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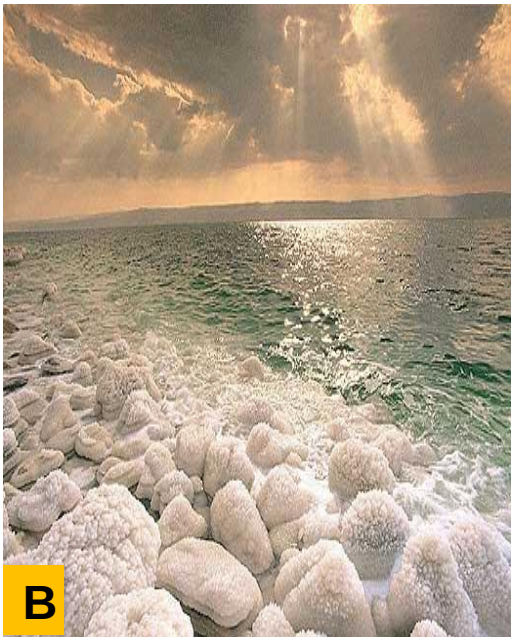
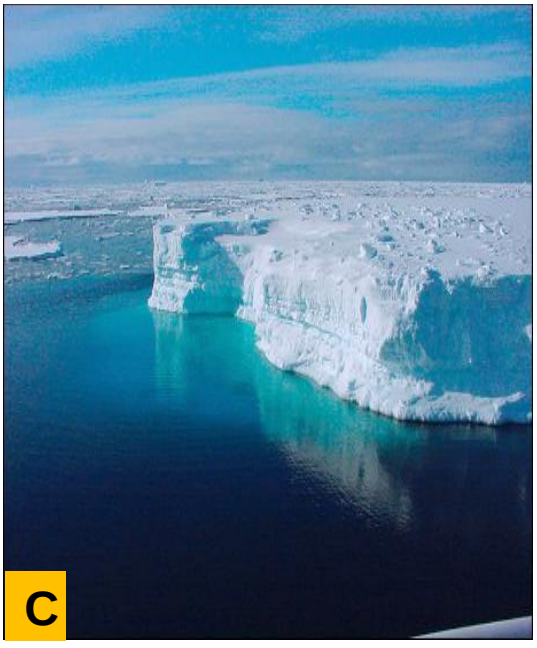
Investigating Extreme Environments

Life on the Edge





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Photography Credits:

A. Picture of Octopus Springs in Yellowstone National Park.
B. Picture of an extreme salty environment
C. Picture of a location in the extreme Arctic Ocean.

National Standards Correlation

Life Science Content Standard C

Regulation and Behavior

All organisms must be able to obtain and use resources, grow, reproduce, and maintain stable internal conditions while living in a constantly changing external environment. Regulation of an organism's internal environment involves sensing the internal environment and changing physiological activities to keep conditions within the range required to survive.

Picture of a plant adapting to its environment.

Diversity and Adaptations of Organisms

Millions of species of animals, plants, and microorganisms are alive today. Although different species might look dissimilar, the unity among organisms becomes apparent from an analysis of internal structures, the similarity of their chemical processes, and the evidence of common ancestry. Biological evolution accounts for the diversity of species developed through gradual processes over many generations. Species acquire many of their unique characteristics through biological adaptation, which involves the selection of naturally occurring variations in populations. Biological adaptations include changes in structures, behaviors, or physiology that enhance survival and reproductive success in a particular environment.

Picture of DNA structure

Earth and Space Science Content Standard D

Structure of the Earth System

Water is a solvent. As it passes through the water cycle it dissolves minerals and gases and carries them to the oceans. The atmosphere is a mixture of nitrogen, oxygen, and trace gases that include water vapor. The atmosphere has different properties at different elevations.

Picture of Earth from space

Purpose
To teach students how to think independently by carrying out an inquiry-based experiment.

Key Concepts
- Define the question and create a scientific approach to answering the question.
- Actively learn the components of experimental design such as: types of variables, treatments, and experimental units.
- Share their experimental findings with others via a Prezi presentation.

Common Misconceptions
- Students have a difficult time formulating a complete hypothesis. Often times they stop after "if…then", and do not provide justification for their reasoning.
- Students are aware of a control treatment, however they do not understand its importance.
- Students don't realize that scientists must present their findings in engaging presentations.

Overview
Astrobiology is the study of the evolutionary and chemical history of life on Earth, and whether such a history could take place in other parts of the universe. The exact set of conditions that have given rise to life on Earth is extraordinarily rare among planets! Earth is the perfect distance from its sun to have a moderate climate and has an abundance of water (which is necessary for life as we know it). However, astronomers do know of several planets and moons that have environments resembling a particular place on Earth. Even though these "extreme" environments are not the most comfortable, the enormous variety of living things on Earth has found ways to adapt to nearly every condition on the planet. The word "extremophile" is used to describe organisms that survive in inhospitable environments. The question is: if life can find a way to survive in harsh conditions on Earth, is it possible for life to begin on other planets with a similar set of conditions? Astrobiologists are also interested in studying what adaptations these extremophiles use to survive, because this could give us clues about what might allow for life on other planets.

Prep Time for Teachers- 2 hours
Class Time- 2 hours 30 minutes (over 4 days)

Objectives

- Students will learn how to design experiments.
- Students will learn how to make qualitative and quantitative observations.
- Students will learn how to collect data.
- Students will learn how to present their findings.

Skills

1. *Conducting* Internet research
2. *Predicting* the outcome of an experiment
3. *Controlling* variables
4. *Conducting* an experiment
5. *Collecting, recording,* and *graphing* data
6. *Drawing* conclusions and *communicating* them to others

Materials For Parts B and C(for a group of 3-4 students)

1. 12 centrifuge tubes (50 mL, sterile)
2. Disposable 2 mL serological pipets (sterile)
3. Rubber pipet bulb or plunger
4. Liquid growing media
5. Live cultures of Thermus *thermophiles*, *Psychrobacter urativorans*, and *Halomonas halodenitrificans*
6. Spectrophotometer

Procedure

Part A- Researching Extreme Environments

Step1- As a group, choose 4 different environments (one for each member of the group) from the list of extreme environments below. Each group member should pick a different condition such as: pH, temperature, salinity, location, seasonal variation, pressure, organism that live there, etc.

List of extreme environments:

- The Dead Sea
- Octopus Spring
- Glaciers of Iceland
- Atacama Desert, Chile
- Kamchatka, Russia
- Guerro Negro,Baja California
- La Brea Tar Pits
- Lupin Mine, Canada
- East African Rift Desert, Eastern Africa
- Great Salt Lake, USA
- McMurdo Dry Valleys, Antarctica
- Lake Assal, Djibouti, Africa
- Magic Mountain, Pacific Ocean
- Loki's Castle, Mid-Atlantic Ridge
- Hanford Site, USA
- Lake Vostok, Antarctica

Step2- Answer the following questions about your extreme environments. Each group member will have a different environment on Earth. After you have completed the following questions, share your answers with your group members.

Day1- Researching Extreme Environments

1. My extreme environment on Earth is: _____
2. Where (exactly) is it located?_____
3. List some characteristics that make this environment extreme:

    a)_____

    b)_____

4. Find two extraterrestrial environments that share these characteristics:

    a)_____

    b)_____

5. Give a description of these extraterrestrial environments (where are they located and what the conditions are like)

    a)_____

    b)_____

6. Find three microorganisms ("extremophiles") that live in your extreme environment on Earth. Provide the scientific name.

    a)_____

    b)_____

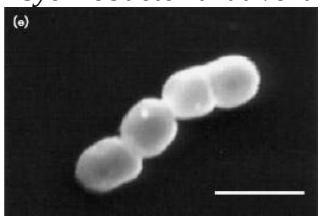
    c)_____

7. Match each one of the extremophiles to the conditions it can tolerate.

Thermus thermophilus

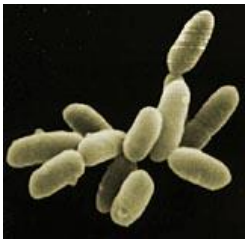
High salt concentrations

Psychrobacter urativorans



Extremely hot temperatures

Halomonas halodentrificans



Extremely cold temperatures

Part B- Identifying your extremophile

*****Before reading on, please familiarize yourself with the following vocabulary terms.**

Vocabulary

1. **Growing medium** is a liquid solution in which bacteria can grow. It contains all the nutrients and minerals that bacteria need to multiply.

2. **Sterile** means that a liquid or surface has absolutely no microorganisms growing in or on it. Most of the equipment in this lab has been sterilized by boiling it to kill all the microorganisms inside. You can keep the insides of laboratory containers sterile by exposing them to the air as little as possible. It's important to keep your equipment sterile so that when you do put a microorganism in a container, you can be sure that it's the only type of microorganism in that container.

3. **Salinity** is a measurement of how much salt is in a fixed volume of solution. It is related to how salty a solution would taste if you drank it. Imagine adding a spoonful of salt to a glass of water: it would taste really salty! But if you added the same spoonful to a bathtub full of water, the salinity would be much lower (it would taste much less salty). Therefore, salinity is related not just to the actual amount of salt, but to the concentration of salt in a solution. In this experiment we will measure salinity in grams of salt per 1 liter of solution (g/L).

4. **pH** is a measurement of how acidic or basic a solution is. You have interacted with acids and bases if you have ever tasted something sour (acidic) or bitter (basic). pH values usually range from 1 (very acidic) to 14 (very basic), with 7 being neither acid nor base. Most organisms survive best in solutions that are somewhere near a pH of 7.

5. **Optical density (OD)** is a measurement technique used to determine how much cells have grown. To measure OD, light is shined through a sample of liquid, and then measured as it comes out the other side. As more cells grow, the liquid becomes cloudy and more light is blocked from passing through. This is often done with a device called a spectrophotometer, which gives an exact measurement of how much light passes through. You can measure OD in other ways, which your teacher may show you.

6. **Blanking** is a technique that is very important when measuring OD. To use a blank, you compare your sample with bacteria growing in it to a sample of growing medium with no bacteria growing. For example, what if you were asked to look at the tube below and determine whether it was cloudy or not?

Part B-Identifying your Extremophile

Step 1- In the following section of the lab, you will be given an unknown liquid culture of either a halophile psychrophile, or thermophile. Your task is to test 3 growth medias, with each media corresponding to a particular environment suitable for bacterial growth. The media bottles will be labeled as A,B, or C.

Based on your research and knowledge of the three extremophiles:

Design a hypothesis: IF an unknown organism grows in _____ (the selected media), THEN it can be identified as _____ _____ BECAUSE _____ _____ _____.

Designing your Experiment

Step 2- Now, obtain your materials.
Step 3- Label each 50ml conical tube with either A, B or C.
Step 4- Next, add 20 mL of your growing medium into each of the tubes A, B, and C.
Step 5- Obtain your unknown bacterial culture X from the teacher and using a micropipet, take 200 µL of Unknown X and add this to your centrifuge tube labeled "A." Repeat this step for medias B and C, using a new pipet tip each time.
Step 7- Give your tubes to the teacher. The teacher will ensure that the tubes are kept in the correct conditions overnight.
Step 8- Look at your samples the next day. One should look cloudier than the others. A visual observation such as this is known as a qualitative observation where you observe things such as a change in color, shape, size, etc.

Fill in the following:

Which media (A, B, or C) was conducive to the growth of your unknown extremophile culture?_____
Therefore, your unknown extremophile is,
_____.

Part C- Manipulating variables in order to see maximum bacterial growth

Now that you know which bacteria culture you have, it is time to design your own experiment where you get to decide what variables are manipulated!

Based on your research and knowledge of the three extremophiles:

1. Design a hypothesis: IF (the name of your extremophile) is grown in (your extreme conditions with manipulated variables), THEN it can be identified as _____ BECAUSE _____ _____.

2. Define the following terms:
    1. **Dependent Variable-**
    2. **Independent Variable-**
    3. **Control Variable-**

ii. In your experiment, what is your dependent variable?
iii. In your experiment, what is your independent variable?
iv. In your experiment, what is your control variable?

Procedure

Step1- Label four 50 mL centrifuge tubes with your choices of growing condition. Also label one of your tubes as control.
Step 2-Label another four tubes with the same labels, but write "blank" on tem to use for your optical density test.

1. What is OD?_____
2. Why is it important to use a blank when measuring the OD?_____ _____

Step 3- When performing this step, be very careful to keep the tubes labeled "blank" sterile.
If you are testing temperature:
Pour 20 mL of your growing medium into each of your six tubes.
If you are testing pH or salinity:
Step 4- Your teacher will give you three different growing medias. Pour 20 mL of each one into the appropriately labeled blank and non-blank tubes.
Step 5- Use a micro pipette to add 200 µL of culture containing your extremophile to each non-blank tube. Swirl each tube to mix the culture thoroughly.
Step 6- You will now take an OD measurement of your samples. Your teacher will show you how to do this using either a spectrophotometer or a visual method. Record your OD readings in the table below.
Step 7- Take OD readings over the next two days. Use these data to make a graph showing how the extremophile grew over time in the three conditions.

Record the OD of your three samples. Don't forget your control!

	Sample 1	Sample 2	Sample 3	Control
Day 1				
Day 2				
Day 3				

You will graph the four samples on a single graph. What will go on the y-axis (vertical axis)? On the x-axis (horizontal axis)?

Photo Credits

A. http://geology.missouri.edu/research/schulteresearch.html
B. http://www.atlastours.net/jordan/deadsea.html
C. http://www.ok4me2.net/2011/01/07/mysteries-of-lake-vostok-on-brink-of-discovery/
D. http://serc.carleton.edu/microbelife/extreme/hypersaline/general.html
E. http://www.kscnet.ru/ivs/lavdi/staff/belousov/uzon.html
F. http://www.daviddarling.info/encyclopedia/T/thermophile.html
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Extremophile Investigation: Preparation Guide

Student materials (for each group):

- 12 50 mL centrifuge tubes (sterile, with caps)
- 6 2 mL serological pipets (sterile)
- Rubber pipet bulb

Teacher materials

- 50 mL centrifuge tubes
- Mason jars with lids
- Sharpie permanent markers
- Yeast extract
- Polypeptone peptone
- Beef extract
- Peptone
- Sodium Chloride (NaCl)
- Sodium hydroxide (NaOH), 0.01 M solution
- Hydrochloric acid (HCl), 0.01 M solution
- pH probe (or pH indicator paper if not available)
- Refrigerator space
- Hot plates
- Large beakers (600 mL or 1000 mL)
- Thermometers
- Spectrophotometer (or visual scale; see below)

Preparing growing media

General tips:

- Use the purest water available (microfilter purified if possible; if not available, use deionized; if not available, use distilled; if not available, use tap)
- All growing media must be sterilized after preparation. If available, an autoclave is the most reliable way to sterilize media. If not, put media in centrifuge tubes or Mason jars, seal tightly, and place in a pot of salted boiling water for 30 minutes.
- These instructions cover how to make media for both identifying the unknown bacteria (Part 1) and determining the best growing conditions (Part 2).

Medium for *Thermus thermophilus*

1. To 1 L of water, add 4 g of yeast extract and 8 g of polypeptone peptone. Stir until completely dissolved.
2. Using a pH probe or indicator paper, make sure the pH is as close to 7.0 as possible. Adjust with sodium hydroxide or hydrochloric acid as

needed (sodium hydroxide increases pH, hydrochloric acid decreases it).

3. Set aside 300 mL of the medium in a Mason jar and label it for use in Part 1.
4. Set aside another 200 mL of the medium in a Mason jar and label it for use in Part 2 if a group is changing the growing temperature.
5. Put 50 mL of the remaining medium into each of 4 50 mL centrifuge tubes. Label one tube pH 6.0, another pH 6.5, another pH 7.5, and the last pH 8.0. Using a pH probe or indicator paper, adjust the pH of each tube to its labeled value by adding drops of sodium hydroxide or hydrochloric acid. (If neither a pH probe or pH paper is available, add about 3 drops of NaOH to the tube labeled 7.5 and 6 drops to the tube labeled 8.0, then add about 3 drops of HCl to the tube labeled 6.5 and 6 drops to the tube labeled 6.0)
6. To the remaining 300 mL of the original medium, add 1.5 g of NaCl. Stir until completely dissolved. Remove 50 mL of this medium and label it 5 g/L NaCl.
7. To the remaining 250 mL of medium, add 1.25 g of NaCl. Stir until completely dissolved. Remove 50 mL of this medium and label it 10 g/L NaCl.
8. To the remaining 200 mL of medium, add 1.0 g of NaCl. Stir until completely dissolved. Remove 50 mL of this medium and label it 15 g/L NaCl.
9. To the remaining 150 mL of medium, add 0.75 g of NaCl. Stir until completely dissolved. Remove 50 mL of this medium and label it 20 g/L NaCl. Discard the remaining 100 mL of medium.
10. Label all medium produced in this section as *Thermus thermophilus*.

Medium for *Psychrobacter urativorans*

1. To 1 L of water, add 3 g of beef extract and 5 g of peptone. Stir until completely dissolved.
2. Follow steps 2 through 9 for *Thermus thermophilus* medium. Label all medium produced in this section as *Psychrobacter urativorans*.

Medium for *Halomonas halodenitrificans* (Note: this one is tricker!)

1. To 1 L of water, add 3 g of beef extract, 5 g of peptone, and 80 g of NaCl. Stir until completely dissolved.
2. Using a pH probe or indicator paper, make sure the pH is as close to 7.0 as possible. Adjust with sodium hydroxide or hydrochloric acid as needed (sodium hydroxide increases pH, hydrochloric acid decreases it.)
3. Remove 300 mL of the medium. To this 300 mL, add 10.5 g of NaCl and stir until dissolved. Place in a Mason jar and label for use in Part 1.
4. Remove another 400 mL from the original medium. (Save the remaining 300 mL for step 6.) To the 400 mL you removed, add 8.0 g of NaCl and stir until dissolved. Divide this 400 mL in half (two 200 mL portions). Place one portion in a Mason jar and label for use in Part 2 if

a group is changing growing temperature. Use the other portion for step 5.

5. Divide the 200 mL portion into 4 50 mL portions in separate 50 mL centrifuge tubes. Label one tube pH 6.0, another pH 6.5, another pH 7.5, and the last pH 8.0. Using a pH probe or indicator paper, adjust the pH of each tube to its labeled value by adding drops of sodium hydroxide or hydrochloric acid. (If neither a pH probe or pH paper is available, add about 3 drops of NaOH to the tube labeled 7.5 and 6 drops to the tube labeled 8.0, then add about 3 drops of HCl to the tube labeled 6.5 and 6 drops to the tube labeled 6.0)
6. Take the 300 mL remaining from step 4. Remove 50 mL of this medium and label it 80 g/L NaCl.
7. To the remaining 250 mL of medium, add 2.5 g of NaCl. Stir until completely dissolved. Remove 50 mL of this medium and label it 90 g/L NaCl.
8. To the remaining 200 mL of medium, add 4.0 g of NaCl. Stir until completely dissolved. Remove 50 mL of this medium and label it 110 g/L NaCl.
9. To the remaining 150 mL of medium, add 1.5 g of NaCl. Stir until completely dissolved. Remove 50 mL of this medium and label it 120 g/L NaCl.
10. Label all medium produced in this section as *Halomonas halodenitrificans*.

Place all centrifuge tubes and Mason jars in an autoclave or salted boiling water for 30 minutes.

<u>Determining optical density (OD)</u>

If a spectrophotometer is available, follow the user guide to operate it correctly. Don't forget to use a blank and a clean cuvette for each measurement. If a spectrophotometer is not available, a procedure to use a visual scale is given here. Print out the following page and cut along each solid line. Give each group one strip and demonstrate how to use it to measure a sample:

1. Take your centrifuge tube with the appropriate blank and hold it at eye level with the paper strip behind it. Make sure there is liquid in front of the paper.
2. Determine which is the smallest "e" that can be clearly seen as the letter "e" (and not a blob or dot). Write the number below this "e" down as the blank measurement.
3. Do the same for your sample. Subtract the number of the blank measurement from the number of the sample measurement to get the OD of your sample.

Remind the students that the values they get from either method are for comparison; they are seeing whether one set of conditions grows faster or slower than another. For example, a reading of 6 doesn't mean there are 6 cells growing; it just means that there are more cells than in a tube with a reading of 5.

To hold samples at elevated temperatures

1. Fill a large beaker with tap water and place on a hot plate. Tape a thermometer inside the beaker so that the bulb is near the middle of the water instead of the bottom.
2. Turn up the hot plate and give it several minutes to equilibrate. You will have to adjust it slowly until the thermometer reads the desired temperature.
3. Seal samples tightly in their centrifuge tubes and place in the water.
4. Cover the beaker tightly with saran wrap to prevent evaporation.
5. Note that several groups can share a beaker if they are testing the same temperature.