EYE

Extreme Yellowstone Expedition

Student Activity Book

MONTANA STATE UNIVERSITY Thermal Biology Institute

NESSP Northwest Earth and Space Science Pipeline

MONTANA STATE UNIVERSITY Academic Technology & Outreach

LESSON 1

EXPEDITION BRIEFING

Expedition: Yellowstone

Yellowstone National Park is home to a supervolcano, thousands of wild animals, and half (more than 10,000) of the world's hydrothermal features. It was established as the world's first national park in 1872.

When most people think of Yellowstone National Park, they think of bison, bears, and the world-famous geyser called Old Faithful, but there is even more to Yellowstone than that. You are going to follow along on the Extreme Yellowstone Expedition (EYE) as scientists and one lucky high school student journey to Yellowstone's remote Heart Lake region to explore some of Yellowstone's undiscovered secrets.

Before we learn more about the research team's mission, let's get to know Yellowstone a bit better.

Take a look at these maps and fill in the information listed below. *Your teacher will provide the information you need to mark these spots on the map.*

On the map of the continental US:

1. Color in and label the location of Yellowstone National Park.
2. Color in and label the location of Glacier National Park.

Lemonade Creek

Whirligig Geyser

"Gabby's Spring"

Mud Pot

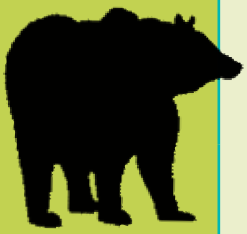
On the map of Yellowstone:

3. Using two colors, shade in and label the parts of Yellowstone in Idaho and Montana. Label the total percentage of the Park in those states – 3% in Montana and 1% in Idaho. The rest is in Wyoming.
4. Mark the Heart Lake region on the map.
5. Use a line to connect the photos of Whirligig Geyser, "Gabby's Spring," Mud Pots and Lemonade Creek to their locations on the Yellowstone map. You'll be studying these thermal features in depth in other lessons.
6. Place an X in the general vicinity of the most remote area in the lower 48 states.

Yellowstone by the Numbers

Yellowstone National Park is massive. At 3,472 square miles, it is larger than Rhode Island and Delaware combined. Here's a guessing game for you to get to know Yellowstone better.

Choosing from the mixed up numbers at left, enter your guesses for the correct numbers to complete the table of Yellowstone facts.



1,000–3,000		Earthquakes (annually)
~ 290		Waterfalls
322		Archeological sites
67		Historic buildings
~ 1,000		Species of mammals
~ 1,600		Species of birds
900+		Miles of back country trails

The Extreme Yellowstone Expedition (EYE) Mission

The EYE team consists of a group of Montana State University (MSU) researchers from the Thermal Biology Institute (tbi.montana.edu). They hiked into the extremely remote area of Heart Lake in order to collect samples and research the microbial organisms living in hot springs there.

Why study microbes?

Microbes are living organisms smaller than your eyes can see. They are the oldest form of life on Earth, and they live just about everywhere–including in, and on, your body. They can even live in really hostile environments like icy glaciers, the bottom of the ocean, and hot springs in Yellowstone National Park.

Microbes that live in extreme environments, called ***extremophiles***, play an incredibly important role in our ecosystems and in biodiversity. They also have many potential applications in biotechnology, medicine, and industry that are only beginning to be discovered.

Microbes also offer an amazing window into astrobiology (the search for life beyond Earth) and questions such as, *How did life form on Earth?*

Scientists at MSU are researching how microbes from Yellowstone may help us find alternatives for gas and fossil fuels, clean up toxic waste, help to cure diseases, or help us find life on other planets.

NASA supports research on the extremophiles from Yellowstone and other places, because scientists think these organisms might resemble some of the first life on Earth. These microbes can help us understand how life started on our planet and how it might form on other planets, so NASA is using research from Yellowstone to help in the search for life on other planets and moons. Scientists think if we discover life elsewhere in the Universe, it's more likely to be an extremophile than, for example, a little green man.

Rare organisms hiding in Yellowstone

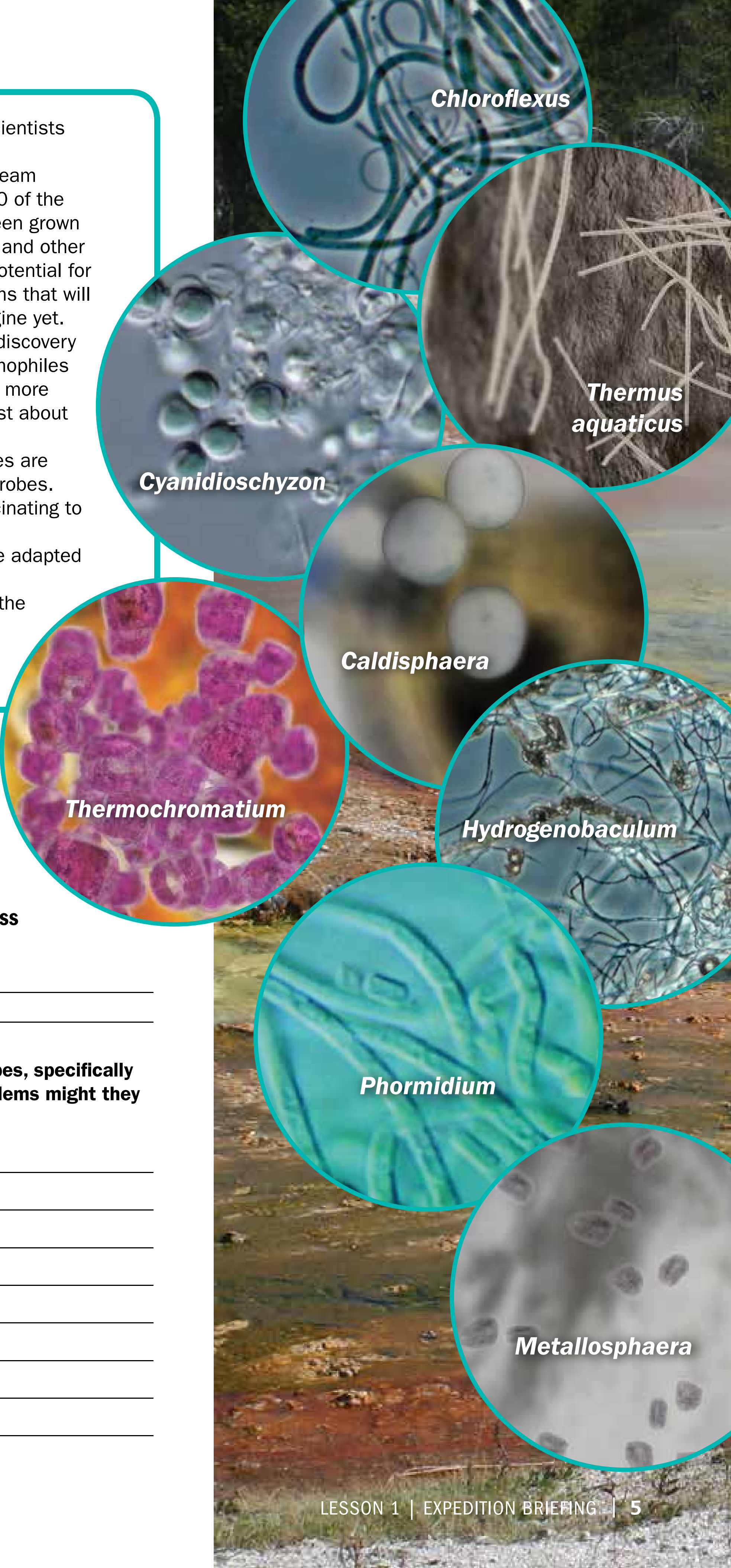
The researchers from EYE have traveled to the Heart Lake area many times, often bringing back water samples containing micro-organisms. Half of the organisms they find are so different from anything already discovered that scientists can't even name them yet!

In examining their samples, the team has found that less than 1 in 1,000 of the organisms they collect has even been grown in a lab. Expeditions to Heart Lake and other areas of Yellowstone have a high potential for the discovery of new microorganisms that will help us in ways we can't even imagine yet.

Scientists get excited about the discovery of ANY new living things, but extremophiles have caused more excitement, and more radical changes in science, than just about any other forms of life.

Why? After all, most extremophiles are single-celled creatures, measly microbes. What is it that makes them so fascinating to scientists?

It's because these microbes have adapted to live in environments of extreme temperatures, pH and toxicity, and the mechanisms they use to do that could unlock the secrets to all kinds of discoveries.



A CLOSER LOOK: Here are some pictures of real microbes that live in extreme environments such as Yellowstone National Park.

Check your expedition preparedness

What is a microbe?

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Why is it important to study microbes, specifically extremophiles? What kinds of problems might they help us solve?

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Planning the expedition

Planning a trip to the Heart Lake region of Yellowstone is extremely tricky. Any time a scientific team goes to Yellowstone, they need a research permit that is issued from the National Park Service. Scientists must explain what research they are conducting and why it is important. They also must have training and obey special rules if they are going to collect any samples from hot springs.

Hot springs can be dangerous. If you fall into a hot spring, you have a high likelihood of dying. One of the hot springs in the Heart Lake Geyser Basin contained the carcass of an elk that had fallen in and died in the boiling water! *If you ever visit Yellowstone, make sure you stay on boardwalks or trails at all times.*

The researchers are not allowed to go to the Heart Lake region from April 1 through June 30. The area is closed every year during that time due to bear activity. The scientists have seen bears in the area many times and were evacuated once because of a bear that kept approaching people. All the researchers carry bear spray. The area can also be thick with mosquitoes, which can be extremely unpleasant.

The Heart Lake region is very remote. It's far from any roads, and cell phones don't work there, so if a member of the team got hurt, it would be dangerous. The hike to Heart Lake is approximately seven miles one way, so it is a long way to bring food, water and gear. Sometimes the researchers use horses to carry their equipment.

If you continue traveling east, past the Heart Lake region, you will reach the most remote area in the lower 48 United States. Located on the Continental Divide, it's called the Two Ocean Plateau and features an unusual site: some of the water that falls here as rain or snow flows to the west and ultimately the Pacific Ocean, and some flows easterly and eventually ends up in the Atlantic Ocean. (Glacier National Park has a site called Triple Divide, where water makes its way to *three* different oceans: the Atlantic, the Pacific and the Arctic.)

BEARS, BURNS AND BUG BITES: This is the arm of an EYE team member after only one day in the field. She was itching miserably for days!

Researchers traveling to Heart Lake must plan for all kinds of weather. It can be really hot or really cold. Once during a trip in September, the temperature was 24°F (-4°C) as the researchers began to hike to the lake. During the summer, they have encountered intense heat, sunburn, and even snow.

To guard against dehydration they must carry lots of water. As the research team reaches Heart Lake, they can use a water purifier to treat the water or boil it before drinking it, but along the trail to the lake, they can't drink any water from Witch Creek—even with a water filter—because it has high amounts of arsenic, which is highly poisonous to humans.

A perfect trip

When researchers plan a trip to Heart Lake they must take many factors into account. From the discussion of expedition planning that you just read, list as many factors as you can.

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If you were going on the expedition, which of the factors in the list above would worry you the most? Why?

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Meet the team

Several Montana State University researchers and students traveled to Heart Lake on the EYE expedition. Here you can meet two of the scientists as well as the Montana high school student who assisted them.

Brent Peyton

Brent Peyton is director of the Thermal Biology Institute and professor of chemical and biological engineering at Montana State University. Dr. Peyton works on finding ways to clean up toxic waste with microbes and methods for creating biofuels – fuel made from living organisms like algae. He has published more than 95 scientific articles and book chapters on these topics. His dad was a chemist, so Dr. Peyton grew up being interested in science. Then Dr. Peyton's high school chemistry teacher, Mrs. Gilmore, suggested that he go into chemical engineering since he liked both math and chemistry, and he's never regretted it. Everything he does now is related to the engineering applications of biotechnology. As for hobbies, Dr. Peyton loves to hike (he tries to hike over 100 miles in the mountains each summer), fly fish, garden, raft, cross-country ski, and hunt antelope, deer and elk.

Dana Skorupa

Dana Skorupa is a post-doctoral researcher in Brent Peyton's lab. Dr. Skorupa hails from Wisconsin. She earned her PhD in microbiology at Montana State University and studied acid-loving algae that inhabit some of Yellowstone National Park's hot springs. Her current research looks at using microorganisms to clean up pollutants produced by the mining industry. During her senior year of high school, Dr. Skorupa took an advanced biology class that sparked her interest in microbiology. She was amazed at how important microorganisms were not only to the wellbeing of our planet but also to the health of our own bodies. When she's not working in Yellowstone or in her lab at MSU, Dr. Skorupa is outside training for 50-mile mountain bike races, skiing with her friends, or bagging peaks with her dog, Rumi.

Gabby Michel

Gabby Michel was born in the Chicago area but moved to Big Sky, Montana with her parents and twin brother when she was four years old. She is a senior at Lone Peak High School and has an interest in the outdoors and the Yellowstone Area. She enjoys her science classes and was very excited to have the opportunity to participate in this field study with Dr. Peyton and several other biologists and engineers. She is interested in pursuing a pre-med track in college, and is looking into becoming a surgeon. Gabby's favorite hobbies are reading, hiking, fly-fishing, and traveling.

Picture yourself as a Yellowstone scientist

Name any hobbies that you have in common with Dr. Peyton, Dr. Skorupa or Gabby Michel.

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Would you be willing to travel on an expedition to the Heart Lake region of Yellowstone National Park? Why or why not? ______________________________

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If you could ask the research team one question, what would it be?

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EXTREMOPHILE HUNTERS: MSU researchers and Big Sky high school student Gabby Michel (center) pause during their hike to the Heart Lake region.

SCIENCE AND YOU...

The word ***science*** comes from an ancient Latin word that means knowledge. Science is more than just a class; it is a process—a way of thinking and learning about things—in which you test predictions about the world around you. Science usually leads to new questions and can never be finished, because there always will be more to discover.

Take a moment and think about science and your everyday life

Can you think of a scientific question or two that you would really like to know the answer to? For example, it could be a big question like, *Is there any such thing as alien life?* Or it could be a question with a smaller scope, like, *Whose mouth has more germs in it, mine or a dog's?*

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Can you name a few movies, TV shows, websites, books or apps you like that feature science or science fiction? List a few here.

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What scientific invention or breakthrough are you most thankful for, and why?

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What science career interests you the most? Why?

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LESSON 2

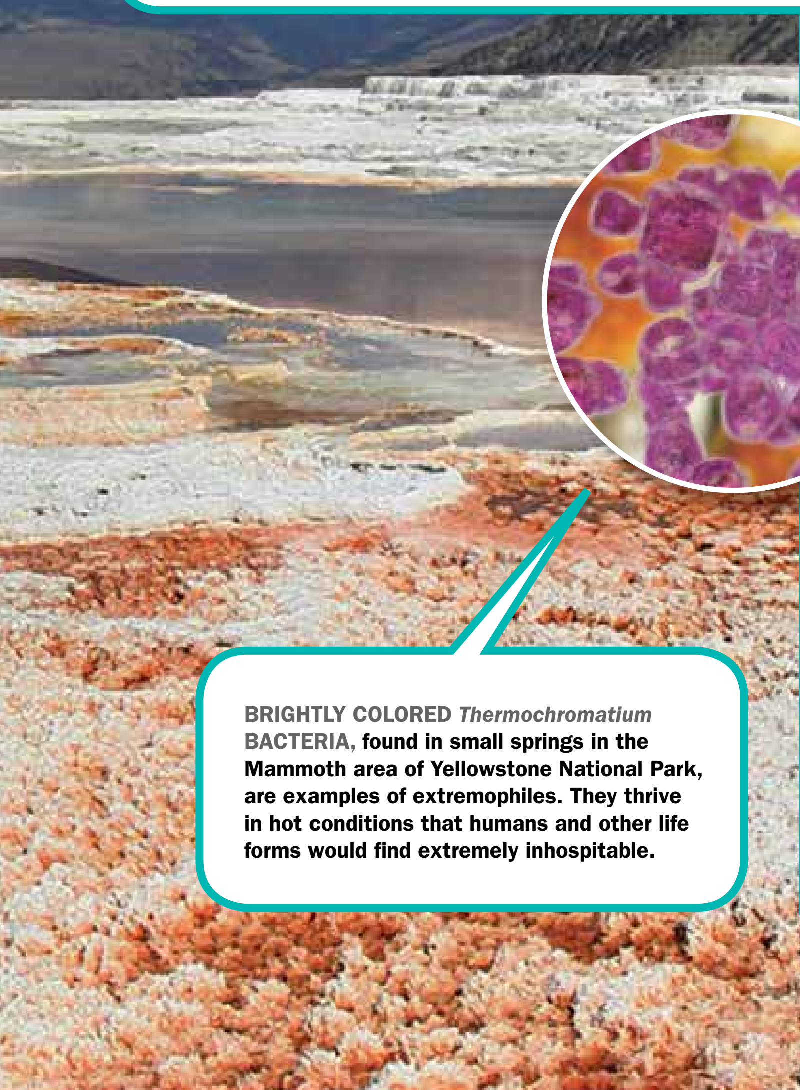
LIMITS OF LIFE

Living on the Edge...

In the past few decades scientists have discovered that life can thrive in places we never imagined it could—such as hundreds of feet below ground, underneath glaciers, at the bottom of the ocean, and in boiling hot springs. Life is a lot tougher than we thought.

Life in extreme conditions

Now we know that there are many forms of life that thrive in environments that we think of as extreme: salty, acid or alkaline, very hot or cold, poisonous, high or low pressure, and even radioactive. Organisms that live in these places are called ***extremophiles,*** and the environments they live in—what we consider extreme on Earth—might be similar to what is normal on other planets or moons. Many scientists think that if we find life elsewhere in the universe, it may resemble these organisms living in Earth's most extreme environments, not the little green men often shown in cartoons and movies.



BRIGHTLY COLORED *Thermochromatium* BACTERIA, found in small springs in the Mammoth area of Yellowstone National Park, are examples of extremophiles. They thrive in hot conditions that humans and other life forms would find extremely inhospitable.

They're not extremely rare

When we talk about life in extreme environments, you might think these life forms are rare, because we think of extreme things as being uncommon. Remember, though, that what we call extreme just means places where *we* couldn't live. Humans (and most plants and animals) only thrive in a narrow range of temperature, pressure, salinity and pH.

There are a lot more extreme environments than one would think. In fact, extremophiles are present all around us. Most of them are single-celled organisms and many are in the domain Archaea.

What's a domain?

Now you are probably wondering, *What's a domain? And what's Archaea?*

When scientists arrange living things into groups that have similarities, that is called ***taxonomy***. The broadest category that scientists use is called a ***domain***. Scientists group all living things into three domains:

Eukaryota **- plants, animals, fungi and some single-celled organisms**

This is the only domain that contains life with multiple cells that work together to do different jobs.

Bacteria **- single-celled organisms that lack a membrane-bound nucleus**

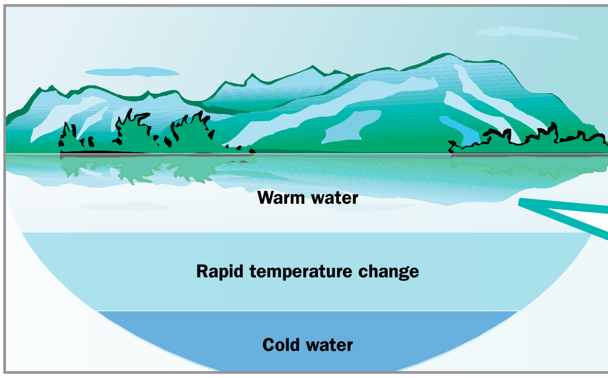
They live all over the Earth—from deep inside rocks to inside the intestines of most eukaryotes.

Archaea **- single-celled organisms that lack a membrane-bound nucleus**

They were originally categorized as Bacteria, but now scientists realize their cells have unique properties separating them from the other two domains of life: they have a different genetic and chemical make up. Many thrive in extreme environments and they evolved in early Earth's harsh environment (hot with no oxygen).

What are the limits of life?

Researchers are working to establish what the environmental limits are for life to exist. Establishing these limits will help scientists in the search for



ENVIRONMENTAL GRADIENTS

An environmental gradient can be a smooth, even change or a more sudden change. This is an example of a basic temperature gradient in a mountain lake. The water gets colder as you go deeper into the lake. Gradients of water pressure and oxygen in the lake would follow a similar pattern. As you go deeper, oxygen levels would diminish, but pressure would increase.

life on other planets and in the search for as yet undiscovered life on Earth.

All this brings us to our next point: organisms function well (thrive) only within particular ranges of an environment. No single organism could survive the entire range of surface temperatures of Earth, but there are certainly those adapted to very hot climates, while others are adapted to very cold climates. Every species has its "preferred" temperatures, acidity, moisture levels, nutrients, and so on. Some species can tolerate a broader range of some of these ***gradients***; others have a very narrow survival range.

The ranges that an organism can tolerate help define its ***niche***—where it can live, and with what other organisms. Human beings are unique in that we have developed technology that lets us expand our niche and live in places that we couldn't even visit otherwise.

Finding an organism's niche

Here's one way to help find an organism's niche. When we take measurements of any ***abiotic*** (non living) factor along some geographic or time axis and see a change (gradual or sudden) in the value, we are measuring an ***environmental gradient***.

Gradient just means "a continuous change in a value." An environmental gradient can be a smooth, even change (for example, pressure varies predictably with water depth) or a more sudden change (like air pressure before a storm).

Gradients can be found on the land and in the air as well. At higher altitudes on a mountain, the average temperature drops significantly, creating a climate gradient on the mountain's slopes. For some mountains, the base can be a tropical rainforest while the top resembles an Arctic tundra. Gradients can be invisible or they can be marked by visible changes such as color or vegetation.

Measuring environmental factors

We can actually measure almost anything in the environment, but let's focus on things that affect life the most.

One obvious measurement is temperature. The Earth's surface experiences temperatures from about -50°C up to 60°C (about -180°F to 130°F), and most life we're familiar with can't survive much outside that range.

Another measurement is humidity (amount of water vapor in air), ranging from nearly 0% up to 100%. In the oceans, we could measure acidity (on the pH scale, from just below 0 up to 14.0), or turbidity (how "muddy" the water is).

We could also measure how much oxygen or CO_2 is in the air. The average concentration of CO_2 was about 396 PPM (parts per million) in 2013, up from less than 320 PPM in the 1960s.

Each of these values can vary, depending on exactly where in the environment we do our measurement. For example, we might measure ocean water temperature at different depths, and we'd find that ocean water becomes colder as we go deeper.

Take a beach for example

A beach is an example of a location with many environmental gradients. In particular, we have the "wetness" of the land: Beyond the tide line is ocean environment, then we have the wet sand where the tides wash in, then the drier beach sand, and finally the dunes.

We can see distinct areas here, each with its characteristic forms of life. These identifiable areas along gradients are called ***zones***, and the process of forming them is called zonation. Each zone has distinct lifeforms. For example, we would rarely find hermit crabs or clams in the dunes area, while beach grass can't take hold in the intertidal zone.

ACTIVITY 1

Analyze this photo looking for gradients.

Identifying Gradients

When you examine this photo, think about how factors in the nonliving environment **(abiotic factors)** affect the living environment **(biotic factors).**

Write in the table below a few of the types of gradients represented in this landscape. (Note that some might be visible and some might be invisible.) The first row in the table shows one example answer, but the gradients, zones and life forms you identify may differ from the example.

Kind of gradient (i.e. temperature, moisture, etc.)

How many zones of this gradient do you see in the picture?

List the different zones *(distinct, identifiable areas within the gradient)* **that you see.**

Name a plant or animal that could live in each zone that you listed. (Hint! There's one living thing that lives almost everywhere!)

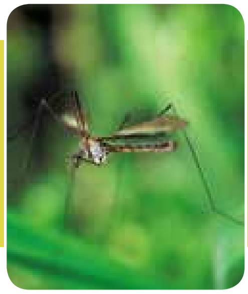
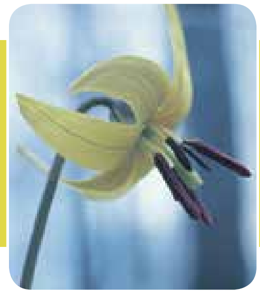
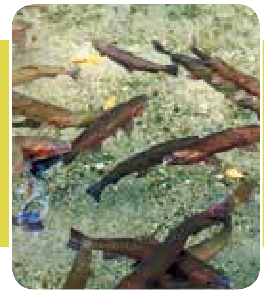
	GRADIENT	# OF ZONES	DESCRIBE THE ZONES	EXAMPLES OF LIFE FORMS
1.	moisture	5	1. ocean, 2. tidal zone, 3. dry sand 4. grass dunes, 5. air	1. fish, 2. clams, 3. crab, 4. snake, 5. bird
2.				
3.				
4.				
5.				

Analyze this photo looking for gradients.

Write in the table below a few of the types of gradients represented in this landscape. (Note that some might be visible and some might be invisible.) The first row shows one example answer, but the gradients, zones and life forms you identify may differ from the example.

	GRADIENT	# OF ZONES	DESCRIBE THE ZONES	EXAMPLES OF LIFE FORMS
1.	human land use	2	1. valley floor, 2. mountains	1. sage brush, 2. deer
2.				
3.				
4.				
5.				
6.				
7.				

ACTIVITY 2

Who's more extreme? You, an insect, a plant or a fish?

What are the toughest organisms on our planet? What kind of life form could survive the world's coldest or hottest temperatures? Could humans survive hotter or colder temperatures than most other life forms?

Think about all these questions as you make predictions below. Base your predictions on the temperature limits you think each type of organism could *withstand,* not what its *optimal* living conditions might be.

Vascular plants have specialized tissues for moving water and minerals throughout the plant, which includes almost all plants except for a few types such as mosses and algae.

HEAT SURVIVORS

Which two types of organisms do you think can survive **hotter** temperatures than the rest? (Color in a dot in the #1 column to mark your first choice. Then color in a dot in the #2 column to mark your second choice.)

COLD SURVIVORS

Which two types of organisms do you think can survive **colder** temperatures than the rest? (Color in a dot in the #1 column to mark your first choice. Then color in a dot in the #2 column to mark your second choice.)

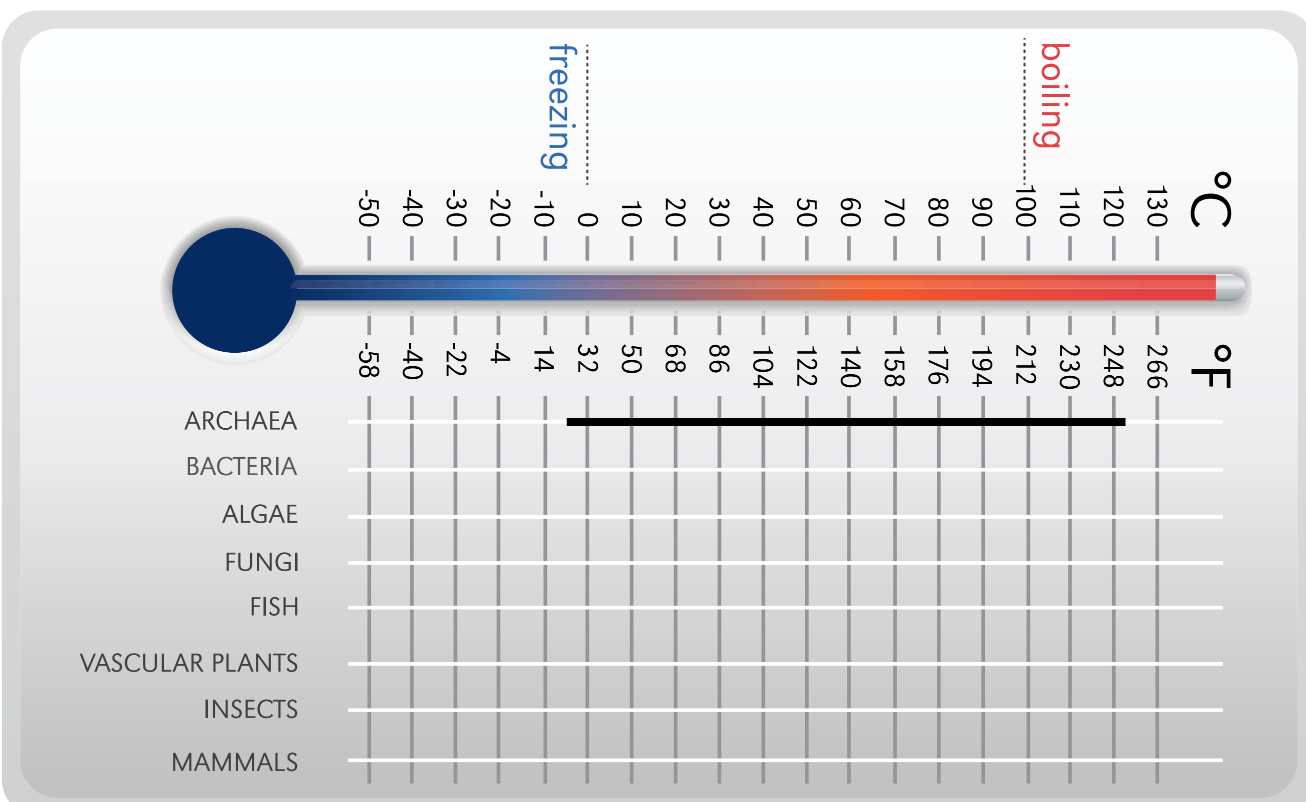
Organism	#1	#2		#1	#2
Mammals	❍	❍	Mammals	❍	❍
Insects	❍	❍	Insects	❍	❍
Vascular Plants	❍	❍	Vascular Plants	❍	❍
Fish	❍	❍	Fish	❍	❍
Fungi	❍	❍	Fungi	❍	❍
Algae	❍	❍	Algae	❍	❍
Bacteria	❍	❍	Bacteria	❍	❍
Archaea	❍	❍	Archaea	❍	❍

Now look at the temperature limits of organisms.

Calculate the middle temperature of the range and write it in the table below.
(Ask your teacher whether to work with the data using Fahrenheit or Celsius.)

ORGANISM	TEMPERATURE RANGE		MIDDLE TEMP. OF RANGE
Mammals	-58°F to 122°F	(-50°C to 50°C)	32°F (0°C)
Insects	32°F to 118°F	(0°C to 48°C)	
Vascular Plants	32°F to 118°F	(0°C to 48°C)	
Fish	-20°F to 104°F	(-29°C to 40°C)	
Fungi	-20°F to 140°F	(-29°C to 60°C)	
Algae	-20°F to 140°F	(-29°C to 60°C)	
Bacteria	-22°F to 203°F	(-30°C to 95°C)	
Archaea	23°F to 250°F	(-5°C to 121°C)	

Draw lines to indicate the temperature range for each organism on the graph below.
(The first one is done for you.) When you have finished your graph, go back to the predictions you made on the previous page and mark whether or not they were correct.



ACTIVITY 3

Yellowstone's Extreme Life

Use the information in this table to graph the niches of all of the microbes listed. *(Your teacher will tell you whether to use Fahrenheit or Celsius values.)*

Microbes that use photosynthesis	pH	Temperature range
Cyanidioschyzon	0–4	104–131°F (40–55°C)
Synechococcus	7–9	126–165°F (52–74°C)
Thermochromatium	6–9	93–135°F (34–57°C)
Zygogonium	0–4	90–131°F (32-55°C)
Microbes that don't use photosynthesis		
Hydrogenobaculum	3–5.5	131–162°F (55–72°C)
Metallosphaera	2–4	122–176°F (50–80°C)
Sulfurihydrogenibium	6–8	140–167°F (60–75°C)
Thermocrinis	7–9	131–195°F (55–91°C)

Directions:

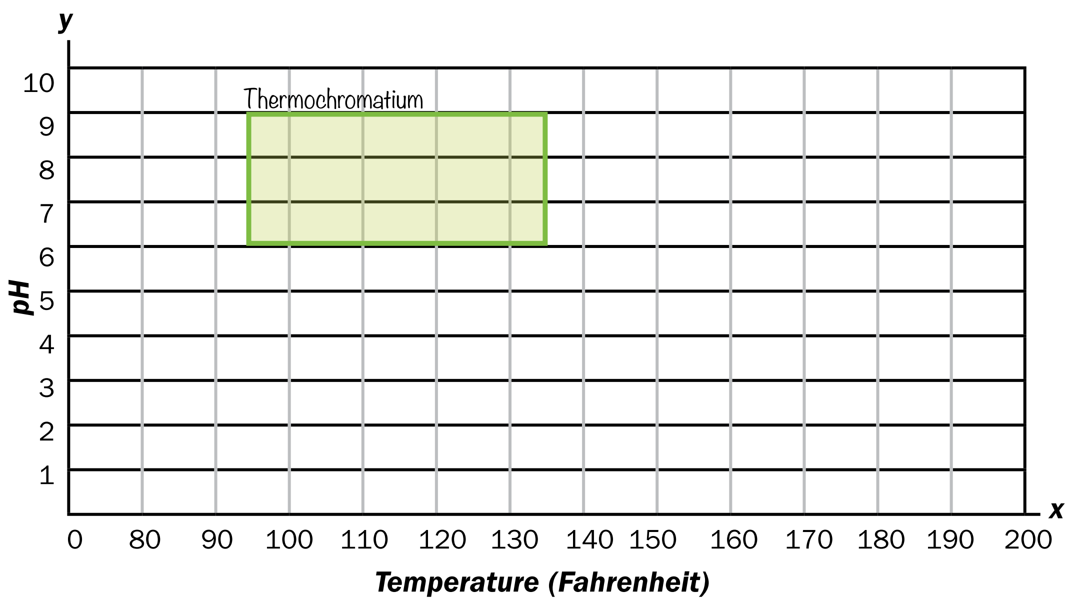
Use two colors: One to graph microbes that use photosynthesis and one to graph those that don't.

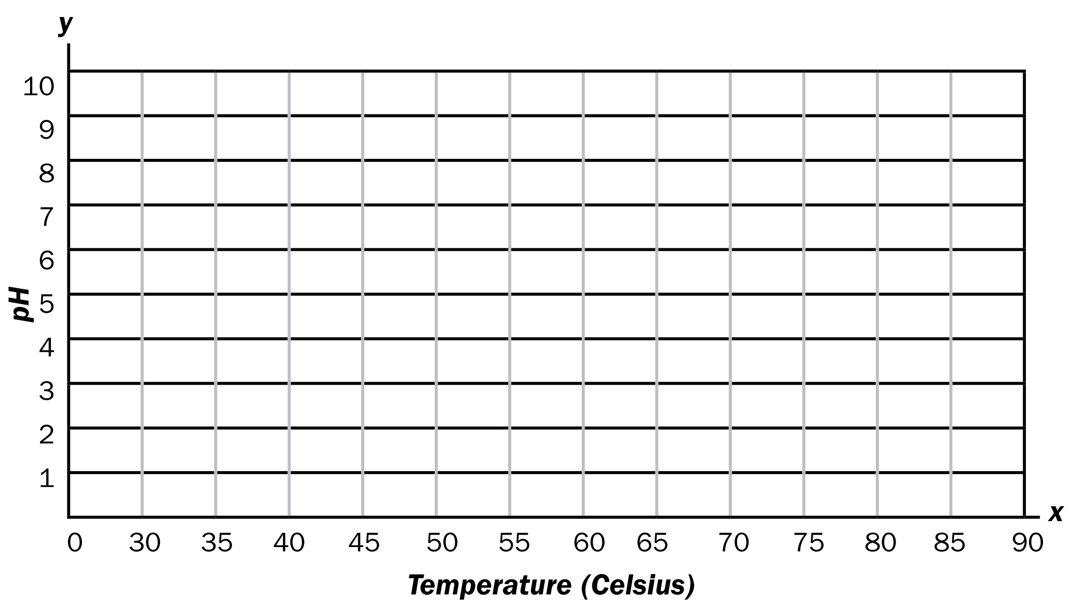
Graph each microbe's **pH range on the *Y axis*** and its **temperature range on the *X axis*.** *(Since each organism can live in a range of pH and temperature, you will end up with a box that represents its niche. One is done for you as an example.)*

Label each niche with the organism's name, abbreviating if necessary.

After you have plotted temperature and pH ranges for all of the organisms, draw a dividing line between those that use photosynthesis and those that don't.

Then, answer questions 1-4 on the next page.





Questions about extreme life

1. Now that you have finished your graphing, what pattern do you notice?

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2. Based on the data you have graphed, what do you think is the upper temperature limit for organisms to live who use photosynthesis? ______________________

3. Do you think this would be enough data for a scientist to make a prediction about the upper temperature limit of photosynthesis? ______________________

Extreme life in Whirligig Geyser

Now look at this photo of Whirligig Geyser in Norris Geyser Basin. It is pH 3.4 and 154°F (68°C) at its source. It is full of iron and arsenic. Arsenic is very poisonous to most multicellular life. (Arsenic contamination of groundwater is a problem that affects millions of people across the world.)

Label the photo

Using the data you graphed on p.15 and the information below, find and label where each of the three following microbes are living in the photo.

- ***Zygogonium*** is a purple or black colored type of algae and it performs photosynthesis.
- ***Metallosphaera*** is orange. It is resistant to many toxic metals and even uses some for energy. It oxidizes iron (turning it to rust).
- ***Cyanidioschyzon*** is a type of green colored algae and it performs photosynthesis.

Whirligig Geyser, Norris Hot Springs

4. For each microbe you labeled in the Whirligig Geyser photo, explain what environmental factors could be influencing where it lives. Focus on temperature and metabolism.

Zygogonium ____

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Metallosphaera ____

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Cyanidioschyzon ____

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ACTIVITY 4

"Gabby's Spring," Heart Lake region, was given this unofficial nickname by MSU researchers because Gabby Michel, a high school student from Big Sky helped them research the spring.

Exploring Yellowstone's Gradients

Yellowstone is one of the few places in the world where an amazing diversity of microbes can been seen without a microscope.

Large microbial ***mats*** and ***filaments*** with distinct white, yellow, green, pink, orange, brown and black colors are visible to the naked eye. Remember that Yellowstone is home to half (more than 10,000) of the world's hydrothermal features and they have a wide range of pHs, temperatures, and chemical compositions that yield a diversity of microbes that scientists are just beginning to discover.

Microbial colors can act as a living thermometer. Millions of tiny microbes band together into groups and form filaments or mats that are often distinct colors. The colors indicate where the water changes temperature, providing a giant map of temperature gradients, or occasionally chemical or pH gradients.

Hint: Finding Signs of Photosynthesis

Photosynthesis is a process used by plants and other organisms to convert light energy, normally from the Sun, into chemical energy. All photosynthesizing organisms have a substance called chlorophyll that is necessary to conduct photosynthesis. It is a green color, but it can sometimes be masked by other pigments that range in color from yellow to orange, or red to purple. If you see something green, that's usually a good indicator of photosynthesizing life.

Find the gradients in "Gabby's Spring"

1. Draw lines between the zones of the thermal gradients on the photo above. How many zones do you think there are? ______________

2. Draw an X where you think the hottest part of the spring is.

3. Do you think this thermal features contains life? ________

Why or why not? ____

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(If you think it contains life, answer the following questions. If not, skip to the photo on the next page.)

4. Describe what kind of microbes you think live in the spring and where they live. Are there multiple zones of life?

____

____

____

5. How do you think the microbes get food? Do they all get food the same way? ____________________

____

____

Note: Because of the high altitude of Yellowstone, water only needs to be approximately 199°F (93°C) to boil whereas at sea level water must be 212°F (100°C) to boil. Sometimes a bubbling hot spring is just an indicator of gases such as carbon dioxide escaping and not a sign of boiling from heat.

Lemonade Creek is fed by hot springs and filled with algae that eat arsenic, which is toxic to humans.

Find the gradients in Lemonade Creek

1. Draw lines between the zones of the thermal gradients on the photo above. How many zones do you think there are? ______________

2. Do you think this thermal features contains life? ________

Why or why not? ____

____

(If you think it contains life, answer the following questions. If not, skip to the photo on the next page.)

3. Describe what kind of microbes you think live in the spring and where they live. Are there multiple zones of life?

____

____

____

____

4. How do you think the microbes get food? Do they all get food the same way? ______________________

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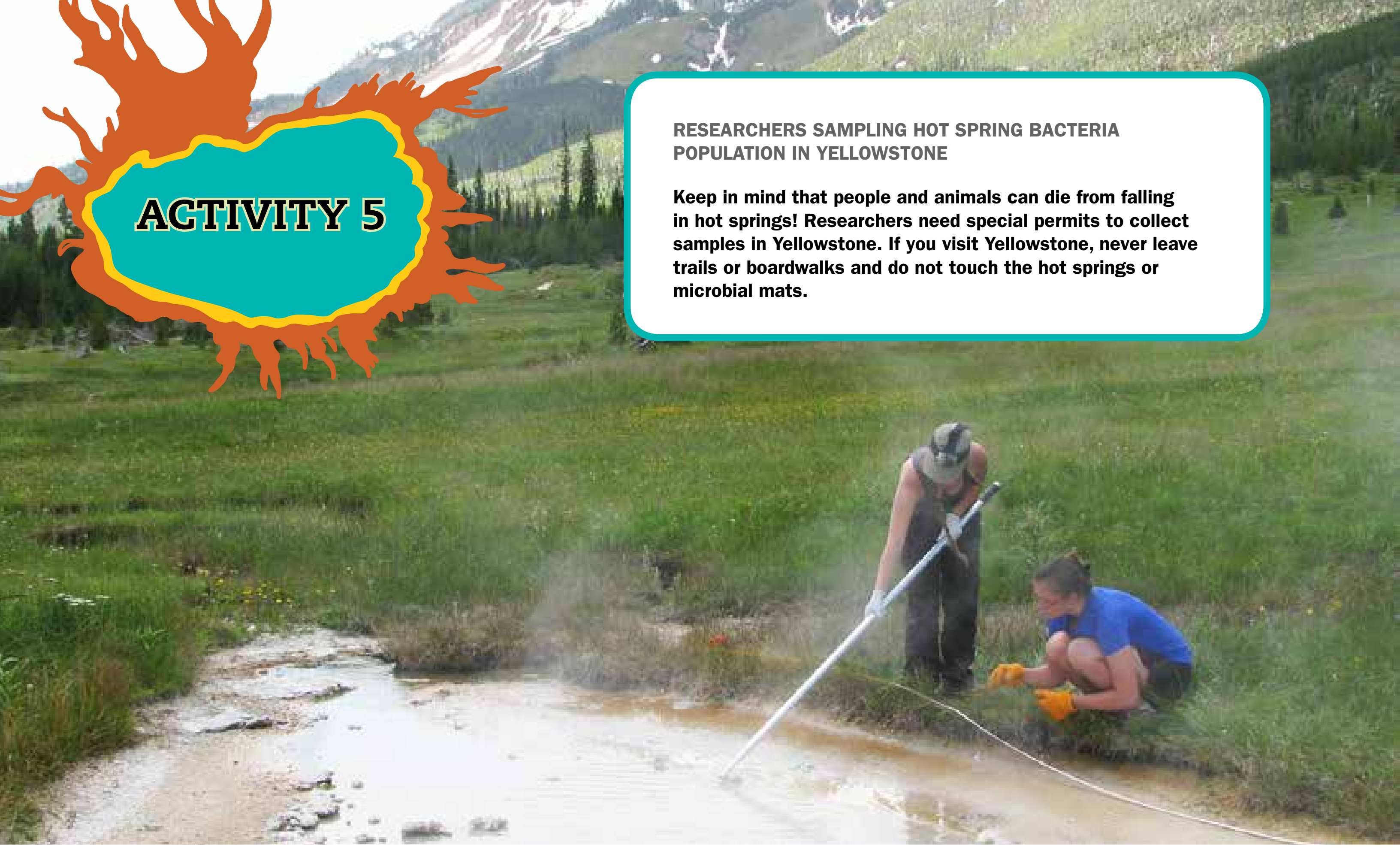
Mud pots are created by very wet, acidic conditions that dissolve rock.

Find the gradients in this mud pot

1. Draw lines between the zones of the thermal gradients on the photo above.
   How many zones do you think there are? ______________
2. Draw an X where you think the hottest part of the spring is.
3. Do you think this thermal features contains life? ________
   Why or why not? ____
   ____

   (If you think it contains life, answer the following questions. If not, skip to the photo on the next page.)
4. Describe what kind of microbes you think live in the spring and where they live. Are there multiple zones of life?
   ____
   ____
   ____
   ____
5. How do you think the microbes get food? Do they all get food the same way? ______________________
   ____
   ____
   ____
   ____

ACTIVITY 5



RESEARCHERS SAMPLING HOT SPRING BACTERIA POPULATION IN YELLOWSTONE

Keep in mind that people and animals can die from falling in hot springs! Researchers need special permits to collect samples in Yellowstone. If you visit Yellowstone, never leave trails or boardwalks and do not touch the hot springs or microbial mats.

Sweet Population Sampling

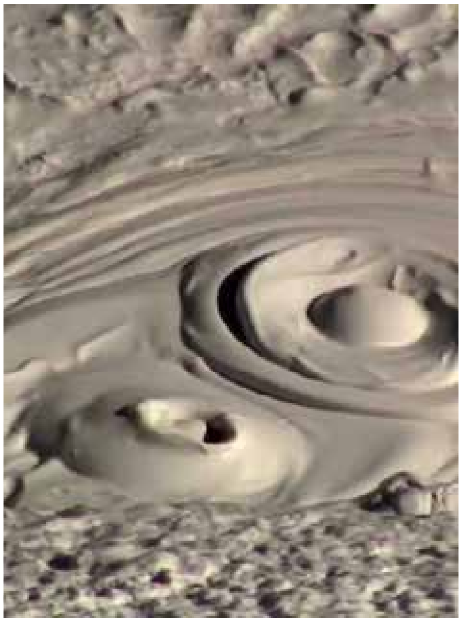
There are three simulations of the hot springs you studied in Activity 4 set up around your classroom. You are going to move around the room and count samples of microbes (that are represented by different colors of candy) from three Yellowstone thermal features.

Note that when scientists collect real data from Yellowstone hot springs there are many more types of microbes living in the hot springs than specimens they see in their sample. They may see numerous shapes and sizes of microbes in a microscope but may only be able to identify a few because of the difficulties of growing and cultivating extremophiles in a lab.

Copy in the box below what candy your teacher is using to represent each microbe.

Organism	Candy Color
Sulfolobus	
Caldisphaera	
Cyanidioschyzon	
Chlorella	
Thermus	
Chloroflexus	
Pseudoanabaena	

1. Fill each square of the grid with drawings of the symbols (noted below the grid) that represent each organism (represented by a colored candy) you see.
2. Draw the symbols in the exact order and place you see them in the virtual spring, then total the numbers of each kind of microorganism you find in each box.
3. Once you have found a total number of specimens for each box, calculate the total number of specimens in the whole sample area (in other words in the whole grid).
4. Then, read the descriptions of the microorganisms found in that hot spring and answer the questions about them.



Mud Pot:

***Sulfolobus*, 65-87°C (149-188°F), pH 2-4**

This organism was first discovered in Yellowstone in 1972. Since then, species of *Sulfolobus* have been found in hot springs on Mount St. Helens and in Italy, Russia, Chile, Japan and Papua New Guinea. It was one of the first hyperthermophiles—organisms that optimally grow above 176°F (or 80°C)—to be discovered. It gets its energy from metabolizing sulfur or sulfur compounds.

***Caldisphaera*, 65-75°C (149-167°F), pH 2.5-5.5**

This microorganism converts sulfur into hydrogen sulfide: a gas that smells like rotten eggs and is flammable, very poisonous and corrosive. They have been found in hot springs in Yellowstone, the Philippines, Russia, and California. Hydrogen sulfide was used by the British Army as a chemical weapon during WW I. If someone is poisoned by inhaling too much hydrogen sulfide, a clue can be the discoloration of copper coins in the pockets of the victim. A bit of hydrogen sulfide is also present in the gas humans produce and is part of the cause of its smell.

Total *Sulfolobus* _____ Total *Caldisphaera* _____ Total # of microbes _____	Total *Sulfolobus* _____ Total *Caldisphaera* _____ Total # of microbes _____	Total *Sulfolobus* _____ Total *Caldisphaera* _____ Total # of microbes _____
Total *Sulfolobus* _____ Total *Caldisphaera* _____ Total # of microbes _____	Total *Sulfolobus* _____ Total *Caldisphaera* _____ Total # of microbes _____	Total *Sulfolobus* _____ Total *Caldisphaera* _____ Total # of microbes _____

X = *Sulfolobus*, **O** = *Caldisphaera*

Total of *Sulfolobus* in the grid __________

Total of *Caldisphaera* in the grid __________

1. Which of these organisms helps create smells like human gas? ______________________________

2. If someone dies of exposure to hydrogen sulfide gas, what might happen to any pennies in his or her pocket?

____

3. Which of these organisms has been found in sites across the world? ______________________________

4. Does either of these organisms use photosynthesis for energy? ______________________________

Lemonade Creek

***Cyanidioschyzon*, 40-55°C (104-131°F), pH 0-4**

Cyanidioschyzon is a spherical type of algae found in acidic hot springs. It uses sunlight for energy and performs oxygen photosynthesis. It is one of the most heat- and acid-tolerant algae known.

***Chlorella*, 20-35°C (68-95°F), pH 0.5-4**

Chlorella, like *Cyanidioschyzon*, are a form of algae and they use photosynthesis for energy. They are a group of eukaryotes, meaning the interior of their cell contains both a nucleus as well as other compartments within the cell, called organelles. This makes them distinct from prokaryotic microorganisms, which lack both of these features. Interestingly, *Chlorella* are packed full of protein, fat, fiber, vitamins, and minerals, making them a very potent superfood that many people use as a daily vitamin.

Total *Cyanidioschyzon* _____ Total *Chlorella* _____ Total # of microbes _____	Total *Cyanidioschyzon* _____ Total *Chlorella* _____ Total # of microbes _____	Total *Cyanidioschyzon* _____ Total *Chlorella* _____ Total # of microbes _____
Total *Cyanidioschyzon* _____ Total *Chlorella* _____ Total # of microbes _____	Total *Cyanidioschyzon* _____ Total *Chlorella* _____ Total # of microbes _____	Total *Cyanidioschyzon* _____ Total *Chlorella* _____ Total # of microbes _____

X = *Cyanidioschyzon*, **O** = *Chlorella*

Total of *Cyanidioschyzon* in the grid _____

Total of *Chlorella* in the grid _____

1. Which of these organisms do some people use as a vitamin? ______________________________

2. What do both of these organisms do to get energy? ______________________________

"Gabby's Spring"

Clear center channel: *Thermus*, 40-79°C (104-174°F), pH 5-9

Thermus is a rod-shaped bacterium that sometimes forms bright red or orange streamers. It contains pigments called cartenoids that act as a sunscreen and protect it from high levels of sunlight. A species of *Thermus* found in Yellowstone was the original source material for an enzyme that allows scientists to make many copies of DNA. Reproducing DNA quickly has allowed for breakthroughs in solving crimes, diagnosing diseases and identifying genes.

Green channel: *Chloroflexus*, 35-85°C (95-185°F), pH 7-9

This bacteria is rod shaped and forms filaments. It uses light for energy but does not produce oxygen as a byproduct. Scientists are studying *Chloroflexus* because they think it may shed light on the evolution of photosynthesis.

Orange channel: *Pseudoanabaena*, 30-50°C (86-122°F), pH 7-9

This microorganism is a type of cyanobacteria, an important group of bacteria that are nearly 2.8 billion years old. Cyanobacteria conduct photosynthesis, meaning they use a combination of sunlight, water, and carbon dioxide to produce food and oxygen. In fact, these ancient organisms are so good at photosynthesis they're thought to have created the oxygen atmosphere that we humans depend on to survive. Sometimes cyanobacteria can grow so fast in water bodies that they form large blooms. These blooms can threaten lakes and streams by blocking sunlight from penetrating down the water column or by producing harmful toxins that can make animals and/or people sick.

Total *Thermus* _____ Total *Chloroflexus*_____ Total *Pseudoanabaena* _____ Total # of microbes _____	Total *Thermus* _____ Total *Chloroflexus*_____ Total *Pseudoanabaena* _____ Total # of microbes _____	Total *Thermus* _____ Total *Chloroflexus*_____ Total *Pseudoanabaena* _____ Total # of microbes _____
Total *Thermus* _____ Total *Chloroflexus*_____ Total *Pseudoanabaena* _____ Total # of microbes _____	Total *Thermus* _____ Total *Chloroflexus*_____ Total *Pseudoanabaena* _____ Total # of microbes _____	Total *Thermus* _____ Total *Chloroflexus*_____ Total *Pseudoanabaena* _____ Total # of microbes _____

X = *Thermus*, **O** = *Chloroflexus*, **V** = *Pseudoanabaena*

Total of *Thermus* in the grid ____, Total of *Chloroflexus* in the grid ____, Total of *Pseudoanabaena* in the grid _____

1. Which one of these organisms has a pigment that it uses like sunscreen? ______________________________

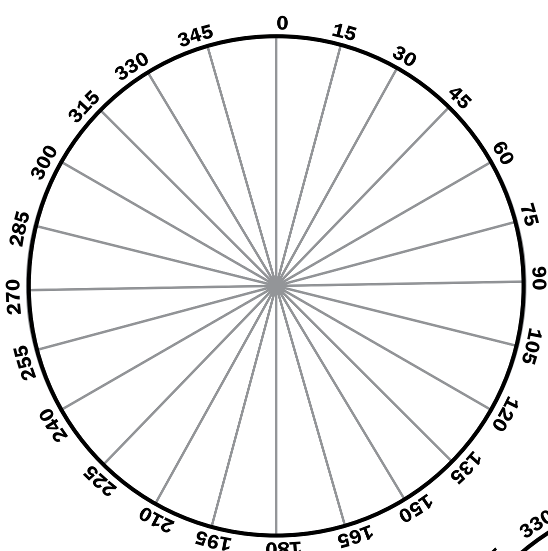
2. Which type of organism is similar to the ones scientists think may have been responsible for producing the oxygen in Earth's atmosphere?____

3. Which type of organism are scientists studying to try to understand the origins of photosynthesis? ____________

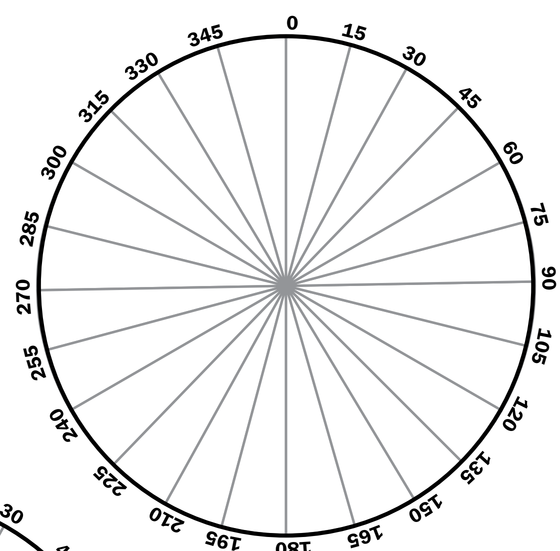
Taking it Further: Population Pie Charts

Using your Sweet Sampling data, calculate the fraction, decimal and percent of each microbe in Mud Pot, Lemonade Creek and “Gabby’s Spring.” Color and label the pie charts to show which microbes are most prevalent in each feature.

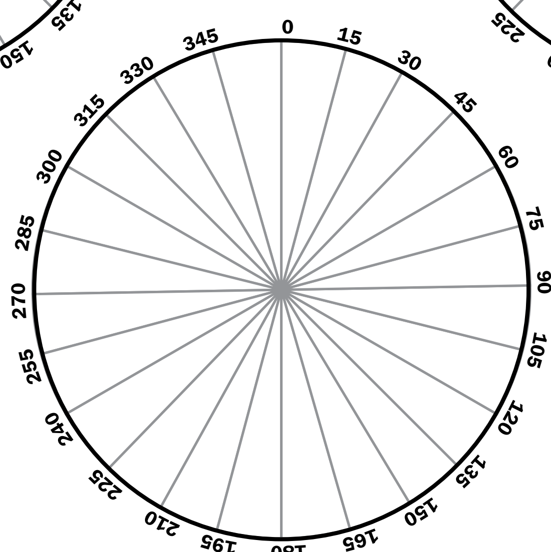
Feature	**Microbe**	**Fraction** numerator/ denominator	**Decimal** numerator ÷ denominator	**Percent** decimal X 100	**Degrees** decimal X 360	**Color** representation on pie chart
Mud pot	*Sulfolobus*					
Mud pot	*Caldisphaera*					
Lemonade Creek	*Cyanidioschyzon*					
Lemonade Creek	*Chlorella*					
“Gabby’s Spring”	*Thermus*					
“Gabby’s Spring”	*Chloroflexus*					
“Gabby’s Spring”	*Pseudoanabaena*					



Mud pot



Lemonade Creek



“Gabby’s Spring”

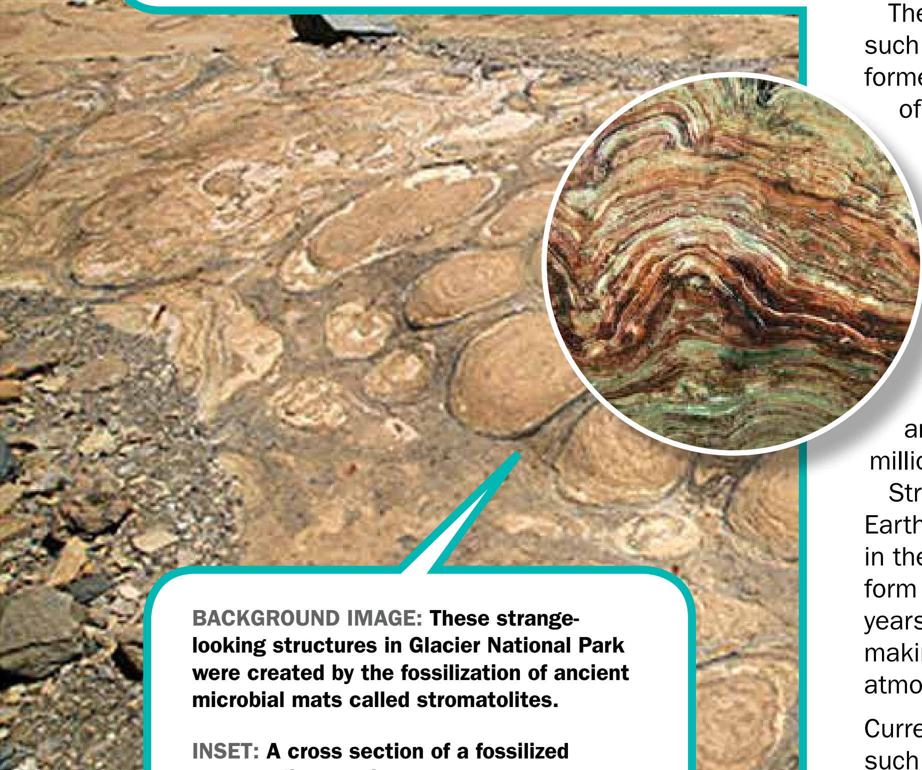
LESSON 3

MICROBIAL MATS AND ALIENS!

Aliens Among Us?

We imagine alien life as looking much different than what we encounter every day on Earth. Why is that? If you are looking for life on Mars or another planet you're almost certainly not going to find a tree or a bear. That's because our planet now has an oxygen-rich atmosphere, lots of sunshine, liquid water and moderate temperatures, but most other planets do not. Our planet is home to birds, reptiles, flowers, grass and many other types of life that could not exist on any other planet in our solar system because the resources they need to survive are not available on those planets.

Though we have not yet discovered living organisms on another planet, the types of life that could survive on other planets would have to survive in conditions that are considered extreme on Earth. Scientists think that if we find life elsewhere in the Universe it is more likely to be an extremophile (a microbe that lives in an "extreme" environment) than a little green man.



BACKGROUND IMAGE: These strange-looking structures in Glacier National Park were created by the fossilization of ancient microbial mats called stromatolites.

INSET: A cross section of a fossilized stromatolite specimen.

Fossilized microbes

Have you ever been to Glacier National Park? Imagine walking on a high mountain trail and seeing a strange pattern of circles on the ground. ***(See background photo.)*** Would you have any idea what you are looking at? They are actually a type of fossil that NASA is searching for on other planets!

The circles are ancient fossilized microbial mats that lived in this area when it was a shallow ancient sea more than 1.3 billion (1,350 million) years ago. Millions of years of Earth's changing climate and shifting tectonic plates have moved what was once a warm shallow sea floor to the top of a modern-day cold mountain.

Old timers of the Earth

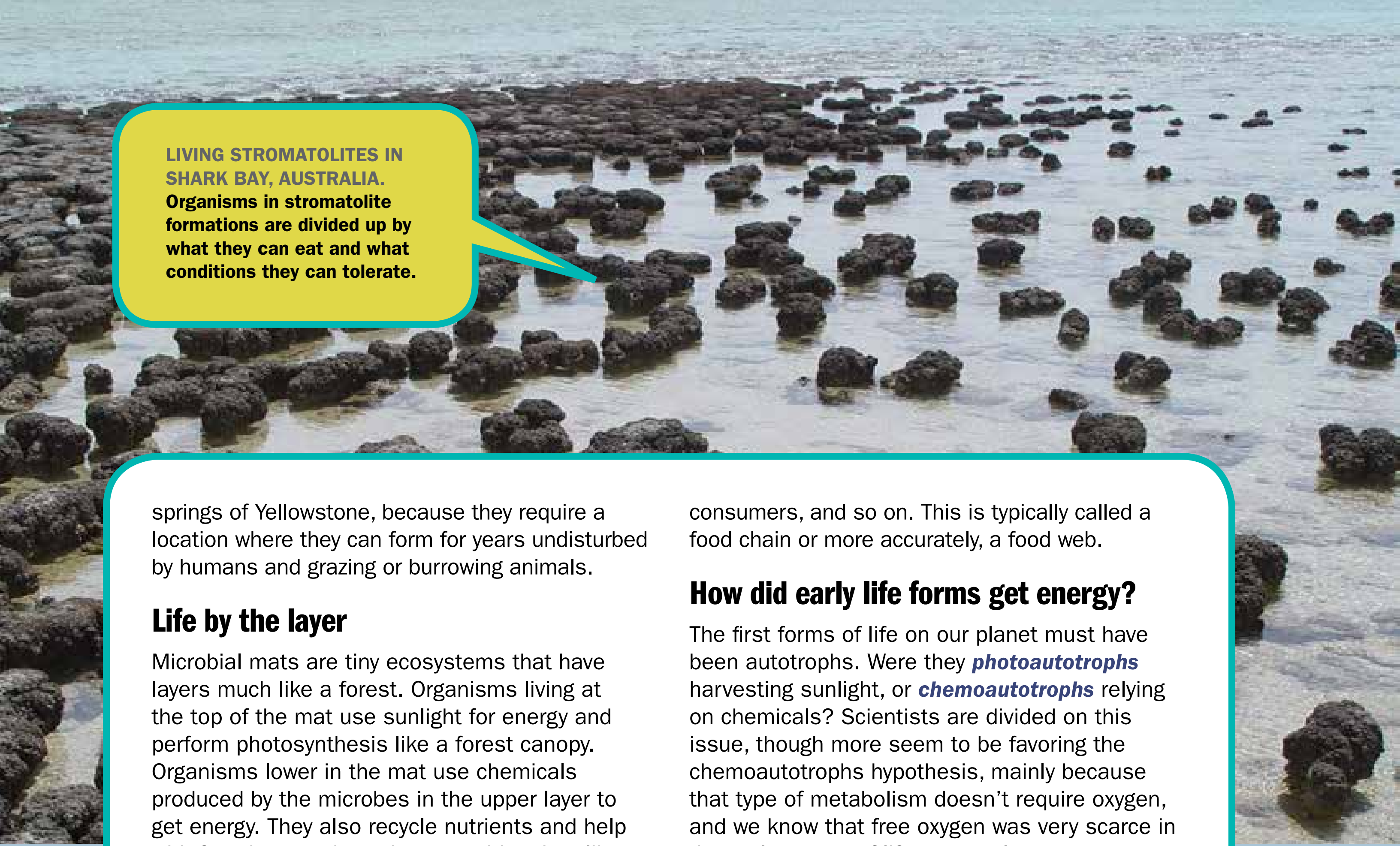
These circular structures are fossils of a ***stromatolite.*** A stromatolite is a structure created by a microbial mat in which a rock-like layer of either sand or precipitated minerals has been trapped.

The microbial mat consists of microorganisms such as bacteria and algae. Stromatolites are formed by microbial communities that consist of many species with different metabolisms competing for or sharing resources. ***(See the fossilized cross section of an ancient Glacier National Park specimen at left.)***

Stromatolites provide one of the most ancient records of life on Earth. Scientists have found fossil remains of stromatolites which date from more than 3.5 billion years ago. If stromatolites are a record of early life on our planet, they might be a record of life on another planet too. Imagine if life formed on Mars millions of years ago when Mars had shallow seas.

Stromatolites used to be much more common on Earth than they are today and they are often found in the ancient fossil record. They were the dominant form of life on Earth for more than two billion years and are thought to be largely responsible for making our atmosphere oxygen-rich (Earth's early atmosphere had little or no oxygen).

Currently, stromatolites occur in only a few places, such as Shark Bay, Australia or in some of the hot



LIVING STROMATOLITES IN SHARK BAY, AUSTRALIA. **Organisms in stromatolite formations are divided up by what they can eat and what conditions they can tolerate.**

springs of Yellowstone, because they require a location where they can form for years undisturbed by humans and grazing or burrowing animals.

Life by the layer

Microbial mats are tiny ecosystems that have layers much like a forest. Organisms living at the top of the mat use sunlight for energy and perform photosynthesis like a forest canopy. Organisms lower in the mat use chemicals produced by the microbes in the upper layer to get energy. They also recycle nutrients and help with functions such as decomposition, just like some of the organisms in a forest's understory.

Each layer of a microbial mat is dominated by microorganisms that thrive in the conditions of that layer and outperform other kinds of life. Organisms compete and cooperate and are divided up by what they can eat and what conditions they can tolerate.

A web of energy

All living things need a way to get food, which gives them energy. Plants can create their own energy using sunlight, but we humans can't, so we have to eat plants, or eat animals that eat plants. We are ***heterotrophs*** (*heteros*=different, *troph*=nutrition) because we must eat other organisms to obtain energy and organic carbon. All animals are heterotrophs, along with some single-celled creatures.

Any organism that can harvest energy directly from the nonliving environment and fix CO_2 into biomass is called an ***autotroph***, a word that means "self feeding" (*autos*=self, *troph*=nutrition).

We can think of energy as "flowing" through lifeforms, starting with the autotrophs (also called producers) and then moving to the heterotrophs (also called consumers), and then maybe to other consumers, and so on. This is typically called a food chain or more accurately, a food web.

How did early life forms get energy?

The first forms of life on our planet must have been autotrophs. Were they ***photoautotrophs*** harvesting sunlight, or ***chemoautotrophs*** relying on chemicals? Scientists are divided on this issue, though more seem to be favoring the chemoautotrophs hypothesis, mainly because that type of metabolism doesn't require oxygen, and we know that free oxygen was very scarce in the early stages of life on our planet.

Different strokes for different trophs

autotrophs – produce food from inorganic (non-living) things such as minerals (*autos*=self, *troph*=nutrition)

photoautotrophs – use sunlight to create food/energy (photosynthesis)

chemoautotrophs – use chemicals to create food/energy (chemosynthesis)

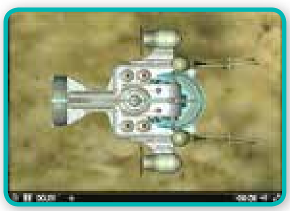
heterotrophs – eat other organisms or organic carbon to obtain energy (*heteros*=different, *troph*=nutrition)

CHEMO VS PHOTO Being a photoautotroph (using photosynthesis) is a much more productive and efficient way to get energy than being a chemoautotroph. It is estimated that the appearance of oxygenic photosynthesis increased biological productivity by a factor of between 100 and 1,000!

YOUR MISSION

Watch the stromatolite video and use the transcript of it below to answer the questions on your worksheet. Soon you will be analyzing your own microbial mat sample and you'll need the information contained in the section you just read as well as facts from the video you are about to watch.

Stromatolite Video Transcript



Imagine an exploration mission through a strange and hostile environment. Now place yourself inside the imaginary vehicle, the .1 mm long Stromatolite Explorer. The mission is to probe a microbial mat; a complex and very ancient assemblage of microorganisms less than 5 mm thick. Automated transponders will take chemical readings every quarter of a millimeter as the vehicle descends.

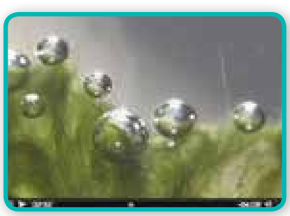
[MUSIC]

Diving into a living mat is like going back in time to explore some of the earliest forms of life on Earth. Microbial mats have been around for over 3 billion years making them Earth's earliest biological communities. Finding the remains of microbial mats on other planets would be powerful evidence that life once thrived there. That's why understanding microbial mats is of such interest to NASA.

In the uppermost region of the mat, diatoms reign supreme. These single cells are encased in houses made of glass. Diatoms convert sunlight and carbon dioxide into fuel molecules and oxygen through the process of photosynthesis.

Now the explorer and crew enter the realm of filamentous cyanobacteria. Each strand is a chain of single celled prokaryotes. No nucleus in these cells, just a long strand of DNA.

DEPTH: .5 MM;
OXYGEN: 600 MICROMOLARS;
HYDROGEN SULFIDE: NEGLIGIBLE
PH 9.7

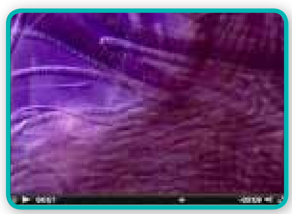


Like other solar-powered photosynthesizers, cyanobacteria produces dissolved oxygen as a byproduct of photosynthesis. Here we see how Earth's atmospheric oxygen originated bubble by bubble.

DEPTH: 2.6MM AND DESCENDING;
OXYGEN: NEGLIGIBLE;
HYDROGEN SULFIDE: 75 MICROMOLARS;
PH 7.2

Because the only light that gets down into the deeper part of a mat is in the form of red to near infrared radiation, the bacteria here, purple sulfur bacteria, use that radiation and the hydrogen sulfate present to perform a kind of photosynthesis that doesn't produce oxygen: anoxygenic photosynthesis.

DEPTH 4.3 MM;
HYDROGEN SULFIDE: 125 MICROMOLARS
OXYGEN: 0



The mat, though relatively thin, shows a steep chemistry gradient from top to bottom. The explorer has descended through layers that contain diatoms, then cyanobacteria, purple sulfur bacteria, and finally sulfate reducing bacteria. Oxygen from photosynthesis at the surface of the mat never makes it to the bottom. So the bacteria down here have evolved to use sulfate to break down or burn organic matter.

ALERT: RESERVE POWER AT CRITICAL LEVEL. INSUFFICIENT SOLAR RADIATION FOR SUCCESSFUL RECHARGE… WARNING.

The mat community contains filamentous cyanobacteria that move between the sulfur-rich deeper layers and the oxygen producing upper layers in a daily cycle. If the crew can remember this, they might have a chance to hitch a free ride back to the surface.

When the sun goes down, the chemical output of the mat community adjusts for life in the dark. A peek at the updated transponder data reveals a new set of readings.

A microbial mat is a community that changes dramatically in the first few millimeters. Descending, the mat changes from a photosynthetic oxygen producing layer to a murky realm where sulfur munching bacteria prevail.

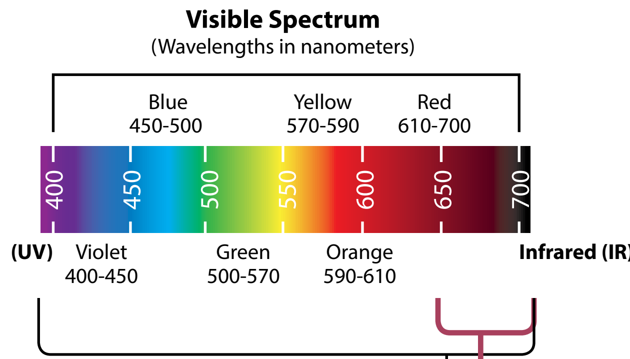
The activities of these simple life forms create an alkaline environment where minerals accumulate and over time a stromatolite will form — one of the telltale signs to look for in our search for life on other worlds.

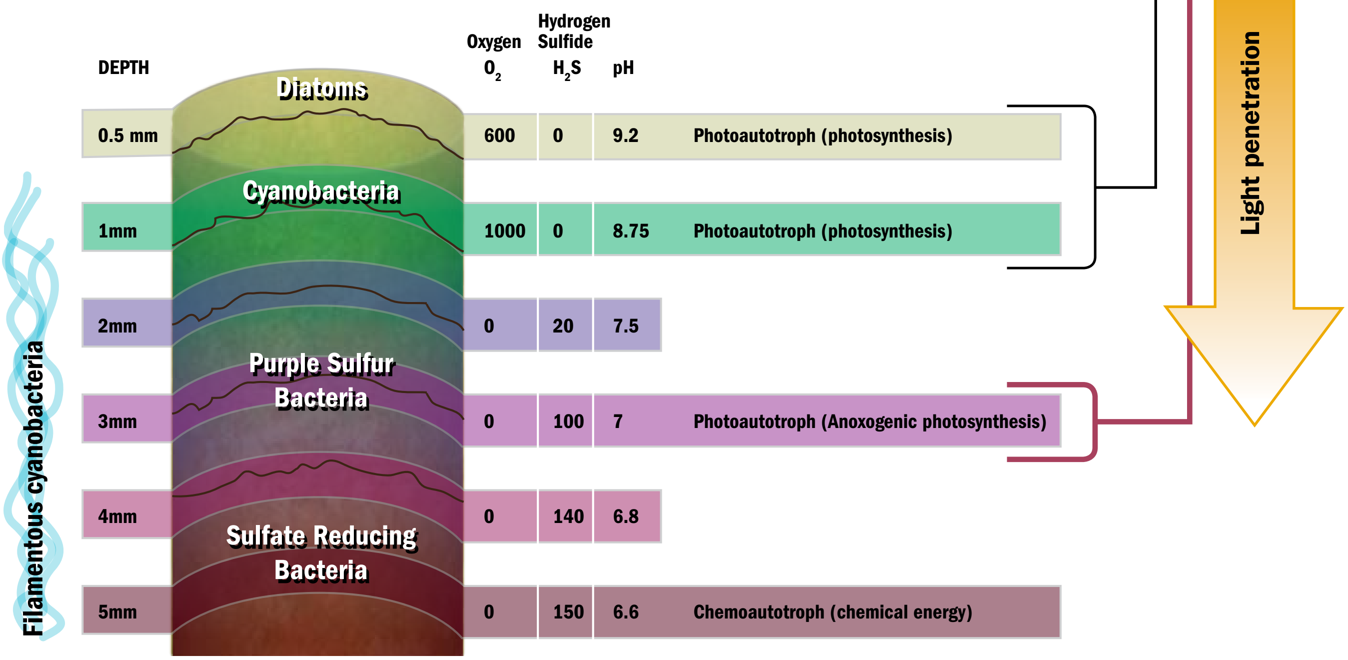
Someday, not so far away when an exploration vehicle from Earth begins probing the soil on a planet such as Mars, it will carry instruments for analyzing biosignatures, minerals and organic matter that may have been left behind by mats that once grew on the shore of Martian seas.

Stromatolite Worksheet

Use the information from the stromatolite video and the graphics below to answer the questions on the next page.

Stromatolites and microbial mats contain energy and light gradients (in a gradient there are increasing or decreasing changes in a variable). The microbes living towards the top of the mat have more resources available to them compared to the lower levels.





How do they make a living?

diatoms
cyanobacteria — **(photoautotrophs)** — photosynthesis

light energy (visible)

$CO_2 + H_2O \rightarrow CH_2O + O_2$ (carbon dioxide + water → sugar + oxygen)

purple sulfur bacteria — **(photoautotroph)** — anoxygenic photosynthesis

light energy (infrared radiation)

$H_2S + CO_2 \rightarrow SO_4 + CH_2O$ (hydrogen sulfide + carbon dioxide → sulfate + sugar)

fermentation

$CH_2O \rightarrow C_2H_3O_2 + H_2$ (sulfate → acetate + hydrogen)

sulfate reducing bacteria — **(chemoautotroph)**

chemical energy

$SO_4 + C_2H_3O_2 \rightarrow H_2S + CO_2$
(sulfate + acetate (fermented sugar) → hydrogen sulfide + carbon dioxide)

1. What is a microbial mat? ______________________________

2. Why do we describe microbial mats as "ancient?" ______________________________

____

3. If you were examining a stromatolite, would the actual bacteria living in it be ancient? ______________

____

4. How thick is this microbial mat? ______________________________

5. How long is the imaginary Stromatolite Explorer vehicle? ________

6. To get a sense of the actual size of the stromatolite in the video, draw a square to represent the stromatolite using your answer for Question 4, and a dot to represent the Stromatolite Explorer using your answer for question 5.

7. Why are microbial mats and stromatolites of interest to NASA? ______________________________

____

____

8. The conditions in the mat change dramatically as you get deeper. What happens to pH, oxygen, and hydrogen sulfide levels as you go deeper into the mat?______________________________

____

____

9. Describe the cycling of matter and flow of energy in a stromatolite (how are the microbes making a living) and mention specifically what the organisms are exchanging. (Use the arrows at the bottom of p.28 to help you answer this question.) ______________________________

____

____

____

____

10. Stromatolites are a complex community that form their own little ecosystem. What genetic and environmental factors shape the distribution and growth of organisms in a stromatolite? If you are having problems coming up with ideas, think about whether or not sulfate reducing bacteria could live at the top levels of the mat — why or why not?

____

____

____

____

11. How did ancient stromatolites and the bacteria in them help Earth's atmosphere to become full of oxygen?

____

____

12. It can take a stromatolite 100 years to grow 5 cm. Some of the stromatolites in Shark Bay, Australia are 2,000-3,000 years old. What would be the size range of stromatolites that old?

____

MAT SAMPLE
OBSERVATIONS

Directions

- Use your core sampler (straw) to take a sample of your microbial mat (cupcake).
- Insert the straw slowly and gently into the center so you do not disturb the composition of your mat.
- Remove the sample by slowly squeezing the empty end of the straw.
- If the sample is not intact, take another.
- Complete the observation form and pretend that your sample was taken from a real living stromatolite.

1. How many layer colors make up your mat? _________
2. Make a sketch of your core in the box at right. Measure the height of each section and label your drawing with your measurements.
3. If this were a real microbial mat, give some reasons why the layers of the mat are different colors.

____

____

____

____

____

4. Assuming that this stromatolite was similar to the one in the previous activity, explain whether or not the sample could have come from any of the following places.

Place	**Could the sample have come from here? (Yes/No)**	**Why or why not?**
A dark cave		
A dry desert		
A fast flowing river where lots of animals swim, drink and cross		
A reservoir of toxic mine waste in Butte, Montana called the Berkeley Pit (pH 2.5)		

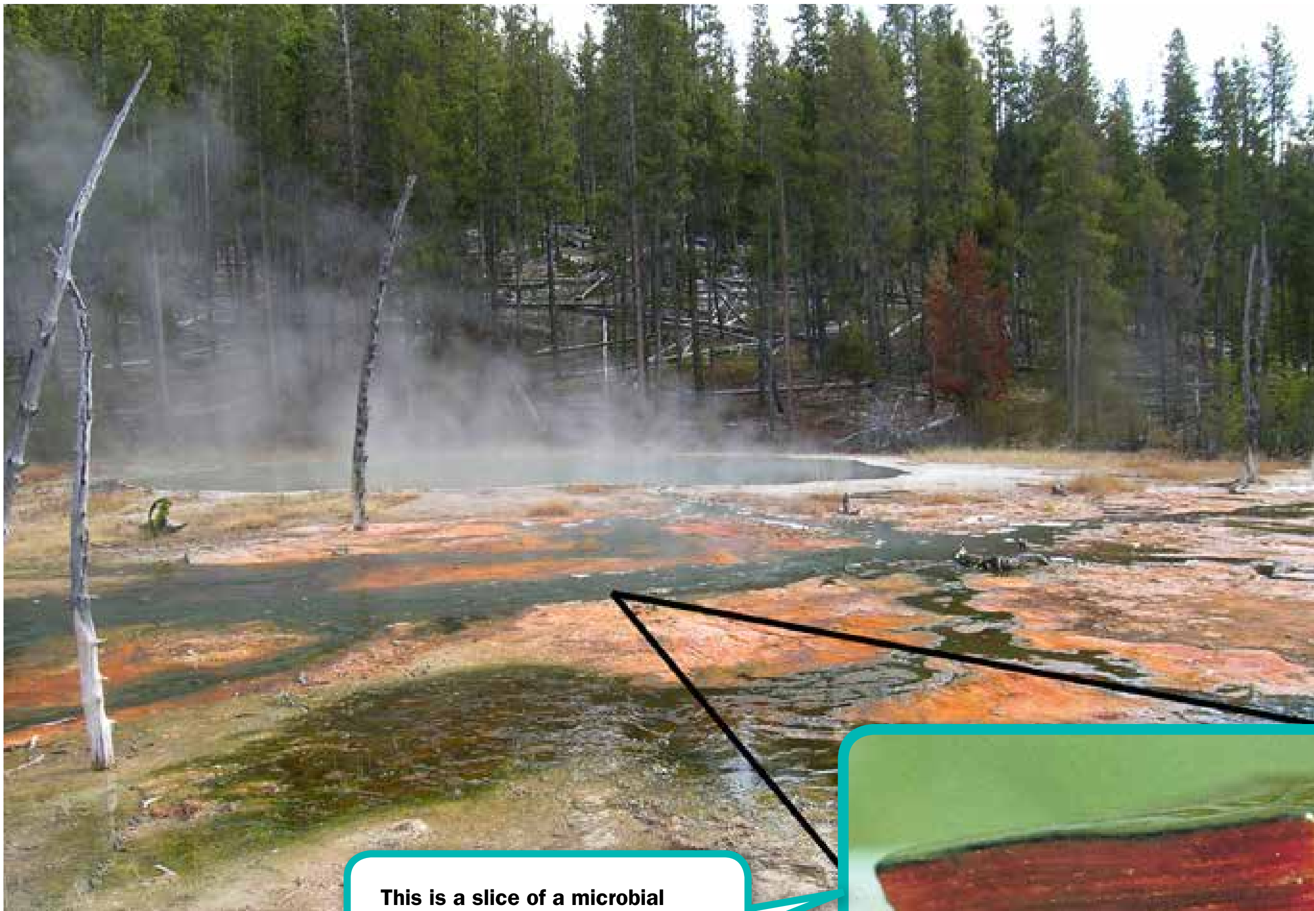
5. Why do you think that your mat might have different sized layers than a classmate's mat?

Note: *When answering, think about resources and remember that you are answering the question as if it were a real stromatolite, not a piece of cake.* ______________________________________

6. Oxygen can be toxic to purple sulfur bacteria and sulfate reducing bacteria, although both bacteria can sometimes tolerate small levels. Before the Earth had oxygen, these types of bacteria could have done fairly well living by themselves. Once the Earth's atmosphere became oxygen rich, what advantage would living in a microbial mat or stromatolite give to the sulfate reducing bacteria?

Hint: *Think about what would have happened to the population of microorganisms that use photosynthesis once Earth had oxygen and how would that impact sulfate reducing bacteria?*

In 2007 the Mars Rover named Spirit, while traveling backwards across the Gusev Crater, dragged its locked wheel, uncovering a white rock layer under the dust. When x-ray spectroscopy looked at this rock, the data showed that the rock was 90% silica. Scientists believe that this kind of rock could have been deposited by a volcanic vent or a hot spring; similar to the low sulfur area of Mushroom Spring in Yellowstone National Park.



This is a slice of a microbial mat collected by scientists from Mushroom Spring in Yellowstone (pH 7.9, temp 154°F (68°C))

The cyanobacteria *Phormidium* forms bacterial mats in strange shapes and can be found in many Yellowstone hot springs such as Grand Prismatic Spring.

LESSON 4

THE UNSEEN WORLD AROUND YOU

An unseen world

All around you is a microscopic world of life. A single gram of soil (a penny weighs 2.5 grams) can contain more than a million bacterial cells. A liter of ocean water contains up to 1 billion bacteria and there are about ten times more bacteria living inside your body as there are "human" cells.

The majority of these critters are bacteria. Bacteria can be harmful to us, but without it we definitely couldn't survive. They help us digest and gather energy from our food.

What are bacteria?

Bacteria are single celled organisms that exist in a wide variety of environments on our planet. They are classified as prokaryotes, meaning they lack a "true" nucleus that is enclosed in a membrane.

Strength in numbers

Bacteria reproduce by cloning themselves and replicating their DNA, then dividing. They are really good at reproducing this way and it's one of the reasons they are so successful on our planet and in our bodies. For example, bacteria common in your intestinal tract, under optimal conditions, can reproduce extremely rapidly, dividing every 20 minutes. That means one bacterium in a petri dish can become two in 20 minutes, those two can become four in another 20 minutes, and so on. After four hours you can end up with 4,096 bacteria!

Mystery bacteria may be useful

Scientists estimate that they have studied less than 1% of the number of microbe species. Many types of microbes are yet to be discovered and may have important characteristics that could make them useful for solving problems. For example, the Extreme Yellowstone Expedition visited Yellowstone to collect samples of microbes from hot springs. The researchers hope that microbes that can live in extreme environments such as hot springs may have the potential to help improve medical cures for diseases, or clean up hazardous waste sites, or be a source for alternative fuels such as hydrogen or biodiesel.

BACKGROUND IMAGE: Whirligig Geyser in Yellowstone National Park.

INSET: *Hydrogenobaculum* bacteria is a rod-shaped bacterium that uses hydrogen, hydrogen sulfide, carbon dioxide (and in some cases, arsenic!) for energy. It is found in acidic streams and pools in places like Ampitheater Springs and Norris Geyser Basin.

YOUR MISSION

Microbes are all around us.

It's your turn to be the scientist and see what you can discover around you. You are going to collect some samples of microbes, but first you need to think of a question or hypothesis you want to explore.

Write a hypothesis or question about an idea you want to investigate and be precise. A hypothesis is an educated guess that you can test to see if it is correct.

Each time you collect a sample, you will actually collect *two* samples. One that you will place in the dark for a few days to see what grows, and one that you will place in sunlight for a few days.

Write your question or hypothesis

Hypothesis: ____

____

For example, "I think samples taken from outdoor areas will have less microbial growth than samples taken from indoors after being left in the dark for a few days, because samples from outside will have more microbes that use photosynthesis and rely on the sun for energy. Therefore many or all of the microbes from the outside samples will not be able to grow in the dark."

Describe your methods

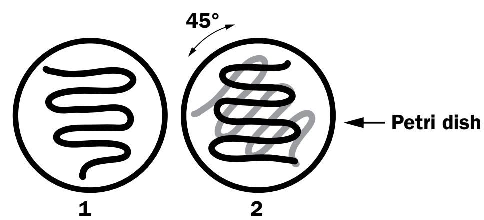
Where you are going to take samples from and why you are choosing those locations?

Methods: ____

____

____

Directions:



1. Listen closely to your teacher's directions.
2. Collect any supplies you will need such as cotton swabs and agar plates. An agar plate is a petri dish that contains a jello-like substance upon which it is very easy for many types of microbes to grow. If possible wear gloves while working with the cotton swabs and agar plates to avoid contamination of the samples. If gloves are not available, wash your hands thoroughly before gathering samples.
3. Plan where you are going to collect samples and take two from each site: one that will be kept in the dark for a few days, and one that will be kept in the light for a few days. Try not to take your samples from the exact same spot because your first effort to sample might have removed many of the microbes.
4. To collect a sample, run your cotton swab over the site several times for approximately 10 seconds. Make sure you use a new cotton swab for each sample.
5. Open the petri dish and rub the cotton swab gently across agar plate many times in a zigzag pattern (as shown above). Rotate the plate 45 degrees and swab the agar plate again for maximum coverage. Repeat as needed to cover the entire surface area of the agar. Make sure the agar plate is exposed to the air for the least amount of time possible to avoid airborne microbes falling onto the agar.
6. After each plate has been streaked, it should be taped with scotch tape on each side to seal it. Flip the plate upside down so that the air and moisture are on the bottom (this helps prevent the agar from drying out and promotes bacterial growth).
7. Once you have sealed the dish closed with tape, do not open it again.
8. Each plate should be labeled with masking tape of the sample location and name(s) of group members.
9. Place each plate in a clear plastic sealed sandwich bag and leave it in the bag for the duration of the experiment.
10. Place one plate of each sample in a totally dark area in the classroom (such as in a box), and place the other in a light area.
11. Return any extra materials to your teacher.

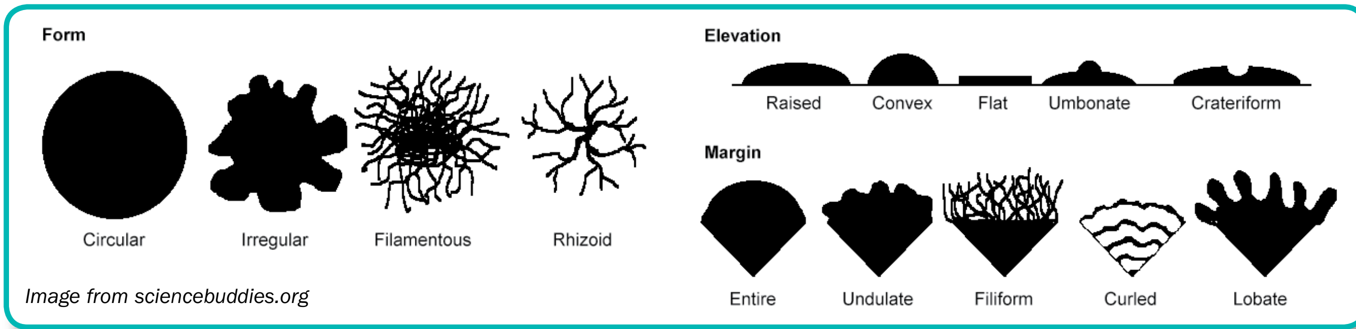


Image from sciencebuddies.org

Draw and label your first observations here.

FIRST OBSERVATION OF SAMPLES:

***WARNING:* Keep your samples sealed at all times.**
The microbes growing in the dishes may be hazardous to your health! Most of what you see growing in your samples will be bacteria, but you might also have yeast, mold, and types of fungi. Many of these microbes have the potential to be toxic to humans.

1. How many days have your samples been growing?

____

2. Draw what you see in each of the petri dishes. Make sure to write labels about which petri dish you are drawing (where was the sample taken and has the sample been in a light area or dark area for the past few days).

3. Estimate what percentage of each plate is covered with growth. ________________________________

4. How many distinct types of growths (microbial colonies) can you see? The more colonies you have, the more diverse your sample is.

____

5. Number each colony on your sketch and describe its morphology (morphology means the form and structure of organisms which includes shape, structure, color, pattern, and texture). Use the drawings above to describe the form, elevation, and margin of each colony.

6. What is the difference between the growth in the samples incubated in the dark vs. those incubated in light?

Petri Dish #1 (incubated in the light)

Petri Dish #2 (incubated in the dark)

7. Are all the microbial colonies you see multiplying (getting bigger) at the same rate? Why or why not?

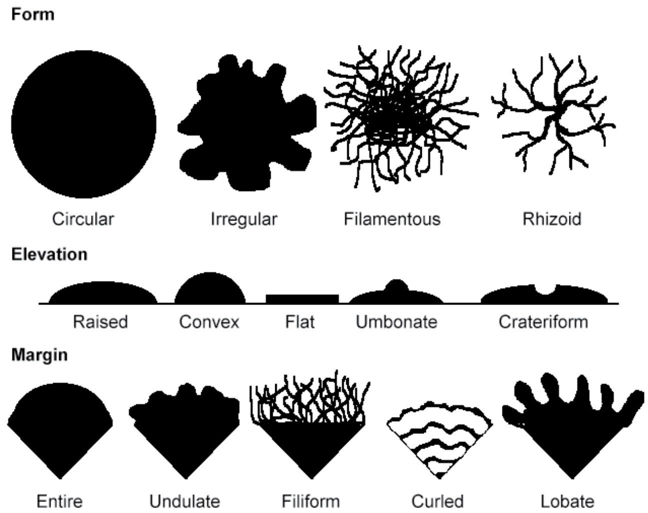


Image from sciencebuddies.org

Draw and label your second observations here.

Petri Dish #1 (incubated in the light)

Petri Dish #2 (incubated in the dark)

SECOND OBSERVATION OF SAMPLES:

***WARNING:* Keep your samples sealed at all times.**

1. How many days have your samples been growing? _______
2. Draw what you see in each of the petri dishes. Make sure to write labels about which petri dish you are drawing (where was the sample taken and has the sample been in a light area or dark area for the past few days).
3. Estimate what percentage of each plate is covered with growth. ________________________________
4. How many distinct types of growths (microbial colonies) can you see? The more colonies you have, the more diverse your sample is. ________________________
5. Number each colony on your sketch and describe its morphology (morphology means the form and structure of organisms which includes shape, structure, color, pattern, and texture). Use the drawings above to describe the form, elevation, and margin of each colony.
6. Put a star next to the sample that has the most diversity. Why do you think this sample might have been the most diverse? *(Think about where the sample was taken, the conditions the sample was left in over the last few days, the possible metabolisms of the microbes, the resources available to the microbes during the last few days, etc.)*

7. What is the difference between the growth in the samples incubated in the dark vs. those incubated in light?

8. Do you think the microbes growing in the dark use light for energy? Why or why not?

9. Do you think all the microbes growing in the light use light for energy? Why or why not?

10. Are all the microbial colonies you see multiplying (getting bigger) at the same rate? Why or why not?

RESULTS:

What conclusions can you draw based on your results? Was your hypothesis correct or was your initial question answered?

Were there any results or conclusions you can make that were not included in your hypothesis or question?

Do you think you might have collected samples of microbes that didn't grow in the dishes? Why or why not?

What surprised you most about this experiment?

What would you do differently if you had the chance to redo the experiment?

Microbes such as bacteria and fungi are some of the oldest forms of life on Earth and microbes account for the majority of the Earth's biomass (the total weight of all living things). If we find life on another planet, scientists think it will most likely be microbial life. Why do you think microbes are so successful?

WARNING: **Follow your teacher's directions about how to discard of the samples. They may be very hazardous!**

battery acid	pH 0
	pH 1
	pH 2
	pH 3
	pH 4
black coffee	pH 5
	pH 6
water	pH 7
	pH 8
	pH 9
	pH 10
	pH 11
bleach	pH 12
	pH 13
liquid drain cleaner	pH 14

BACKGROUND IMAGE: This Yellowstone thermal feature was unofficially nicknamed "Gabby's Spring" by Montana State University (MSU) researchers because Gabby Michel, a student at Lone Peak High School in Big Sky Montana, accompanied the MSU Extreme Yellowstone Expedition scientists and helped them gather data from the spring.

You will be using the data that she collected to learn more about Yellowstone's thermal features.

INSET: Gabby took the samples collected by the MSU team and helped examine them in a lab.

INVESTIGATING HOT SPRINGS

A wild place to study

Yellowstone National Park is a beautiful but wild place. Of the millions of tourists who visit the Park each year, a few are injured or killed in rare incidents: being attacked by an animal, being struck by lightning, drowning, falling off cliffs or other accidents.

One of the most dangerous features of the Park are its hot springs. If you fall in one, you will be severely burned and it is likely that you will die. Therefore, when you visit the Park it is critical that you stay on the boardwalks and paths and never touch the hot springs. When scientists go to Yellowstone to research the hot springs, they have to get special permits from the National Park Service and receive training on how to gather samples.

A virtual research expedition

Since it's much too dangerous (and illegal) for anyone but fully trained and properly permitted individuals to gather data from a Yellowstone hot spring, today you are going to examine a virtual hot spring. Either your teacher or you and your classmates will put together the pieces of a virtual hot spring on your classroom floor.

The pieces will form a picture of a hot spring found in the Heart Lake region of Yellowstone National Park unofficially nicknamed "Gabby's Spring." Today you will be using the data from "Gabby's Spring" to learn more about Yellowstone's thermal features.

What organisms could live here?

You will be working with data on pH and temperature. These two variables impact what kind of organisms can live in a certain place. If you know the temperature and pH of an environment, then you can guess what kinds of organisms might be living there.

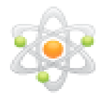
For example, the pH of water can vary slightly due to the minerals found the soil and rocks around it because of pollution, the amount of plant growth and organic material found in the water and other factors.

The average pH of surface ocean water is around 8, whereas the average pH of clean rain (not polluted acid rain) is around 5.5. If the pH of water goes below 5, fish reproduction (breeding) is affected. If the pH of water goes below 4, fish die. So if you test water from a stream and it has a pH of 4 or lower, there will be no fish living that area.

What is pH?

pH is a measure of a measure of how many hydrogen ***ions*** are in a solution. The strength of an acid or base is measured on a scale called the pH scale.

- The scale ranges from 0 to 14 with the middle value (7) being neutral (neither acidic nor basic).
- Zero is the strongest acid and 14 is the strongest base.
- Every time you go *down* a number on the scale (e.g., from 7 to 6) there are 10 times more hydrogen ions.
- Every time you go *up* a number on the scale (e.g., from 7 to 8) there are 10 times fewer hydrogen ions



An *ion* is a charged atom or molecule. It is charged because the number of electrons do not equal the number of protons in the atom or molecule. An atom can acquire a positive charge or a negative charge depending on whether the number of electrons in an atom is greater or less then the number of protons in the atom.

This means that your stomach acid (pH 2) is *100,000 times more acidic than neutral water (pH 7),* not just five times more acidic, as you might assume.

In agriculture, pH is one of the most important single properties of the moisture associated with a soil, since that indication reveals what crops will grow readily in the soil and what adjustments must be made to adapt it for growing any other crops. For example, most agricultural crops will not grow in acidic soils and need alkaline soil, whereas some evergreen trees and shrubs like acidic soils.

Your teacher may have asked you to test the pH of samples you brought in from home or samples taken from around your school.

If you are testing pH, fill in the names of the liquids you tested and their pH values on the scale at left.

YOUR MISSION

Start your virtual research expedition to Yellowstone

Look at the photo of "Gabby's Spring" pictured here or the large version of the hot spring which should be on your classroom floor. There are microbes that can live in very hot water living in the spring. The microbes and minerals cause the vivid colors of the hot spring.

In the green and orange areas, the microbes are so thick that they form mats and are visible to the naked eye. The white areas of the spring are mostly mineral deposits. The microbes living in the blue part and center clear channel of the spring are not visible to the naked eye.

Predict: Which part of "Gabby's Spring" do you think will be hottest and which part will be coolest (the blue, the green, or the orange parts)? Mark your predictions in the table below.

Test: Take temperature readings of the liquid samples in your class, following your teacher's directions, and write the results below. ***NOTE:*** *the temperatures you collect will not accurately reflect the temperatures of the hot spring. You will work with the real temperature data in the graphing activity.*

	Your prediction: Blue? Green? or Orange?	**Explain the reasons behind your prediction**	**Your results: (Blue, Green or Orange?)**	**Mark a ✓ if your prediction was correct, or an x if it was incorrect**
Hottest colored area				
Mid-range				
Coolest colored area				

Now measure the pH of "Gabby's Spring."

1. What pH did you measure? ____________________________

2. Add the pH of "Gabby's Spring" to your pH chart on the previous page as well as the pH values for surface ocean water, which is around 8, and clean rain (not polluted acid rain), which is around 5.5.

3. Is "Gabby's Spring" acidic or alkaline? ____

4. What are some of the possible causes for water from different sources to have different pH values?

____

____

5. What are two important variables in this hot spring that change and impact the visible zones you see, and therefore the kind of organisms that live in those zones?

____

Center channel (clear)	
ft.*	Temp °F (°C)
0	______°F (______°C)
3	______°F (______°C)
6	______°F (______°C)
9	______°F (______°C)
12	______°F (______°C)
15	______°F (______°C)
18	______°F (______°C)

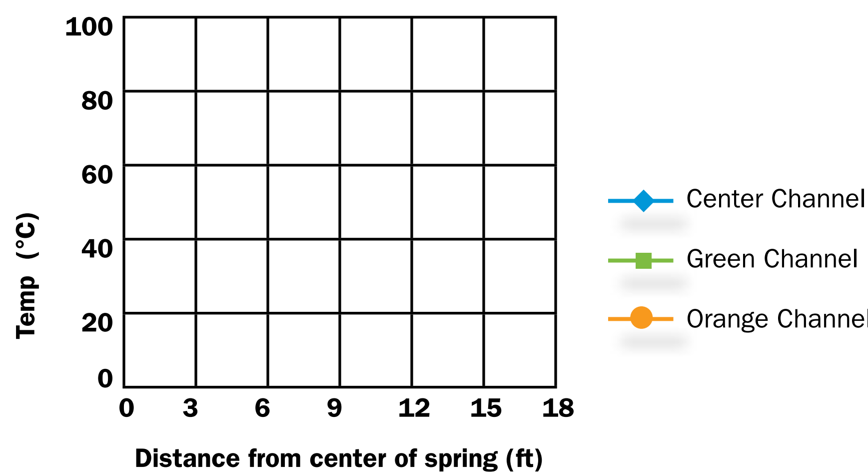
Green outer channel	
ft.*	Temp °F (°C)
0	–
3	–
6	______°F (______°C)
9	______°F (______°C)
12	______°F (______°C)
15	______°F (______°C)
18	______°F (______°C)

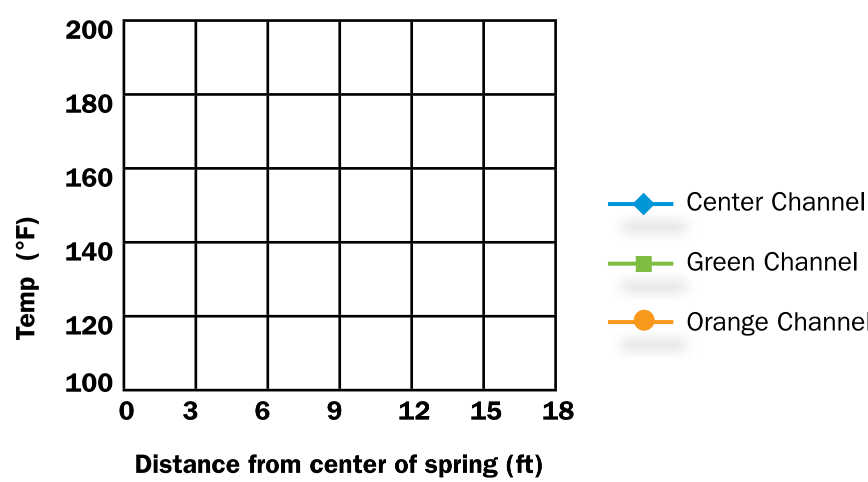
Orange outer channel	
ft.	Temp °F (°C)
0	–
3	–
6	–
9	______°F (______°C)
12	______°F (______°C)
15	______°F (______°C)
18	______°F (______°C)

* Distance from center of spring in ft.

Gather temperature data

Use the "Gabby's Spring" map on your classroom floor to virtually sample temperatures in the clear, green and orange channels at 3 foot intervals from the spring's center and mark them in the chart at left. *(Your teacher can tell you whether to fill in Fahrenheit or Celsius values.)*





Graph your temperature data

Now that you have gathered the temperature data, graph the distance on the X axis and the temperature on the Y axis above. Use a different color for each channel (ideally use ink that matches the color of each channel: blue, green and orange) and connect the data points of each channel when you have plotted them all. *(Your teacher can tell you whether to graph Fahrenheit or Celsius values.)* After you are finished, answer questions 1-5 (below and on the next page).

1. Almost all the data you graphed should illustrate a basic trend. What is that trend?

____

2. There is one data point that very visibly doesn't follow the same basic trend as the rest. On which channel line is the data point? Make some guesses as to why it is so different than the others?

3. When you look at the picture of the spring, why do you think the clear center channel gets so narrow?

4. What do you think happens to the spring's channels beyond the frame of the picture? Do the three zones go on for miles?

5. Looking at the graph you made on the previous page, what could you infer about the temperature zones where the organisms in the green and orange channels can live? Why?



Zones of life

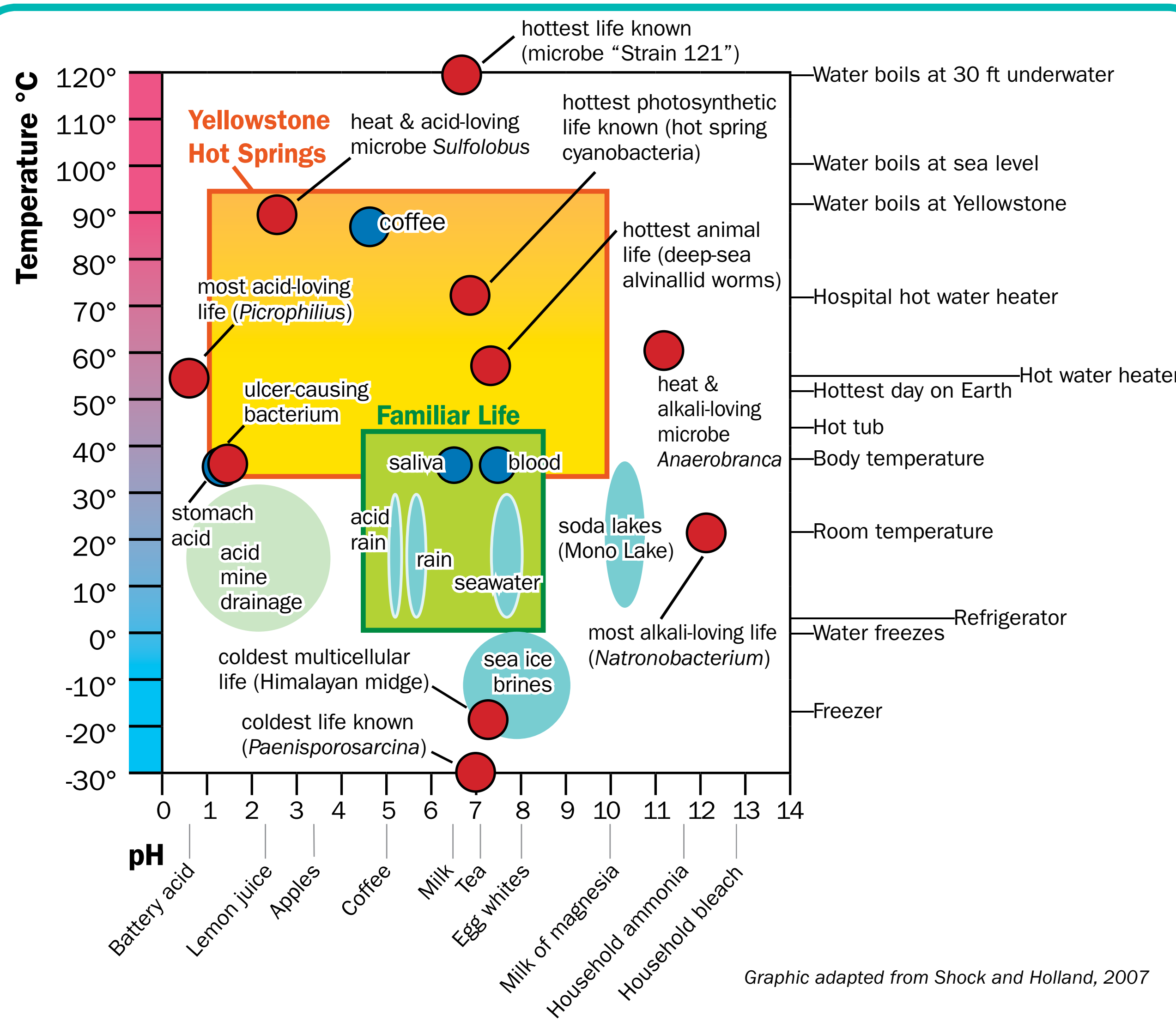
The graphic on the next page illustrates some of the concepts we have been talking about. It depicts the temperature and pH niches where various organisms can live (in other words, the environmental conditions that are suitable for them to thrive). Answer the questions below by looking at the graphic.

NOTE:

- The red dots are specific types of life.
- The blue dots are non-living things.
- The large shapes are niches or types of environments.

1. In what area of the graphic would you find a tree? ______
2. According to the graphic, what kind of animal life can survive the hottest temperatures?

LIFE ON EARTH



Graphic adapted from Shock and Holland, 2007

3. Which zone on the graphic is larger: The one representing life in Yellowstone hot springs, or the one representing familiar life?

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4. Clearly illustrating scientific data so that it is easy to understand is very difficult. Does this graphic seem effective to you? In other words, does it help you understand and visualize how different forms of life inhabit different niches? Explain why you find the graphic helpful or unhelpful.

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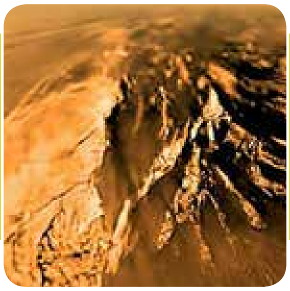
5. If you look at the types of life that survive in the hottest temperatures, the most alkaline conditions, the most acidic conditions, and the coldest temperatures, what kinds of life are they all, and therefore what kind of life is the most robust and able to survive what we consider to be extreme conditions?

Exploring for Life

Imagine that you have to advise NASA as to which of the following places to go look for alien life. If you want to rule out places to look for life, which variable (temperature or pH) do you think is the better one to use? In other words, if you had data for pH levels on other planets and temperatures on other planets, which data would be more helpful to use to tell NASA where not to go?

____

____

____

Mark in the chart below if you think NASA should look for life in any of the following places in our solar system.

Location in solar system	**Explore? (Yes or No)**
Mercury: temperature ranges from 800°F (430°C) to -280°F (-170°C)	
Venus: average temperature 864°F (462°C)	
Jupiter: average temperature in the clouds -234°F (-145°C)	
Europa (one of Jupiter's moons): -260°F at the equator's surface (-160°C), possible liquid water under the ice that could reach the boiling point in places 212°F (100°C)	
Neptune: average temperature of -328°F (-200°C)	

What about Mars?

The temperatures on Mars can range from -287°F (-177°C) to 86°F (30°C). Scientists think it is highly unlikely there is any life on Mars currently but that we might find signs of past life that existed when Mars had more of an atmosphere, less extreme temperatures, and liquid water on the surface.

Will we ever find aliens?

Even if we never find life on other planets or moons in our own solar system, there is probably life somewhere else in our giant universe. NASA's Kepler mission is constantly finding new planets. To learn more about the mission and find out how many planets have been discovered visit ***kepler.nasa.gov.***

EYE

Extreme Yellowstone Expedition

MONTANA STATE UNIVERSITY | Thermal Biology Institute

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