Lessons from the Deep:

Exploring the Gulf of Mexico's Deep-Sea Ecosystems

Education Materials Collection

Image captions/credits on Page 2.

lesson plan

What Killed the Seeds?

(adapted from the *Okeanos Explorer* Education Materials Collection)

Focus
Bioassays

Grade Level
7-8 (Life Science)

Focus Question
How can the biological effects of chemicals be studied?

Learning Objectives
- Students will be able to explain and carry out a simple process for studying the biological effects of chemicals.
- Students will be able to explain ways in which deep-sea ecosystems may be directly beneficial to humans.
- Students will be able to infer why organisms such as sessile marine invertebrates appear to be promising sources of new drugs.

Materials
- Copies of *Bioassay Inquiry Guide*, one for each student group
- 5 Radish seeds; at least 60 for each student group (ten seeds for each replicate)
- 10% household bleach solution, about 50 ml for each student group
- Kitchen strainer; may be shared among several student groups
- Zip-top plastic freezer bags, 1-quart size, or disposable plastic petri dishes, 100 mm x 10 mm (Carolina Biological Supply No. WW-74-1248); at least six for each student group
- Felt tip markers
- Paper towels
- Disposable plastic pipettes with rubber bulb or aspirator, one for each student group

- Ruler graduated in millimeters
- Distilled water
- Clean glass containers with stoppers or caps for collecting water samples; minimum capacity about 100 ml
  (NOTE: Mention of proprietary names does not imply endorsement by NOAA.)

Audio/Visual Materials

- Marker board, blackboard, or overhead projector with transparencies for group discussions

Teaching Time

One or two 45-minute class periods, plus time for student observations over several class periods

Seating Arrangement

Groups of 2-3 students

Maximum Number of Students

32

Key Words

Natural products
Drugs from the sea
Bioassay
Gulf of Mexico
Deep-sea coral
Cold-seep

Background Information

Deepwater ecosystems in the Gulf of Mexico are often associated with rocky substrates or "hardgrounds." Most of these hard bottom areas are found in locations called cold seeps where hydrocarbons are seeping through the seafloor. Microorganisms are the connection between hardgrounds and cold seeps. When microorganisms consume hydrocarbons under anaerobic conditions, they produce bicarbonate which reacts with calcium and magnesium ions in the water and precipitates as carbonate rock. Two types of ecosystems are typically associated with deepwater hardgrounds in the Gulf of Mexico: chemosynthetic communities and deep-sea coral communities. Hydrocarbon seeps may indicate the presence of undiscovered petroleum deposits, so the presence of these ecosystems may indicate potential sites for exploratory drilling and possible development of offshore oil wells. At the same time, these are unique ecosystems that may be important in other ways as well.

Images from Page 1 top to bottom:

A close-up mussel aggregation with *Chirodota heheva* sea cucumbers. Image courtesy of Expedition to the Deep Slope 2007.
http://oceanexplorer.noaa.gov/explorations/07mexico/logs/july3/media/cuke_600.html

A CTD rosette being recovered at the end of a cast. Note that the stoppers on the sample bottles are all closed. Image courtesy of INSPIRE: Chile Margin 2010.
http://oceanexplorer.noaa.gov/explorations/10chile/logs/summary/media/2summary.html

A methane hydrate mound on the seafloor; bubbles show that methane is continuously leaking out of features like this. If bottom waters warmed, this entire feature may be destabilized and leak methane at a higher rate.
http://oceanexplorer.noaa.gov/explorations/10chile/background/methane/media/methane4.html

Lophelia pertusa create habitat for a number of other species at a site in Green Canyon. Image courtesy of Chuck Fisher.
http://oceanexplorer.noaa.gov/explorations/08lophelia/logs/sept24/media/green_canyon_lophelia.html

Deepwater chemosynthetic communities are fundamentally different from other biological systems, and there are many unanswered questions about the individual species and interactions among species found in these communities. These species include some of the most primitive living organisms (Archaea) that some scientists believe may have been the first life forms on Earth. Many species are new to science, and may prove to be important sources of unique drugs for the treatment of human diseases.

Despite the many advances of modern medicine, disease is still the leading cause of death in the United States. Cardiovascular disease and cancer together account for more than 1.5 million deaths annually (40% and 25% of all deaths, respectively). In addition, one in six Americans have some form of arthritis, and hospitalized patients are increasingly threatened by infections that are resistant to conventional antibiotics. The cost of these diseases is staggering: $285 billion per year for cardiovascular disease; $107 billion per year for cancer; $65 billion per year for arthritis. Death rates, costs of treatment and lost productivity, and emergence of drug-resistant diseases all point to the need for new and more effective treatments.

Most drugs in use today come from nature. Aspirin, for example, was first isolated from the willow tree. Morphine is extracted from the opium poppy. Penicillin was discovered from common bread mold. To date, almost all of the drugs derived from natural sources come from terrestrial organisms. But recently, systematic searches for new drugs have shown that marine invertebrates produce more antibiotic, anti-cancer, and anti-inflammatory substances than any group of terrestrial organisms.

Particularly promising invertebrate groups include sponges, tunicates, ascidians, bryozoans, octocorals, and some molluscs, annelids, and echinoderms. Organisms from hydrothermal vent communities have proven to be useful in a variety of ways, including treatment of bone injuries and cardiovascular disease, copying DNA for scientific studies and crime scene investigations, and making sweeteners for food additives.

Many of animals that provide useful natural products do not appear particularly impressive. Many are sessile, and live all or most of their lives attached to some sort of surface. Several reasons have been suggested to explain why these animals are particularly productive of potent chemicals. One possibility is that they use these chemicals to repel predators, since they are basically "sitting ducks." Since many of these species are filter feeders, and consequently are exposed to all sorts of parasites and pathogens in the water, they may use powerful chemicals to repel parasites or as antibiotics against disease-causing organisms. Competition for space may explain why some of these invertebrates produce anti-cancer agents—if two species are competing for the same piece of bottom space, it would be helpful to produce a substance that would attack rapidly dividing cells of the competing organism. Since cancer cells often divide more rapidly than normal cells, the same substance might have anti-cancer properties.

The potential for discovering important new drugs from deep-ocean organisms is high, because most of Earth's seafloor is still unexplored, and deep-sea explorations routinely find species that have never been seen before. In 2003, the Ocean Explorer Deep Sea Medicines Expedition visited the Gulf of Mexico to search for new resources with pharmaceutical potential. The expedition collected selected benthic invertebrates from deep-water bottom communities in the Gulf of Mexico (sponges, octocorals, molluscs, annelids, echinoderms, tunicates), and tested extracts of these organisms to identify those that may be useful in treatment of cancer, cardiovascular disease, infections, inflammation, and disorders of the central nervous system. Because their potential importance is not yet known, it is critical to protect deepwater ecosystems from adverse impacts caused by human activities.

This lesson guides student inquiries into bioassays, which are tests that use biological organisms to study the action of chemicals or physical changes in the environment.

Learning Procedure

1. To prepare for this lesson:

   (a) Review the following essays:

   Chemosynthetic Communities in the Gulf of Mexico (http://oceanexplorer.noaa.gov/explorations/02mexico/background/communities/communities.html);
   The Ecology of Gulf of Mexico Deep-sea Hardground Communities (http://oceanexplorer.noaa.gov/explorations/06mexico/background/hardgrounds/hardgrounds.html);
   Medicines from the Deep Sea: Discoveries to Date (http://oceanexplorer.noaa.gov/explorations/03bio/background/); and
   What is a Natural Product? (http://oceanexplorer.noaa.gov/explorations/03bio/background/products/products.html)

   (b) You may also want to review the following visual resources and consider presenting some of these to your students:

   - Image collections from Sulak, *et al.* (2008). Master Appendix D of this large report contains many images of deep-water coral communities. Download the pdf files "Master Appendix

D - Megafaunal Invertebrates of Viosca Knoll, *Lophelia* Community Investigation," and "Key to Plates in Master Appendix D" from http://fl.biology.usgs.gov/coastaleco/OFR_2008-1148_MMS_2008-015/index.html

- Video showing some of the extraordinary biological diversity of the Gulf of Mexico (http://oceanexplorer.noaa.gov/explorations/03mex/logs/summary/media/ngom_biodiversity_cm3.html)

- Videos of deepwater corals and coral communities (http://oceanexplorer.noaa.gov/explorations/09lophelia/logs/photolog/photolog.html)

- Virtual tour of a cold-seep community (http://www.bio.psu.edu/cold_seeps)

- Slideshow of highlights from Expedition to the Deep Slope 2006 (http://oceanexplorer.noaa.gov/explorations/06mexico/background/media/slideshow/slideshow.html)

- Slideshow of images from the Expedition to the Deep Slope 2007 (http://oceanexplorer.noaa.gov/explorations/07mexico/logs/summary/media/slideshow/html_slideshow.html)

(c) Review procedures on the *Bioassay Inquiry Guid*e, and assemble materials listed on Page 1 of this Lesson Plan. To prepare a 10% bleach solution, mix 50 ml household bleach with 450 ml tap water. Keep the solution away from sunlight. The *Guide* instructs students to prepare at least three replicates for each solution being tested and for each control solution. This is the minimum number of replicates needed for statistical analysis; more is better, if time and materials permit.

2. Briefly introduce the concept of chemosynthetic communities, and describe the two types of deep-sea ecosystems found in the Gulf of Mexico. Discuss the potential of these ecosystems as sources of new drugs for treating of cardiovascular disease, cancer, inflammatory diseases, and infections, as well as other natural products. Ask students to list some reasons that these kinds of drugs might be found primarily among sessile invertebrates.

3. Tell students that they will be learning to use a technique for studying the effects of chemicals on living organisms. Explain that a bioassay uses a biological organism to study the effects of chemicals or physical environmental change (such as radiation or heat). When toxicity is being studied, bioassays provide an

integrated measure of all changes to which a test organism is suggested, and provide a different type of understanding than would be obtained from direct measurements of specific chemical or physical factors.

Tell students that they will be using radish seeds as a bioassay organism. Two responses will be investigated: germination and growth rate. Lead a discussion to identify one or more substances (liquids are easiest) whose toxicity is to be tested. Runoff water from a street (usually contaminated with vehicle emissions) or a nearby water body suspected of being polluted are common test subjects. Have students collect the substances to be tested. A sample of 100 ml is adequate for the test. Remind students to wash their hands thoroughly after handling water that is suspected of being contaminated.

(Washing hands is a good idea after ANY laboratory procedure!)

4. Have students perform bioassays using the procedure described on the *Bioassay Inquiry Guide*.

5. Lead a discussion of students' results. Students should realize that different organisms are not equally sensitive to chemical agents. For example, the concentration of copper in water that would kill algae or a snail is harmless to most fish. When choosing a bioassay organism, investigators need to consider which compounds or organism responses are of most concern. Seed bioassays are very sensitive to herbicides and fairly sensitive to metals. They are less sensitive than fish or invertebrate assays to industrial chemicals like polychlorinated biphenyls (PCBs) or solvents. A full evaluation of a sample's biological activity requires performing several different bioassays. Bioassays for drug screening, for example, often include bacteria (to screen for potential antibacterial activity) and specific tissue cultures (to screen for anti-cancer activity).

The Bridge Connection

www.vims.edu/bridge/ – Click on "Ocean Science" in the navigation menu to the left, then "Habitats," then "Deep Sea" for resources on deep-sea communities. Click on "Human Activities" then "Technology" for resources on biotechnology.

The "Me" Connection

Have students write a short essay on how bioassays might be of personal benefit.

Connections to Other Subjects

English/Language Arts, Mathematics (Statistics)

Assessment

Written reports offer opportunity for assessment.

Extensions

1. See the "Resources" section of *Lessons from the Deep: Exploring the Gulf of Mexico's Deep-sea Ecosystem Education Materials Collection Educators Guide* for additional information, activities, and media resources about deepwater ecosystems in the Gulf of Mexico.

2. Visit http://www.woodrow.org/teachers/bi/1993/ for more activities related to biotechnology from the 1993 Woodrow Wilson Biology Institute.

3. Visit http://oceanexplorer.noaa.gov/explorations/03bio/welcome.html to find out more about the Deep Sea Medicines 2003 Expedition.

4. Visit http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon09no1.pdf for more examples and ideas for using bioassays.

Multimedia Discovery Missions

http://www.learningdemo.com/noaa/ Click on the links to Lessons 3, 5, 6, and 12 for interactive multimedia presentations and Learning Activities on Deep-Sea Corals, Chemosynthesis and Hydrothermal Vent Life, Deep-Sea Benthos, and Food, Water, and Medicine from the Sea.

Other Relevant Lesson Plans from NOAA's Ocean Exploration Program

Monsters of the Deep (6 pages, 464 KB)

http://oceanexplorer.noaa.gov/explorations/07mexico/background/edu/media/monsters.pdf

Focus - Predator-prey relationships between cold-seep communities and the surrounding deep-sea environment (Life Science)

Students describe major features of cold seep communities, list at least five organisms typical of these communities, and infer probable trophic relationships among organisms typical of cold-seep communities and the surrounding deep-sea environment. Students also describe the process of chemosynthesis in general terms, contrast chemosynthesis and photosynthesis, and describe at least five deep-sea predator organisms.

One Tough Worm (8 pages, 476 KB)

http://oceanexplorer.noaa.gov/explorations/07mexico/background/edu/media/worm.pdf

Focus - Physiological adaptations to toxic and hypoxic environments (Life Science)

Students explain the process of chemosynthesis, explain the relevance of chemosynthesis to biological communities in the vicinity of cold seeps, and describe three physiological adaptations that enhance an organism's ability to extract oxygen from its environment. Students also describe the problems posed by hydrogen sulfide for aerobic organisms, and explain three strategies for dealing with these problems.

Microfriends (Grades 5-6; 6 pages, 420k)

http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_microfriends.pdf

Focus: Beneficial microorganisms (Life Science)

Students will be able to describe at least three ways in which microorganisms benefit people, describe aseptic procedures, and obtain and culture a bacterial sample on a nutrient medium.

Living by the Code (5 pages, 400k)

http://oceanexplorer.noaa.gov/explorations/03bio/background/edu/media/meds_livingcode.pdf

Focus: Functions of cell organelles and the genetic code in chemical synthesis (Life Science)

In this activity, students will be able to explain why new drugs are needed to treat cardiovascular disease, cancer, inflammation, and infections; infer why sessile marine invertebrates appear to be promising sources of new drugs; and explain the overall process through which cells manufacture chemicals. Students will also be able to explain why it may be important to synthesize new drugs, rather than relying on the natural production of drugs.

Other Links and Resources

The Web links below are provided for informational purposes only. Links outside of Ocean Explorer have been checked at the time of this page's publication, but the linking sites may become outdated or non-operational over time.

http://oceanexplorer.noaa.gov/ – Ocean Explorer Web site

Rathbun, J. 1996. A Simple Bioassay Using Lettuce Seeds. The Volunteer Monitor. Spring, 1996. Available online at http://www.epa.gov/owow/monitoring/volunteer/newsletter/volmon09no1.pdf

Mayer, A. M. S. and K. R. Gustafson. 2003. Marine pharmacology in 2000: Antitumor and cytotoxic compounds. Int. J. Cancer 105:291-299. Available online at http://marinepharmacology.midwestern.edu/docs/MP2000_Anticancer_Mayer_Gustafson.pdf

Tim Batchelder, T. 2001. Natural products from the sea: Ethnopharmacology, nutrition and conservation. Townsend Letter for Doctors and Patients, Feb, 2001. Available online at http://www.findarticles.com/p/articles/mi_m0ISW/is_2001_Feb/ai_70777319/pg_1

http://www.woodrow.org/teachers/bi/1993/ – Background and activities from the 1993 Woodrow Wilson Biology Institute on biotechnology

http://www.piersystem.com/go/site/2931/ – Main Unified Command Deepwater Horizon response site

http://response.restoration.noaa.gov/deepwaterhorizon – NOAA Web site on Deepwater Horizon Oil Spill Response

http://docs.lib.noaa.gov/noaa_documents/NESDIS/NODC/LISD/Central_Library/current_references/current_references_2010_2.pdf – Resources on Oil Spills, Response, and Restoration: a Selected Bibliography; document from NOAA Central Library to aid those seeking information concerning the Deepwater Horizon oil spill disaster in the Gulf of Mexico and information on previous spills and associated remedial actions; includes media products (web, video, printed and online documents) selected from resources available via the online NOAA Library and Information Network Catalog (NOAALINC)

http://www.gulfallianceeducation.org/ – Extensive list of publications and other resources from the Gulf of Mexico Alliance; click "Gulf States Information & Contacts for BP Oil Spill" to download the Word document

http://rucool.marine.rutgers.edu/deepwater/ – Deepwater Horizon Oil Spill Portal from the Integrated Ocean Observing System at Rutgers University

http://www.darrp.noaa.gov/southeast/deepwater_horizon/ index.html – Information about damage assessments being conducted by NOAA's Damage Assessment Remediation and Restoration Program http://response.restoration.noaa.gov/ – Click "Students and Teachers" in the column on the left for information, fact sheets, and activities about oil emergencies, habitats, and other ocean issues

http://www.noaa.gov/sciencemissions/bpoilspill.html – Web page with links to NOAA Science Missions & Data relevant to the Deepwater Horizon/BP Oil Spill

http://ecowatch.ncddc.noaa.gov/jag/data.html – Data Links page on the Deepwater Horizon Oil Spill Joint Analysis Group Web site

http://ecowatch.ncddc.noaa.gov/jag/reports.html – Reports page on the Deepwater Horizon Oil Spill Joint Analysis Group Web site

http://www.education.noaa.gov/Ocean_and_Coasts/Oil_Spill.html - "Gulf Oil Spill" Web page from NOAA Office of Education with links to multimedia resources, lessons & activities, data, and background information

http://www.geoplatform.gov/gulfresponse/ - Web page for GeoPlatform.gov/gulfresponse—an online map-based tool developed by NOAA with the EPA, U.S. Coast Guard, and the Department of Interior to provide a "one-stop shop" for spill response information; includes oil spill trajectory, fishery area closures, wildlife data, locations of oiled shoreline and positions of deployed research ships

Fisher, C., H. Roberts, E. Cordes, and B. Bernard. 2007. Cold seeps and associated communities of the Gulf of Mexico. Oceanography 20:118-129; available online at http://www.tos.org/oceanography/issues/issue_archive/20_4.html

Sulak, K. J., M. T. Randall, K. E. Luke, A. D. Norem, and J. M. Miller (Eds.). 2008. Characterization of Northern Gulf of Mexico Deepwater Hard Bottom Communities with Emphasis on *Lophelia* Coral - *Lophelia* Reef Megafaunal Community Structure, Biotopes, Genetics, Microbial Ecology, and Geology. USGS Open-File Report 2008-1148; http://fl.biology.usgs.gov/coastaleco/OFR_2008-1148_MMS_2008-015/index.html

National Science Education Standards

Content Standard A: Science As Inquiry

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

Content Standard C: Life Science

- Behavior of organisms

Content Standard D: Earth and Space Science
- Structure of the Earth system

Content Standard F: Science in Personal and Social Perspectives
- Personal and community health
- Natural resources
- Enviromnental quality

Ocean Literacy Essential Principles and Fundamental Concepts

Essential Principle 1.
The Earth has one big ocean with many features.
Fundamental Concept h. Although the ocean is large, it is finite and resources are limited.

Essential Principle 3.
The ocean is a major influence on weather and climate.
Fundamental Concept f. The ocean has had, and will continue to have, a significant influence on climate change by absorbing, storing, and moving heat, carbon and water.

Essential Principle 5.
The ocean supports a great diversity of life and ecosystems.
Fundamental Concept c. Some major groups are found exclusively in the ocean. The diversity of major groups of organisms is much greater in the ocean than on land.
Fundamental Concept d. Ocean biology provides many unique examples of life cycles, adaptations and important relationships among organisms (such as symbiosis, predator-prey dynamics and energy transfer) that do not occur on land.
Fundamental Concept g. There are deep ocean ecosystems that are independent of energy from sunlight and photosynthetic organisms. Hydrothermal vents, submarine hot springs, and methane cold seeps rely only on chemical energy and chemosynthetic organisms to support life.

Essential Principle 6.
The ocean and humans are inextricably interconnected.
Fundamental Concept b. From the ocean we get foods, medicines, and mineral and energy resources. In addition, it provides jobs, supports our nation's economy, serves as a highway for transportation of goods and people, and plays a role in national security.
Fundamental Concept g. Everyone is responsible for caring for the ocean. The ocean sustains life on Earth and humans must live in ways that sustainthe ocean. Individual and collective actions are needed to effectively manage ocean resources for all.

Essential Principle 7.
The ocean is largely unexplored.
Fundamental Concept a. The ocean is the last and largest unexplored place on Earth—less than 5% of it has been explored. This is the great frontier for the next generation's explorers and researchers, where they will find great opportunities for inquiry and investigation.
Fundamental Concept b. Understanding the ocean is more than a matter of curiosity. Exploration, inquiry and study are required to better understand ocean systems and processes.
Fundamental Concept d. New technologies, sensors and tools are expanding our ability to explore the ocean. Ocean scientists are relying more and more on satellites, drifters, buoys, subsea observatories and unmanned submersibles.
Fundamental Concept f. Ocean exploration is truly interdisciplinary. It requires close collaboration among biologists, chemists, climatologists, computer programmers, engineers, geologists, meteorologists, and physicists, and new ways of thinking.

Send Us Your Feedback

We value your feedback on this lesson.
Please e-mail your comments to: oceanexeducation@noaa.gov

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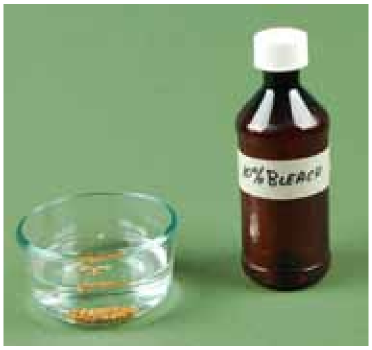
Bioassay Inquiry Guide

(Adapted from an article by Joe Rathbun in the Spring 1996 issue of the Volunteer Monitor)

Step 1a:



Step 1b:

Step 3:

Step 4:

Step 6:

1. Soak seeds for 20 minutes in a 10% solution of household bleach in distilled water, then rinse thoroughly under running tap water. The solution kills fungi, which could interfere with seed germination.

2. Cut paper towels into pieces approximately 11″ x 6″. You will need at least three pieces for each solution being tested, as well as at least three pieces for each control solution.

3. Place 10 seeds in the middle of each paper towel, leaving about 1/2″ space between the seeds. Fold the edges of the paper towel over to cover the seeds.

4. Place each paper towel with the seeds into a zip-top plastic freezer bag or disposable plastic petri dish. Pipette enough undiluted sample solution into the bag or dish to saturate the paper towel. Prepare at least three replicates for each sample being tested, as well as at least three controls using distilled water instead of sample water. Use the same volume in each bag or dish.

5. Incubate bags or dishes at room temperature, in the dark, for five days. (It is OK to briefly check the dishes during incubation. If the paper seems dry, pipette a few ml of distilled water onto the paper.)

6. When incubation is complete, record the number of seeds that germinated in each bag or dish, and measure (to the nearest mm) the length of the root that has emerged from each germinated seed (the image shows a seed after 24 hours of incubation). If fewer than 80% of the seeds in the control sample germinate, this indicates a problem with the assay (e.g., bad seeds, poor incubation conditions). If this happens, the test should be re-run.

7. For each sample (including the controls), calculate the mean and standard deviation of root lengths. Comparisons can be made by using the Student's t-test. A more approximate method is to compare the mean ±1 standard deviation of each sample to the control. If a sample's mean plus 1 standard deviation is less that the mean of the control minus 1 standard deviation, there is a strong likelihood that the sample is significantly more toxic than the control. Prepare a written report of your results, including a discussion of the outcome.