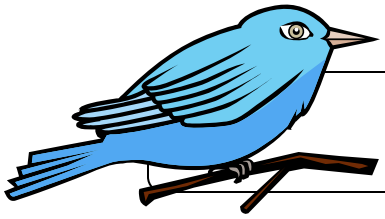

Bioluminescence

Summary:
Students will use bioluminescent dinoflagellates to study photosynthesis, circadian rhythms and dinoflagellates. They will also compare and contrast the ecosystems of the Bioluminescent Bay and the Fox River through research and field trips.

Background Information:

Dinoflagellates are unicellular protists and most exhibit the following characteristics:

- They are planktonic.
  Ninety percent of all dinoflagellates are marine plankton.
- They are small.
  Although many of them are microscopic, the largest, *Noctiluca*, may be as large as 2 mm in diameter!
- They are mobile.
  Dinoflagellates swim by means of two flagella, movable protein strands which propel the cell through the water. The longitudinal flagellum extends out from the sulcal groove of the hypotheca (posterior part of cell); when it whips back and forth it propels the cell forward. The flattened flagellum lies in the cingulum, the groove that extends around the equator of the cell. Its motion provides maneuvering and forward movement. As a result of the action of the two flagella the cell spirals as it moves.
- Many are covered by cellulose plates.
  The cell is surrounding by a series of membranes called the amphiesma. In "armored" species cellulose deposited between the membranes forms rigid plates called thecae. "Naked" cells lack thecae.
- Their chromosomes are always condensed.
  In addition, the DNA is not associated with histones as in other eukaryotic cells. Dinoflagellates contain a lot of DNA, which explains the large size of the nucleus. The metabolic requirements of supporting the large amount of DNA may explain the low growth rates of dinoflagellates

Grade Level: 10th

Goal: For students to understand the importance of dinoflagellates to the marine and freshwater environment.

Key Concepts: Students will expand their knowledge of ecosystems and eukaryotic and prokaryotic cells by comparing and contrasting two different ecosystems.

Objectives: Upon completion of this lesson, students will:
1) Understand Circadian Rhythm.
2) Understand Dioflagellates and their importance to marine and freshwater environments.
3) The difference between Mosquito Bay (Bioluminescent Bay) ecosystem and Fox River ecosystem.
4) Other ways to determine water quality.

Teaching Location: In the classroom, lab and field.

Lesson Time: 1-2 weeks

Subject Areas of Infusion:
Environmental Education, Science and Social Studies

Standards:
Social Studies
A.12.1
A.12.2

Science
B.12.2
B.12.4
C.12.3

Environnemental Education
B.12.7

compared to other unicellular protists.

- Not all dinoflagellates are photosynthetic.
  Many dinoflagellates are photosynthetic, manufacturing their own food using the energy from sunlight, and providing a food source for other organisms. The photosynthetic dinoflagellates are important primary producers in coastal waters.

Some photosynthetic dinoflagellates are symbiotic, living in the cells of their hosts, such as corals. Called zooxanthellae, they are found in many marine invertebrates, including sponges, corals, jellyfish, and flatworms, as well as within protists, such as ciliates, foraminiferans, and colonial radiolarians. Approximately half of all species are heterotrophic, eating other plankton, and sometimes each other, by snaring or stinging their prey. Non-photosynthetic species of dinoflagellates feed on diatoms or other protists (including other dinoflagellates); *Noctiluca* is large enough to eat zooplankton and fish eggs. Some species are parasites on algae, zooplankton, fish or other organisms.

Reproduction:

The most common form of reproduction is asexual, where daughter cells form by simple mitosis and division of the cell. The daughter cells will be genetically identical to that of the original cell. The thecal plates may either be divided or completely shed and then reformed. Under some conditions sexual reproduction may occur. Motile gametes are formed as a result of mitosis, because dinoflagellates are usually haploid. When two gametes fuse a motile planozygote may be formed.

Dinoflagellate Bioluminescence:

The dinoflagellates are also known as Pyrrhophyta, meaning "fire plants". Some species produce bioluminescence, which is light produced as a result of a chemical reaction within specialized structures in the dinoflagellate cell. Agitation of seawater containing dinoflagellates will stimulate light flashes. The phenomenon was first noted in the genus *Noctiluca* in the 1800's, but the ability to produce bioluminescence is now known to occur in several marine species of dinoflagellates. Light is produced as a result of a chemical reaction. Therefore, light production in dinoflagellates and all other luminescent organisms involves a chemiluminescent reaction in which a substrate, luciferin, is oxidized, releasing a large amount of energy in the form of light. Unlike light produced by light bulbs, in which a portion of the energy is wasted as heat, most of the energy released from the chemiluminescent oxidation of luciferin occurs in the form of light. Hence bioluminescence is commonly called "cold light". All bioluminescent reactions occur in the presence of oxygen.

Circadian Rhythm:

In most dinoflagellates the expression of bioluminescence is controlled by an internal biological rhythm. Towards the end of the day the luminescent chemicals are packaged in

vesicles called scintillons, which then migrate into the cytoplasm. During the night, light emission is triggered by mechanical stimulation of the cell according to the following hypothesized sequence of events: Following an unknown mechano-chemical transduction process, an action potential is generated in the internal vacuole membrane. The action potential propagates throughout the cell, allowing protons to pass from the vacuole, where they are sequestered, into the cytoplasm. As a result the cytoplasm is acidified, and the chemiluminescent process is activated in the scintillons according to the steps previously described.

Because of the circadian rhythm, most dinoflagellates produce much less bioluminescence during the day compared to the light produced at night. Even if you turn off the lights during the day, you won't get luminescence produced. This is because there are fewer scintillons present during the day, and possibly an uncoupling of the sensory and effector pathways. Bioluminescence reaches maximum levels approximately 2 hours into the dark. Bioluminescence can be mechanically stimulated by stirring or bubbling, chemically stimulated by an acid solution, and also stimulated by ultrasound.

Mosquito Bay (Bioluminescent Bay):

This unique bay contains up to 720,000 single-celled bioluminescent dinoflagellates per gallon of water. These half-plant, half-animal organisms emit a flash of bluish light when agitated at night. The high concentration of these creatures (Pyrodimium *bahamense*) can create enough light to read a book.

The bioluminescent glow is produced by a physico-chemical reaction that begins when the single celled dinoflagelate organism is disturbed. The defense mechanism starts a chemical chain reaction that results in a blue green glow that lasts as long as the organism is disturbed. This is a reaction that occurs because a very specific set of nutrients are available in the water to sustain these little bioluminescent organisms. There is more than one bioluminescent bay on the Island of Vieques, the brightest being Mosquito Bay.

Several things had to come together all in one spot for bioluminescence in Mosquito Bay and elsewhere to have evolved:

- First of all, Mangroves, or their ancestors, had to surround the bay and provide a habitat for specific bacterium which produces vitamin B12 in large quantities as a metabolic by-product.
- Secondly, the mouth of the bay had to be narrow to keep its waters from being washed out to sea, diluting the dinoflagelate population, and the location had to be remote and without pollution.
- The temperature must also stay warm, and constant within a very narrow range.
- Next, the water had to remain calm enough so that most of the saltier water could sink to the bottom and eventually be carried away back into the ocean

by mild undercurrents, since the organisms cannot thrive in the saltier ocean water.

- Lastly, the population of natural predators for the organism had to remain low.

Here is a more scientific explanation of the phoneme:

The reaction producing bioluminescence is the direct conversion of chemical energy into light energy. In the case of the bioluminescent bay organisms (dinoflagelates), the luminosity is thought possibly to be a defense mechanism.

The reaction begins with the creation of a large packet or quantum of light energy (photon, or energy quantum), from a highly specialized physico-chemical process. The process is a recombination of radicals (oxide, hyroperoxide) involving a specific molecule that undergoes a chemical charge when affixed by an enzyme (substrate - D-luciferin), ATP or adenosine triphosphate, the energy storing molecules of all living organisms, and oxygen. The reaction is controlled by the enzyme, luciferase, an enzyme or biological catalyst that accelerates and controls the rate of chemical reactions in cells. The chemical energy excites a specific molecule resulting in the photon, which produces the light. The light emitted is independent of any light absorbed by the organism (as would be the case with a "glow in the dark" object which requires charging by direct contact with light). This whole reaction takes less than 1/1000th of a second but the light will last as long as the organism is in a state of excitability, provided the organism can absorb enough nutrients from the water to sustain it.

Photons are discrete energy “quanta” or “packets”, and their energy is constant. The number released (as well as the intensity with which that release is perceived by our senses) may vary. Different types of substrates (luciferins) in organisms produce different colors. Organisms such as the dinoflagelates emit light ranging from blue to yellow green (~490nm is the frequency of the light wave). For those of you with a degree or two in chemistry - here's the mind numbingly concise explanation:

$$FMNH_2 + RCHO + O_2 \longrightarrow FMN + RCOOH + H_2O + light\ (490nm)$$

Fox River

Please visit The Fox River Group Website for natural history of the Fox River at http://www.foxrivergroup.org/fox_treasured.htm for a look at the history of the Fox River, early environmental issues, PCBs, recent improvements and ongoing changes, and the progress of the last 30 yrs.

Description of PCB's:

PCB's are a class of chemicals known as polychlorinated biphenyls. They are entirely man-made and do not occur naturally. They were first manufactured commercially in 1929 by Monsanto, their sole U.S. manufacturer. They were used in many different types of products including hydraulic fluid, casting wax, pigments, carbonless copy paper, plasticizer, vacuum pumps, compressors, heat transfer systems and others. Their primary use, however, was as a dielectric fluid in electrical equipment. Because of their stability and resistance to thermal breakdown as well as their insulating properties they were the fluid of choice for transformers and capacitors. As a matter of fact, because of their fire resistance, they were required by some fire codes.

During the 1970's, the health risks associated with PCB's became a major consideration due to several well publicized incidents. The most noted of these is known as the Yusho Incident. It took place in Japan when a rice oil plant had an equipment leak of PCB fluid into the product. The rice oil was sold and consumed resulting in many people being adversely affected.

Among the health affects of PCB's are skin ailments called chloracne, reproductive disorders, liver disease and others. PCB's are a suspected human carcinogen and a known animal carcinogen. They are resistant to degradation and therefore persist for many years in the environment. Furthermore, they bioaccumulate in the foodchain and are stored in the body fat of animals and humans. Because of the health and environmental risks associated with PCB's, an Act of Congress, the Toxic Substances Control Act of 1976, directed the U.S. Environmental Protection Agency (EPA) to ban the manufacture of PCB's and regulated their use and disposal. EPA accomplished this by the issuance of regulation in 1978. The State of Connecticut, however, banned the manufacture of PCB's and began regulating them effective July 1, 1976. Both EPA and Connecticut, although banning their manufacture, allowed equipment containing PCB's to continue in use for the remainder of their useful lives.

PCB contamination from historic uses and dumping is widespread throughout the U.S. and the world. Disposal into waterways has caused PCB contamination of rivers, oceans, soils and even the polar ice cap. As a result, many forms of wildlife have become contaminated with PCB's. There have been bans on fishing in various locations. The U.S. Food and Drug Administration has issued an advisory against eating fish with more than 2 parts per million PCB's and recommended a maximum weekly intake. In addition, the U.S. EPA and the State of Connecticut have established a maximum contaminant level of 0.5 parts per billion of PCB's in drinking water.

Introduction

Dinoflagellates are an important part to any marine environment. They are important primary producers, and therefore an important part of the food chain. We can also use them as an indicator to the quality of the water and the ecosystem that it is part of. By looking at the number of dinoflagellates that are present in a given amount of water, we can tell the general water quality in that particular ecosystem.

Activity 1. (2 Weeks)
Growing Dinoflagellates

Background

Dinoflagellates are incredibly easy to grow at home, requiring as little care as a houseplant except these "plants" produce bright blue light when shaken at night! These dinoflagellates have a circadian rhythm, which controls their bioluminescence and photosynthesis on a 24-hour basis. They "think" its day, and they only produce bioluminescence or flash when they "think" it's night. The bioluminescence that a dinoflagellate can produce reflects how healthy it is. In a similar way that we perceive being touched, this is the type of stimulation that causes a dinoflagellate to produce a flash of light. Dinos are highly sensitive to anything that pushes on their cell walls. Even the seawater they live in can cause them to flash if it becomes very rough and stirred up. That is what is happening whenever you shake the container that your dinos live in. The water becomes very turbulent and pushes on their cell walls causing them to flash. It takes about a week for them to get used to the new light cycle (their circadian rhythms are getting use to their new home).

Materials:

- *Pyrocystis fusiformis* (bioluminescent dinoflagellates
- Microscope
- Aquarium/terriarium light
- Timer
- Culture flasks
- Alga-Gro seawater medium (nutritional needs of Dinoflagellates) found at Carolina Biological Supply Co. [800-334-5551 for Eastern U.S.]

Set-Up:

1. Buy bioluminescent dinoflagellate (Sunnyside Sea Farms [email: sunnyside@seaFarms.com)
2. Set up area for growing dinoflagellate. This must include a timer for the 12 hour on and 12 hour off cycle of light.
3. Microscopes for viewing of dinoflagellates.

Vocabulary

Photosynthesis: The process in plants and algae that allows them to produce their own food sources and continually regenerate oxygen in the environment.

Circadian rhythms: Biological process that oscillate with an approximate 24-hour periodicity when there are no external timing cues. Basically, a circadian rhythm is an internal daily biological clock.

Bioluminesce: production of light by living organisms.

Dinoflagellate: any of numerous one-celled, aquatic organisms bearing two dissimilar flagella and having characteristics of both plants and animals. Most are microscopic and marine.

Introduction

Introduce what the students will do in the lesson. Give background information on bioluminescent dinoflagellates, photosynthesis, circadian rhythms and dinoflagellates. In addition provide general information on the ecosystems of the Bioluminescent Bay and the Fox River.

Procedure:

1. Quantity of dinoflagellates
2. Dependable light source, bright but not too hot that has a timer (The only way they can grow is by producing their own food using photosynthesis, just like plants!)
3. A room that is not too hot or cold. Windows are not ideal, and one should avoid handling containers since the body temperature of humans could and often causes problems with dino growth.
4. Set up a "strict schedule" for light applications. Start schedule so that the dinoflagellates are in their day stage during the school day. After one week change schedule so that the dinoflagellates are in their night stage during the school day.
5. Clear container

Activity 2. (One Day)

Websites Materials

- Internet access
- Information on history of the Fox River (found at http://www.foxrivergroup.org/fox_treasured.htm)
- Information on Biolumence Bay (found at http://www.biobay.com/ and at http://www.viequesinformation.com/glow.html)
- Information about PCB's and the clean up (Cleanup plan can be downloaded at http://www.dnr.state.wi.us/org/water/wm/lowerfox/proposedplan.html)

Introduction to Fox River and Mosquito Bay
Procedure:

1. Talk about the history and future of the Fox River and the upcoming PCB clean up.

2. Take video/internet field tip to Mosquito Bay and discuss the importance and future of the bay.
3. Compare how humans have influenced both areas.

Activity 3. (One Day)
Field Trip to Fox River

Materials:

- Water Action Volunteers Worksheets (see attachments for worksheets, fact sheets and keys)
- Water Testing Kit
- Nets
- Magnifying Glasses
- White Ice Cube Trays
- Collection Tub
- Tweezers
- Thermometer
- Safety goggles, disposable plastic/latex gloves
- Measuring tape
- Markers/flags/ties for trees
- Watch with second hand/stopwatch
- Float (an orange works best)
- Calculator
- Yardstick or marked Dframe net pole
- Bucket
- Stirring stick
- Turbidity tubs

Introduction.
Introduce the equipment to students and familiarize them with how they function and their purpose. Describe the lesson and give any specific field trip instructions.

Procedure:

1. Take students to areas of the Fox River in Appleton that will be watch areas for the PCB clean up and have DNR representative talk with the students.
2. Break students into groups to work on:
    - Temperature, dissolved oxygen, and turbidity tests,
    - Biotic Index
    - Stream Flow
    - Habitat Information
3. Take river samples for examination in the lab.

Activity 4. (Two Days in LMC, One Day for presentations)
Research in LMC for Fox River, Mosquito Bay and Bioluminescent Dinoflagellates

Materials:

- Have LMC pull any information on Bioluminescent Dinoflagellates, Mosquito Bay, and Fox River.
- Internet access.
- Paper and markers to make posters

Introduction:
Describe the research activity to students and describe why bioluminescent bays are important to study and how they differ from aquatic life found in Wisconsin.

Procedure:

1. Show students where print materials are located.
2. Provide students with the following internet addresses as a start for their research:

   The Bioluminescence Web Page:
   http://www.lifesci.ucsb.edu/~biolum/organism/dinohome.html#LM
   http://www.lifesci.ucsb.edu/~biolum/
   Science Background Information from Berkley:
   http://www.ucmp.berkeley.edu/protista/dinoflagellata.html
   http://www.ucmp.berkeley.edu/glossary/gloss3/photosyn/index.html
   SCRIPPS Institution for Oceanography:
   http://www.sio.ucsd.edu/explorations/biolum/
   Island Adventures Bio Bay:
   http://www.biobay.com/
   Bioluminescent Bay:
   http://www.viequesinformation.com/glow.html
   Calgary Information Website on Dinoflagellates:
   http://www.geo.ucalgary.ca/~macrae/palynology/dinoflagellates/dinoflagellates.html
   Fox River Website:
   http://www.foxrivergroup.org/fox_treasured.htm
   http://www.foxriverwatch.com/
   Department of Natural Resources: Fox River
   http://www.dnr.state.wi.us/org/water/wm/lowerfox/proposedplan.html

3. Break students into groups of three.
4. Give each group the Fox River, Mosquito Bay or Bioluminescent Dinoflagellates to research. Students may use Internet or print resources.

5. Students will make posters of their findings. They must include information about human interaction and the problems that have been caused by this interaction. Also included should be an educated prediction of the future whatever they are researching.
6. Have students present their findings to the class.

Activity 5. (Two Days)
Water sample testing and experimenting with bioluminescence dinoflagellates

Materials:

- Water samples from Fox River and Samples of Bioluminescence Dinoflagellates
- Microscopes
- Slides

Introduction.
Introduce lesson to students. Describe what to look for when examining the dinoflagellates.

Procedure:

1. Break class into lab groups.
2. Set up lab with microscopes and samples of bioluminescent dinoflagellates and a sample of Fox River water.
3. Have students set up wet mounts of each sample.
4. Have students answer the following questions:

Experimenting with the bioluminescence dinoflagellates:
During Day Stage

1. What do the dinoflagettates look like under the microscope?
2. Draw and label the parts of the cell.

During Night Stage:

1. What different types of stimulation cause them to flash?
2. How much stimulation can the dino withstand before their bioluminescence is exhausted? How long would it take them to recover?
3. What immediate effects do you see if you put dinos in their night phase into the light?
4. What difference do you notice between a cell in the middle of its day phase and one in the middle of the night phase? Hint: the chloroplasts are the golden brown bodies with the cell. How might you explain this?

Examine the different stages in the life cycles of these asexually reproducing cells. (The entire life cycle takes 5-7 days.)

Fox River Water:

1. Identify the different organisms that you see. (Use River Key from Water Action Volunteers Packet)
2. What is your opinion of the quality of the water based on the organisms that you identified?
3. What organisms would you expect to see for a high quality water sample?

Conclusion:

There are many ways that the quality of a marine or freshwater ecosystem can be identified. Although looking at the number of dinoflagellates is one way many other organism can be used for this purpose. Protecting water will be one of the most important environmental issues that our students will face and it is important that they understand the reason behind this.

Assessment:

Students will write proposals on how and why Mosquito Bay and the Fox River should be protected. Students will present these proposals to a panel made up of their peers. The wining proposal will receive a prize.

Adaptations:

By taking out some of the researching that is involved in this lesson, students of any age and ability will be able to enjoy this lab. The hands on activity will grab student interest and will give them background information to further study the subject.

References:

The Bioluminescence Web Page:
http://www.lifesci.ucsb.edu/~biolum/organism/dinohome.html#LM
http://www.lifesci.ucsb.edu/~biolum/
Science Background Information from Berkley:
http://www.ucmp.berkeley.edu/protista/dinoflagellata.html
http://www.ucmp.berkeley.edu/glossary/gloss3/photosyn/index.html
SCRIPPS Institution for Oceanography:
http://www.sio.ucsd.edu/explorations/biolum/
Island Adventures Bio Bay:
http://www.biobay.com/
Bioluminescent Bay:
http://www.viequesinformation.com/glow.html
Calgary Information Website on Dinoflagellates:
http://www.geo.ucalgary.ca/~macrae/palynology/dinoflagellates/dinoflagellates.html
Fox River Website:
http://www.foxrivergroup.org/fox_treasured.htm
http://www.foxriverwatch.com/
http://www.dnr.state.wi.us/org/water/wm/lowerfox/proposedplan.html