



Gulf of Mexico Exploration

Biochemistry Detectives

FOCUS

Biochemical clues to energy-obtaining strategies

GRADE LEVEL

9-12 (Chemistry)

FOCUS QUESTION

How can researchers determine energy and nutritional strategies used by organisms in cold-seep communities?

LEARNING OBJECTIVES

Students will be able to explain the process of chemosynthesis.

Students will be able to explain the relevance of chemosynthesis to biological communities in the vicinity of cold seeps.

Students will be able to describe three energy-obtaining strategies used by organisms in cold-seep communities.

Students will be able to interpret analyses of enzyme activity and $\delta^{13}\text{C}$ isotope values to draw inferences about energy-obtaining strategies used by organisms in cold-seep communities.

ADDITIONAL INFORMATION FOR TEACHERS OF DEAF STUDENTS

In addition to the words listed as key words, the following words should be part of the vocabulary list.

Hydrothermal vent
Hydrogen sulfide
Tectonic plate
Chemosynthetic bacteria

Hydrocarbon gases
Continental margins
Polychaete worm
Salinity
Tubeworm
Pogonophora
Tentacles
Hemoglobin
Organism
Organic
Benthic
Pelagic organisms
Hydrocarbons
Decaying
Isotope
Delta values
Enzymes
Inferences
Bivalves

Trophosome tissue

There are no formal signs in American Sign Language for any of these words and many are difficult to lipread. Having the vocabulary list on the board as a reference during the lesson will be extremely helpful. It would be very helpful to copy the vocabulary list and hand it out to the students to read after the lesson. Have the virtual tour of a cold seep community up on the classroom computer or assign it the night before for homework so that the students come in with some background content knowledge.

MATERIALS

- Flip chart, chalk board, or marker board
- Copies of "Cold Seep Organism Analysis Results," one or more sheets for each student group

AUDIO/VISUAL MATERIALS

None

TEACHING TIME

One 45-minute class period

SEATING ARRANGEMENT

Groups of four students

MAXIMUM NUMBER OF STUDENTS

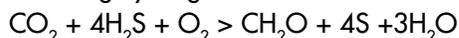
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KEY WORDS

Cold seeps
Methane hydrate ice
Chemosynthesis
Brine pool
Vestimentifera
Trophosome
Detritus
Isotope analysis
 $\delta^{13}\text{C}$
Enzyme analysis

BACKGROUND INFORMATION

One of the major scientific discoveries of the last 100 years is the presence of extensive deep sea communities that do not depend upon sunlight as their primary source of energy. Instead, these communities derive their energy from chemicals through a process called chemosynthesis (in contrast to photosynthesis in which sunlight is the basic energy source). Some chemosynthetic communities have been found near underwater volcanic hot springs called hydrothermal vents, which usually occur along ridges separating the Earth's tectonic plates. Hydrogen sulfide is abundant in the water erupting from hydrothermal vents, and is used by chemosynthetic bacteria that are the base of the vent community food web. These bacteria obtain energy by oxidizing hydrogen sulfide to sulfur:



(carbon dioxide plus hydrogen sulfide plus oxygen yields organic matter, sulfur, and water). Visit <http://www.pmel.noaa.gov/vents/home.html> for more information

and activities on hydrothermal vent communities.

Other deep sea chemosynthetic communities are found in areas where hydrocarbon gases (often methane and hydrogen sulfide) and oil seep out of sediments. These areas, known as cold seeps, are commonly found along continental margins, and (like hydrothermal vents) are home to many species of organisms that have not been found anywhere else on Earth. Typical features of communities that have been studied so far include mounds of frozen crystals of methane and water called methane hydrate ice, that are home to polychaete worms. Brine pools, containing water four times saltier than normal seawater, have also been found. Researchers often find dead fish floating in the brine pool, apparently killed by the high salinity.

As is the case with hydrothermal vents, chemosynthetic bacteria are also the base of the food web in cold seep communities. Bacteria may form thick bacterial mats, or may live in close association with other organisms. One of the most conspicuous associations exists between chemosynthetic bacteria and large tubeworms that belong to the group Vestimentifera (formerly classified within the phylum Pogonophora; recently Pogonophora and Vestimentifera have been included in the phylum Annelida). Pogonophora means "beard bearing," and refers to the fact that many species in this phylum have one or more tentacles at their anterior end. Tentacles of vestimentiferans are bright red because they contain hemoglobin (like our own red blood cells). Vestimentiferans can grow to more than 10 feet long, sometimes in clusters of millions of individuals, and are believed to live for more than 100 years. They do not have a mouth, stomach, or gut. Instead, they have a large organ called a trophosome, that contains chemosynthetic bacteria. Hemoglobin in the tubeworm's blood transports hydrogen sulfide and oxygen to bacteria living in the trophosome. The bacteria produce organic molecules that provide nutrition to the tubeworm. Similar relationships are found in clams and mussels that have chemosynthetic bacteria living

in their gills. A variety of other organisms are also found in cold seep communities, and probably use tubeworms, mussels, and bacterial mats as sources of food. These include snails, eels, sea stars, crabs, isopods, sea cucumbers, and fishes. Specific relationships between these organisms have not been well-studied.

This activity focuses on different energy-obtaining strategies that have been found in organisms living in cold seep communities. There are two basic options for obtaining energy (food) in the deep-sea environment. One is to feed on organic material that originates from photosynthetic organisms living in the upper water column. In benthic (sea bottom) communities, this organic material is present primarily as detritus (bits of dead plant and animal tissue) that settles from the upper water column to the bottom. Some organic material may also be present in the form of pelagic organisms that move vertically through the water column.

The other basic option for obtaining energy (food) is through chemosynthesis. The energy for chemosynthesis may come from a variety of chemical sources. In cold seep communities methane and hydrogen sulfide appear to be the primary energy sources. Chemosynthesis also requires a source of dissolved carbon (CO_2 in the equation above). This carbon may come from the surrounding seawater, or from hydrocarbons (oil and gas) seeping from beneath the sea floor, or from limestone, or from decaying organic matter in the sediment. Knowing the source of carbon and energy that are used by a particular species gives important clues about the structure of food webs and the functioning of biological communities. Researchers often obtain these clues through studies of carbon isotope ratios.

Carbon isotope studies are based on the fact that the amount of the stable carbon isotope ^{13}C varies depending upon the source of the energy and carbon. Isotope content is typically compared with a standard, and the results are expressed as delta values, abbreviated $d(x)$ in parts-per-thousand

(‰; also called “parts-per-mille”). Scientists have found that $\delta^{13}\text{C}$ of carbon in photosynthetically-derived detritus is -18 to -20‰; $\delta^{13}\text{C}$ in carbon derived from seawater is -0‰; $\delta^{13}\text{C}$ in carbon from organisms that feed on methane is -40‰ or less; $\delta^{13}\text{C}$ in carbon from organisms that depend upon sulfur as an energy source is between -30 and -40‰.

Scientists also obtain clues about energy-obtaining strategies from other biochemical studies on tissues from cold-seep community organisms. The enzymes adenosine triphosphate sulfurylase (ATPS), adenosine-5-phosphosulfate reductase (APR), and sulfide oxidase (SuO) are commonly found in organisms that use sulfur, while ribulose-bisphosphate carboxylase (RuBP) is common in autotrophic organisms, and methanol dehydrogenase (MeD) is found in organisms that use methanol as an energy source.

In this activity, students will analyze the results of biochemical analyses on organisms from cold seep communities as a basis for drawing inferences about the energy-obtaining strategies used by these organisms.

LEARNING PROCEDURE

1. Lead a discussion of deep-sea chemosynthetic communities. Contrast chemosynthesis with photosynthesis. Point out that there are a variety of chemical reactions that can provide energy for chemosynthesis. Visit http://www.bio.psu.edu/cold_seeps for a virtual tour of a cold seep community.

Review the various options available to organisms in cold-seep communities for obtaining energy (food). Briefly discuss the use of $\delta^{13}\text{C}$ isotope analysis, and enzyme analysis for obtaining clues about specific energy-obtaining strategies. You may want to construct a chart showing what the presence of certain enzymes or $\delta^{13}\text{C}$ values tells researchers about the probable source of energy and carbon.

2. Distribute one or more “Cold Seep Organism Analysis Results” sheets to each student group.

Explain that these are results of biochemical studies on gill tissues of bivalves and trophosome tissues of vestimentiferans. These tissues are used because they are the sites of energy-producing activity within the organisms. Groups receiving "Bivalve $\delta^{13}\text{C}$ Analysis" sheets should plot these data as histograms, and draw inferences about the energy-obtaining strategy used by each group represented in their data. Groups receiving "Enzyme and $\delta^{13}\text{C}$ Analysis" sheets should be assigned data from one of the three organisms (all three groups are included on the data sheet for comparative purposes), and be asked to draw inferences about the energy-obtaining strategy used by each group represented in their data.

3. Have each group present their results, and summarize these on a flip chart, chalk board, or marker board. Lead a discussion of these results. Students who examined $\delta^{13}\text{C}$ isotope data for bivalves should recognize three distinct groups: mussels with $\delta^{13}\text{C}$ values of -40 or less, suggesting a methane-based strategy; clams with $\delta^{13}\text{C}$ values of -20 to -40, suggesting a sulfur-based strategy; and clams with $\delta^{13}\text{C}$ values of -14 to -20, suggesting a heterotrophic strategy.

Students who examined data from the vestimentiferan *Lamellibrachia* sp. and the clam *Pseudomilthia* sp. should recognize that the enzyme activity and $\delta^{13}\text{C}$ values suggest a sulfur-based strategy, probably involving bacterial symbionts. Similarly, results for the unidentified mussel suggest a methane-based strategy.

THE BRIDGE CONNECTION

www.vims.edu/bridge/vents.html

THE "ME" CONNECTION

Have students write a short essay on their personal strategy for obtaining energy, and how their strategy might involve some form of chemosynthesis. What chemical energy source(s) would their chemosynthetic strategy utilize?

CONNECTIONS TO OTHER SUBJECTS

English/Language Arts, Biology, Earth Science

EVALUATION

Have students prepare individual written statements of their conclusions prior to oral presentations in Step #3. You may wish to create a grading rubric that includes the group (oral) and individual (written) components.

EXTENSIONS

Have students investigate other chemosynthetic communities (e.g., hydrothermal vents) and contrast the energy-obtaining strategies found in these communities with those found in cold-seep communities.

RESOURCES

<http://oceanexplorer.noaa.gov> – Follow the Gulf of Mexico Expedition daily as documentaries and discoveries are posted each day for your classroom use.

<http://www.bio.psu.edu/People/Faculty?Fisher/thome.htm> – Web site for the principal investigator on the Gulf of Mexico expedition

<http://www.rps.psu.edu/deep/> – Notes from another expedition exploring deep-sea communities

<http://www.ridge.oce.orst.edu/links/edlinks.html> – Links to other deep ocean exploration Web sites

<http://www-ocean.tamu.edu/education/oceanworld/resources/> – Links to other ocean-related Web sites

Paull, C.K., B. Hecker, C. Commeau, R.P. Feeman-Lynde, C. Nuemann, W.P. Corso, G. Golubic, J. Hook, E. Sikes, and J. Curray. 1984. Biological communities at Florida Escarpment resemble hydrothermal vent communities. *Science* 226:965-967 – early report on cold seep communities.

Brooks, J. M., M. C. Kennicutt II, C. R. Fisher, S. A. Macko, K. Cole, J. J. Childress, R. R. Bidigare, and R. D. Vetter. 1987. Deep-Sea

hydrocarbon seep communities: Evidence for energy and nutritional carbon sources. Science 238:1138-1142. – Technical journal article upon which this activity is based.

NATIONAL SCIENCE EDUCATION STANDARDS

Content Standard A: Science As Inquiry

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

Content Standard B: Physical Science

- Chemical reactions
- Interactions of energy and matter

Content Standard C: Life Science

- Interdependence of organisms
- Matter, energy, and organization in living systems

Content Standard D: Earth and Space Science

- Energy in the Earth system

FOR MORE INFORMATION

Paula Keener-Chavis, National Education
Coordinator/Marine Biologist
NOAA Office of Exploration
Hollings Marine Laboratory
331 Fort Johnson Road, Charleston SC 29412
843.762.8818
843.762.8737 (fax)
paula.keener-chavis@noaa.gov

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<http://oceanexplorer.noaa.gov>

Student Handout
Cold Seep Organism Analysis Results
Bivalve $\delta^{13}\text{C}$ Analysis

Animal	$\delta^{13}\text{C}$ (‰)	Animal	$\delta^{13}\text{C}$ (‰)	Animal	$\delta^{13}\text{C}$ (‰)
mussel	-56	clam	-18	mussel	-46
clam	-32	clam	-34	clam	-18
mussel	-56	clam	-32	mussel	-46
mussel	-56	clam	-16	mussel	-46
clam	-36	mussel	-50	clam	-16
clam	-32	mussel	-50	mussel	-44
mussel	-52	clam	-30	mussel	-44
mussel	-52	mussel	-50	clam	-16
clam	-32	clam	-34	mussel	-44
clam	-36	clam	-18	mussel	-44
mussel	-52	clam	-36	clam	-38
clam	-34	clam	-32	mussel	-44
clam	-32	mussel	-50	mussel	-44
clam	-18	clam	-36	clam	-16
mussel	-52	mussel	-48	mussel	-44
clam	-36	clam	-30	mussel	-42
mussel	-52	mussel	-48	mussel	-40
clam	-36	clam	-32	clam	-38
clam	-32	clam	-34		
clam	-30	clam	-18		
mussel	-50	mussel	-46		
mussel	-50	clam	-30		
clam	-14	mussel	-46		
clam	-34	clam	-38		
clam	-32	mussel	-46		
mussel	-50	clam	-16		
clam	-18	mussel	-46		
mussel	-50	mussel	-46		
clam	-36	clam	-36		
clam	-16	mussel	-46		
clam	-30	mussel	-46		
clam	-32	clam	-36		
mussel	-50	mussel	-46		
clam	-36	mussel	-46		

Student Handout
Cold Seep Organism Analysis Results
Enzyme and $\delta^{13}\text{C}$ Analysis

Enzyme Activity

Animal	RuBP	ATPS	APR	MeD	SuO	$\delta^{13}\text{C}$ (‰)
<i>Pseudomilthia</i> sp. (clam)						
sample #1	0.43	12.86	0.83	nd	2.1	-33.5
sample #2	0.41	2.47	0.66	nd	1.94	-33.6
sample #3	0.44	15.43	1.36	nd	2.04	-32.5
Unidentified Mussel						
sample #1	0.011	nd	nd	0.66	0.7	-51.8
sample #2	0.017	nd	nd	0.53	0.75	-52.0
sample #3	0.021	nd	nd	0.4	1.09	-52.6
<i>Lamellibrachia</i> sp. (vestimentiferan)						
sample #1	0.24	4.24	0.70	nd	1.77	-36.6
sample #2	4.03	1.03	nt	nd	3.15	-36.8
sample #3	4.97	0.51	0.78	nd	5.47	-37.4

nd = not detected

nt = not tested