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Cnidarian-Microalgae Symbioses

Prep Time: ~40-60 minutes
Lecture Time: ~40 minutes
Activity Time: ~50-60 minutes
Total Teaching Time: ~90 minutes

Grade Level: High School

Materials Needed: Powerpoint slides, preserved specimens (**RECOMMENDED**; jellyfish, sea anemones, coral skeletons [check Carolina Biological or other biology classroom suppliers])

Activity 1 (5.3)

- one dissecting microscope per group
- one *Aiptasia pallida* per group/person (available at http://www.carolina.com/marine-and-saltwater-animals/sea-anemone-aiptasia-living/162865.pr?question=aiptasia)
- tropical flake fish food OR hatched brine shrimp
- one pair of forceps per group
- small clear cups/bowls of sea water (low clearance is best [short in height])
- plastic wrap
- salt to mix seawater (http://petoverstock.com/instant-ocean-sea-salt-mix-10-gal.html?utm_source=Google+Products&utm_campaign=Google+Products+Datafeed&utm_medium=Comparison+Shopping&gclid=CjwKEAjwz4u9BRCbioK3stnBznESJADA75xbGTc_Bps_eDLbS2xsEV0cB0ziC6ZH1Qukr3cD9kz9-RoC2Nfw_wcB)

Activity 2 (6.3):

- one compound microscope per group (objectives 5x, 10x and 40x)
- two or three *Aiptasia pallida* per group (available at http://www.carolina.com/marine-and-saltwater-animals/sea-anemone-aiptasia-living/162865.pr?question=aiptasia)
- one pair of small dissecting scissors (option: http://www.carolina.com/dissecting-scissors/dissecting-scissors-stainless-steel-straight-4-12-in/621760.pr)
- small cup of seawater
- one microscope slide and cover slide per person
- white vinegar (acetic acid)
- one medium beaker per table
- several 1.5 mL disposable pipets per group
- one paper towel per person
- salt to mix seawater (http://petoverstock.com/instant-ocean-sea-salt-mix-10-gal.html?utm_source=Google+Products&utm_campaign=Google+Products+Datafeed&utm_medium=Comparison+Shopping&gclid=CjwKEAjwz4u9BRCbioK3stnBznESJADA75xbGTc_Bps_eDLbS2xsEV0cB0ziC6ZH1Qukr3cD9kz9-RoC2Nfw_wcB)

Objectives

1. Define symbiosis and three subtypes.
2. Describe the relationship between microalgae and their host cnidarians, and why some sea anemones are good models for studying some aspects of coral-microalgae symbioses.
3. Define coral bleaching and communicate why the changing climate is affecting coral reef ecosystems.

1. Symbiosis (~10 minutes)

1.1 Can anyone define symbiosis?

a. **Symbiosis**: an intimate, long-term relationship between two or more different organisms where at least one partner benefits from the interaction.

1.2 Does anyone know of an example of a symbiotic relationship?

a. Sea anemone and clown fish (mutualism)
b. Human and malaria (parasitism)
c. Cow and bird (commensalism)

1.3 There are many examples of symbiotic relationships, but symbiosis is a very broad term that can be used to refer to many different types of relationships. Let's define three common subtypes of symbiosis.

a. **Mutualism**: A symbiotic relationship in which both partners benefit from the interaction.
b. **Commensalism**: A symbiotic relationship in which one partner benefits while the other is unaffected.
c. **Parasitism**: A symbiotic relationship in which one partner benefits while the other is harmed.

1.4 Let's figure out how to characterize a few interactions from the list of examples.

Questions to ask students for each interaction: Which partners benefit? Which partners are harmed? Which partners are unaffected? How would you characterize this relationship?

a. Sea anemone and clownfish (**mutualism**): clownfish lives among the tentacles of the sea anemone.
   i. What does each partner get from this relationship?

1. Clown fish uses the anemone and its stinging tentacles for protection from predators, and as a home.
2. The sea anemone gets bits of food that the clownfish drops, and is able to utilize waste products from the clownfish.

b. Cow and bird (**commensalism**): cow lives in a field and the bird lands on the ground in the field.
   i. What does the cow get from this relationship?
      1. Nothing.
   ii. What does the bird get from this relationship?
      1. The bird picks bugs and undigested bits of food out of the cow's feces.
      2. **<u>CRITICAL THINKING</u>**: Can anyone think of a situation when this relationship would become a mutualism? How does each partner benefit from the relationship now?
         a. If the bird was on the cow, picking bugs/parasites off of the cow. The cow gets parasites or annoying bugs removed and the bird gets some food.

c. Human and malaria (**parasitism**): malaria lives in the bloodstream of a human.
   i. What does the human get from this relationship?
      1. Severe blood loss (anemia) and clogged blood vessels
   ii. What does malaria get from this relationship?
      1. Blood and nutrients and a place to reproduce

2. Cnidarians and Microalgae (~7 minutes)

2.1 What are cnidarians?

a. **Cnidaria** is a phylum that includes corals, sea anemones, and jellyfish.
b. All cnidarians are:
   - radially symmetrical
   - have two tissue layers
   - have one body opening
   - jelly-like
   - have a nerve net
   - have a digestive cavity that forms the main body

2.2 Cnidarians and symbiosis

a. Corals, sea anemones, and some jellyfish join in mutualistic relationships with **microalgae** (symbionts).

   a. **Microalgae**: microscopic algae that live in the **water column**, in the **sediment**, or **on other organisms**. Microalgae can live in **freshwater** or **saltwater**, and are important **primary producers** (they undergo photosynthesis to produce sugars for larger organisms).

b. In cnidarian-microalgae symbioses:
   - the microalgae undergo photosynthesis
   - pass sugars to their cnidarian host
   - the host provides a place for the algae to live
   - inorganic carbon (used for photosynthesis)
   - nitrogenous waste (used in algal growth)

c. These are endosymbiotic relationships. What does endosymbiotic mean?
   a. **Endosymbiotic**: A **mutualistic** relationship in which one organism lives **inside** another organism; in time the **two organisms become one organism**. The process of becoming one organism takes millions, possibly billions of years.

3. Coral Bleaching (~10 minutes)

3.1 Relationship breakdown – Does anyone know what we call the breakdown of the symbiotic relationship between corals and microalgae?

a. **Coral Bleaching:** The breakdown of the coral-microalgae symbiotic relationship and subsequent loss of microalgae from the coral tissue.

b. The process can be observed in symbiotic sea anemones and jellyfish too; generally termed **bleaching.**

3.2 Why is coral bleaching so detrimental to corals and to the reef ecosystem?

a. Some corals rely exclusively on sugars from their symbionts to survive. Unless these corals are able to uptake more microalgae after bleaching has occurred, then they will die of starvation.

b. Corals build the foundation of reef ecosystems; their calcium carbonate skeleton builds reef structures. These reef structures are what support the incredible diversity of life on coral reefs. Reefs also provide many ecosystem services to society:
   - tourism
   - coastal protection
   - source of food
   - nutrient cycling
   - nursery space
   - habitat space

When corals bleach and die, there is not maintenance of the reef structure anymore. Diversity decreases and many organisms that once inhabited the reef either leave or die.

3.3 What causes bleaching to occur?

a. Temperature shock.
   i. High temperature
   ii. Low temperature
b. Prolonged light shock.
   i. High light
   ii. Low light
c. Low pH conditions can lead to bleaching.
d. Disease can lead to bleaching.

4. Climate Change and Ocean Acidification (~5 minutes)

4.1 Defining climate change.

a. **<u>Climate change</u>:** An anthropogenic global problem that is causing higher maximum temperatures, increased sea surface temperature, and is predicted to cause more intense storms and more unpredictable weather systems. Climate change is caused by excess greenhouse gases (water, CO_2, and methane) in the atmosphere trapping heat in the atmosphere.

4.2 Defining ocean acidification.

a. **<u>Ocean acidification</u>:** a global problem that will affect all marine life. Ocean acidification is caused by excess atmospheric CO_2 dissolving into sea water, making the pH of seawater more acidic.

5. Sea Anemones versus Corals (~20 minutes)

5.1 Corals

a. It is important to study corals and their relationship with microalgae so that we can better **protect** reefs and the **abundance and diversity** of organisms that live on them. However, corals are becoming increasingly difficult to study:
   - Many are **endangered** and are **protected** from collection. Even if permits are obtained to study some of these corals, they are **difficult to care for in the laboratory**.
   - Corals need **specific water chemistry**, **optimal temperature**, and **high light** conditions. For these reasons, corals are often very **expensive to maintain and study**.

- Corals can only be maintained in a **bleached state** for a short period of time before they **must be recolonized with symbionts or die**.

5.2 Why we study sea anemones instead

a. Sea anemones on the other hand are **not endangered**, are **easy to collect** from the field, **do not require specific water chemistry**, can be maintained under **low light** conditions, and can survive under a **wider range of temperature** conditions.
   - Sea anemones can be maintained in a **bleached state** for an **unlimited amount of time** as long as they are fed.
   - Some sea anemones join in a symbiotic relationship with **microalgae from the same genus as corals**. This makes some sea anemones an **ideal model for studying some aspects of cnidarian-algae symbioses**.
   - Sea anemones are **not ideal for all studies**, because they **do not produce a calcium carbonate skeleton** like corals.

b. Examples of symbiotic sea anemones:
   - *Aiptasia pallida*
   - *Anthopleura elegantissima*

5.3 **Activity 1**: Viewing and feeding *Aiptasia pallida*.

a. <u>At least 4 hours before class:</u>
   - If the anemones are attached to the side of a larger container, you can remove them by scraping them off of the container with a flat-head screwdriver, or your fingernail.
   - Fill the bowls up with sea water and place enough anemones in the cup/bowl (either one per group or one per person). You can also put one anemone per cup/bowl. This will allow the anemones to attach to the bottom of the cup/bowl, making observation and feeding easier.
   - Avoid moving the bowls around so that the anemones can settle.

b. Cover the bottom of the dissecting microscope with a sheet of plastic wrap to protect from corrosive saltwater and make clean-up easier.

c. Allow each student to observe their anemone for a minute or two. Start with the microscope light off and then turn the light on. The anemone will likely react to the light. If the students zoom in far enough, they may be able to see individual algal cells (small brownish/yellowish spheres) in the anemone.
   - **<u>GUIDING QUESTIONS</u>**:
     - What do the anemones do when the light is off?
       - Probably not a lot of movement. The anemones may be closed up or just relaxed open.
     - Hypothesize what the anemones will do when you turn the light on (example hypothesis structure below).

- I hypothesize that the anemone will ___________ when the light is turned on because _____________.

- What do the anemones do when the light is turned on?
  - The anemones will probably orient their tentacles in a way such that the most surface area is receiving direct light. The anemones may move around more.
- Why do you think the anemone react like this when the light is turned on?
  - The anemones are re-orienting their tentacles so that the microalgae inside are able to capture optimal light. More light for the algae, means more photosynthesis, and more sugar for the anemone (This reaction is what we expect for most cases. You could also provide the anemones with too much light, which may cause the anemone to close up to prevent photo inhibition in their algae).
- Based on your observations of how *Aiptasia* moves, make a prediction about how this movement affects how *Aiptasia* captures food.

d. Next, give each student a single flake of fish food. Have each student in turn, use the forceps to pick up the flake fish food, and while looking through the microscope, feed their anemone by placing the flake on the tentacles of the anemone. Tell the students to observe how the anemone puts the food into its mouth with its tentacles. Ask the students to record observations about anemone feeding from when the food contacts the tentacles to when the food particle is completely ingested.

e. If time permits, the students can feed the anemones more than once.
   - **LEAD IN FOR NEXT SECTION**: What makes the tentacles of cnidarians able to capture food that may be larger or stronger than themselves?

6. **Nematocysts** (~35 minutes)

6.1 What are on cnidarian tentacles that allows them to capture food?

a. **Nematocysts:** specialized cells containing a harpoon-like projection and a trigger hair. When the trigger is stimulated, the harpoon fires, typically injecting toxins in to the target.

6.2 What are nematocysts used for?

a. Nematocysts come in a variety of shapes and sizes, and are used for multiple purposes. **Basotrichs** are used in food capture and defense and **spirocysts** are used to attach to a substrate.

6.3 **Activity 2:** Observation of microalgae and nematocysts firing (NOTE: You can also use hydra to observe nematocyst firing; if so, use a whole small hydra on each slide and put the coverslip over the whole organism as if it were a tentacle).

a. This activity can be prepped by the teacher or by the students. Using small sharp scissors, quickly clip a tentacle from one of the anemones. Try to clip the tentacle as close to the oral disk of the anemone as possible. Using the scissors or a disposable pipet, carefully pick up the tentacle from the water. If you miss the first time or lose the tentacle, wait a couple of minutes until the anemone expands (This is why having multiple anemones per student is optimal for this activity).
b. Place the tentacle onto a microscope slide with no more than one or two drops of seawater. Carefully place a cover slip over the tentacle. Using a pencil with an eraser, carefully push down just a little bit on the cover slip, directly on top of the tentacle. You will probably see the tentacle squish a little bit.
c. Make sure that the microscope is on the lowest magnification (< 10X). Clip the microscope slide onto the stage of the microscope and center the slide so that the tentacle is in the center of the viewing area.
d. Looking through the microscope and adjust the focus of the scope until you can see the tentacle. Move the stage towards the base (end that you clipped) of the tentacle. When you find the end of the tentacle, you should be a bunch of microalgae (brown spheres; *Symbiodinium*) oozing from the end of the tentacle. Increase the magnification to 10X and allow each student in the group to observe the tentacle at this magnification. Then, encourage the students to increase the magnification to 40X to observe the symbionts and the tentacle closer up. Students may be able to see nematocysts in the tentacle tissue or mixed in with the algae that are oozing from the base of the tentacle. The nematocysts may be discharged or unfired (Unfired nematocysts look like long ovals, and discharged nematocysts look like long ovals on a string!).
   - **<u>GUIDING QUESTIONS</u>**:
     - Have students draw an unfired and a discharged nematocyst that they observed under on the microscope.
     - How is the location of the nematocysts on the tentacle related to the function that they serve for the animal?
       - Nematocysts used for prey capture are located on the tentacle of the animal. They are concentrated around the edges of the tentacles, especially near the tip of the tentacle.
     - Why do you think nematocysts are found in higher concentration near the tip of the tentacle?
       - If a prey item contacts the tip of the tentacle, there is less of a chance that the prey will come in to contact with another tentacle, so the anemone may need more nematocysts in order to ensure a successful capture

event. If a prey item contacts the middle of the tentacle, there is a likely chance that the prey item will also contact additional tentacles, so the anemone may not need as many nematocysts in order to ensure a successful capture event.

- Based on what you know about nematocysts and their many functions, where else would you predict to find nematocysts on a sea anemone? Hint: Not all nematocysts are used for prey capture. Some nematocysts are used to attach to substrate and others are used for defense.
    - The column of the anemone – defense nematocysts
    - The foot of the anemone – attachment nematocysts

e. In order to observe the nematocysts firing, each student will need to prepare their own microscope slide as described above (Or the teacher can prepare a couple of slides and show the whole class if you have a microscope with a camera attached that can be projected on a screen).

f. With the microscope slide on the stage, focus on an edge near the tip of the tentacle (opposite end from the cut). Increase magnification to 10X and refocus and then 40X and refocus.

g. Carefully place a drop or two of white vinegar next to but not touching the cover slip. Have one student look through the microscope, and another student use the disposable pipet to carefully push the drop of vinegar towards the edge of the cover slip. As soon as the vinegar mixes with the water, have the student pushing the vinegar tell the student looking through the microscope. The student looking through the microscope should see nematocysts fire (NOTE: this process can be instantaneous or can take a couple of minutes as the vinegar mixes with the water). If no nematocysts fire, have the student not looking through the microscope take a small corner of paper towel and touch the water under the edge of the cover slip on the opposite side that the vinegar was added, to draw the vinegar across the slide. Sometimes you may have to add an additional drop of vinegar. The key to the student seeing the nematocysts fire is to continue watching the tentacle while another student moves/adds the vinegar. Be patient!

Discussion/Wrap-Up Questions

1. Why did the vinegar cause the nematocysts to fire? Hint: The nematocysts did not physically contact anything like they normally would.
2. Can anyone think of an example of an endosymbiotic relationship that has become permanent (i.e. the two organisms have become one)?
    a. Mitochondria
    b. Chloroplast
3. How do nematocysts relate to cnidarian feeding mode, lifestyle, and defense?
4. What role do microalgae play in cnidarian nutrition?

Definitions

1. **Symbiosis**: an intimate, long-term relationship between two or more different organisms where at least one partner benefits from the interaction.
2. **Mutualism**: A symbiotic relationship in which both partners benefit from the interaction.
3. **Commensalism**: A symbiotic relationship in which one partner benefits while the other is unaffected.
4. **Parasitism**: A symbiotic relationship in which one partner benefits while the other is harmed.
5. **Microalgae**: microscopic algae that live in the water column, in the sediment, or on other organisms. Microalgae can live in freshwater or saltwater, and are important primary producers (they undergo photosynthesis to pass energy up the food chain).
6. **Endosymbiotic**: A mutualistic relationship in which one organism lives inside another organism; in time the two organisms become one organism. The process of becoming one organism takes millions, possibly billions of years.
7. **Coral Bleaching:** The breakdown of the coral-microalgae symbiotic relationship and subsequent loss of microalgae from the coral tissue.
8. **Climate change:** An anthropogenic global problem that is causing higher maximum temperatures, increased sea surface temperature, and is predicted to cause more intense storms and more unpredictable weather systems. Climate change is caused by excess greenhouse gases (water, CO_2, and methane) in the atmosphere trapping heat in the atmosphere.
9. **Ocean acidification:** a global problem that will affect all marine life. Ocean acidification is caused by excess atmospheric CO_2 dissolving into sea water, making the pH of seawater more acidic.
10. **Nematocysts:** specialized cells containing a harpoon-like projection and a trigger hair. When the trigger is stimulated, the harpoon fires, typically injecting toxins in to the target.

Other Resources

- Digital Lesson Plan and Powerpoint: https://www.dropbox.com/sh/il55p60n07xb4v9/AABnPPWyY3fWYDFatJkBqOQHa?dl=0
- Endosymbiosis: http://evolution.berkeley.edu/evolibrary/article/endosymbiosis_01
- Symbiosis: https://www.youtube.com/watch?v=hncA8KsSqdc
- *Aiptasia* feeding: https://www.youtube.com/watch?v=HmfLP6qDHJU
- *Aiptasia* feeding under fluorescent lighting (Red fluorescence is caused by the chlorophyll in the algal symbionts. Pause at 0:08 at 1080p and see if you can point

out individual microalgae in the bottom right corner of the sea anemone.): https://www.youtube.com/watch?v=T-fqcK_WRBc

- Nematocysts firing: https://www.youtube.com/watch?v=6zJiBc_N1Zk
- Nematocysts firing: https://www.youtube.com/watch?v=IJidAI1WASo
- Sea anemones and clownfish: https://www.youtube.com/watch?v=eWXOurnVTYg
- TED-Ed video about symbiosis: https://www.youtube.com/watch?v=2AM3ARs9MMg&list=PL45DD1CA57AA3122A
- Nematocyst firing mechanism: https://www.youtube.com/watch?v=-Tp38DUjUnM
- Nematocyst firing mechanism: https://www.youtube.com/watch?v=YF9CP8rQ-Pc
- Nematocyst firing mechanism (0:52 – 1:19): https://www.youtube.com/watch?v=xQNxXUtRjzg
- Unfired nematocysts: http://magnified-world.tumblr.com/image/96920385739