Aiptasia pallida as a Model for Coral Reef Bleaching

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Abstract

Using *Aiptasia pallida*, the tropical pale sea anemone, students can study coral reef bleaching by measuring the effects of various environmental factors on zooxanthellae, photosynthetic symbiotic dinoflagellate algae that inhabit corals and *Aiptasia*. *Aiptasia pallida*, like corals, can lose their endosymbionts in response to various stimuli. In this experiment students manipulate several factors which have been associated with coral bleaching, such as changes in temperature and light intensity. By measuring endosymbiont numbers before and during exposure to various temperatures and light intensities, students monitor the effects of these factors over time. Students also look at the role that food plays in preservation of endosymbionts by *Aiptasia pallida* during the different environmental treatments. Students work collaboratively in their labs and pool their data with data from other lab sessions that run both concurrently and throughout the day and evening, giving them a comprehensive look at endosymbiont numbers during the course of the day. Skills such as experimental design, collecting, organizing, and representing data, and communicating results are an important component of the lab. Students also gain procedural knowledge as they learn how to handle the anemones, identify, and quantify endosymbionts. This laboratory experiment provides the flexibility for customizing to the needs of the course and the availability of resources. It lends itself well for studies in ecological or species relationships, and can be taught using different strategies, ranging from traditional laboratory instruction to inquiry-based learning.

Introduction

Aiptasia pallida is a small sea anemone found in the Caribbean and along the east coast of the United States. It is most abundant from North Carolina to the Florida Keys. *A. pallida* has a mutualistic symbiotic relationship with dinoflagellate algae of the genus *Symbiodinum*. This dinoflagellate is called an endosymbiont, because it lives inside the sea anemone, its host. The sea anemone receives oxygen and photosynthetic products from the dinoflagellate, whereas the dinoflagellate's photosynthetic ability is enhanced by the carbon dioxide and other molecules produced by the anemone. Most of these molecules are waste products resulting from the sea anemone's metabolic processes.

Sea anemones are not unique in exhibiting this type of relationship. As a matter of fact, other cnidarians (the group to which sea anemones belong) like hydra and coral reefs have the exact same symbiotic relationship with the same genus of dinoflagellate. In coral reefs, the endosymbiont is particularly important because it helps the coral host obtain calcium carbonate, which it needs for building the colorful, structural framework of the coral ecosystem. It is also one of the primary producers of the coral reef ecosystem.

If the coral hosts lose significant amounts of endosymbionts, the coral reefs become whitened in a process called coral bleaching. Loss of endosymbionts is thought to be mainly due to the increased temperatures and solar radiation that has been correlated with global warming. Numerous studies have looked at the effects of environmental stresses on the cnidarian-dinoflagellate relationship, as well as food abundance and other factors that may enhance or promote this symbiotic relationship. Scientists are working hard to study the process of coral bleaching, yet experimental findings to date have not shed much light on how to resolve this global issue.

In experiments that analyze the coral bleaching process, the sea anemone is used as the study animal, because it is very easy to keep in the lab and also undergoes bleaching when it loses its endosymbionts. *A. pallida*, in particular, is very hardy and is readily available from local suppliers. This species of sea anemone is actually considered a pest by keepers of marine aquaria, because it multiplies easily, is voracious, and outcompetes most other aquarium life. We will be using *A. pallida* in our laboratory studies.

Student Outline

Assess the overall concentration of endosymbiont cells in *A. pallida* individuals exposed to various environmental factors.

Protocol

Week 1: Examining the anemones and endosymbionts

1. Examine one anemone under a dissecting scope by placing it in a watch glass with some saltwater. Note the tank from where you took your anemone so that you can return it once you are finished. The sea anemone will probably collapse. Allow it some time to expand fully before making your observations. NOTE: Keep the microscope light off as the anemone recovers.
    - **a.** Make a drawing and write down your observations in the space provided at the end of this lab.
    - b. Measure the oral disc diameter (Figure 1) of your organism with a clear plastic grid ruler. Slide the ruler under the watch glass and count the squares taken up by the oral disc diameter (each square is 1 mm across). NOTE: You need to learn to measure the sea anemone, but your lab instructor will be measuring the sea anemones you will be sampling each week. He/she will provide those measurements for you to enter into Table 1. (Oral disc diameter can also be taken by using calipers.)

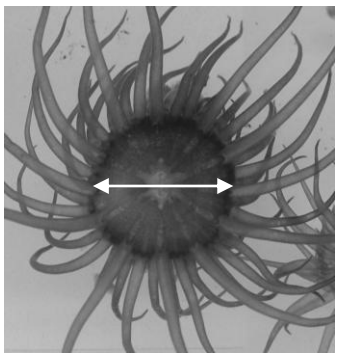

Figure 1. Measuring oral disc diameter.

2. The group with the largest sea anemone will cut their sea anemone into six pieces using a scalpel from a dissecting kit. (All other groups will return the anemones to their respective tanks. Be careful with the organisms. They may look like they have collapsed, but they will resume their shape once they return to their aquaria.) The table with the large sea anemone will use a pair of tweezers to separate the pieces. One person from each table will then take a piece of anemone to prepare a wet mount (see next step).
3. Each table will make a wet mount slide of a piece of anemone.
    a. View under the microscope to locate the endosymbionts. Make sure that you observe and can identify endosymbionts, because you will be counting these cells for the duration of this experiment.
    b. Make a drawing and write down your observations in the space provided at the end of this lab.

Week 1-3: Collecting cell count and absorbance data
Procedure A

1. Four tables will each receive one bullet tube with one fourth of a sea anemone from each of the experimental aquaria.
2. Using a pipette, add enough distilled water to your bullet tube to reach the 1.5 ml line. Use a motorized tissue grinder to form a sea anemone homogenate (an even mixture of sea anemone, endosymbiont cells, and distilled water).
3. Centrifuge for approximately 30 seconds in a microcentrifuge.
4. Observe your sample to make sure that you have supernatant (the fluid suspended at the top) and a solid pellet at the bottom of the tube. If you don't see a clear separation, then centrifuge again.
5. Using a pipette, remove the supernatant and discard, being careful not to disturb the pellet at the bottom of the tube (the pellet represents the endosymbiont cells).
6. Using a pipette, add enough distilled water to fill the bullet tube containing the solid pellet to the 1.5 ml line. Vortex for approximately 10 seconds to resuspend the cells (to put cells back into solution).
7. At your table, break up into pairs:
    a. *Pair #1*: Set a micropipette fitted with a yellow tip to 100 µl. Take 100µl of your sample and follow step #8 below. Before using the micropipette, pay careful attention as your laboratory instructor demonstrates this technique. Pipetting can be difficult, particularly when using such small portions. It is important to be able to properly measure each sample.
    b. *Pair #2*: Transfer the remaining amount of sample into a cuvette that can be used in a spectrophotometer. Add more distilled water as needed to fill the cuvette sufficiently. Using a Kimwipe®, wipe off any fingerprints from the cuvette prior to putting it in the spectrophotometer.
    Take the absorbance reading for your sample in a Spectrophotometer set to 610 nm. Your lab instructor will assist you with this procedure. Record your measurement in Table 1.
8. Place the sample you extracted with the micropipette in step #7a on a grid slide, place a cover slip over the sample.

a. Using a compound light microscope, count the number of endosymbiont cells. (NOTE: you will be using a random sample approach to choose one square of the grid slide to sample. Your lab instructor will assist you.)
    i. Record your count in Table 1 for your experimental condition (i.e., control, treatment 1, 2, or 3). Make sure to get counts from the other groups.

Procedure B

1. Initial set up: Place each sea anemone in a perforated Petri dish (see Figure 2) suspended in a large tank by an aquarium sleeve (see Figure 3). Sea anemones will acclimate in this tank before they are exposed to control and experimental conditions. Leave sea anemones undisturbed in the original aquarium water for approximately one week

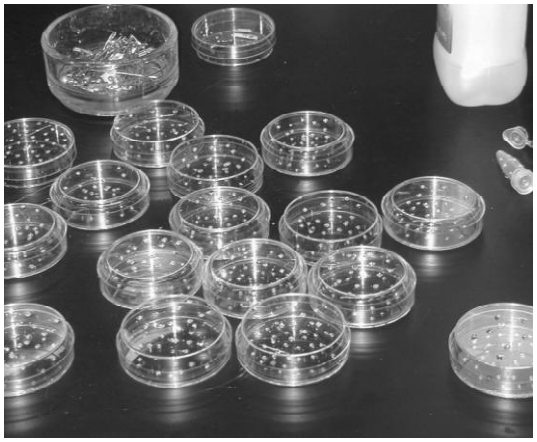

Figure 2. Petri dishes used

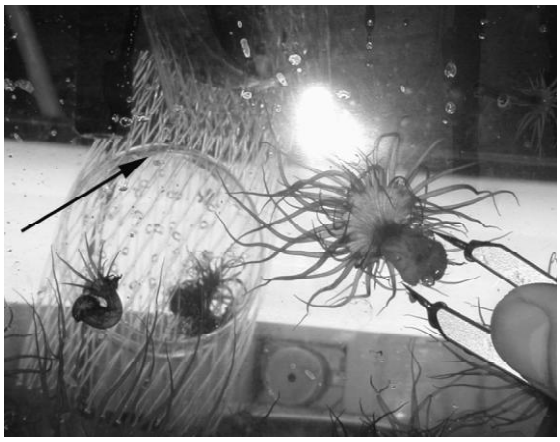

Figure 3. Anemones floating in sleeve in aquarium.

2. Remove the Petri dishes with the sea anemones from the aquarium sleeves and place the perforated dish in a lid without holes. Using calipers, measure the "base" (see Figure 4) and "head" (see Figure 5) of each specimen. Record in the Excel® file prepared for your treatment. Place in the appropriate control and experimental conditions.

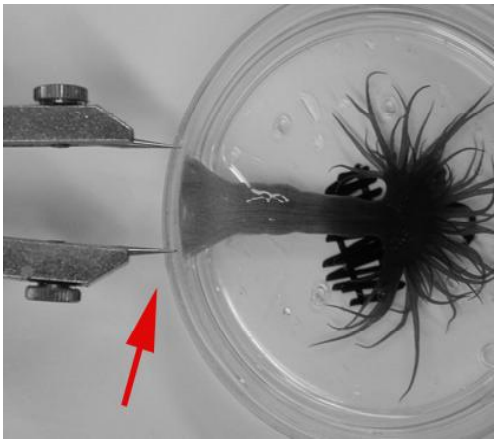

Figure 4. Measuring base.

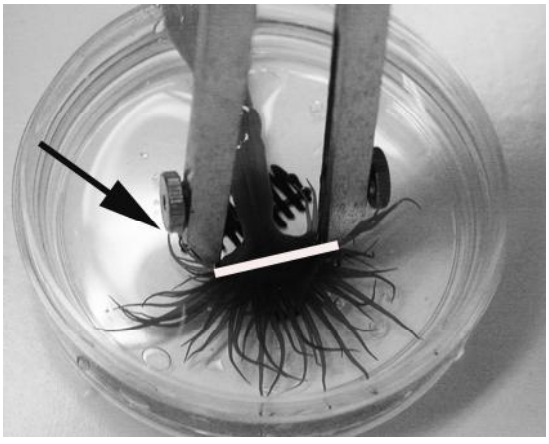

Figure 5. Measuring head.

3. Measure endosymbionts for each condition. Transfer the water from the Petri dish into a cuvette that can be used in a spectrophotometer. Using a Kimwipe®, wipe off any fingerprints from the cuvette prior to putting it in the spectrophotometer.
    a. Take the absorbance reading for your sample in a Spectrophotometer set to 610 nm. Your lab instructor will assist you with this procedure. Record your measurement. Repeat for each condition. NOTE: Ensure that endosymbionts are present in the water before transferring to the cuvette. Make a slide with a sample of the Petri dish water to ensure that you have endosymbionts.

NOTE: You will need to record data obtained from all of the lab groups in your section as well as from other lab sections. Your laboratory instructor will facilitate the collection of data.

Table 1. Lab section _____ sea anemone data.

Week (Date)	Experimental Condition	Oral Disc Size (mm)	Cell Count	Absorbance
One:				
Two:				
Three:				

Instructor's Notes

This lab is designed so that you can use either procedure "A" or "B" as fits best with your lab conditions. It is also designed for large courses, so you may adapt as necessary. If running a large course with many lab sections, you can pool data from all lab sections so that students have more data to analyze (you can provide data tables in each lab room to record data throughout lab sections, but students should record all of their data in their lab book/notebook as well). If conducting this experiment in a smaller class, students can use more than one fourth of a sea anemone in when processing sea anemones as described in procedure "A."

Prior to lab, ask students to answer the following questions as part of a "pre-lab" assignment.

1. If you were part of a research team assigned to finding ways of preserving the fragile coral reef ecosystem, which factors would you be most interested in examining?
2. Based on the observation that sea anemones lose their endosymbionts due to various factors, write a hypothesis that considers only one of these factors.

3. Describe the experimental design you would use to test your hypothesis. What are the dependent and independent variables?
4. How would you set up your data table to record the data you plan to collect? Draw your table below.

Also, provide students with a place to make their observations and drawing for the duration of the lab. For the first part of either procedure, provide students with a diagram or picture of the internal anatomy of sea anemones, so that they become familiar with their model animal. When students have completed the lab, they can answer the following questions:

1. What general trend do you observe in your cell count data?
2. Why did we measure absorbance? What general trend do you observe in your absorbance data?
3. How would you graph your data?
4. What general conclusions can you make about the environmental factor you observed and its effects on endosymbiont concentration in anemones?

When students are viewing the endosymbionts in *A. pallida*, they should see golden algae (Fig. 6):

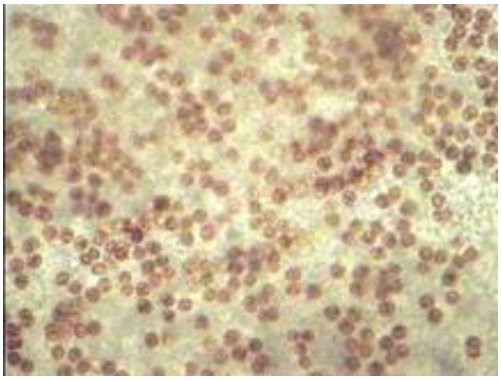

Figure 6. Sea anemone endosymbionts(100x).

If the lab is equipped with video microscopy equipment, encourage students to take pictures of the endosymbionts.

Experimental conditions to use with students include: varied temperatures, direct UVA and UVB light, no light, dietary changes (including food deprivation). All of these conditions allow students to make observations about endosymbiont cell quantity and overall sea anemone health. Students can make various predictions and manipulate a number of variables. These experimental conditions may be carried out in small aquaria where students can place the sea anemones with or without the perforated petri dishes.

When sea anemones lose their endosymbionts, they will appear pale to completely bleached (Fig. 7).

Figure 7. Unbleached (left) and bleached (right) sea anemones.

Notes for Procedure B

1. We have successfully kept sea anemones for up two months in the conditions specified under procedure "B". However, note that eventually individuals do find their way out. The advantage of the Petri dishes is that individuals show no stress response when placed in the dishes, since animals are removed gently from the tank, usually by placing a larger container under the Petri dish and removing it with its contents along with some sea water. Animals will not retract tentacles or if they do, they will only do so momentarily. Students do not have to wait to measure animals when using this technique.
2. Petri dishes can be kept in larger Petri dishes containing water (because there are holes in the smaller dishes) on window sills, in heated incubators, etc. for weeks. We have kept dishes on window sills for two weeks with little care except to add some new sea water every two to three days for two weeks. Animals show an initial stress response, becoming darker and shrinking and then after about a week (when you are convinced they are dead), they begin to expand to normal size. These individuals appear very light obviously having lost many endosymbionts.

Also consider using these animals for any lab in which you would normally use *Hydra*. Students can more easily see the endosymbionts in a small sample, even in one tentacle of a specimen. The same is true for nematocysts. The cnidocytes are larger in this group and one specimen can furnish enough cells (since there are so many tentacles) for a whole class. Clearer individuals (and they vary) can be fed, and since they are so large as compared to *Hydra*, students can easily observe food entering the alimentary tract. They are easily kept in an aquarium with a bubbler for months. We have had a colony for two years kept in a salt water aquarium with a fresh water filtering system (no protein pumps necessary). Be careful of substrate, since these organisms will burrow into any small crevice and may be impossible to take out. We keep live rock in the colony's tank because live rock adds so much stability to the system and helps to maintain the colony. However, for a few weeks before experiments, we remove individuals that happen to move on to tank walls and transfer them to a tank with only a little sand on the bottom.

We have not been successful in finding a way to extract intact individuals from the live rock. If you simply wish to observe individuals or speed the process of obtaining free individuals, Petri dish bottoms (with no holes) that have been scratched can be

suspended in the colony tank. Individuals are attracted to high and rough surfaces (we suspect out of competition for food and light; we feed fish food and raise colonies under full spectrum lights). We suspect the attraction for being high in the aquarium close to lights and floating fish food is what keeps individuals, which can easily leave Petri dishes with holes, from leaving the dishes. Individuals that become attached (and they will, even to the minute ridges created by the scratches) to the Petri dish bottoms can easily be observed in a larger container of sea water, or scraped off with a blunt narrow object (we use old credit cards) for examination of nematocysts, for placement in floating Petri dishes with holes, or for feeding studies.

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About the Authors

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