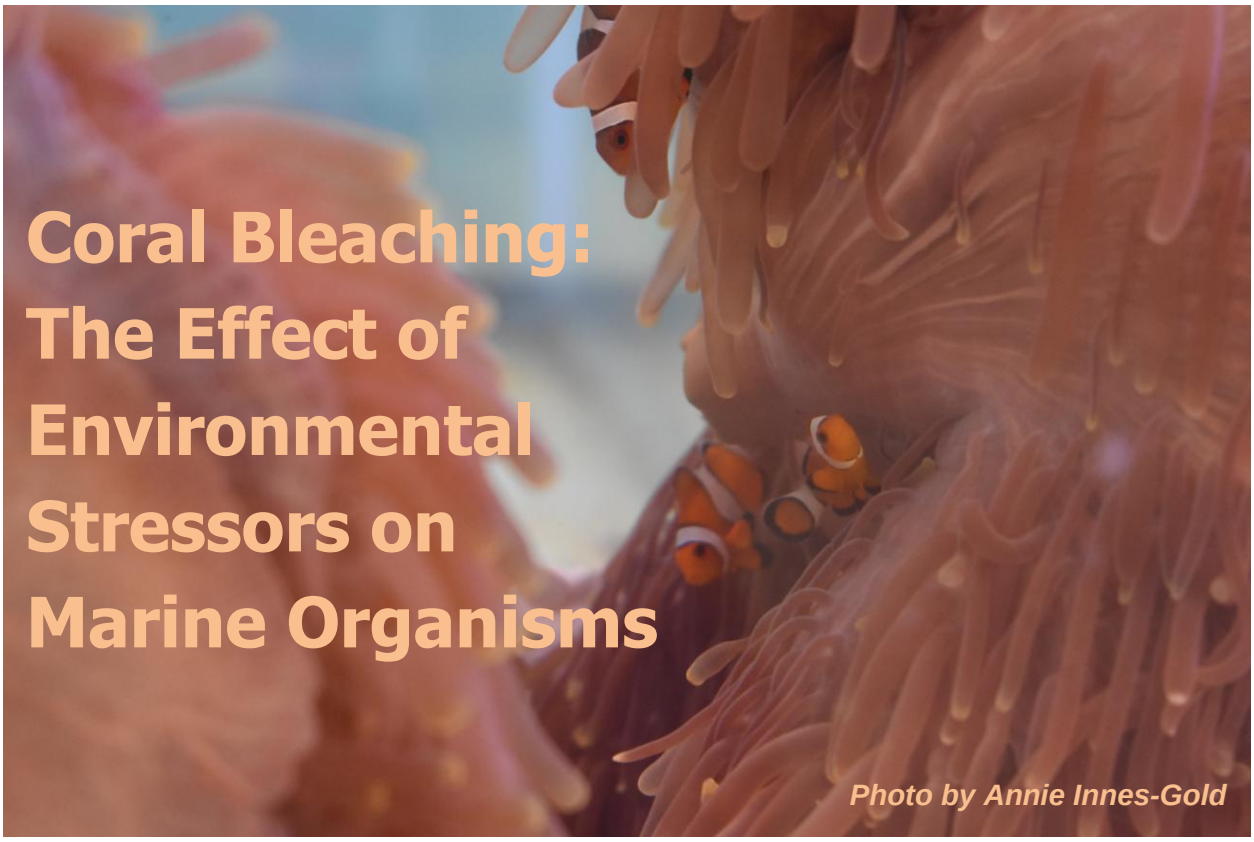

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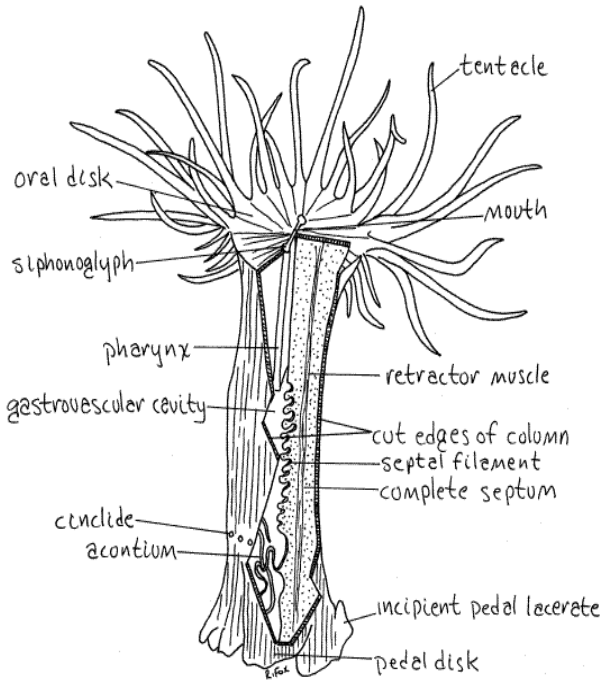
Objective

Students will study the effects of changing environmental conditions, using *Aiptasia pallida* as a model organism for coral bleaching.

Standards Met: NGSS

HS-LS1-3. Plan and conduct an investigation to provide evidence that feedback mechanisms maintain homeostasis.

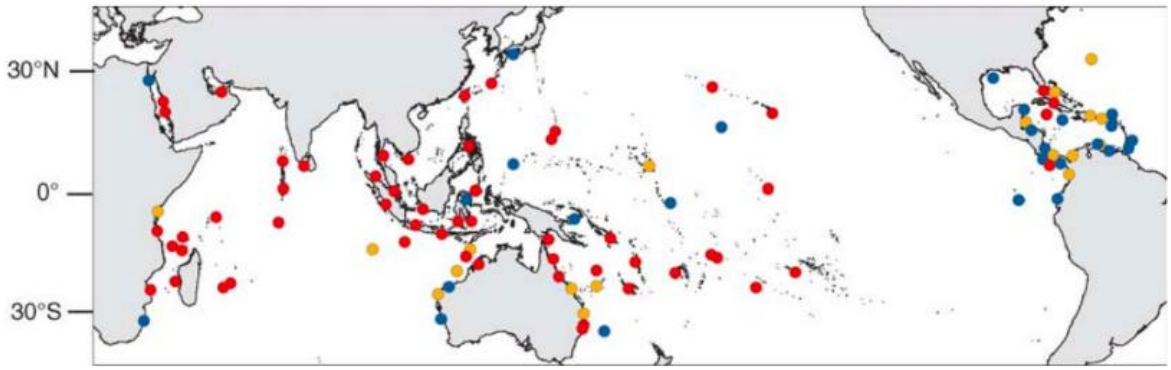
HS-LS2-7. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity



Background

Coral bleaching is the process by which corals, when stressed, expel the symbiotic algae that provides it with essential compounds. This is because under stress, the endosymbionts produce toxic levels of oxygen. When they are expelled, this causes the coral to lose its color, and appear bleached. This can happen because of a variety of stressors, such as acidification, lack of light, chemical pollution, and mainly, rising temperatures. Corals can recover from bleaching events. However, this is rare, and their health is severely compromised. Here is an article about a mass bleaching in Hawaii and the reef's recovery:
https://coralreef.noaa.gov/aboutcrcp/news/featuredstories/feb15/cbleachhawaii.html

Because of rising ocean temperatures and other environmental stressors, bleaching events have become more common. The 1998 El Niño event triggered an underwater heatwave that killed 16% of the world's corals, and was considered the first global bleaching event. The second mass bleaching event came in 2010. The third, and the worst to date, spanned three years (2014-2017), and hit every major coral region in the world. Studies have found that mass bleaching events have become five times more common today than they were 40 years ago. It is important that research focuses on the causes of bleaching, so effective policies can be put in place to protect reefs from these factors.



The global extent of coral bleaching from 2015-2016. Each circle represents one of 100 reefs, with red indicating severe bleaching, yellow indicating moderate bleaching and blue showing no bleaching. Source: Hughes et al. (2017)

Aiptasia pallida, a sea anemone, is often used as a model organism to study coral bleaching. It also has a symbiotic relationship with algae, and is easy to maintain in a lab setting. In this lab, students will study *Aiptasia* and their symbionts, as well as how these organisms fare under common environmental stressors.

Experimental part

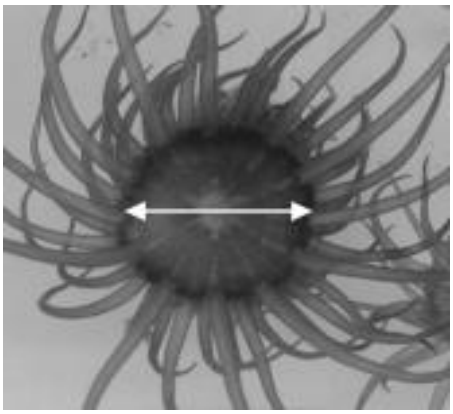
Materials

- *Aiptasia pallid (PVCNSI can provide the specimen)*
- Glass dishes
- Seawater (available in pet stores)
- Microscope
- Glass slides & cover slips
- Brine shrimp
- Scalpel
- Tweezers
- Materials to vary environmental conditions (i.e. heat plate, lamp, HCl & pH probe, fertilizer, sunscreen)

Procedure

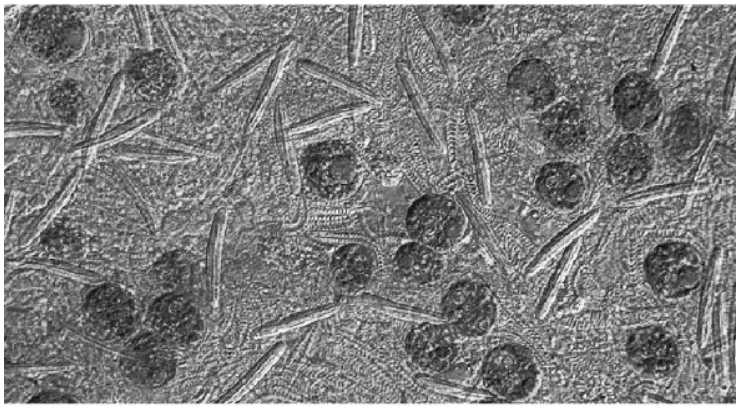
Week 1: Observe the anemones under a microscope

Part 1: Keeping your anemone in the dish, observe it under the microscope. Start at the lowest magnification, and then increase. Draw what you see at the varying magnifications. Measure the diameter of the oral disc, using a clear ruler or calipers. Record this value.



Source: http://www.ableweb.org/volumes/vol-31/v31reprint.php?ch=24

Part 2: Each group should use dissecting scissors and tweezers to cut off a large tentacle from one anemone. Each group will make a wet mount of the tentacle. Use tweezers to place the anemone piece on a glass slide, then put a plastic cover piece over it. Each group should look at their slide under the microscope. Locate the endosymbionts, and draw a diagram of what you see. Write down any observations.



Source: https://www.researchgate.net/figure/Tentacle-squash-from-temperate-symbiotic-sea-anemone-Anthopleura-elegantissima-at-40x_fig1_284163284

Endosymbionts will look like the small circles. The longer shapes are nematocysts, or specialized cells used for prey capture.

Week 2: Exposure to environmental stressors

Each group will focus on a different environmental stressor. Groups should research possible environmental conditions to manipulate before coming to lab. If you were part of a coral conservation research group, what environmental stressor would you find it most interesting to study? Possible ideas include: temperature, pH, light exposure, chemicals (i.e. from fertilizer, sunscreen). Each group should create a hypothesis of what they think could happen, and record their independent and dependent variables.

Each group should put one anemone per dish, and make at least two replicates of their environmental treatment, and two replicates of a control treatment (with regular seawater). Anemones should be fed brine shrimp every day, and water should be changed every other day (and replaced with new treatment water, for the experimental treatments). Groups should coordinate who in the group will have these responsibilities each day.

Weeks 3 & 4: Microscope observation after stress

In weeks 3 and 4, groups should cut off a tentacle from each anemone in both the environmental and control treatments. Prepare wet mounts of these tentacles, making sure to keep track of which mount corresponds to each treatment. Observe the tentacles under a microscope, and draw what you see. Make notes of the density of endosymbionts, as well as any change in color or behavior.

If during the course of the experiment any anemones appear unhealthy (i.e. detach from the dish, smell like they are decomposing), either lower the extremity of your treatment, or end the experiment early and return the anemone to regular seawater. If they appear white, that is okay. Lighter color means bleaching has occurred, and *Aiptasia* can survive without their endosymbionts.

Questions

1. Did you see any differences in the experimental vs. control anemones? Why or why not might this have occurred? What would you expect to see if you continued this experiment for a longer duration?
   Hint for Teacher! This could go either way. If there were differences, it is likely that one of the treatments put the anemone under stress, and caused a loss of symbionts. The treatment may have mimicked a real environmental stressor, and caused a decline in the health of the coral. If there was no difference between treatments, maybe it was not run for a long enough time for the anemone to be

affected, or maybe the treatments were not extreme enough to see a difference in a relatively short amount of time. This doesn't mean that a reef wouldn't degrade in the presence of this stressor; Aiptasia is a less sensitive organism than many corals, which is why it is easier to maintain in lab settings.

2. Are there any improvements you would make to this experiment? Would you do anything differently next time?
   Hint for Teacher! Answers will vary depending on stressor used. They may include more treatments, more replicates, more/less extreme conditions, a more accurate way to count symbionts.
3. How might you make this experiment more realistic?
   Hint for Teacher! One idea would be to use a combination of stressors in addition to individual treatments. In nature, reefs are exposed to many stressors at once, not just one isolated one.

Take Home Messages

Human activities have a huge impact on marine life. In this lab, each group studied the effect of one stressor on coral bleaching. However, in the ocean, there are many different types of stressors interacting and degrading coral reefs which we can help decrease.

Lesson Adopted From:

- http://www.ableweb.org/volumes/vol-31/v31reprint.php?ch=24
- http://www.eosmithcoralproject.org/projects/2008/ph.html
- https://oceanservice.noaa.gov/facts/coral_bleach.html
- https://coral.aims.gov.au/info/bleaching.jsp
- https://www.theguardian.com/environment/2017/jun/20/worst-global-coral-bleaching-event-eases-as-experts-await-next-one
- http://www.globalcoralbleaching.org/

- https://www.carbonbrief.org/severe-coral-reef-bleaching-now-five-times-more-frequent-than-40-years-ago
- https://coralreef.noaa.gov/aboutcrcp/news/featuredstories/feb15/cbleachhawaii.html
- Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., … & Bridge, T.C. (2017). Global warming and recurrent mass bleaching of corals. *Nature*, *543*(7645), 373.