Math Lesson Plan:

Cyanobacteria Races: Cyanobacteria Motility Experiment for a Classroom

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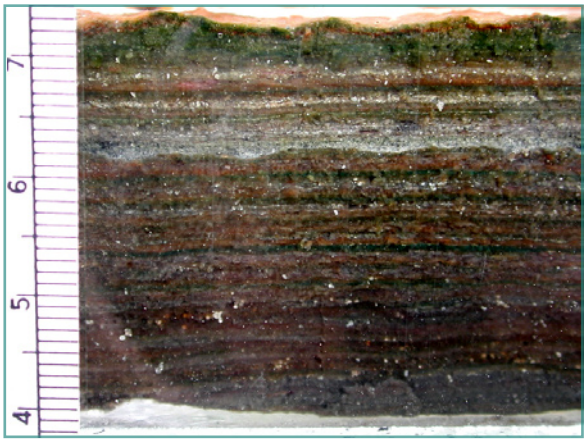
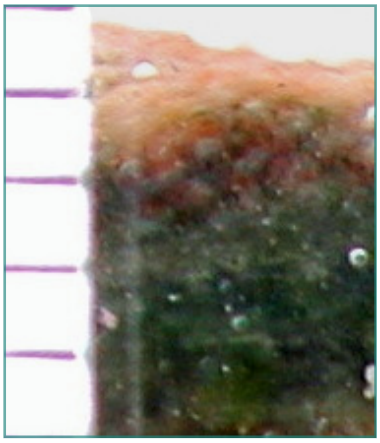
Background

Cyanobacteria living in microbial mats can move, both up and down, and sideways. They may orient themselves, and find the optimal depth at which to live, using chemical, light or gravity clues[1]. In this experiment, students will expose cultures of freshwater cyanobacteria to a directional light source, measuring their movement toward this light source with a ruler and recording measurements. Through analyzing cyanobacteria motility data, students will determine how cyanobacteria respond to light clues. The structures produced by the movement of cyanobacteria can be preserved in fossil records. Studying cyanobacteria movement and cultures can help scientists interpret those fossil records. By studying fossil records of cyanobacteria motility on earth, scientists are better able to identify fossil records in their search for life in the universe and beyond.





Microcoleus mat from Baja California

Notice the different colored layers of organisms living in the mats. Organisms orient themselves in the mats in response to light, nutrient, and chemical

Main Concept

Explanations of nature can be formulated, tested and evaluated using observation, experiments, and mathematical models.

Scientific Question

How important are light cues in affecting cyanobacteria motility?

[1] Bebout, Brad. "Cyanobacterial Motility Experiment." Microbial Mat Education Page. NASA. 1 April 2004. http://microbes.arc.nasa.gov/cyanobacterialmotlity.cfm

Objectives

1. The student will formulate and test a hypothesis explaining cyanobacteria movement using observation, experimentation and mathematical models.
2. The student will develop a better understanding of factors affecting movement in cyanobacteria.

Abstract of Lesson

Students will conduct an experiment and test their hypothesis on how light affects cyanobacteria movement. Experimental data will be graphed for analysis so students can draw conclusions about the results of the experiment.

Prerequisite Concepts

1. Photosynthesis
2. Structure of cyanobacteria cell

Major Concepts

1. Experimental design, formulating a hypothesis, testing explanations of nature using observation, experiments, and theoretical and mathematical models, evaluating investigations
2. Biological adaptations of cyanobacteria
3. 3. Function of cyanobacteria in an ecosystem

Misconceptions

1. Students may have trouble identifying variables in an experiment.
2. Controlling more than one variable in the experiment may be troublesome for some students.
3. Students may have trouble recognizing the effects of different variables in an experiment.
4. Students may rely on personal beliefs and concepts rather than scientific evidence to explain experimental results. learn to look at the graph and the scale of the graph to interpret the graph correctly.

National Education Standards

Fully Met	Partially Met	Addressed
NSES A1 (5-8): Abilities Necessary to do scientific inquiry d, e, g, h **NSES A2 (5-8):** Understanding about scientific inquiry c **NSES C6(5-8):** Regulation and Behavior c	**NSES A1 (5-8):** Abilities Necessary to do scientific inquiry a, b, c, **NSES A2 (5-8):** Understanding about scientific inquiry e, f **NSES C6(5-8):** Regulation and Behavior a, d **NSES C7(5-8):** Populations and Ecosystems c	**NSES A2 (5-8):** Understanding about scientific inquiry a **NSES B6(5-8):** Transfer of Energy f **NSES D5(5-8):** Earth in Solar System d
2061: 1B (6-8) #1 **2061: 1C (6-8) #7** **2061: 12D (6-8) #1, 2**	**2061: 2C (6-8) #2** **2061: 12A (6-8) #1**	**2061: 5E (6-8) #3** **2061: 12A (6-8) #2**

California Science Standards

Fully Met	Partially Met	Addressed
Grade 6: Investigation and Experimentation #7 a, b, c, d, e Grade 7: Investigation and Experimentation #7 a, c, e Grade 8: Investigation and Experimentation #9 a, b, c, e	Grade 6: Ecology #5 a	Grade 7 Physical Principles in Living Systems #6 a

Reading on Topic Related to Study

Cyanobacterial Motility experiment on the Microbes @ NASA webpage http://microbes.arc.nasa.gov

Materials List

Cyanobacterial Motility experiment data set on the Microbes @ NASA webpage http://microbes.arc.nasa.gov

For classroom Lab for 30 students:

1. Agar[2]
2. Media[3]: 3 quarts: Alga-Gro Freshwater Medium (ER-15-3752 Carolina Biological Supply)

[2] If you do not want to mix agar and alga-Gro medium to make your plates, Carolina Biological can provide custom agar formulations. The order needs 1 to 2 weeks for delivery after receipt of order. (800)227-1150.

[3] If you choose to mix media from Alga-Gro concentrate, separate instructions are available.

Materials List cont.

3. Cyanobacteria cultures:

   Lyngbya (ER-15-1830 Carolina Biological Supply), *Oscillatoria* (ER-15-1830 Carolina Biological Supply)

   If dissecting microscopes are available, additional cyanobacteria cultures may be ordered:

   Anabaena (ER-15-1710 from Carolina Biological uses Alga-Gro Freshwater Medium-order an additional quart), *Spirulina major* (ER-15-1900 from Carolina Biological uses Alga-Gro Seawater Medium ER-15-3754 from Carolina Biological-order one quart of Seawater Medium)
4. 5 cm x 2.5 cm clear plastic boxes to use for plates
5. Black electrical tape
6. Pipette
7. Tweezers
8. Metric ruler
9. Black poster board to cover the top and bottom of the plates
10. Plastic boxes with lids
11. Light source: an aquarium light with a cool white or full spectrum light bulb (for each set of class experiments)
12. Two sheets of stiff black poster board (foam-filled) for each class period.
13. Scissors
14. Fine-tip permanent markers (one for each lab group in a class period)
15. Duct tape
16. Balance
17. Autoclave or pressure cooker
18. Spatula for measuring agar
19. Stirring rod
20. Oven for maintaining the temperature of the agar
21. Erlenmeyer flasks (2000 ml)
22. Stoppers for flasks
23. Graduated cylinder
24. Thermometer
25. Incubator or warm location near a window or full spectrum light source
26. Dissecting microscopes or hand-lenses
27. Time-lapse video of cyanobacteria motility experiment in our lab, access through the Microbes @ NASA website http://microbes.arc.nasa.gov

Handouts

1. Lab notebook
2. Rubric for Lab evaluation

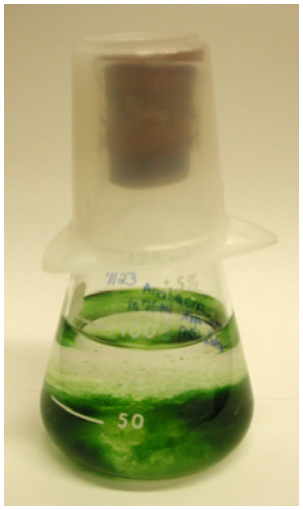
Preparation

1. Order two quarts of Alga-Gro Freshwater Medium (ER-15-3752 Carolina Biological Supply) and cyanobacteria Cultures: *Lyngbya* (ER-15-1830 Carolina Biological Supply), *Oscillatoria* (ER-15-1830 Carolina Biological Supply) so they arrive at least seven days in advance of your in-class laboratory experience.

MATERIALS NEED TO ARRIVE AT LEAST SEVEN DAYS BEFORE YOUR IN-CLASS LAB

SEVEN DAYS BEFORE YOUR IN-CLASS LAB

2. When cultures arrive, transfer each culture from its container into 100 ml of Alga-Gro medium to grow cultures. Reserve remaining Alga-Gro medium to use in making agar plates. Keep each culture in a separate flask and close with a stopper.



Label all flasks with the date, media type, and bacteria species

You will be growing the cyanobacteria for seven days before your classroom experiment. Growing the bacteria in media produces filaments that are more visible to the naked eye. See the following example:

On 7/6/04, Cyanobacteria were inoculated on an Alga-Gro and Agar plate in Box 3. The cyanobacteria were grown in the Alga-Gro medium from 6/25/04 to 7/6/04 before they were inoculated on the plates on 7/6/04. The box was placed 19 cm from the light source. The left end is where the bacteria were inoculated. Notice the Oscillatoria filament loops that were photographed on 7/13/04.

Preparation cont.

3. Once the cyanobacteria culture is suspended in the medium, place the flask containing the mixture in an incubator containing a light source that is set at 27ºC. If an incubator is not available, place the flasks containing cyanobacteria cultures by a full spectrum light source or window. Cool white fluorescent tubes of 200 to 400 foot candles work best for growing cultures. If bacteria are cultured by a window, tissue paper should be placed on the glass to diffuse the sunlight and keep the temperature from being too hot.[4] The temperature should be at least 22ºC but not greater than 27ºC.

4. Check the flasks filled with cyanobacteria cultures daily to make certain the temperature is between 22ºC-27ºC, the cultures are growing, and the medium is not evaporating. If media is evaporating, add media so that there is 100 ml of liquid in the flask. If the culture is growing rapidly, transfer 50 ml of culture and media into another flask and add 50 ml of Alga-Gro media. Label all flasks with the date, media type, and bacteria species. Remember that freshwater cyanobacteria such as *Lyngbya, Oscillatoria* and *Anabaena* should be cultured in Alga-Gro Freshwater Medium. If you have ordered the marine cyanobacteria, *Spirulina*, it should be cultured in Alga-Gro Seawater Medium.

SEVEN DAYS BEFORE YOUR IN-CLASS LAB

5. One day before the in-class laboratory experience, prepare agar plates for students to inoculate in class.

6. Prepare Alga-Gro Media to mix with agar. For Alga-Gro and agar plates:

   Measure 3.2 grams of agar and add to 400 ml of Alga-Gro medium. (This yields an agar and alga-Gro solution with 8 grams of agar to 1 liter of Alga-Gro.) Place a magnetic stirrer in the flask and place on stir plate. This will yield enough media to fill 40 plates with 10 ml of agar.

7. Autoclave or pressure-cook the solution for 15 minutes at 15 lbs pressure. Keep mixtures at a temperature of 45ºC (113ºF) until ready to pour the agar-medium into the plastic boxes to form plates.

8. Set out 40 clear plastic boxes and a sterile pipette of 10 ml. You are pouring additional plates to allow for classroom calamities. You will need one box for each lab group, as well as a control box for each cyanobacteria species for each row in the experimental set-up. There will be four rows for each experimental set-up, so four additional plates of Alga-Gro are needed for each cyanobacteria species in each experimental set-up. Do not autoclave the plastic boxes or they will melt. Keep agar mixture heated and stirring on stir plate so the agar does not set. Fill each plastic box with 10 ml of agar and media solution. Cover and set aside in a cool, dark place for use in lab tomorrow.

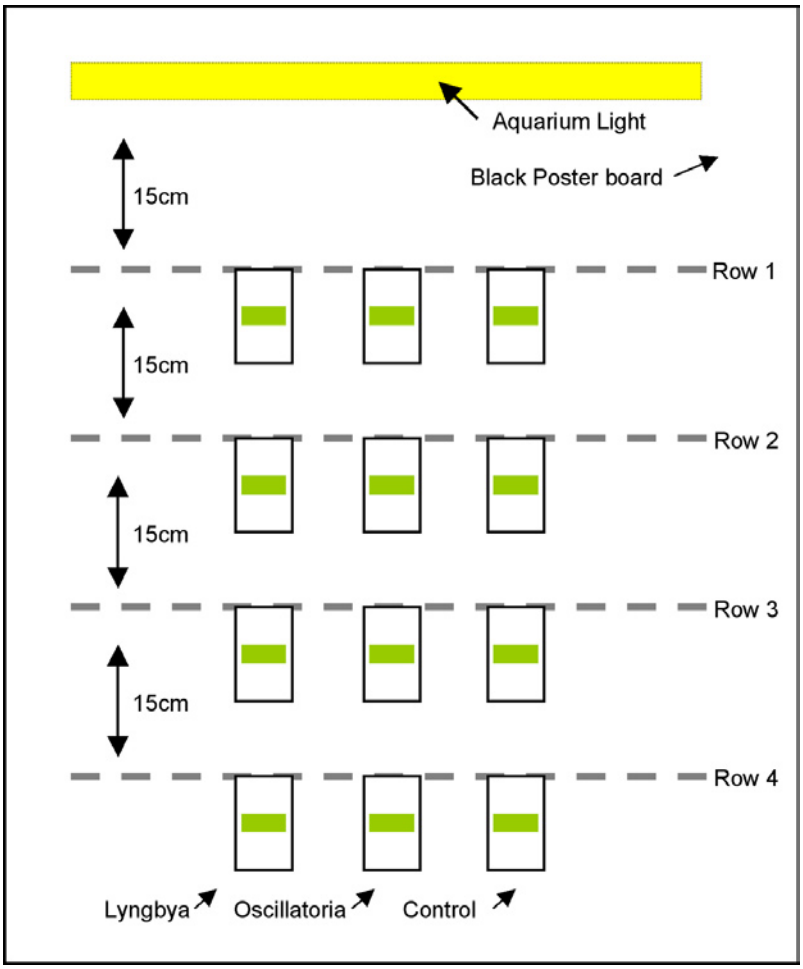
CAN BE DONE IN ADVANCE OF DAY NINE:

[4] For more information, read: N/A. Culturing Algae. Burlington, N.C.: Carolina Biological Supply Company, 1978.

Preparation cont.

9. Prepare one light set-up for each class period. Take an aquarium light with a close to full-spectrum light tube and duct tape it at the end of the table. Take one sheet of stiff black poster board approximately 71 cm X 60 cm (the stiff kind with the foam in-between works best) and divide it into four sections. Starting with the first section 15 cm from the light source, draw a line horizontally across the poster board and label it Row 1. Measure 15 cm from the Row 1 line and draw another line horizontally across the paper. Label this line Row 2. Measure 15 cm from the Row 2 line and draw a line horizontally across the paper. Label this line Row 3. Finally, measure 15 cm from the Row 3 line, draw a line horizontally across the paper and label it Row 4.

See Diagram below: [5]



Place the poster board on a flat surface, with the labeled rows showing. The end closest to the Row One label (This is the top of the board.) should be placed as close to the light source as possible. Secure the board to the table with duct tape. Take two small clear boxes and set them at the top far corners of the poster board. These boxes will hold up the poster board enough to let the light through. Place the poster board on top of the boxes. At the opposite end of the top sheet of poster board, use duct tape to secure it to the table. Students will place their inoculated agar plates at different intervals on the rows on the first sheet of poster board, and then use the top sheet to cover the plates so extra light does not change the results of the experiment.

[5] Diagram courtesy of Heather Hunsperger

Preparation cont.

10. Obtain the following materials for each laboratory group:

- Agar plate (Lab groups will be given one plate)
- A species of cyanobacteria to inoculate on the plate
- Pipette
- Inoculating loop
- Metric ruler with millimeter measurements
- Black electrical tape (enough to go around three sides of the width of the box)
- Black paper to cover the top and bottom of the box
- Masking tape to label experiment
- Scissors
- One fine-tip permanent marker

Classroom Lab Procedure

1. Student lab groups should include 2-4 students.
2. Give the background information on the experiment.

Background

Cyanobacteria living in microbial mats can move, both up and down, and sideways. They may orient themselves, and find the optimal depth at which to live, using chemical, light or gravity clues[6]. In this experiment, you will expose cultures of freshwater cyanobacteria to a directional light source, measuring their movement toward this light source with a ruler and recording measurements. Even though cyanobacteria are oriented vertically in a microbial mat, horizontal placement reduces variables in the experiment. When cyanobacteria are flat, they have equal access to carbon dioxide, oxygen and nutrient agar making light the only variable. Through analyzing cyanobacteria motility data, you will determine how cyanobacteria respond to light clues. The structures produced by the movement of cyanobacteria can be preserved in fossil records. Studying cyanobacteria movement and cultures can help scientists interpret those fossil records. By studying fossil records of cyanobacteria motility on earth, scientists are better able to identify fossil records in their search for life in the universe and beyond.

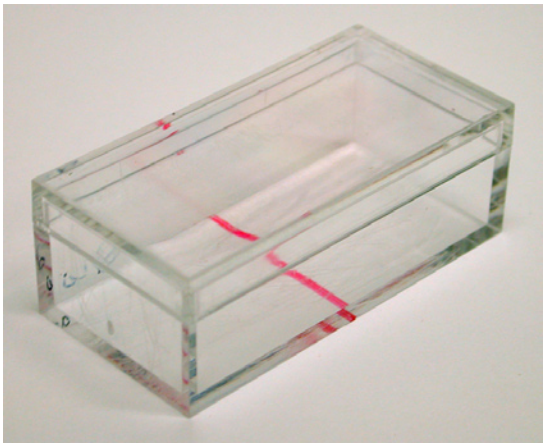
3. Discuss possible outcomes of the experiment with the class.
   - What do you think that the cyanobacteria will do in general?
   - How do you think the distance from the light source corresponds to cyanobacteria motility?
   - What do you think causes your proposed outcome?

4. Have students formulate a hypothesis and write it in their lab notebooks. The hypothesis should be a prediction of the results of the experiment and should be testable. Sometimes an "if…then" statement is a good format for creating a hypothesis.

[6] Bebout, Brad. "Cyanobacterial Motility Experiment." Microbial Mat Education Page. NASA. 1 April 2004. http://microbes.arc.nasa.gov/cyanobacterialmotility.cfm

Classroom Lab Procedure cont.

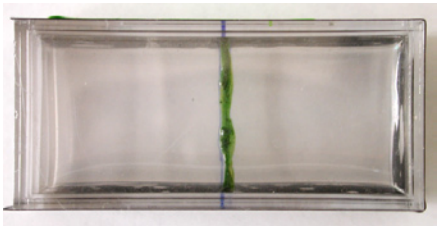
5. Have students take a fine-tip permanent marker and ruler and draw a line across the bottom width of the box, dividing it into two equal sections. See diagram right:



This line will be used as a guide to inoculate the cyanobacteria. Warn students not to open the plates or mutilate the agar when turning the box upside down.

6. If dissecting microscopes are available, have students look for any signs of bacterial or other microbial life on the agar plate. Have students record their initial observations.

7. Model how to inoculate the plate. Remove lids from the plates. Students will be placing a line of cyanobacteria in the middle of the plate on top of the line that they have drawn. The line of bacteria that the student is inoculating needs to be as close to the middle of the box as possible and not be more than 2 mm thick.
See example:



Note that the culture was inoculated on a line drawn in the middle of the bottom of the box. The other areas of the box are kept free of culture at the start, so that motility can be observed.

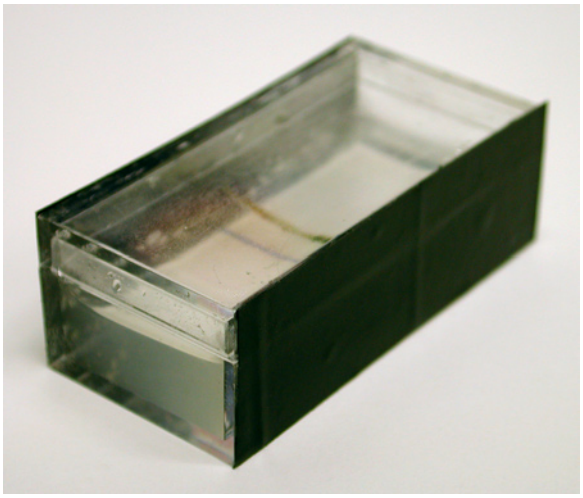
8. Plates need to be inoculated and placed on the Rows in the following manner:
   - For the group conducting Part 1: Place *Oscillatoria* plate on Row 1
   - For the group conducting Part 2: Place *Oscillatoria* plate on Row 2
   - For the group conducting Part 3: Place *Oscillatoria* plate on Row 3
   - For the group conducting Part 4: Place *Oscillatoria* plate on Row 4
   - For the group conducting Part 5: Place *Lyngbya* plate on Row 1
   - For the group conducting Part 6: Place *Lyngbya* plate on Row 2
   - For the group conducting Part 7: Place *Lyngbya* plate on Row 3
   - For the group conducting Part 8: Place *Lyngbya* plate on Row 4
   - For the group conducting Part 9: Place *Oscillatoria* Control plate on Row 1
   - For the group conducting Part 10: Place *Oscillatoria* Control plate on Row 2
   - For the group conducting Part 11: Place *Oscillatoria* Control plate on Row 3
   - For the group conducting Part 12: Place *Oscillatoria* Control plate on Row 4
   - For the group conducting Part 13: Place *Lyngbya* Control plate on Row 1
   - For the group conducting Part 14: Place *Lyngbya* Control plate on Row 2
   - For the group conducting Part 15: Place *Lyngbya* Control plate on Row 3
   - For the group conducting Part 16: Place *Lyngbya* Control plate on Row 4

If there are additional lab groups, additional plates cultured with Lygnbya and Oscillatoria can be placed on each row. If Anabaena and Spirulina cultures are used, one plate of each culture must be placed on each row.

The control plates will have black electrical tape on all four sides. One *Lyngbya* Control plate and one *Oscillatoria* Control plate will be placed on each row in the experimental set-up.

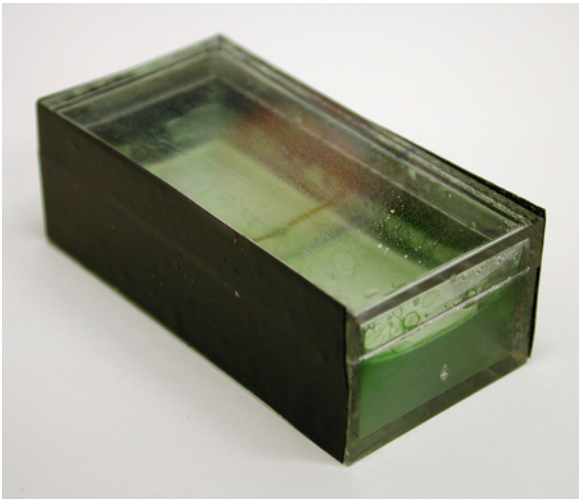
Classroom Lab Procedure cont.

9. Once students have inoculated their cultures, have them cover the boxes with the lids. Tape around the two length sides of the box with black electrical tape.



The two ends need to be open so light can enter the plate and flow to the plate behind it when placed in the experimental light box.

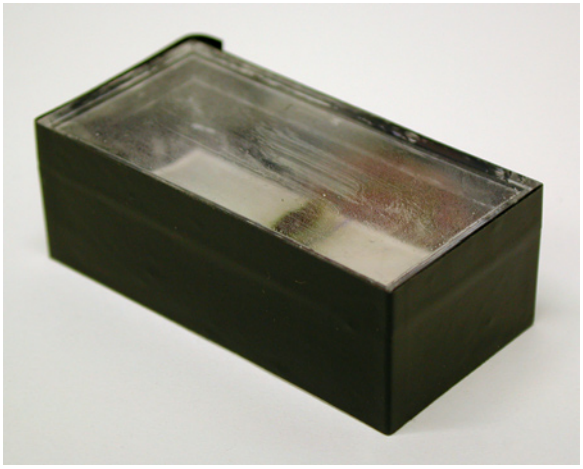
If the box is to be placed on row four, three sides of the box need to be taped to block ambient light flow. The open side will face the light. See example:



Mark the date, group name, time, and type of media and culture on masking tape and place on the side of the box. An arrow needs to be made showing the direction the cyanobacteria should be migrating toward the light. The arrow should point away from the inoculation line and toward the end of the box that is facing the light source. The masking tape should only be placed over black electrical tape.

Classroom Lab Procedure cont.

10. Student groups that are inoculating control boxes need to cover the edges of the box on all sides with black electrical tape. See example:



Control box with all four sides covered in black electrical tape.

Students should mark the date, group name, time, and type of media and culture on masking tape and place on the side of the box. An arrow needs to be made showing the direction the cyanobacteria should be migrating toward the light. The arrow should point away from the inoculation line and toward the end of the box that is facing the light source. The masking tape should only be placed over black electrical tape.

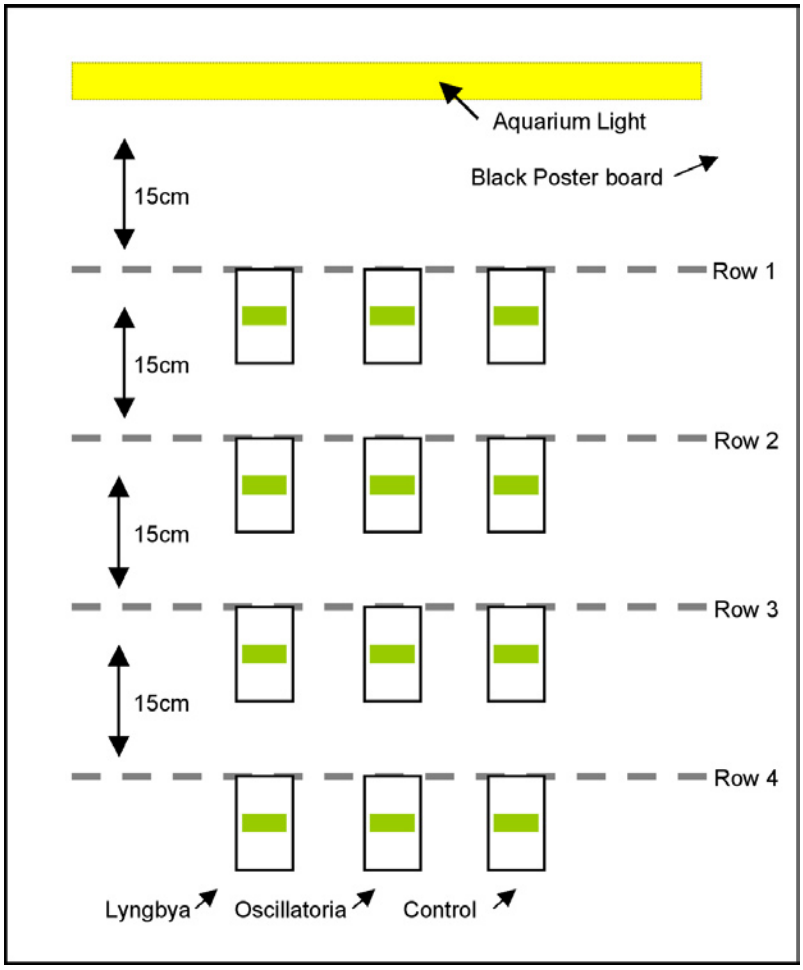
11. Control boxes for each medium placed on each row will allow students to compare the difference in motility between the cyanobacteria in the boxes exposed to the light and the cyanobacteria that were not exposed to any light.

Student boxes need to be placed in the experimental set-up in the following locations:

- For the group conducting Part 1: Place *Oscillatoria* plate on Row 1
- For the group conducting Part 2: Place *Oscillatoria* plate on Row 2
- For the group conducting Part 3: Place *Oscillatoria* plate on Row 3
- For the group conducting Part 4: Place *Oscillatoria* plate on Row 4
- For the group conducting Part 5: Place *Lyngbya* plate on Row 1
- For the group conducting Part 6: Place *Lyngbya* plate on Row 2
- For the group conducting Part 7: Place *Lyngbya* plate on Row 3
- For the group conducting Part 8: Place *Lyngbya* plate on Row 4
- For the group conducting Part 9: Place *Oscillatoria* Control plate on Row 1
- For the group conducting Part 10: Place *Oscillatoria* Control plate on Row 2
- For the group conducting Part 11: Place *Oscillatoria* Control plate on Row 3
- For the group conducting Part 12: Place *Oscillatoria* Control plate on Row 4
- For the group conducting Part 13: Place *Lyngbya* Control plate on Row 1
- For the group conducting Part 14: Place *Lyngbya* Control plate on Row 2
- For the group conducting Part 15: Place *Lyngbya* Control plate on Row 3
- For the group conducting Part 16: Place *Lyngbya* Control plate on Row 4

Classroom Lab Procedure cont.

The boxes should be oriented so that one end of the box is facing the light and is touching the line. Make certain that the end of the box that faces the light source corresponds to the direction that the arrow indicates on the masking tape in the box. See diagram:



12. Turn on aquarium light and cover with the second sheet of black poster paper.

Daily Observations

The number of days needed to take motility measurements varies according to the conditions that the cyanobacteria are under. Motility measurements may be taken from three to five days after inoculation and placement in the experimental set-up depending on the speed at which the cyanobacteria move.

Classroom Lab Procedure cont.

Daily Observation Procedures

1. If dissecting microscopes are available, have students view and measure cyanobacteria motility under a dissecting microscope. If microscopes are not available, use a hand-lens. Large *Oscillatoria* sp. and *Lyngbya* sp. filaments should be visible with the naked eye.

2. Have students record measurements toward the light and away from the light each day in their lab notebook for their plate. Use the inoculation line as zero when measuring movement in millimeters.

3. Students should record observations about the appearance of the filaments. Any changes in the direction of movement should also be recorded in the lab journal. Drawings and diagrams can be made to illustrate observations.

4. When students complete recording measurements in their lab notebook, have them record measurement on a class data sheet.

5. Daily discussion can include:
   - Did the cyanobacteria respond to light?
   - Did the different species respond in the same way?
   - How far did the cyanobacteria move toward the light?
   - How far did the cyanobacteria move away from the light?
   - Is your species of cyanobacteria moving at the same speed as it did on previous days?
   - Compare your cyanobacteria species with another species on your row, which were faster or slower?

Conclusion of Experiment and Analysis of Data

(Bar Graphing Activity to be completed when final data measurements have been recorded.)

1. Students graph the data they collected for their plate from their science experiments on cyanobacteria motility in the following ways:
   a. Create a line graph plotting the daily movements of cyanobacteria over time. One line should be made connecting the measurements of movement toward the light. Another line should be made connecting the measurements of movement away from the light. Be certain that the y-axis (distance) does not start at zero so that movement away from the light can be marked in negative numbers. See the graphs for the Cyanobacteria Motility Experiment performed at Ames Research Center on the Microbes @ NASA website in the For Educators section. This page can be accessed at: http://microbes.arc.nasa.gov.

   Note: Students need to graph their data on the same scale to compare data. If the graphs are made on overhead transparencies, graphs can be overlaid to compare results and analyze relationships between experimental groups.

   b. Create a summary bar graph for the total movement cyanobacteria made toward and away from the light. Handout the Rubric: Cyanobacteria Motility Experiment for guidelines. For each plate:
   - Average the total distance the cyanobacteria made toward the light
   - Average the total distance the cyanobacteria made away from the light.

Conclusion of Experiment and Analysis of Data cont.

c. On a class bar graph, plot the distance each culture of bacteria moved. Again, the y-axis does not start with zero, but allows negative numbers to be plotted that signify movement away from the light. Label each bar with the plate number and Row number. Use separate colors to distinguish movement away from the light and toward the light. See the graphs for the Cyanobacteria Motility Experiment performed at Ames Research Center on the Microbes @ NASA website in the For Educators section. This page can be accessed through the Microbial Ecology/Biogeochemistry Research Laboratory at http://microbes.arc.nasa.gov

2. Using the data displayed in the graphs, ask the following questions at the conclusion of the lab:
   - Did the cyanobacteria respond to light?
   - Did the different species respond in the same way?
   - Did they move at the same speed over the course of the experiment?
   - Which were faster or slower?
   - After looking at your graph, what questions do you have for further research?
   - Why do you think the cyanobacteria responded in the way that they did?

   Possible explanations may include the following. Research by Niels B. Ramsing and Lee Prufert-Bebout reported in *Motility of Microcoleus chthonoplastes subjected to different light intensities quantified by digital image analysis*(190), suggests several potential strategies *Microcoleus* use to find optimal light conditions including speed, direction of movement, and frequency of movement. If light conditions are favorable for *Microcoleus*, movement is very slight and they move less often. In favorable light conditions, the speed of *Microcoleus* is slower. *Microcoleus* change direction by curling and bending and reverse direction of movement more when conditional are favorable. However, factors other than light may be responsible for movement.[7] Nutrient variability in the agar medium may be another reason cyanobacteria move.

3. Have students complete a written summary interpreting their bar graph. Use the Rubric: "Cyanobacteria Motility Experiment" for guidelines in completing the summary.

4. Compare student experiment data to data from Baja Stromatolite Cyanobacteria on the web site for the Cyanobacterial Motility Experiment.

Questions

1. Did the Baja cyanobacteria have a different response to light than our classroom cultures?

2. Which were faster or slower?

3. What other differences or similarities do you notice between the Baja Cyanobacteria Motility Experiment and our classroom experiment?

[7] Ramsing, N.B. and Prufert-Bebout, L. (1994) *Motility of Microcoleus chthonoplastes* subjected to different light intensities quantified by digital image analysis. In: Microbial Mats: Structure, Development and Environmental Significance, edited by L.J. Stal and P. Caumette, Springer-Verlag, New York, pp. 183-191.

Follow-up lab questions

1. What do you know about cyanobacteria motility?
2. What other questions do you have about factors affecting cyanobacteria motility?
3. What do you want to learn?
4. How would you find it out? (What factors would you consider when designing your experiment?)

Evaluation

Use the Rubric for the Cyanobacteria Motility Experiment (found in the Math section) to assess student graphs and graph interpretation summaries.

Credits

Lesson Plan:

Art, Graphic Design & Layout: C. Triano & T. Esposito, TopSpin Design Works