



## 2004 Deep-Scope Expedition

# Where is That Light Coming From?

### FOCUS

Bioluminescence

### GRADE LEVEL

9-12 (Chemistry & Life Science)

### FOCUS QUESTION

What is the basic chemistry responsible for bioluminescence, and how does this process benefit deep-sea organisms?

### LEARNING OBJECTIVES

Students will be able to explain the role of luciferins, luciferases, and co-factors in bioluminescence, and the general sequence of the light-emitting process.

Students will be able to discuss the major types of luciferins found in marine organisms.

Students will be able to define the "lux operon?"

Students will be able to discuss at least three ways that bioluminescence may benefit deep-sea organisms, and give an example of at least one organism that actually receives each of the benefits discussed.

### MATERIALS

None

### AUDIO/VISUAL MATERIALS

(Optional) Images of deep-sea environments and organisms that use bioluminescence (see Learning Procedure)

### TEACHING TIME

One 45-minute class period, plus time for student research

### SEATING ARRANGEMENT

Classroom style or groups of 3-4 students

### MAXIMUM NUMBER OF STUDENTS

30

### KEY WORDS

Chemiluminescence  
Bioluminescence  
Fluorescence  
Phosphorescence  
Luciferin  
Luciferase  
Photoprotein  
Counter-illumination  
Lux operon

### BACKGROUND INFORMATION

Deep-sea explorers face many challenges: extreme heat and cold, high pressures, and almost total darkness. The absence of light poses particular challenges to scientists who want to study organisms that inhabit the deep ocean environment. Even though deep-diving submersibles carry bright lights, simply turning these lights on creates another set of problems: at least some mobile organisms are likely to move away from the light; organisms with light-sensitive organs may be permanently blinded by intense illumination; even sedentary organisms may shrink back, ceasing normal life activities and possibly becoming

ing less noticeable; and small cryptic organisms may simply be unnoticed. In addition, some important aspects of deep-sea biology simply can't be studied with ordinary visible light. Many marine species are known to be capable of producing light, and it is reasonable to suppose that ability to produce and detect light might be particularly important to organisms that live in near-total darkness.

The primary purpose of the 2004 Ocean Exploration Deep-Scope Expedition is to study deep-sea biological communities using advanced optical techniques that provide new ways of looking at organisms that make their home in the blackness of the deep ocean. These techniques are based on a number of basic concepts that can be summarized under the general heading of "bioluminescence."

Bioluminescence is a form of chemiluminescence, which is the production of visible light by a chemical reaction. When this kind of reaction occurs in living organisms, the process is called bioluminescence. It is familiar to most of us as the process that causes fireflies to glow. Some of us may also have seen "foxfire," which is caused by bioluminescence in fungi growing on wood. Bioluminescence is relatively rare in terrestrial ecosystems, but is much more common in the marine environment. Marine organisms producing bioluminescence include bacteria, algae, coelenterates, annelids, crustaceans, and fishes.

The fundamental chemiluminescent reaction occurs when an electron in a chemical molecule receives sufficient energy from an external source to drive the electron into a higher-energy orbital. This is typically an unstable condition, and when the electron returns to the original lower-energy state, energy is emitted from the molecule as a photon. Lightning is an example of gas-phase chemiluminescence: An electrical discharge in the atmosphere drives electrons in gas molecules (such as  $N_2$  and  $O_2$ ) to higher-energy orbitals.

When the electrons return to their original lower-energy orbitals, energy is released in the form of visible light.

Chemiluminescence is distinctly different from fluorescence and phosphorescence, which occur when electrons in a molecule are driven to a higher-energy orbital by the absorption of light energy (instead of chemical energy). Both processes may occur in living organisms. Atoms of a fluorescent material typically re-emit the absorbed radiation only as long as the atoms are being irradiated (as in a fluorescent lamp). Phosphorescent materials, on the other hand, continue to emit light for a much longer time after the incident radiation is removed (glowing hands on watches and clocks are familiar examples). Chemiluminescent reactions, on the other hand, produce light without any prior absorption of radiant energy. Another light-producing process known as triboluminescence occurs in certain crystals when mechanical stress applied to the crystal provides energy that raises electrons to a higher-energy orbital.

The production of light in bioluminescent organisms results from the conversion of chemical energy to light energy. The energy for bioluminescent reactions is typically provided by an exothermic chemical reaction. The light-emitting molecules in bioluminescent reactions are known collectively as luciferins. The light-emitting reactions typically involve a group of enzymes known as luciferases. Several different luciferins have been found in marine organisms, suggesting that bioluminescence may have evolved many times in the sea among different taxonomic groups. Despite these differences, almost all marine bioluminescence is green to blue in color. These colors travel farther through seawater than warmer colors. In fact, most marine organisms are sensitive only to blue light.

**LEARNING PROCEDURE**

1. If you want to include demonstrations of chemiluminescence, bioluminescence, fluorescence, and phosphorescence, see the ‘Cool Lights’ lesson plan for suggestions. The following web sites are useful resources if you want to show images of deep-sea environments and organisms that use bioluminescence:

<http://www.biolum.org/>

[http://oceanexplorer.noaa.gov/gallery/livingocean/livingocean\\_coral.html](http://oceanexplorer.noaa.gov/gallery/livingocean/livingocean_coral.html)

<http://www.europa.com/edge.of.CyberSpace/deep.html>

<http://www.europa.com/edge.of.CyberSpace/deep2.html>

<http://www.pbs.org/wgbh/nova.abbyss/life.bestiary.html>

<http://biodidac.bio.uottawa.ca/>

<http://www.fishbase.org/search.cfm>

2. Ask students to describe characteristics of deep-sea environments (depth = 1,000 meters or more). You may want to show images of various deep-sea environments and organisms that use bioluminescence.

Focus the discussion on light in the deep ocean. Students should realize that light is almost completely absent. Ask whether plants and animals are ever able to produce their own light. Most students will be familiar with fireflies, and may mention bioluminescence in other species.

Review the basic concept of chemiluminescence, and contrast this process with fluorescence and phosphorescence. Students should understand that every light-producing process requires a source of energy (chemical, electrical, mechanical, or light). Tell students that bioluminescence is a form of chemiluminescence that occurs in living organisms, but do not explain the details of bioluminescence at this point. Students may ask about incandescence, in which light is produced by combustion reactions (thermal energy). Review the concept of the visible and near-visible light spectrum. Students should understand that light at the blue end of the spectrum (including ultraviolet light)

has higher energy than light at the red end of the spectrum (including infrared).

3. Tell students that their assignment is to investigate the chemistry of bioluminescence, and prepare a report that answers the following questions:
  - What is the role of luciferins, luciferases, and co-factors in bioluminescence, and what is the general sequence of the light-emitting process?
  - What are the major types of luciferins found in marine organisms?
  - Different groups of marine organisms have different luciferases and co-factors.
  - How much light is emitted by bioluminescence, and how long is a single burst of light emission?
  - What is the “lux operon?”
  - What are three ways that bioluminescence may benefit deep-sea organisms?

4. Have each student or student group present and discuss the results of their research. Points that should emerge during these discussions include:
  - **The role of luciferins, luciferases, and co-factors in bioluminescence, and the general sequence of the light-emitting process:**

The generalized steps in a bioluminescent reaction are:

- Release of a large quantum of energy involving oxide or hydroperoxide radicals;
- Transference of this energy to a substrate (known collectively as luciferins), causing an electron to be driven to a higher-energy orbital (a less stable state); and
- Return of the electron to its original orbital (a more stable state), accompanied by release of energy as a photon of light.

These reactions are typically catalyzed by enzymes known as luciferases. In some species, the luciferin and luciferase are bound together in a “photoprotein” which is activated by a co-factor (such as calcium ions).

• **The major types of luciferins found in marine organisms:**

- Bacterial luciferin involves a long chain aldehyde and a reduced riboflavin phosphate.
- Dinoflagellate luciferin (also found in euphausiid shrimp) is a tetrapyrrolic compound whose molecular structure is similar to chlorophyll and may have been derived from chlorophyll.
- Ostracod luciferin (known as vargulin, and also found in some fishes) is an imidazolopyrazine consisting of modified molecules of tryptophan, isoleucine and arginine.
- Coelenterazine is the luciferin found in cnidaria, ctenophores, squid, copepods, decapod shrimp, mysid shrimp, and some fishes. This luciferin is an imidazolopyrazine consisting of modified molecules of two tyrosines and a phenylalanine.

In contrast to the marine luciferins, firefly luciferin is a relatively simple benzothiazole assembled from the amino acids tyrosine and cysteine.

• **Different groups of marine organisms have different luciferases and co-factors:**

Marine luciferases are complex proteins consisting of very long chains of amino acids. Amino acid sequences have been determined in luciferases from a bioluminescent bacterium, a firefly, a jellyfish and a dinoflagellate and they are all very different structures. Required cofactors differ as well. Cnidaria require calcium ions to trigger bioluminescence, while dinoflagellates require a change in pH, and at least one marine annelid worm requires ferrous iron.

• **The amount of light emitted by bioluminescence, and how long is a single burst of light emission:**

Reports vary. Light emission from dinoflagellates is on the order of  $6 \times 10^8$  photons in a flash lasting only about 0.1 second. The intensity of bioluminescence from dinoflagellates is strongly dependent on the intensity of sunlight the previous day; the brighter the sunlight, the brighter the flash. Jellyfish are reported to emit about  $2 \times 10^{11}$  photons per second for periods measured in tens of seconds. Light emission from a single bioluminescent bacterium is on the order of  $10^3$  to  $10^4$  photons per second. By comparison, the light emitted from a 100-watt light bulb is about  $10^{18}$  photons per second.

• **The “lux operon:”**

A group of genes containing the information needed to synthesize enzymes needed for bioluminescence.

• **Ways that bioluminescence may benefit deep-sea organisms:**

Some organisms seem to use bioluminescence to locate other members of the same species, and we infer that this would be useful for mating activities. Almost any bioluminescent organism may be inferred to derive this benefit.

Bioluminescence may also be useful for feeding. Some organisms (such as the angler fish) use bioluminescence to attract prey species. Others (such as fishes in the malacosteid family) have a “floodlight” system that allows them to see nearby organisms. These fishes have organs that produce red light (an exception to the “blue only” rule), as well as eyes that can detect red light. Since most other species (so far as we know) cannot see red light, the malacosteids can sneak up on their prey without being detected.

A third potential use for bioluminescence is camouflage. It may not be immediately

obvious how emitting light could make an organism less visible, yet this is the strategy involved in counter-illumination. You can illustrate this by holding a white index card against a window in a darkened room. The card will block out light coming through the window and be visible as a darker object against the bright background. If you shine a flashlight on the card, the illumination on the “dark” side of the card will be closer to that of the background, making the card less visible. Counter-illumination could thus be a useful strategy to a swimming organism (such as a squid or fish) trying to be less visible to a potential predator swimming below.

Bioluminescence may be used for defense in a different way. Some tube-dwelling worms (such as the volcano worm) spew out clouds of glowing blue material when they are threatened. The strategy is similar to the fear scream of monkeys or birds, which are intended to attract the attention of higher order predators that may attack the threatening predator. So a glowing cloud produced by the worm exposes the threatening invader and makes the invader vulnerable to attack by a higher order predator. A similar glowing cloud might be used by swimming species to temporarily blind an attacker. This strategy is used by squid, shrimp, and some fishes.

5. Discuss how light-producing processes and the ways they are used by deep-sea organisms could be useful to scientists exploring deep ocean environments. The 2004 Deep-Scope Expedition will use several techniques to make observations that have never been made before. A new deep-sea observatory called Eye-in-the-Sea can be placed on the bottom and left alone to observe sea life without interference from a submersible vessel. The observatory is capable of capturing video images using only red light (which should be invisible

to many organisms) and can be programmed to acquire time-lapse images (one minute of recording every fifteen minutes) over several days. The video recording system can also be programmed to respond to bioluminescence, so that whenever a bioluminescent event is detected, the recorders will start to capture additional bioluminescent images, then will turn on the red illumination to capture an image of organism producing the bioluminescence.

Other observations will use ultraviolet light to search for fluorescent organisms that may be less visible under “white” light. A related study will measure the spectral reflectance of captured organisms, to determine what kinds of illumination will make these organisms most visible to observers. Yet another series of studies will investigate whether polarized light is used by deep-sea organisms, how polarized light is changed by these organisms and deep-sea water, and whether these changes can be detected and recorded.

#### THE BRIDGE CONNECTION

[www.vims.edu/bridge/](http://www.vims.edu/bridge/) – Click on “Biology” in the navigation menu to the left, then “Plankton,” then “Phytoplankton” for resources on ocean food webs. Click on “Ecology” then “Deep Sea” for resources on deep sea communities.

#### THE “ME” CONNECTION

Have students write a short essay on how bioluminescence might be potentially important to their own lives.

#### CONNECTIONS TO OTHER SUBJECTS

English/Language Arts, Earth Science, Life Science

#### EVALUATION

Written reports prepared in Step 3 provide opportunities for assessment.

## EXTENSIONS

1. Have students visit <http://oceanexplorer.noaa.gov> to find out more about the 2004 Deep-Scope Expedition and about opportunities for real-time interaction with scientists on current Ocean Exploration expeditions.
2. Bioluminescence can be demonstrated with several organisms. Dinoflagellates are widely used; see [http://siobiolum.ucsd.edu/Biolum\\_demos.html](http://siobiolum.ucsd.edu/Biolum_demos.html) and <http://www.lifesci.ucsb.edu/~biolum/organism/dinohome.html> for sources and demonstration ideas. Fotodyne, Inc. offers kits for demonstrating bacterial bioluminescence (see <http://www.fotodyne.com/education/safelumi.php>)

## RESOURCES

- [http://www.geocities.com/bioluminus/how2detect\\_bioluminescence.html](http://www.geocities.com/bioluminus/how2detect_bioluminescence.html)  
– Web page on how to detect and record bioluminescence of small animals photographically
- <http://www.lifesci.ucsb.edu/~biolum/> —The Bioluminescence Web page
- <http://www.nightsea.com/> – Web site offering products for studying fluorescence underwater
- <http://www.flinnsci.com> – Web site for Flinn Scientific, Inc., source for materials for demonstrating chemiluminescence; Phone 1-800-452-1261
- <http://www.biolum.org/> – Harbor Branch Oceanographic Institution Web site on bioluminescence
- <http://ice.chem.wisc.edu/materials/light/lightandcolor7.html> – Web site with links to other activities involving fluorescence and phosphorescence
- [http://oceanexplorer.noaa.gov/gallery/livingocean/livingocean\\_coral.html](http://oceanexplorer.noaa.gov/gallery/livingocean/livingocean_coral.html)  
– Ocean Explorer photograph gallery
- <http://oceanica.cofc.edu/activities.htm> – Project Oceanica Web site, with a variety of resources on ocean exploration topics

## NATIONAL SCIENCE EDUCATION STANDARDS

### Content Standard A: Science as Inquiry

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

### Content Standard B: Physical Science

- Structure of atoms
- Structure and properties of matter
- Chemical reactions

### Content Standard C: Life Science

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

### Content Standard E: Science and Technology

- Abilities of technological design
- Understandings about science and technology

### Content Standard F: Science in Personal and Social Perspectives

- Natural resources

## FOR MORE INFORMATION

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<http://oceanexplorer.noaa.gov>