# Modeling the Dynamic Oyster Microbiome

## Teacher Guide

## Activity Overview

Microbes play essential roles in the health and livelihoods of all plants and animals. In the ocean, microbes form tight relationships with a variety of invertebrates like corals, sponges, and molluscs. This hands-on activity demonstrates the dynamics of microbial ecology using dried pasta and beans to model disturbance events in the oyster microbiome. This activity teaches students about (1) the importance of niche availability and habitat space and (2) the influence of disturbance events, like ocean acidification, on the oyster microbiome community diversity and structure. Throughout this exercise, students discuss differences in the habitat space available and microbial community diversity.

### Learning Level

High School (9th - 12th)
Undergraduate

### Duration

Class time: 30 - 40 minutes
Teacher preparation: 15 -30 minutes

### Next Generation Science Standards

**HS-LS2-6 Ecosystems: Interactions, Energy, and Dynamics**
Evaluate the claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem.

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## Focus Question

How does the microbiome of a healthy oyster compare to that of an oyster impacted by ocean acidification?

## Objectives

**Students will be able to:**
- Understand that animals, like oysters, are tightly linked to their associated microbes
- Understand that in a healthy oyster, native microorganisms fill all available habitats restricting opportunistic pathogens from invading
- Explain how ocean acidification may affect the microbiome of oysters

## Attributions

This activity, created by Andrea Unzueta-Martinez, is based on research conducted in Dr. Jennifer Bowen's Lab at the Northeastern University Marine Science Center. The development of this activity was funded by NSF-OCE 1635423 and NSF-DEB 1655701 to Dr. Katie Lotterhos.

# Background

An **ecosystem** is a community of living things that interact with each other and their physical environment. Rainforests, deserts, and forests are all ecosystems. Each contains living components, like plants and animals, as well as non-living components, like rocks, air, and water. These ecosystems are large-scale, making it easy to think about different organisms living in these ecosystems - frogs, snakes, squirrels for example. However, ecosystems occur on microscopic scales too! Microorganisms like bacteria, viruses, and fungi form intricate communities in all sorts of habitats, even on animals themselves. For example, oyster tissues are home to diverse microbial communities, known as the oyster **microbiome**.

Micorbiomes play important roles in the health of marine invertebrates by providing them with specialized functions. For example, deep-sea molluscs that live in hydrothermal vents depend on chemosynthetic bacteria to convert $CO_2$ and $CH_4$ into organic compounds and provide necessary nutrients. Another way in which microbiomes can help their animal hosts is by providing protection against harmful pathogens through **competitive exclusion**. When beneficial microbes occupy all the available **niche** space, there is no room for harmful bacteria to colonize the animal. Healthy marine organisms are in balance with their microbiome so that beneficial microbes prosper while harmful microbes are controlled. This delicate balance can be destabilized by external stressors and can cause the onset of disease.

Disturbances like **ocean acidification** can destabilize the oyster microbiome by changing its composition and structure. Ocean acidification occurs when carbon dioxide ($CO_2$) in the atmosphere dissolves into the ocean. As it dissolves, it reacts with water ($H_2O$) to form carbonic acid ($H_2CO_3$). This reaction releases protons ($H^+$), which lowers the pH of the water. The equation for these reactions is shown below.

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

A more acidic ocean poses a problem for some marine life because the released protons then react with carbonate ions ($CO_3^{2-}$) found in the water to form bicarbonate ions ($HCO_3^-$), shown in the equation below. This reduces the concentration of carbonate ions in the water column.

$$2H^+ + CO_3^{2-} \rightarrow H^+ + HCO_3^-$$

Marine organisms that build hard shells from calcium carbonate, like oysters, need a high concentration of calcium and carbonate ions in seawater to successfully build their shells. As a result, when faced with ocean acidification, oysters struggle to build their shells and undergo a stress response.

In addition to this, ocean acidification alters the oyster microbiome. Some members of the microbiome are not able to survive the harsh conditions of an acidified ocean while other members are resilient and able to persist. These changes in microbiome composition result in a change in **species diversity**. Species diversity is defined as a measure that combines the number of species in a community and their relative abundances compared with one another. Therefore, a decrease in the number of species in the oyster microbiome due to ocean acidification will cause a decrease in species diversity.

However, the lowered species diversity does not last long, as niches within the microbiome are now open. Environmental microbes have the opportunity to colonize, resulting in reorganization of the microbiome. Colonization of new microbes may pose a problem if **opportunistic pathogens** rather than native microbes settle in open niches. If this occurs, it may be detrimental to the host health and can cause disease.

Ocean acidification threatens the livelihood of oysters and their microbiomes, posing a threat to the ecosystems that are supported by oysters and the economies that depend on the oyster fishery.

# Materials

**For each student:**

- 8.5 x 11 sized picture of a shucked oyster (provided in student packet)
- Oyster microbiome bags
    - Clear, sandwich sized resealable bags
    - Pasta: rotelle, medium shells, ditalini, ziti, occhi di pernice, macaroni, tri colored farfalle, tri colored fusilli
- Environmental microbe bags
    - Clear, sandwich sized resealable bags
    - Dried beans: black beans, black-eyed peas, red beans, garbanzo beans, green lentils
- Bacteria (pasta type) keys
    - One key of which pasta type represents which bacteria
    - One key of which bacteria (pasta types) are sensitive to ocean acidification

# Teacher Preparation

1. Gather all materials
    a. Print out student packet for each student
    b. Purchase or obtain 8 different types of pasta and 5 different types of beans
    c. Purchase or obtain clear, sandwich sized bags
2. Create a key for the 'oyster microbiome' pasta and label it "Figure 1. Oyster microbiome key. Each pasta type represents a bacterial Order found on oyster tissues. Pasta color represents bacterial Genera."
    a. Each type of pasta is associated with one bacterial order found in oyster tissues, which are as follows: Pseudomonadales, Alteromonadales, Campylobacterales, Rhizobiales, Flavobacteriales, Salinisphaerales, Spirochaetales, and Rhodobacterales
    b. An example of a key is on the following page (p. 4)
3. Create a key for which bacteria (pasta types) are sensitive to ocean acidification and label it "Figure 2. Ocean acidification sensitive bacteria (pasta shapes)"
    a. Choose four different types of pasta to be sensitive
    b. An example of a key is on the following page (p. 4)
4. Assemble oyster microbiome bag
    a. Randomly put different kinds of pasta in clear, sandwich sized bags
5. Assemble environmental microbes bag
    a. Randomly put different kinds of beans in clear, sandwich sized bags

## Examples of Oyster Microbiome Keys

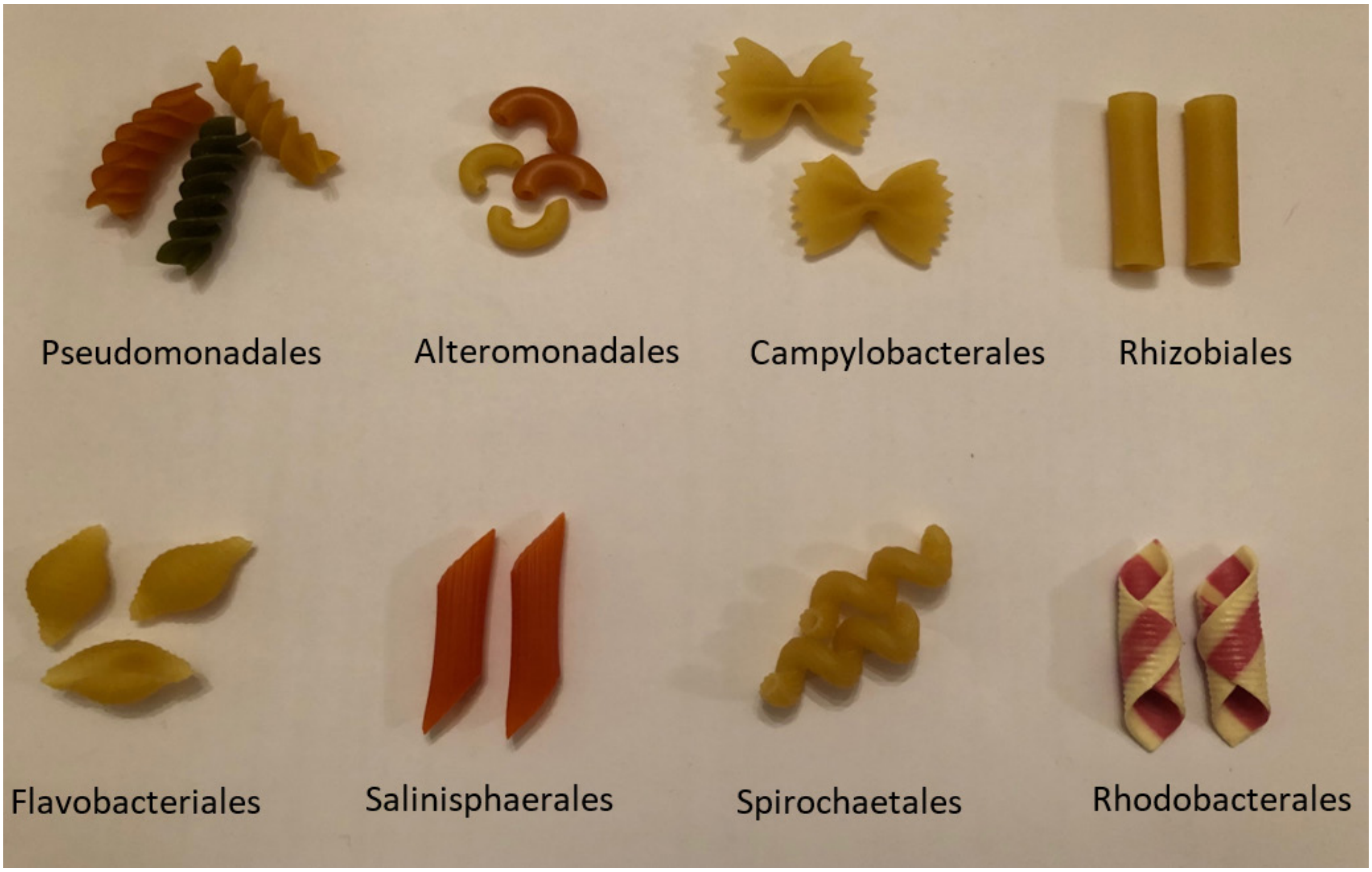


**Figure 1.** Oyster microbiome key. Each pasta type represents a bacterial Order found on oyster tissues. Pasta color represents bacterial Genera.

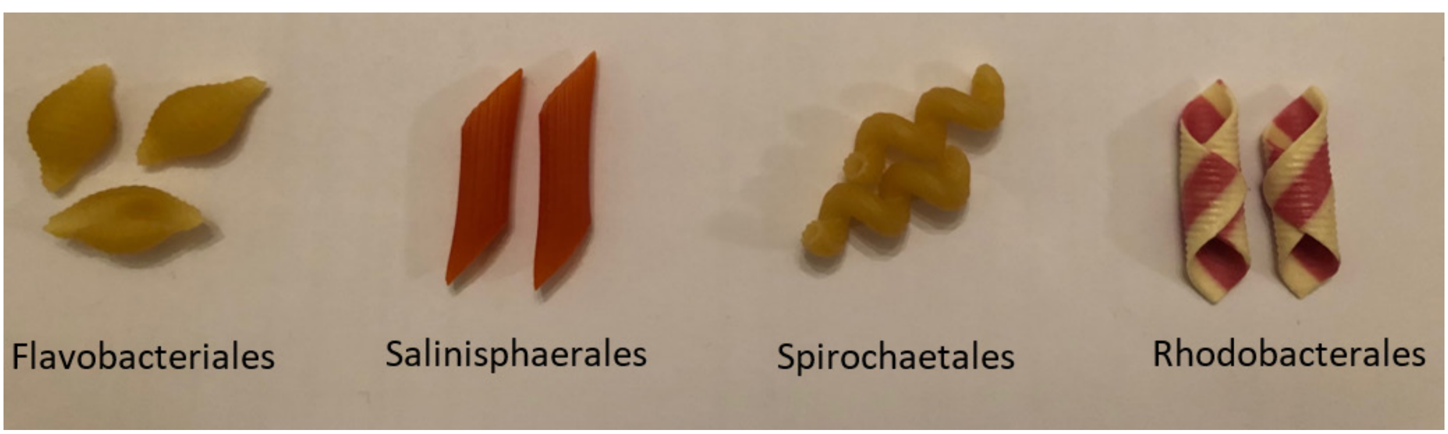


**Figure 2.** Ocean acidification sensitive bacteria (pasta shapes)

# Procedure

**Step 1. Introduce focus question to the class** (5 minutes)
Pose the focus question, *"How does the microbiome of a healthy oyster compare to that of an oyster impacted by ocean acidification?"*, to the class and begin the lesson with a short discussion. Do any students have predictions or educated guesses about the differences between a healthy oyster microbiome and one affected by ocean acidification? What makes them think this?

**Step 2. Provide context for the activity topic** (5 minutes)
Once the short discussion finishes, begin introducing the topic of the activity: microbiomes and how they are affected by environmental factors. Information about this topic can be found in the 'background' section earlier in this packet.

Explain that inside an oyster, there is a whole ecosystem of microbes including bacteria and viruses. These microbes make up what is called the oyster's microbiome, which has evolved alongside the oyster for hundreds of thousands of years. Major disturbance events can alter the microbiome and change its species diversity. This activity will explore the effects of such a disturbance.

**Step 3. Set up the activity** (2 minutes)
Provide the materials to each student: shucked oyster picture, 'oyster microbiome' pasta bag, and 'environmental microbes' bean bag.

**Step 4. Conduct the activity** (20 minutes)

a. **Introduction:** Tell the students that they have been given a model of an "oyster microbiome" in a bag and a picture of an oyster. The oyster tissue in the image is the entire habitat of the oyster microbiome. The pasta are different Orders of bacteria, while the different colors of pasta are different Genera of bacteria.

b. **Preparing the oyster microbiome:** Tell the students to empty their microbiome bag (pasta) onto the oyster picture. They should be sure that the microbes (pasta) fit within the oyster tissue habitat. Any extra microbes (pasta) should be left in the bag.

c. **Assessing the native diversity:** Have students estimate the species diversity of their microbiome. On their worksheet it asks several questions about species diversity.
   i. How many different species are present?
   ii. Is one species more abundant than another?
   iii. Which species is most abundant?
   iv. Which species is least abundant?
   v. Can additional pasta fit into the oyster tissue habitat without spilling over? What does this mean about the habitat?
   vi. Compare your oyster microbiome with your neighbors'. How are they similar? How are they different?

d. **Ocean acidification disturbance:** Flash forward to the year 2100 where the oceans are predicted to have a pCO2 of 900ppm. The oceans will be more acidic than they are today (400ppm). To simulate the effects of this change in ocean chemistry on the oyster microbiome, take 3 minutes to remove the types of pasta in Figure 2. Ask the students to reexamine their microbiome and answer the following questions on their worksheet.
   i. How does the original oyster microbiome diversity differ from post ocean acidification diversity?
   ii. Are there spaces left open in the oyster tissue habitat?
   iii. Is there room in the oyster tissue habitat for more bacteria?
   iv. Why did some species survive but others were killed by ocean acidification?
   v. When native bacteria are killed, what bacteria might fill the open space?

e. **Colonization by environmental bacteria:** Have the students add between one and three handfuls of environmental bacteria to their disturbed microbiome depending on how much space is available in their oyster tissue habitat. Have the students reexamine their microbiome and answer the following questions on their worksheet.
   i. How does the diversity now differ from the original diversity?
   ii. How much of that diversity is from the original microbiome and how much is environmental?
   iii. How full is the oyster tissue habitat?
   iv. Is there room in the habitat for more bacteria?
   v. How does the diversity in your microbiome compare to that of other groups around you?

f. **End of activity discussion:** With the people around them, have the students discuss possible ecological implications of this shift in the oyster microbiome.
   i. In an undisturbed microbiome, can opportunistic bacteria invade the oyster?
   ii. What would happen to the oyster if the opportunistic environmental bacteria were pathogenic?
   iii. Like ocean acidification, what other disturbances could have an effect on the oyster microbiome?

g. **Clean up:** Have students put their oyster microbiome pasta and environmental bacteria beans into their respective bags.

**Step 5. Discuss main takeaways and evaluate understanding of the topic** (5 minutes)
Return back to the focus question mentioned at the beginning of the lesson, *"How does the microbiome of a healthy oyster compare to that of an oyster impacted by ocean acidification?"* Ask students to use the knowledge that they have gained from the activity to answer this question either in groups or as a class. To evaluate understanding, you could go over the last three questions on the worksheet and ask the students what they discussed in their small groups.

As a conclusion to the lesson, explain that ocean acidification threatens the health of oysters and their microbiomes, which could then negatively affect the ecosystems oysters live in.

# Vocabulary

**competitive exclusion:** two species using a limiting resource in the same way cannot coexist indefinitely

**ecosystem:** all the organisms in a given area as well as the physical environment in which they live; an ecosystem can include one or more communities

**keystone species:** a strong interactor that has an effect on energy flow and community structure that is disproportionate to its abundance or biomass

**niche:** the abiotic and biotic conditions that a species needs to grow, survive, and reproduce

**ocean acidification:** a reduction in the pH of the ocean over an extended period of time, caused primarily by uptake of carbon dioxide ($CO_2$) from the atmosphere

**opportunistic pathogen:** an infectious microorganism that is normally commensal or does not harm its host but can cause disease when the host's resistance is low

**microbiome:** a community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment

**species diversity:** a measure that combines the number of species in a community and their relative abundances compared with one another

# References

Adapted from: Estes, A. 2015. Modeling the dynamic digestive system microbiome. *Journal of microbiology & biology education* **16**: 271-273. doi.org/10.1128/jmbe.v16i2.908

Sakowski, E. G. 2016. The microbiome of the eastern oyster, *Crassostrea virginica*, in health and disease. [Doctoral dissertation, University of Delaware]. ProQuest Dissertations & Theses.

# Student Worksheet Solutions

## Assessing the Native Diversity

1. Estimate the species diversity of your microbiome.
    a. How many different species are present?
    b. Is one species more abundant than another?
    c. Which species is most abundant?
    d. Which species is least abundant?

**Solution:** All of these answers are dependent on the microbiome each student creates.

2. Can additional pasta fit into the oyster tissue habitat without spilling over? What does this mean about the habitat?

**Solution:** No - this means that the microbiome is full and other bacteria cannot colonize the same tissue.

3. Compare your oyster microbiome with your neighbors'.
    a. How are they similar?
    b. How are they different?

**Solution:** These answers are dependent on the microbiome each student creates. However, some similarities could include having the same species of bacteria, having a more abundant species, having the same number of species. Some differences could include having different numbers of a species, containing different species, having a different arrangement inside the microbiome.

## Ocean Acidification Disturbance

4. Reexamine your microbiome and answer the following questions.
    a. How does the original oyster microbiome diversity differ from post ocean acidification diversity?

**Solution:** The post ocean acidification should have a lower diversity than the original.

   b. Are there spaces left open in the oyster tissue habitat? Circle one. Yes No
   c. Is there room in the oyster tissue habitat for more bacteria? Circle one. Yes No
   d. Why did some species survive but others were killed by ocean acidification?

**Solution:** The bacteria species that died from ocean acidification were sensitive to the change in pH and could not survive. There are differences among the survival of bacteria species because there is genetic variation among them. One genotype of a bacteria was better suited to deal with the more acidic conditions than others.

## Colonization by Environmental Bacteria

5. Reexamine your microbiome and answer the following questions.
    a. How does the diversity now differ from the original diversity?

**Solution:** This answer is dependent on the microbiome each student creates. Some answers could include that the diversity is bigger, smaller, or the same.

    b. How much of that diversity is from the original microbiome and how much is environmental?

**Solution:** Again, this solution is dependent on the microbiome each student creates.

    c. How full is the oyster tissue habitat?

**Solution:** The oyster tissue habitat should be full.

    d. Is there room in the habitat for more bacteria? Circle one. Yes No

    e. How does the diversity in your microbiome compare to that of other groups around you?

**Solution:** Again, this solution is dependent on the microbiome each student creates. The diversity could be lower, higher, or the same.

## End of Activity Discussion

With your neighbors, discuss possible ecological implications of this shift in the oyster microbiome.

6. In an undisturbed microbiome, can opportunistic bacteria invade the oyster? Why or why not?

**Solution:** Opportunistic bacteria cannot invade an undisturbed microbiome because all of the available space and niches are already filled. Due to the competitive exclusion principle, two species with identical niches cannot coexist indefinitely. Therefore, the opportunistic bacteria would not be able to colonize and survive.

7. What would happen to the oyster if the opportunistic environmental bacteria were pathogenic?

**Solution:** If the pathogenic bacteria colonized the oyster tissues, the oyster would likely suffer consequences such as illness or death.

8. Like ocean acidification, what other disturbances could have an effect on the oyster microbiome?

**Solution:** Answers may vary, but could include temperature rise, pollution, shifting precipitation patterns, or algal blooms.

**Name:** ______________________________ **Date:** ________________

# Modeling the Dynamic Oyster Microbiome

## Activity Worksheet

## Focus Question

How does the microbiome of a healthy oyster compare to that of an oyster impacted by ocean acidification?

## Objectives

**Students will be able to:**

- Understand that animals, like oysters, are tightly linked to their associated microbes
- Understand that in a healthy oyster, native microorganisms fill all available habitats restricting opportunistic pathogens from invading
- Explain how ocean acidification may affect the microbiome of oysters

## Activity

### Preparing your Oyster Microbiome

Empty the oyster microbiome pasta bag onto the oyster picture. Make sure all the pasta microbes fit within the oyster tissue habitat. If there are extra pasta microbes, leave them in the bag.

### Assessing the Native Diversity

1. Estimate the species diversity of your microbiome.
    a. How many different species are present? ____________________
    b. Is one species more abundant than another? Circle one. Yes No
    c. Which species is most abundant? ____________________________________
    d. Which species is least abundant? ____________________________________

2. Can additional pasta fit into the oyster tissue habitat without spilling over? What does this mean about the habitat?

________________________________________________________________________________
________________________________________________________________________________
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________________________________________________________________________________

3. Compare your oyster microbiome with your neighbor's.
    a. How are they similar?

    ______________________________
    ______________________________

    b. How are they different?

    ______________________________
    ______________________________

## Ocean Acidification Disturbance

Flash forward to the year 2100 where the oceans are predicted to have a $pCO_2$ of 900ppm. The oceans will be more acidic than they are today (400ppm). To simulate the effects of this change in ocean chemistry on the oyster microbiome, remove the types of pasta in Figure 2.

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    a. How does the original oyster microbiome diversity differ from post ocean acidification diversity?

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    ______________________________
    ______________________________
    ______________________________

    b. Are there spaces left open in the oyster tissue habitat? Circle one. Yes No
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    d. Why did some species survive but others were killed by ocean acidification?

    ______________________________
    ______________________________
    ______________________________
    ______________________________

    e. When the native bacteria are killed, what bacteria might fill the open habitat space?

    ______________________________
    ______________________________
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    ______________________________

## Colonization by Environmental Bacteria

Add between one and three handfuls of environmental bacteria to your disturbed microbiome depending on how much space is available in your oyster tissue habitat.

5. Reexamine your microbiome and answer the following questions.
    a. How does the diversity now differ from the original diversity?

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    b. How much of that diversity is from the original microbiome and how much is environmental?

    ______________________________________________________________

    ______________________________________________________________

    c. How full is the oyster tissue habitat? ______________________________
    d. Is there room in the habitat for more bacteria? Circle one. Yes No
    e. How does the diversity in your microbiome compare to that of other groups around you?

    ______________________________________________________________

    ______________________________________________________________

## End of Activity Discussion

With the people around you, discuss possible ecological implications of the shift in oyster microbiome.

6. In an undisturbed microbiome, can opportunistic bacteria invade the oyster? Why or why not?

______________________________________________________________

______________________________________________________________

7. What would happen to the oyster if the opportunistic environmental bacteria were pathogenic?

______________________________________________________________

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8. Like ocean acidification, what other disturbances could have an effect on the oyster microbiome?

______________________________________________________________

______________________________________________________________

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# About the Scientist


Image by Rachel Kara, Northeastern University

Behind this activity is a scientist at the Northeastern University Marine Science Center working on valuable research. **Andrea Unzueta-Martinez** is a doctoral candidate working in the lab of Dr. Jennifer Bowen. Ms. Unzueta-Martinez is interested in understanding the relationship between microbes and their hosts in the context of global change and disease in marine environments. Her research can help to identify ways to make oysters more resilient to changes in the ocean, fight disease, or grow faster. To learn more about Ms. Unzueta-Martinez' research, please follow the information below.

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Google Scholar: https://tinyurl.com/AUMGoogleScholar