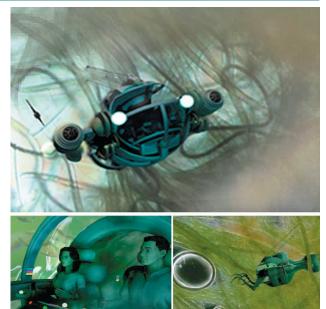
A Teacher's Guide to Stromatolite Explorer

Investigation of a Microbial Mat Community

Robin Bucaria, Dartmouth Middle School Dr. Brad Bebout, NASA Ames Research Center

In this episode, the *Stromatolite Explorer* dives into a living microbial mat less than 5 millimeters thick. As the crew ventures deeper in the mat, the vehicle looses power and lacks solar radiation to recharge the battery. It is only through applying their knowledge of the daily cycles of the bacteria in the mat, that the crew is able to make it back to the surface. During the mission, the crew investigates the organisms that live in the mat and take chemical readings as they descend. The mission details life forms, biogeochemical cycles, and NASA's interest in microbial mats.

The Stromatolite Explorer video describes an imaginary voyage inside a microbial mat. This Teacher's guide contains a more detailed explanation of a number of the concepts discussed in the video, including the graphs presented in the video. Download the Stromatolite Explorer video from http://learn.arc.nasa.gov/microbe.



Contents

- 1. Standards and Objectives
- 2. How to use the movie
- 3. Microbial mat organisms and structures
- 4. Microbial Mat Chemistry
- 5. Learning Activities
- 6. Glossary of Terms
- 7. Discussion Questions
- 8. Follow-up Activities

1. Standards and Objectives:

National Education Standards:

| Fully Met | Partially Met | Addressed |
|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
| NSES C6(5-8): Regulation and Behavior a, b NSES C7(5-8): Populations and Ecosystems a, b, c | NSES C4(5-8): Structure and Function in Living Systems a, b NSES C6(5-8): Regulation and Behavior c, d NSES D4(5-8): Structure of the Earth System h, k | NSES D6(5-8): Earth in the Solar System d |
| 2061: 5A(6-8) #1, #5 2061: 5D(6-8) #2 2061: 5E(6-8) #2 | 2061: 4E (6-8) #2, #4 2061: 5D(6-8) #1 2061: 5E(6-8) #1, #3 | 2061: 5D(6-8) #2 2061: 5D(6-8) #1 |

California Science Standards:

| Fully Met | Partially Met | Addressed |
|---------------------------|--------------------------------------------------------------|---------------------------------------------------------------------------|
| Grade 6: Ecology #5: a, b | Grade 6: Ecology #5: c Grade 7: Earth & Life History #4 a | Grade 7: Cell Biology #1: a Grade 8: Chemistry of Living Systems #6 |

Objectives:

At the end of the movie and discussion, the student will:

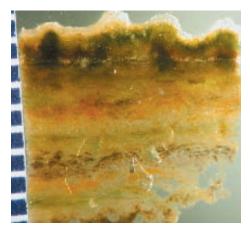
- understand that a microbial mat is composed of layers of different microbes.
- 2 understand that these biological communities have been around over 3 billion years and contain some of the earliest forms of life.
- 3. learn that diatoms convert sunlight and carbon dioxide into fuel molecules and oxygen through the process of photosynthesis.
- 4. learn that cyanobacteria are singled celled organisms without an enclosed nucleus that also conduct oxygenic photosynthesis.
- 5. learn that other forms of bacteria, such as purple sulfur bacteria, conduct photosynthesis that doesn't produce oxygen: anoxygenic photosynthesis.
- 6. know that a microbial mat is a self sustaining ecosystem where one member of the community produces a gas or sugar that another member of the community needs to obtain energy.
- discover that the chemical concentrations of oxygen and other substances change within the layers of the mat (a chemical gradient) due to the amount of light at that level, the community of producers at that layer, and the raw materials (sugar, chemicals, and gases), available at that level.
- 8. learn how NASA uses information about microbial mats to find life on other worlds.

2. How to Use the Movie

- I. Introduce mat organisms and biological structures using the material in this guide.
- 2. Introduce vocabulary from the Glossary of Terms included in this guide.
- 3. Select learning activities to use either before or after the viewing the movie.
- 4. Play the Stromatolite Explorer movie through once uninterrupted for the students to gain an overview of a microbial mat.
- 5. Play the *Stromatolite Explorer* movie again, stopping the film and making explanatory comments. Transparencies of the annotated graphs can be made to use with the class.
- 6. Discuss the film using the questions for discussion included in this guide.
 - Complete desired follow up activities listed in this guide that are available at http://learn.arc.nasa.gov/microbe

3. Microbial Mat Organisms and Structures:

What are Microbial Mats?



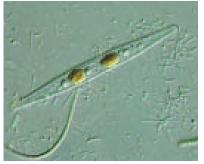
Have you ever noticed green spongy areas by water fountains, drain spouts or gutters? The green areas where water collects are biofilms created by bacteria. Allow the bacteria in those

biofilms to grow for longer periods of time and a microbial mat will form. A microbial mat is composed of layers of different microbes, working together in a self-sustaining ecosystem. Color, smell and location help to identify microbial mats. The top layer of a mat often contains blue green algae, or cyanobacteria, as well as diatoms. White, purple, and black appear can appear in the lower layers of a mat. Because they are attractive as food for animals, mats live in areas that are least attractive to other forms of life. Hot areas, salty areas, dry areas, cold areas and places that are sheltered from animal foragers are the locations where microbial mats grow. It wasn't always that way. Before grazing animals appeared on Earth, about 500 million years ago, microbial mats were far more common, even dominated the biosphere of the early Earth.

A microbial mat is a complex food web. Like all other life forms, each member of this food web requires energy. For photosynthetic microbial mats, energy enters the upper layers of the ecosystem as light, which is captured by the cyanobacteria and diatoms with photosynthesis. Other microbes living in this ecosystem sustain life by using the gases, sugars, and organic matter produced in these upper layers to gain energy. Each member of the community produces something that allows another member of the community to extract energy from the environment. Think of it this way: A microbial mat is like an apartment building, with different microorganisms living on different floors of the building. In the penthouse suit, closest to the sun, are the cyanobacteria. They take the carbon dioxide and produce oxygen and sugars used by themselves and other members of the mat community. On the next floor, other microbes take the sugar and other byproducts for their food. By borrowing that "cup of sugar" from the microbes on the floor above, they gain the energy they need and produce another form of that sugar for the surrounding microbes, which in turn, use these materials and produce yet another form of sugar for other microbes. The chemicals, gasses and sugars that one microbe does not need may be used by another microbe within the community.

What are Diatoms?

Diatoms are one-celled algae with a covering, known as a frustule, of silica (or glass) that makes up their cell wall. Dia-



toms are eukaryotic; they have a membrane-bound nucleus. Notice the nucleus, chloroplasts and other separate organelles inside the cell wall. Diatoms are autotrophs and manufacture their

own food using the sun's energy and water in a process called photosynthesis.

What are Cyanobacteria?

Cyanobacteria is the name giving to a group of bacteria that perform oxygen producing photosynthesis. All cyanobacteria are unicellular but some grow in colonies or filaments, often surrounded by a gelatinous sheath. Cyanobacteria, as with other bacteria, are prokaryotes. Their genetic material is not contained in a nucleus in the cell. While cyanobacteria were once mistaken for algae, they are actually prokaryotic organisms (they have no membrane –bound nucleus). Through the process of photosynthesis, cyanobacteria changed Earth's early atmosphere, creating the oxygen rich environment we know today. Cyanobacteria fossils have been found that are more that 3.5 billion years old. Microcoleus is a filamentus cyanobacteria that produces



food through the process of photosynthesis. In the picture at right, it can be seen that many *Microcoleus* trichomes are enclosed in a common sheath, giving rise to a very characteristic "bundle" appearance. *Mi*-

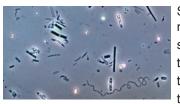
crocoleus is very common in microbial mats from hypersaline environments, and in soils.

Oscillatoria is a type of filamentous cyanobacteria with taper-



ing, unbranched filaments (trichomes), often with a rounded cell. *Oscillatoria* glide by wave movements of tiny fibers. *Oscillatoria* can be found in salt marshes, hot springs, in damp soil, in fresh water and in the sea.

What are Sulfate Reducing Bacteria?



Sulfate reducing bacteria reduce sulfate to hydrogen sulfide. They are bacteria that use sulfate as an electron acceptor (see respiration) to derive energy from

organic matter. They are generally classified as anaerobes because most SRB cannot exist where oxygen is present.

What are Colorless Sulfur Bacteria?



Colorless sulfur bacteria use hydrogen sulfide and oxygen as a kind of battery to synthesize sugars. Because the place where they can get

both oxygen and hydrogen sulfide moves on a daily cycle, they must migrate, too.

What else lives in the mat?

Various protists and other organisms can live in the mat. In the movie, a nematode wiggles through the mat.

Nematode



A nematode is a worm that lives in soil, water or in plants or animals. Nematods can be less than a millimeter in length and range to several meters in length.



Gloecapsa is a unicellular cyano-bacteria that have groups of cells held together by a muscilaginous sheath.

What are Purple Sulfur Bacteria?



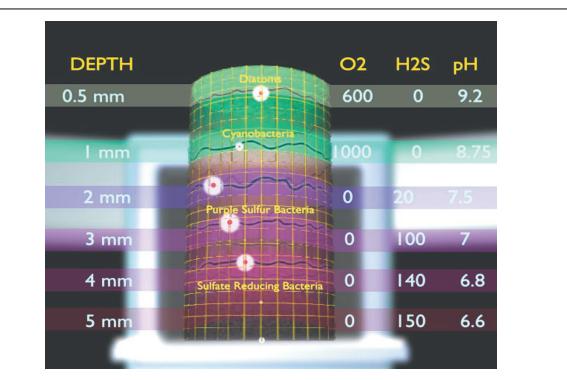
Purple sulfur bacteria use infrared radiation and hydrogen sulfide (the rotten egg smell found in swamps, sulfur springs or stagnant water) to perform anoxygen-

ic photosynthesis, a kind of photosynthesis that doesn't produce oxygen. In doing so, they produce granules of elemental sulfur.

4. Microbial Mat Chemistry

The chemical composition changes within the mat depending on the amount and wavelength of light penetrating the mat. Different types of bacteria live at different layers of the mat, depending on the conditions available to provide the elements needed to conduct the processes needed to survive. In fact, some bacteria move to find optimum conditions. Notice in the annotated graphs that follow how concentrations of oxygen and hydrogen sulfide change with depth, as well as how pH changes. Light and dark conditions are both annotated.

4:22 in the movie: The mat though relatively thin, shows a steep chemistry gradient from top to bottom. The explorer has descended through layers composed predominantly of diatoms, and then cyanobacteria, of purple sulfur bacteria, and finally, sulfate reducing bacteria.



Chemistry Gradient

Notice the variation in the concentration of oxygen and hydrogen sulfide in the mat. This chemistry gradient changes according to depth in the microbial mat. In the first millimeter of the mat, where diatoms and cyanobacteria live, the concentration of oxygen is highest because it is produced by photosynthesis (from 600 μ mol/L at the top of the mat to 1000 μ mol/L at a depth of one millimeter). At a depth of two millimeters the organisms have consumed all of this oxygen, and hydrogen sulfide appears (20 μ mol/L). Hydrogen sulfide is produced from sulfate by sulfate reducing bacteria that use the sulfate to break down organic matter. Purple sulfur bacteria living at a depth of 2-3 millimeters in the mat use that hydrogen sulfide and near infrared radiation ("light") to conduct anoxygenic photosynthesis. Notice that the concentration of hydrogen sulfide is at 150 μ mol/L five millimeters deep in the mat. At that depth, there is no infrared radiation, and the hydrogen sulfide builds up. Because bicarbonate and CO₂ are taken out of the system by photosynthesis, pH increases.

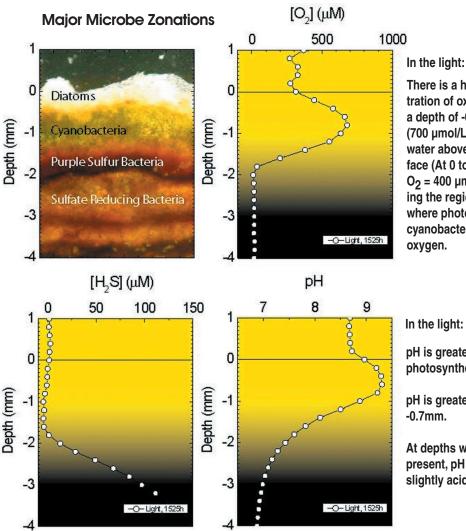
Light Version

(between 4:24-4:37 in the movie) 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.

In the light:

Hydrogen sulfide (H₂S) concentrations increase at a depth of -1.9mm in the mat.

The greatest concentrations of hydrogen sulfide (H₂S)occur at depths where the least amount of light penetrates the mat (-3 mm produces 120 µmol/L of H₂S). At these depths, sulfate reducing bacteria use sulfate to break down organic matter.



There is a higher concentration of oxygen (O₂) at a depth of -0.9 millimeters (700 µmol/L) than in the water above the mat surface (At 0 to 1 millimeter, O₂ = 400 µmol/L) indicating the region of the mat where photosynthesis by cyanobacteria produces

pH is greatest (9.4) where photosynthesis occurs.

pH is greatest at a depth of

At depths where H₂S is present, pH is neutral to slightly acidic.

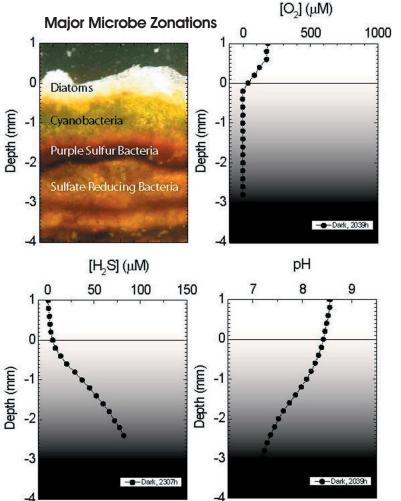
Dark Version

(5:28-5:37 in the movie) 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.

In the dark:

Hydrogen sulfide (H_2S) production occurs closer to the surface of the mat from depths of -0.2mm to -2.5mm).

Organisms using hydrogen sulfide and oxygen move closer to the surface of the mat to get the oxygen they need.



In the dark:

The oxygen concentration level decreases indicating consumption of oxygen through the process of respiration.

Straight lines at zero concentration of O_2 show anoxic areas in the mat. Anoxic areas contain zero oxygen.

In the dark:

pH levels are lower at the top of the mat then when oxygen is being produced (pH 8.5 in the dark instead of 9.45 in the light)

The top –2 mm of the mat are still basic.

5. Learning Activities

Locate Microbial Mats

A microbial mat is formed when different layers of microbes interact to form an ecosystem. The different organisms and chemicals produced by these organisms create a rainbow effect if you look at a cross section of the layers. Because microbial mats are food for animals, they are located in areas where other forms of life cannot survive to eat them. Check high saline areas (salt marshes, intertidal zone, saline lakes and ponds), high temperature areas (hot springs), areas where there is stagnant water, areas of decomposition of organic matter (corn silage in Kansas). Mats can form in sand, dirt, in water and in rocks. Use your sense of smell to locate the rotten egg stench from the sulfur produced by mats. Cut cross sections of thick slime, sand, and rocks to check for the colorful layers that compose a microbial mat.



A microbial mat formed in the sand.



Mats grow where predators cannot eat them.



A mat found on a barrier island in North Carolina.

And area to the summer

A mat growing inside gypsum.

Look at mat organisms under a microscope.

Many forms of Cyanobacteria can be ordered from a biological supply company for students to observe under a microscope. *Gloecapsa, Lyngbya, Oscillatoria,* and *Phormidium* are some of the freshwater cyanobacteria that are available. *Spirulina* is one marine variety that is readily available. Have students

observe the different types of bacteria under a microscope and draw their cell structure. How do they move? What do they look like?

Samples from mats that have been found in the field may also be observed with a microscope.

6. Glossary of Terms

Alkali environment: A high pH (basic) environment, often high in salts.

- **Anoxygenic photosynthesis:** Photosynthesis that does not produce oxygen as a byproduct. Hydrogen sulfide is used as a source of electrons in the same way that water is used as a source of electrons in "normal" (oxygenic) photosynthesis.
- Biosignature: A measurable chemical and/or physical phenomenon that indicates the presence of life.
- **Chemistry gradient:** The variation of the concentration of a chemical substance in a solution through some linear path, usually depth in the case of a microbial mat.
- Cyanobacteria: a single celled bacterium capable of photosynthesis.
- Diatom: A one-celled algae with a covering, known as a frustule, of silica, or glass.
- Filamentous bacteria: An elongated bacterial cell or a chain-like series of cells of bacteria
- <u>H₂S (Hydrogen sulfide)</u>: A colorless, flammable, poisonous gas that has a characteristic rotten-egg odor. Hydrogen sulfide is produced by sulfate reducing bacteria.
- **Microbial Mat**: Microbial mats are layered communities of microorganisms that are only a few millimeters thick. Many biogeochemical processes are conducted by the organisms in the mat to sustain life. The mat can acquire nitrogen through the process of nitrogen fixation. The mat community is a fully functioning ecosystem of producers and consumers. The upper layers of the mat contain cyanobacteria that create their own food though the process of photosynthesis. Light energy is used to split hydrogen molecules from water and hook them onto carbon dioxide molecules. Through photosynthesis, they create organic compounds, such as sugar, needed for their life, as well as produce oxygen. Below this layer, other bacteria that are tolerant of oxygen, take the sugars and oxygen to create energy to survive and produce other substances necessary for the life of other organisms in the mat. At the bottom layer of the mat, where oxygen does not penetrate, live bacteria that cannot survive in the presence of oxygen. They, in turn produce other chemicals necessary for life in the ecosystem.
- Mineral: A naturally occurring, homogeneous inorganic solid substance having a definite chemical composition and characteristic crystalline structure, color, and hardness.¹
- Organic matter: Matter that is derived from living organisms.
- **Oxygenic photosynthesis:** Photosynthesis that produces oxygen as a byproduct.
- **Prokaryote:** A one-celled organism which does not have a nucleus or organelles enclosed by a membrane. Bacteria and cyanobacteria are examples of prokaryotes.
- Photosynthesis: The process of using light as an energy source to convert carbon dioxide and water to carbohydrates.
- **Purple sulfur bacteria:** A bacterium that uses infrared radiation and hydrogen sulfide to perform the kind of photosynthesis that doesn't produce oxygen.
- **Respiration:** Respiration is the process of releasing energy from organic matter. Electrons obtained from the organic matter, are transferred through an electron transport chain, to an electron acceptor. In the most familiar form or respiration (our own), electrons are passed from sugars (what our food is broken down to in our bodies) to oxygen. Microbes are capable of using a wide range of electron acceptors instead of, or in addition to, oxygen. Aerobic respiration is the release of energy from an organic substance in the presence of oxygen. The oxygen is used to accept electrons from the organic matter. Anaerobic respiration also releases energy from an organic substance, however; this energy release occurs when oxygen is not present, other substances, such as nitrate and sulfate are used to accept the electrons form organic matter.
- Stromatolite: Rock-like layers of sand or minerals produced by microorganisms trapping, binding or precipitating minerals.
- **Sulfate reducing bacteria:** Bacteria that use sulfate as an electron acceptor (see respiration) to derive energy from organic matter.

¹The American Heritage® Dictionary of the English Language4th ed., s.v. "mineral," <u>http://dictionary.reference.com/search?</u> <u>q=mineral</u> (accessed August 5, 2005).

7. Discussion Questions

Question: What is a microbial mat?

Answer: A microbial mat is a community of microorganisms that is only a few millimeters thick. Within the community, each organism produces chemical compounds, (gases, sugars or other organic substances) used by other members of the community to gain energy. Different types of microbes are located at different depths within the mat, based on their own chemical and light requirements. This creates the distinctive layers appearance of mats. If one slices a mat and looks at it from the side, it can be seen that the upper brown layer contains diatoms, cyanobacteria conducting photosynthesis compose the green layer, and the pink layer, contains purple sulfur bacteria. The dark areas, where oxygen is not present, is inhabited by fermenters, and sulfate reducing bacteria. Colorless sulfur bacteria are always at the interface of the oxygen containing, and anoxic layers. When light and chemical conditions change, at night for example, colorless sulfur bacteria move to the top of the mat, since oxygen is not present in the lower layers.

Question: What is a Stromatolite?

Answer: A stromatolite is a mineralized microbial mat that can form in several ways. The sheaths of the filamentous cyanobacteria, such as *Microcoleus* and *Oscillatoria* are sticky. As the cyanobacteria move through sand or soil, their sheaths can trap and bind minerals together. Over time, a stromatolite will form. Precipitation of minerals by the chemical processes of the bacteria in the mat can also turn it into a stromatolite.

Question: Why are microbial mats important to NASA?

Answer: Microbial mats are important to NASA for several reasons. First, mats help scientists understand what life was like on early Earth because they have literally been around almost that long. By studying microbial mats, scientists can learn more about how life on earth evolved, including how our atmosphere formed. Studying the production of trace gases by communities of microorganisms may help us to discover life on other planets, if those planets contain similar kinds of organisms. Secondly, if the remains of microbial mats were found on another planet, (remember the characteristic layered appearance of mats) they would indicate that life was once on the planet. Finally, the small size of these ecosystems makes them attractive to send into space to help us understand how the space environment affects life; all of the biochemical processes found in larger earth ecosystems occur in mats but within the space of a few millimeters.

Question: What are some of the most ancient life forms?

Answer: The microbes that compose a microbial mat such as cyanobacteria, purple sulfur bacteria, sulfate reducing bacteria, sulfur bacteria, etc. are some of the most ancient life forms. Microbial mats have existed for over three billion years.

Question: What role did microbial mats play in the development of our planet?

Answer: The biogeochemical processes in microbial mats changed the composition of the gases in our atmosphere. The atmosphere is a mixture of nitrogen, oxygen, and trace gases, the oxygen was added mostly by mats. Remember the photosynthesis within diatoms and cyanobacteria creates oxygen, one of the main gases that compose our atmosphere.

Question: How do the bacteria in the mat make adjustments for changes in light?

Answer: Different bacteria in the mat have different light and chemical requirements. Cyanobacteria use sunlight at the top of the mat to conduct photosynthesis. However, when there is no light present, they respire the sugars that they have made to obtain energy. When oxygen is not present, they can perform fermentation. Therefore, these organisms are very adaptable in adjusting the way they gain energy. Purple sulfur bacteria use red or near infrared radiation, so are located at a place in the mat where they can receive both infrared radiation ("light") and hydrogen sulfide. Other bacteria move in a daily cycle to locate the conditions they need to gain energy.

Remember when the stromatolite explorer ran out of power, it latched onto filamentous bacteria to catch a free ride back to the surface where the solar radiation present would recharge the fuel cell? Those bacteria make that trip every day because they need to follow the oxygen.

Question: How does photosynthesis work in the mat?

Answer: As in plants, which may be more familiar to you, cyanobacteria and diatoms take the energy contained in sunlight and carbon dioxide and turn it into sugars (food) and oxygen. Oxygen is produced as a byproduct of this type of photosynthesis. The sugars become the food for the cyanobacteria or diatom, and some of it is available to other organisms in the mats, as it leaks out of the diatoms and cyanobacteria, or when they die. The photosynthesis equation represents this process:

$6CO_2 + 6H_2O_{----}$ light energy $C_6H_{12}O_6 + 6O_2$

This equation states that six molecules of carbon dioxide are combined with six molecules of water (using light energy) to make one molecule of a simple sugar (glucose) and six molecules of oxygen. Other microbes use different types of light and chemicals to perform photosynthesis that does not produce oxygen.

Question: What type of microbes conduct photosynthesis without producing oxygen?

Answer: Purple sulfur bacteria use red to near infrared radiation and hydrogen sulfide to perform anoxygenic photosynthesis.

8. Follow-up Activities

Interactive Biogeochemical Cycle

A script to have students act out the interactive biogeochemical cycle of a microbial mat is available to download from the For Educators section of the Microbes@NASA website at http://learn.arc.nasa.gov/microbe

Microbial Mat Investigations

A comprehensive microbial mat curriculum including access to a remotely operated Web Lab is located at http://learn.arc.nasa.gov/microbe

Additional questions or comments?

Please contact Dr. Brad Bebout at NASA Ames Research Center at brad.m.bebout@nasa.gov, http://exobiology.arc.nasa.gov/ssx/microecobiogeo/

Credits

Stromatolite Explorer:

Produced by Eric Russell Copyright 2005 BioMedia Associates Art: Bruce Russell, Eric Russell

Teacher's Guide:

Art, Graphic Design & Layout: C. Triano, Boomerang Design Group Photography: Dr. Brad Bebout, Dr. Lee Prufert-Bebout, Mary Hogan, Heather Hunsperger, David J. Patterson

