease. Did Marinus Beijerinck's research on tobacco mosaic disease fit the criteria for identifying a microorganism pathogen according to the Germ Theory of Disease?



Name	Date

Class

A Planet of Viruses by Carl Zimmer Essay Analysis

Introduction (pages 1–6) "A Contagious Living Fluid": Tobacco Mosaic Virus

Analyze: On page 6, Carl Zimmer ends his introduction by saying "This is a planet of viruses." Why does the author believe this?

Evaluate: What influences do viruses have on our lives? What influences will viruses have on our lives in the future?

Create: What would happen if the world did not have any viruses?



Visualizing Viruses Investigation with Teacher Instructions

<u>*Timing*</u> is crucial in this lab. You must have <u>three consecutive class days</u> to perform the experiment. This lab cannot go over a weekend.

Materials Checklist 1 (provided in the EasyPhage[®] kit from Scientific Methods, Inc.):

Sterile medium EasyPhage [®] EP-10 (10 bottles) Store at 4 °C. For best results, warm to 35 °C or at least room temperature before use.
Sterile pretreated Petri dishes (10) Store at room temperature.
E. coli Famp bacterial cells on agar slant (nonpathogenic) Store at 4 °C. Warm to 24-35°C before use.
MS2 (male specific) coliphage virus in a tiny vial in a small, dark bag (nonpathogenic) Store at 4 °C.
Bacterial stain, sterile in a tiny vial in a small, dark bag Store at 4 °C.
Microbiological loop, sterile, plastic, disposable, in a plastic bag Store at room temperature.
Trypticase Soy Broth (TSB) in large centrifuge tube Store at 4 °C. For best results, warm to 35 °C or at least room temperature before use.
Micropipets, sterile (2) Store at room temperature.
Materials Checklist 2 (materials provided by the teacher):
Sterile water
35 °C incubator
Sterile, individually wrapped plastic pipets
Parafilm to seal Petri dishes
Sterile 50 mL conical tubes for sterile water
Peabody Fellows Explorers and Investigators. © 2015 Yale Peabody Museum of Natural History. All rights reserved.

Overview

DAY 1: Culture E. coli overnight.

Transfer *E. coli* from the agar slant into the TSB tube using the sterile plastic loop. Incubate it overnight. A fresh, 24-hour culture of *E. coli* is required. The bacteria must be actively dividing for the virus to infect the bacteria, so the lab will not work with an older culture.

DAY 2: Conduct the lab.

Using aseptic (sterile) technique, inoculate media bottles with *E. coli*, phage, and stain. Pour the media into the pretreated plates and allow them to harden for one hour. Incubate plates overnight in a 35 °C incubator. Plaques will not form at room temperature.

DAY 3: Read results within 24 hours.

E. coli and phage will continue to grow if the plates incubate longer. This will cause plaques to enlarge until they overlap and are no longer distinct. The indicator dye will also fade over time.

Aseptic (Sterile) Technique

- Wear gloves throughout the investigation to protect yourself from contamination. Do not touch any of the lab materials with your bare hands.
- Lab gloves are not sterile. Avoid touching sterile items even when wearing gloves.
- When inoculating the TSB tube with *E. coli*, the loop is sterile until you take it out of the bag. Only touch the handle. Do not let the loop touch anything but the bacteria and the broth.
- Do not allow the lower surfaces of vial lids to touch anything. When you open a vial, place the lid upside down on the counter. Otherwise, you may contaminate the vial.
- Do not breathe or cough into open containers (sterile water, vials, *E. coli* culture tube, or the Petri dish). Air can carry contaminants.
- Do not leave the Petri dish lid open longer than necessary. Dust from the air will fall onto the surface and contaminate the culture. Open the plate with one hand and slowly pour the contents of the bottle onto the surface with the other hand. Quickly replace the lid.
- Do not reuse any items used in this lab.
- Soak contaminated materials in a 10% bleach solution overnight, then discard in the trash the next day.

Procedure

DAY 1: Culture E. coli overnight.

1. Allow *E. coli* agar slant to warm to 24 to 35 °C before use.

2. Wear gloves and use proper aseptic (sterile) technique. Remove the sterile plastic loop from the plastic bag. Only touch the handle. Do not allow the loop to touch any surface.

3. Carefully open the agar slant of *E. coli*. Gently touch the loop onto the bacterial growth. A small amount of visible bacteria on the loop is enough.

4. Open the TSB tube carefully and dip the loop into the broth. Swirl it around a bit without touching the rim. Close the tube and let it incubate upright overnight (18 to 24 hours) at 24 to 35 °C.



Bacteria: *Escherichia coli* bacterial cells must be actively growing for the virus to infect them. The bacteria are the host and the virus is the pathogen. In the stage of bacterial growth called the log or exponential phase, bacteria have enough nutrients to grow very quickly. The entire population can actually double every 20 minutes. This is the *E. coli* generation time. In humans, a generation takes about 20 years.

DAY 2: Conduct the lab. Use aseptic (sterile) technique.

1. Warm media bottles and TSB broth tube to 35 °C or at least room temperature before use. Mix by gently swirling the bottle to avoid bubbles.

2. Each student or group should receive a media bottle and a pretreated plate. Label the bottom of the plate with student name, date and title of the lab by writing along the outer edge of the bottom with a marker. Do not write in the center of the plate to avoid obscuring any plaques that form.

3. Add 6 mL of sterile water to media bottle with a sterile plastic pipet.

4. Add 70 µL (or one drop) of bacterial stain to media bottle with a sterile plastic micropipet.

5. Add 100 μ L (or one drop) of MS2 coliphage to each media bottle with a sterile plastic micropipet.

Viruses, also called phages, are often grouped by the host they infect. Bacteriophages only infect bacteria and coliphages only infect *E. coli* bacteria. Every major group of organisms has a specific group of phages that infects it.

6. Add 300 μ L (or three drops) of the *E. coli* overnight culture to each media bottle with a sterile plastic micropipet.

7. The media bottles now contain water, bacterial stain, MS2 coliphage and *E. coli*. Swirl the bottle very gently to mix reagents without introducing bubbles.

8. Pour contents onto the bottom of the pretreated Petri dish (from kit). Leaving the Petri dish on the table, swirl it gently so that any bubbles that form move to the edge of the plate. Allow the plate to harden for one hour. Incubate overnight (18 to 24 hours) in 35 °C incubator.

DAY 3: Read the results within 24 hours.

You should be able to see "holes" in the colored medium on the Petri dishes. These empty spots are called plaques. Each plaque indicates a place where one virus infected a single bacterium. This virus then replicated and released hundreds or thousands of new viral particles to infect new neighboring bacterial cells, killing the bacteria in the process. This leaves an empty spot because the stain will only color live bacteria.



Yellow Fever virus (Shutterstock/molekuul.be)



Common Cold virus (CDC/ Dr. G. William Gary, Jr.)



Chikungunya virus (Shutterstock/molekuul.be)

Visualizing Viruses Investigation



Dengue virus (Shutterstock/molekuul.be)



Influenza virus (NIAID/Wikimedia Commons)



West Nile virus (Shutterstock/decade3d-anatomyonline)

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Are viruses "living" or "non-living"? Opinions differ among scientists.

Non-Living

- Do not absorb or digest nutrients
- Cannot divide on their own

Living

- Metabolize
- Reproduce inside host cell
- Evolve (mutate) quickly

Because viruses evolve (or mutate) quickly, most scientists consider them to be <u>alive</u>.

Viruses are too small to be seen with the naked eye or even with a compound microscope.



Electron Microscopes



David J Morgan from Cambridge, UK (Wikimedia Commons)



Ernst Ruska Electron Microscope - Deutsches Museum - Munich (Wikimedia Commons)

Electron Micrographs



Common cold virus (Dr. Fred Murphy CDC/Wikimedia Commons)



Dengue fever virus (CDC/Wikimedia Commons)



Ebola virus (Cynthia Goldsmith CDC/Wikimedia Commons)



Chikungunya virus (Cynthia Goldsmith CDC/Wikimedia Commons)



Human Immunodeficiency Virus (HIV) (Dr. Edwin P. Ewing CDC/Wikimedia Commons)



Influenza virus (Cynthia Goldsmith CDC/Wikimedia Commons)

The only way to "see" viruses is to look for evidence of them.



The holes on the plate are called <u>plaques</u> and they show where a virus killed all the surrounding bacteria, leaving a blank spot.

Virus Life Cycle





E. coli bacteria with T4 phage

T4 bacteriophage is a virus that only infects the *Escherichia coli* bacterium. The phage enters the cell, causing the cell to stop its normal functions and make more virus.



E. coli bacteria full of new viruses.

These *E. coli* bacteria are about to burst open and release the newly created viruses to attach and infect new cells. Cells that have already released viruses are also seen.

E. coli bacteria packed with new T4 bacteriophage virus heads and tails





Photos by John E. Wertz, PhD



"Ghost" DNA

Once the *E. coli* bacterium bursts and releases new viruses, some bacteriophages will still bind to the empty plasma membrane and inject DNA. This "ghost DNA" is visible in the empty space.

Effects of Climate Change on Mosquitoes and Viruses

- Mosquitoes are cold-blooded (ectothermic) and their body temperature varies with the temperature of the environment.
- Both the mosquito <u>vector</u> and the viral <u>pathogen</u> living inside the mosquito are affected by changes in weather and climate.

How Viruses Transmit Diseases

- Most viruses do **not** cause disease and are usually very specific for only one host.
- Some disease-causing viruses are transmitted through air, touch or bodily fluids.
- Others are carried from an infected to a non-infected human through an insect bite.





The blood is digested in the gut of the mosquito. The virus begins to replicate in the cells of the gut, then spreads into the body cavity, and finally to all tissues and glands.







Within the mosquito body, even a small change in temperature can have a large biological effect on virus growth.

Visualizing Viruses Lab

Viruses are too small to be seen with compound microscopes.

The only way to actually "see" a virus is to look for evidence of one.

How to "see" viruses?

- **Day 1**, Grow an overnight culture of a safe, nonpathogenic strain of *E. coli*.
- **Day 2**, Grow T4 bacteriophage virus mixed with *E. coli*.
- **Day 3**, Look for evidence of bacteria and viruses.
- As *E. coli* grows, the media will stain dark pink. Areas without pink color show where bacteria have been killed by viruses. These areas are called **plaques**.

Bacterial Life Cycle Phases



Stay Sterile!

Aseptic technique

- Wear gloves the whole time to protect yourself and the lab from contamination. Do not touch any lab materials with bare hands.
- Do not allow tops or lids of vials to touch **anything**. This will contaminate the contents. Open the vial and put the lid upside down on the counter.
- Do not breathe into any open containers such as the sterile water, vials, *E. coli* culture tube, or Petri dish. The air can carry contaminants.
- Do not leave the Petri dish lid open any longer than necessary. Open the plate with one hand. With the other hand, pour the contents of the bottle into the plate slowly so as not to introduce bubbles, then put the lid back on quickly.

Materials

- T4 virus
- E. coli broth
- Sterile medium EasyPhage[®]
- EasyPhage[®] Petri dish
- Pipets and micropipets
- Bacterial stain
- Sterile water
- Sterile 50 mL conical tubes for sterile water
- 35 °C incubator
- Parafilm to seal Petri dish
- Markers for labeling Petri dish
- Gloves and safety goggles

Procedure: Part 1 of 2

- Label underside of Petri dish with name/initials and date.
 Do not write in the center of the plate to avoid obscuring the plaques.
- Add 6 mL sterile water to media bottle.
- Add: -Bacterial stain
 -T4 virus
 -E. coli broth
- Put top back on bottle and swirl **gently** to mix reagents without introducing bubbles.



Procedure: Part 2 of 2

- Pour contents onto bottom of Petri dish and cover immediately. Swirl gently on the table to move any bubbles to the edge of the plate.
- Allow to harden for one hour. Seal plate closed with Parafilm. Incubate inverted (upside down) overnight in 35 °C incubator.




Sterile Technique

Sepsis is a medical term for an infection caused by pathogens or the toxins they can make. **Anti**-means against, so an **antiseptic** spray or ointment fights against pathogens.

When scientists grow bacteria on Petri dishes, they only grow the specific bacteria they want to study. Because bacteria exist almost everywhere, this can be difficult. Scientists are careful to keep the Petri dish **sterile** or free from any microorganisms. Then they place the bacteria to be studied onto the Petri dish, so it can grow by itself.

Fortunately, scientists and doctors have developed a method to keep most bacteria and other pathogens from contaminating a sterile space. This is called **sterile technique**, because it is a way to keeps things as sterile as possible.

Nurses and doctors follow this same method during surgery, so it is also called **surgical asepsis**. Surgical asepsis is used in the operating room, delivery room, during surgical procedures, and when bandages are changed.

When using sterile technique, pay close attention to the way you handle items. These rules will keep an area free from all microorganisms. An object or area is described as sterile or not sterile. Basic rules of sterile technique include the following:

- Only a sterile object can touch another sterile object.
- Open sterile packages carefully. Do not allow the inner surface of a sterile wrapper to touch a non-sterile area.
- Avoid spilling any solution on a cloth or paper used as a field for a sterile set-up.
- Hold sterile objects above the level of the waist. If you can't see it, you might contaminate it.
- Avoid talking, coughing, sneezing or reaching over a sterile field or object.
- Use dry, sterile forceps when necessary.
- Consider the edge (outer one inch) of a sterile field to be contaminated.
- Consider an object contaminated if you have any doubt whether it is sterile.

Name _____

Date Class

Mosquito YOLO (*You Only Live Once*) Part 1: Hatching Eggs Life Cycle and Observations Lab

Place enough distilled water in a jar or a dish to cover ONLY the bottom. Submerge the piece of paper with mosquito eggs under water, note the time, and place the dish under the dissecting microscope.

Count the eggs on your piece of filter paper. How many hatched within a set time period? What percentage of eggs hatched? Compare your results with your classmates.

What factors might influence the number of eggs that hatch?

Describe what you see as the larvae emerge from the eggs.

How long does the process of hatching take?



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Name

Date Class _____

Mosquito YOLO (You Only Live Once) Part 2: Larvae Life Cycle and Observations Lab

Observe larvae hatched from eggs at different times.

Make observations about the differences and similarities between the larval stages.

Label the drawing: Observe and label the following structures below: mouth brush, head, thorax, abdomen, siphon (air tube). What are the functions of these structures?

	Mouth Brush:
JELL HARDEN	Head:
	Thorax:
and the for the contract will as	Abdomen:
	Siphon:

Feeding: You can observe larval mouthparts in action by watching larvae feed under the microscope. Place larvae in a deep-well slide or microaquarium and add a few tiny specks of finely crushed food or activated charcoal.

Describe how the larvae eat: Do they turn toward the food? Do the mouth brush appendages start and stop in response to food?



Name_____

Date _____

Class _____

Mosquito YOLO (You Only Live Once) Part 3: Larvae and Pupae Life Cycle and Observations Lab

Mosquito Larvae Diving Experiment

Prediction 1: How long do you think a larva will stay under water?

Prediction 2: How deep do you think a larva will dive?

Procedures

- 1. Measure 50 mL of distilled water in a graduated cylinder.
- 2. Capture a larva with a plastic pipette.
- 3. Drop the larva in the cylinder of water.
- 4. Record the time when the larva starts to descend.
- 5. Stop the time when the larva returns to the top.

Record your data:

Why do you think larvae hang at the top of the water column?

Why do you think larvae dive under the water?





Look at the diagram of the mosquito life cycle above. List three places where the life cycle could be disrupted and describe what could disrupt the life cycle there.





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NEATO MOSQUITO





Mosquito Life Cycle





Egg



Larva



Pupa







Female laying egg raft on permanent water



Electron Micrograph of Mosquito Eggs

Floodwater eggs







LARVA



Mouth brushes filter food particles from the water









ADULT EMERGING FROM PUPA

Pupal skin splits along top and adult wriggles out





Adult emerging from pupal skin



ADULT MOSQUITO









ADULT MOSQUITO



Male has bushy antennae Female's antennae less hairy



Mosquito Habitats





Floodwater







Container Habitats









Host Location







Mosquito Blood Feeding



Labium (sheath) folds back as stylets enter the skin









MOSQUITO-TRANSMITTED DISEASE

Malaria Yellow Fever engue



Container Habitat Survey Form

Name ______

Container type	Number seen in yard	Total in yard
Treeholes		
Cans (tin cans or pop cans)		
Cups (plastic or Styrofoam)		
Bottles (glass or plastic)		
Pet bowls		
Buckets		
Tires		
Barrels		
Flowerpots (that hold water)		
Toys (that hold water)		
TOTAL		



Container Habitat Survey



Container Habitat Survey



Life Cycle of the Aedes triseriatus Treehole Mosquito Video Transcript, Neato Mosquito Curriculum Division of Vector-Borne Diseases Centers for Disease Control and Prevention (CDC)

EGG

The female treehole mosquito lays eggs inside tree holes and other types of containers that can hold water such as cans, buckets, flowerpots and discarded tires.

When first laid, the small cigar-shaped eggs are soft and white. After a few hours, they harden and darken. The eggshell provides protection for the embryo developing within, which may survive for a year or more until conditions are appropriate for hatching. When it rains and the eggs are covered with water the larvae in the eggs begin to hatch.

The female treehole mosquito may lay up to 150 eggs at one time. During her lifespan of 30 days, she may lay 4 to 5 batches of eggs. One female treehole mosquito is capable of laying up to 1,000 eggs during her brief lifetime.

The hatching process is very slow. The larva pushes off the cap of the egg and gradually works its head out of the small opening. The hatching process may take up to two minutes per egg. Once the head is out, the rest of the worm-like body quickly escapes the eggshell.

LARVA

The mosquito larva is aquatic. It lives in the water and swims very actively. Because of their characteristic swimming motion, mosquito larvae are often called **wrigglers**. The treehole mosquito spends about 10 to 14 days in the larval stage.

The body of the larva consists of the dark brown **head**, the **thorax** and the **abdomen**. The dark spot on the side of the head is the eye, and next to it are the **mouth brushes**. The small, swollen round segment just behind the head is the thorax. Behind the thorax is the long slender abdomen.

The larva functions as a food collector and has a well-developed digestive system. The larva feeds on bacteria, algae and other organic materials suspended as small particles in the water. Mosquito larvae feed almost constantly using the fine mouth brushes to obtain their food. The particles are filtered out as the rapidly moving brushes pass through the water. Particles accumulating on the brushes are then eaten. Like all insects, as the larvae feed and grow they **molt** or shed their skin as they become bigger.

Though the mosquito larva lives in water, it breathes air. At the tip of the abdomen is a specialized breathing structure called the **siphon**, which is a hollow tube that penetrates through the water's surface. The larva breathes air through the siphon just like a skin diver uses a snorkel.

PUPA

After the mosquito larva has reached a large enough size, it transforms from the wriggling worm-like

Climate Change and Vector-Borne Disease

larva to the pupa stage. The pupa also lives in the water and–like the larva–it also breathes air. The pupa gets air through a pair of tubes called **trumpets** that penetrate through the water surface. You can see the trumpets touching the surface of the water on the top of the pupa.

Also like the larva, the pupa swims actively in the water. Instead of a wriggling motion, the pupae appear to be tumbling through the water as they flick the abdomen. Because of this swimming motion, mosquito pupae are often called **tumblers**.

Unlike the mosquito larva, the pupa does not feed. During the pupal stage the larval body is being transformed into the adult form. If you look closely at the pupa, you can see some of the structures of the adult mosquito that are forming inside. The dark spot is the eye. The antenna is the small ridge that wraps around the top of the eye. The legs and wings are folded under the thorax, and the long slender abdomen of the adult mosquito is behind the thorax.

ADULT EMERGENCE

Having spent 3 to 4 days in the pupal stage, the fully formed adult mosquito is ready to emerge from the pupal skin. The pupa finds a quiet spot and rests just below the water's surface. It then begins to swallow air to increase the pressure inside the pupal skin, much like blowing up a balloon. It may take the pupa up to 2 to 3 minutes to inflate its skin.

As the internal pressure increases, the pupal skin splits open along the top and the adult mosquito slowly emerges. As you can see, the adult mosquito's head emerges first with its fully developed wings on the left and the antennae on the right. The six legs are tucked neatly under its body ready to unfold.

This whole process takes about five minutes. After it is free of the pupal skin, the adult mosquito sits quietly on the surface of the water while its wings dry. After a few minutes it is ready to fly away.

ADULT FEMALE AND MALE

The adult female treehole mosquito is larger than the male. One way to separate the sexes is by looking at the **antennae**. The female's antennae have few hairs while the antennae of the male are very bushy and feathery.

BLOOD FEEDING

Only the female mosquito sucks blood, which she uses to make the many eggs she will lay later. The female mosquito locates a blood meal host by smell and vision. After flying to the host and finding a suitable place to land, she begins the feeding process.

First she inserts a hollow needle-like feeding tube into the skin. The feeding tube is called the **stylets**. She probes around in the skin with the stylets searching for a small blood vessel. The stylets are pushed in and pulled out repeatedly as the female tries to locate a source of blood. The sheath-like labium folds back, exposing the stylets as they are inserted into the skin.

While the female mosquito is probing for a blood vessel, she is injecting saliva into the skin. The saliva



contains an anesthetic so you don't feel the bite. It also contains other substances like anticoagulants that prevent the blood from clotting and clogging up the very fine feeding tube, and other substances that make the blood feeding process easier. More importantly, if the female mosquito is infected with a virus or parasite, she may inject these disease-causing agents along with the saliva.

When the female locates a small blood vessel, muscular pumps in the head begin pumping blood into the stomach. The stomach stretches to hold the very large blood meal. A female mosquito can consume about five times her weight in blood. The feeding process takes about three minutes.

When full, she pulls out the stylets and flies away. After she finishes feeding, she will find a quiet place to digest the blood and develop a batch of eggs. In about three days she will find a treehole or other container in which to lay her eggs and will be ready to take another blood meal.

Teacher Notes

CDC Neato Mosquito Curriculum

Click below to download the video (a large 37 MB zipped file) from the archived curricula of the Division of Vector-Borne Diseases, Centers for Disease Control and Prevention (CDC).

Download the QuickTime video "Life-Cycle of the Treehole Mosquito"

To Bite or Not to Bite...

Only female mosquitoes bite, using nutrients in blood to develop eggs. Receptors for attraction to humans are turned off after the female takes a blood meal.



What ATTRACTS Mosquitoes?





NO











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Mosquito Inquiry Experiment Teacher Notes

This inquiry-based lab can be integrated into biology or environmental science in middle or high school life sciences.

Students will investigate aspects of climate change that could affect mosquito development and determine which variables to test. The primary climate-related issue is the effect of temperature increase or decrease on the life cycle of mosquitos, but students may also be allowed to pursue other environmental options. Examples could include density of organisms, food availability, pH of water or water source.

Background

Learning Objectives

- Students will identify variables that affect the growth and development of mosquitoes using observations and recorded data.
- Students will draw conclusions about how global climate change can affect individual species.

Introduction

- Have students brainstorm factors that affect mosquito development.
- Guide the discussion to highlight the theme of climate change.
- Ask probing questions to get students to think about possible variables that can be investigated in the lab. There are many variables to consider and each can be addressed in the design of the experiments.

Time Requirement

- One full class period for students to design and set up their experiments.
- A minimum of four additional days (10 minutes per day) for students to observe larvae develop into pupae and to collect data.
- Additional time required for students to write up results or a lab report.
- Additional time required for teachers to set up a rearing chamber and submerge mosquito eggs two days before the student experiment begins (see Mosquito Rearing Instructions).

Experiment

The following experiment is designed to measure the effect of different temperatures on the development rate of mosquito larvae. If your students choose other variables to test, you can make the appropriate changes to the materials, preparation, and procedures.

Materials

- Mosquito rearing chambers: any clear containers such as canning jars, baby food jars, or a BioQuip Breeding Chamber
- Spring water, distilled water, or conditioned tap water
- Mosquito food (ground dry fish food pellets or flakes)
- Covering for containers: tulle or netting squares, cheesecloth, screen, rubber bands
- Hatched Aedes sp. mosquito larvae (approximately 30 larvae per student group)
- Plastic pipets (with tips cut off to transfer larvae)
- Graduated cylinders
- Microaquaria, Petri dishes, deep-well slides

- Thermometers
- Chick incubator (Hovabator)
- Cooler with ice packs
- Safety equipment as required (goggles, apron, gloves)

See source list for these materials.

Preparation

- See Mosquito Rearing Instructions for information on conditioning water and submerging eggs.
- Submerge the mosquito eggs for two to three days before students begin the experiment. Hatch all eggs in one container so temperature and food are kept constant until the inquiry experiment.
- Refer to Mosquito YOLO for preliminary companion activities that provide experience observing the mosquito life cycle before students begin the experiment:
- See Mosquito Life Cycle Background for a detailed discussion of complete metamorphosis, including scientific illustrations.

Procedure

- Ideally start the experiment two days after submerging the eggs.
- The student experiment usually takes five days. Recommendation: Submerge eggs on a Friday and start the student experiment on Monday. Larvae will be more visible for students to observe after two days of development over the weekend. Adjust schedule accordingly for different class schedules.
- Divide students into lab groups to design and conduct an experiment. Teams of two students are ideal for laboratory work, but circumstances may necessitate teams of three or four students.
- Have lab groups create a hypothesis, procedure, and data table to record observations.
- Distribute larvae in multiples of 10 to simplify estimating averages.
- Allow larvae to grow under experimental conditions such as different temperatures, food supply, or population density.
- Students can test any range of temperatures; however, recommended temperatures for a five-day experiment are as follows: 37 °C, 30 °C, and 22 °C (approximate room temperature). Cooler temperature options such as 10 °C (cold chamber) will inhibit development of the larvae. Temperatures in a refrigerator will prevent further development.
- Individual larvae do not simply grow larger, but develop through four stages called instars. All instars look alike, except the size of the larvae increases with each new stage. In this experiment, mosquito larvae complete the development cycle more quickly at the higher temperature (37 °C), so they reach the next instar stage in a shorter time
- Each day, record the number of pupae and calculate the percentage of total.
- Chart a graph with results.
- Determine at which temperature the larvae become pupae in the shortest amount of time.

Teacher Tip

You may continue the experiment for 7 to 10 days if you wish to observe adults emerge. The experiment measures the number of days needed for larvae to develop into pupae under different environmental conditions. This unit of measurement is used because the time to mature to the adult stage is not as accurate. Development of larvae into pupae is not 100% and development of pupae into adults is not 100%. This discrepancy will be cumulative and affect your results. Students might wait a very long time on larvae that will never become pupae or pupae that will never become adults.

Sources of Mosquito Eggs, Larvae, and Food

Eggs

- State Agricultural Experiment Stations
- Centers for Disease Control and Prevention: www.cdc.gov (Ask for local mosquito supplier.)
- Local researchers or professors

Larvae

Starting with larvae is an alternative if eggs are unavailable.

- Native larvae (collected in the wild)
- Larvae from pet stores (raised to feed fish)

Food

- Pet stores: Ground dry fish food pellets or flakes; ground dog, cat or rodent chow, or a mixture of ground fish food and animal chow.
- Mosquito Diet (Carolina Biological Supply)

Mosquito Rearing Instructions

Mosquito eggs remain viable for different periods of time. It is best to use the eggs as quickly as possible. Eggs can dry out or mildew depending on moisture levels. Refer to storage instructions from the supplier. Some larvae will emerge almost immediately, but others may take 30 to 40 minutes, and from desiccated eggs can take a day or more.

Procedures (for Aedes aegypti and Aedes albopictus)

- 1. Open the mosquito rearing chamber and fill the bottom portion with 400 to 500 mL of water (spring water, distilled water, or conditioned tap water aerated overnight to dissipate chlorine). Add one small pinch of mosquito food to the water and stir to distribute the food. Allow the water to sit for at least an hour before adding the eggs.
- 2. Cut a slice from a filter paper of mosquito eggs; the size depends on how many larvae you need. A small dark patch on the filter paper can contain hundreds of eggs.
 - **Mosquito YOLO (***You Only Live Once***)**: A one-sixth or one-eighth size slice will usually be enough for students to watch the eggs hatch and observe the larva, pupa, and adult stages.
 - **Mosquito Inquiry Lab**: Submerge a half or whole filter paper to hatch many larvae. To avoid overcrowding, you may need to separate them into new chambers or jars within a few days.
- 3. Slip the paper into the water so that it sinks to the bottom of the rearing chamber. If the paper floats, tap it with forceps to dislodge the air bubbles.
- 4. Replace the chamber top, making sure that the funnel portion points upward into the top section, not into the section with the water.
- 5. After 24 to 48 hours, all viable eggs will have hatched. Use forceps to remove and discard the filter paper. Rinse off the filter paper to make sure no larvae are clinging to it. If you do not remove the filter paper, it will disintegrate and cloud the water.
- 6. Open the chamber and add a small pinch of food every day if the larvae have eaten most or all of the food from the previous day. If not, wait and feed the next day. Once there are many pupae, add one final pinch and then do not feed again. Pupae do not eat and will soon emerge as adults.
- 7. It is better to underfeed than overfeed. The water should never look dirty, cloudy, or brown.
- 8. After the experiment is completed, you may let them die naturally and then discard all larvae, pupae, and adults or pour out the live larvae and pupae onto dry ground. Adults can be killed by placing the chamber in a freezer for 15 minutes.
- 9. If you want to keep the adults alive for a while, soak a cotton ball in water sweetened with sugar or honey. Place the cotton ball on the mesh screen. Adults will drink this "nectar" and live for a few weeks. Replace the cotton ball every few days.

DO NOT release adult mosquitoes into the environment!



Climate Change and Vector-Borne Disease

Increasing Temperatures Affect Mosquito Growth



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Birds, Malaria and the Changing Climate



How do birds get avian malaria?

How does climate change affect habitats in Hawaii? Birds get malaria too. As in humans, the microorganism that causes the disease is transmitted by a mosquito bite. In Hawaii, several species of native honeycreepers are endangered by avian malaria and others are already extinct. Higher temperatures in the mountains play a role in this epidemic.

A mosquito vector first bites an infected bird and then transmits the pathogen to an non-infected bird.

Pathogen: A microorganism—such as a bacterium, virus or protozoan —that can cause disease



Bird habitats



Mosquitoes live in low, wet areas. As temperatures warm, birds avoid mosquitoes by migrating upland in the cooler mountains as long as food is available. Avian malaria increases when native birds can no longer move into higher areas than mosquitoes.

What lies ahead for avian malaria?

Malaria—in humans or other animals—is very difficult to eradicate, despite efforts to control the mosquito vector population. Reducing standing water larval habitats can help slow the spread of avian malaria.



The product of the state of the

Aosquito habitats





arge cavities in felled tree ferns collect



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Effect of Temperature on Rate of Reaction Teacher Notes

- 1. **Relevance of this activity to the mosquito life cycle**: Mosquitoes and the pathogens they transmit are directly affected by changes in weather and climate. All vector-borne pathogens spend a part of their life cycle in cold-blooded arthropods and are subject to environmental factors. Marginal changes in temperature can have potentially large biological effects on disease transmission.
- 2. If your students are not familiar with the *Alka-Seltzer*[®] commercials, several versions are available on YouTube.
- 3. You can also use generic brand effervescent tablets.
- 4. You might like to introduce this activity with a review of physical and chemical changes. Comparing tearing paper with burning paper is a good example. Sugar also shows a physical change when dissolved in water, but a chemical change when burned. However, it is both a physical and chemical change to dissolve salt in water. Even though you can recover salt crystals by evaporating the water, the Na⁺ and Cl⁻ ions dissociate when dissolved.
- 5. This lab can be done without timing the reaction. For a quick demonstration, drop the tablets into each of the three water temperature beakers simultaneously and just observe the difference in reaction times.

Extensions (Investigator level)

- 1. Calculate the standard deviation of the reaction times for each temperature. Add error bars to your graph to show the amount of variance in the data.
- 2. Is reaction rate predictable over a larger temperature range? Use higher and lower temperatures to test reaction rate.

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Climate Change and Vector-Borne Disease

Effect of Temperature on Rate of Reaction

Have you seen television commercials for Alka-Seltzer® effervescent tablets? You may have heard its advertising slogan "Plop, plop, fizz, fizz, oh what a relief it is![®]" When you drop the tablets in water, they bubble or effervesce, like an extra-fizzy soda. The bubbles in the soda and coming from the tablets are carbon dioxide gas (CO₂). The CO₂ is already in the soda, but with the Alka-Seltzer[®], the CO₂ is produced by a chemical reaction that occurs when the tablets dissolve in water.

Making Predictions

What factors do you think could speed up the chemical reaction?

What factors do you think could slow down the chemical reaction?

In this experiment, you will measure the time it takes for one effervescent tablet to react completely. In this case, consider reaction time as the time it takes for the tablet to dissolve. You will test how reaction time changes with temperature and determine whether the results support your hypothesis.

Materials (per group)

- Glass beakers (3)
- Thermometer •
- Stopwatch
- Effervescent tablets (3)

Procedure

- 1. Measure 200 mL each of warm water, room temperature water, and cold water.
- 2. Pour the water into three separate glass beakers.
- 3. Measure the water temperature in each beaker and record it in the Data Table.
- 4. Remove one effervescent tablet from the package for each beaker.
- 5. Use only whole tablets! Do not use broken or crushed ones.
- 6. For each temperature, drop one effervescent tablet into the beaker. Start timing immediately with the stopwatch.

Name ___

1





- Room temperature water
- Cold water

- 7. Stop the timer when the tablet has dissolved completely.
- 8. Record the reaction times (in minutes and seconds) in the Data Table.

Results

Data Table

Water Type	Temperature (°C)	Reaction Time (minutes and seconds)
Warm		
Room Temperature		
Cold		

Questions and Conclusions

- 1. What is the independent variable?
- 2. What is the dependent variable?
- 3. Which variables were constant? (List at least two.)
- 4. Why did we use whole tablets for each demonstration? What might happen if we used broken tablets?
- 5. What might happen if some students stirred the water as the tablets were dissolving?
- 6. How can you relate this activity to the life cycle of a mosquito?
- 2) concentration of reactants
- 3) temperature

Climate Change and Vector-Borne Disease

Effect of Temperature on Rate of Reaction



A chemical reaction occurs when there is a chemical change in one or more substances.

Physical Change	Chemical Change
Changes the look or shape of a	Produces new substances;
substance but does not make a	chemical bonds break and new
new substance; no chemical bonds	bonds form.
break or form.	
Example: Crumpling or tearing	Example: Burning a piece of paper
paper into pieces changes the look	uses energy to break chemical
and shape of the paper, but it is	bonds. Cellulose reacts with
still paper.	oxygen in the air to form new
	substances CO_2 and H_2O .

Alka-Seltzer® has been helping people cure indigestion and upset stomach since 1931. The TV commercials featured the jingle "Plop, plop, fizz, fizz, oh what a relief it is®" because the tablets effervesce, or fizz, when dropped into water. This chemical reaction releases CO₂ bubbles.

The fizziness happens when baking soda (sodium bicarbonate) and citric acid react chemically in water. They yield sodium citrate, water, and carbon dioxide gas, which causes bubbles.

$C_6H_8O_7$	+	3 NaHCO₃	\rightarrow	Na₃C ₆ H₅O ₇	+ 3 H ₂ O	+	3 CO ₂ (gas bubbles)
(citric acid)		(sodium bicarbonat	e)	(sodium citrate)	(water)		(carbon dioxide)

In a chemical reaction, reactants (starting materials) yield (change into) products (new chemical substances) when molecules collide with enough energy to break old bonds and make new ones.

Some factors that influence the speed of a chemical reaction are:

- 1) surface area of starting reactants

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Name _____

1

As temperature increases, molecules of water collide with each other faster, allowing reactions to occur faster. What effect might increased or decreased temperature have on the rate of the effervescence reaction? Do you think that the tablet might dissolve faster or slower with temperature change?

Create a hypothesis _____

In this experiment, you will measure the **time** it takes for one effervescent tablet to react completely. In this case, consider "reaction time" as the time it takes for the tablet to dissolve. You will test how reaction time changes with temperature and determine whether the results support your hypothesis.

Warm water

Cold water

Room temperature water

Materials (per group):

- Glass beakers (3)
- Thermometer
- Stopwatch
- Effervescent tablets (3)

Procedure

- 1. Measure 200 mL each of warm water, room temperature water, and cold water.
- 2. Pour the water into three separate glass beakers.
- 3. Measure the water temperature in each beaker and record it in the Data Table.
- 4. Remove one effervescent tablet from the package for each beaker.
- 5. Use only whole tablets! Do not use broken or crushed ones.
- 6. For each temperature, drop one effervescent tablet into the beaker. Start timing immediately with the stopwatch.
- 7. Stop the timer when the tablet has dissolved completely.
- 8. Record the reaction times (in minutes and seconds) in the Data Table.

Results

Data Table

Water Type	Temperature (°C)	Reaction Time
		(minutes and seconds)
Warm		
Room Temperature		
Cold		

Graphing

- 1. Graph the reaction time (minutes) on the y-axis against the water temperature (degrees Celsius) on the x-axis for the entire class.
- 2. How does reaction time change with temperature?
- 3. Do the results support your hypothesis? Why or why not?

Experimental Design

- 1. What is the <u>dependent variable</u>?
- 2. What is the independent variable?
- 3. Which variable(s) are <u>constant</u>?
- 4. If some tablets are whole and some are broken, would the temperature experiment still be valid? Why or why not?
- 5. If some students stirred the water while their tablets were dissolving, would the temperature experiment still be valid? Why or why not?
- 6. If everyone in the class used a different water temperature, could you average the results? Why or why not?
- 7. If you used different brands of antacid tablets, would the results be valid? Why or why not?
- 8. How does this activity relate to the life cycle of a mosquito?

Pathogens: Effect of Climate

Viruses are particles of DNA or RNA, usually with a protein coat. They are found in all living organisms and in all types of environments, even the most extreme.

Most viruses do not cause disease and are usually very specific for only one host. Some disease-causing viruses are transmitted through air, touch or badly fluids. Others are carried from an infected to a non-infected human through an insect bite.



How are insect-borne viruses transmitted?

Mosquitoes are cold-blooded (etathermic) and their body temperature varies with the temperature of its surroundings. Both the mosquito vector and the viral pathogen living inside the mosquito are affected by changes in weather and climate.

Humans are warm-blooded (endothermic) They can adjust to temperature changes in the environment by keeping body temperature and pH levels constant. As a result, humans and the pathogens living inside humans will not be affected by changes in environmental temperature.





Within the mosquito body, even a small change in temperature can have a large





he viral pathogen grows throughout the masquito body. Once it reaches a attitual number is a valuence alongly. It can be arread along with the sales when a new home both is bitten.

Are viruses "living" or "nonliving"?

Opinions differ among scientists. Viruses do not absorb or digest nutrients and cannot divide on their own, but they do metabolize and reproduce inside a host cell. Because viruses evolve (or mutate) quickly, most scientists consider them to be alive.



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