Seagrass Wasting Disease

Teacher Guide

Activity Overview

Seagrass is a foundation species that creates a unique habitat for many different organisms; however, seagrass wasting disease is killing off this important plant. This interactive activity explores the impact of seagrass wasting disease on different genotypes of seagrass. Students will identify and quantify seagrass wasting disease and then learn how to use microsatellites to identify and differentiate among seagrass genotypes.

Learning Level

High School (9th - 12th)

Duration

Class time: 30 - 40 minutes
Teacher preparation: 5 minutes

Next Generation Science Standards

HS-LS4-4 Biological Evolution: Unity and Diversity
Construct an explanation based on evidence for how natural selection leads to adaptation of populations.

HS-ESS3-6 Earth and Human Activity
Use a computational representation to illustrate the relationships among Earth systems and how those relationships are being modified due to human activity.

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Focus Question

How does genetic identity/diversity of a host affect disease outbreaks?

Objectives

Students will be able to:
- Identify and quantify seagrass wasting disease in eelgrass, a genus of seagrass
- Use microsatellites to identify and differentiate among eelgrass genotypes
- Explain why genotypic identity and genetic diversity of eelgrass is important for the management of wasting disease

Attributions

This activity, created by Dr. Torrance Hanley, is based on research conducted in Dr. Randall Hughes' 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

Seagrasses are flowering plants that have adapted to live entirely underwater. Like all plants, they uptake carbon dioxide and release oxygen through photosynthesis. Through the uptake of carbon dioxide, seagrasses are able to provide organic carbon to an environment that would otherwise be food-limited. The excess organic carbon is then buried within the sediment instead of being released into the atmosphere, contributing to a major portion of carbon sequestration. In addition, seagrass structure provides many ecological services, such as sediment stabilization and enhanced biodiversity. Because seagrass dominates the ecosystem, increases diversity, and modulates nutrients and energy, it is defined as a **foundation species**.

Populations of these important seagrasses, however, are being infected by a common marine pathogen, *Labyrinthula zosterae*, a slime mold-like protist. In the 1930s, this protist decimated eelgrass, a genus of seagrass, on both sides of the North Atlantic through wasting disease. This disease is spread via direct contact with infected leaves and causes black-brown lesions on the infected plant. As the infection develops, the lesions increase in size, and ultimately results in death. The characteristic lesions make it possible to determine the presence or absence of *Labyrinthula* infection by simply looking at the seagrass leaves. In addition, lesion area is correlated with infection level, meaning analysis of lesion size can serve as a proxy for pathogen intensity.

Though many eelgrasses are affected by this disease, some may have a **genotype** that is more resistant. If you look at a seagrass meadow, you may think that the plants all look virtually the same (i.e. have similar traits or **phenotypes**), which often leads to the conclusion that they have low **genetic diversity** or low **intraspecific variation**. However, there is variation present in seagrasses, though it is not very obvious. Some plants with different genotypes are shorter with more leaves or taller with fewer leaves. In the same way, seagrasses can have genotypes that vary in resistance to disease.

To measure genetic diversity, or the number of unique genotypes, scientists can use microsatellite markers. **Microsatellites** are short, tandem, repetitive segments of DNA (e.g., TATATATA) that are found at high frequency in the genomes of most organisms. These segments are highly variable, which makes it possible to measure genetic diversity within populations and sites. In order to detect these microsatellites and measure how long the repeats are, DNA is amplified using **polymerase chain reaction** (PCR). On each side of the microsatellite, there are regions of DNA from which locus-specific **primers** are created. These primers will be used to create copies of the region containing the microsatellite sequence using PCR. Once the DNA is amplified, it can be separated by either **gel electrophoresis** or capillary electrophoresis. By using these methods, the investigator can determine differences in the number of repeats within each sequence.

For example, a 54 base pair (bp) fragment that contains 10 copies of a 2 base pair repeat (e.g. TATATATATATATATATATA) is 8 base pairs longer than a fragment that contains only 6 copies of the same repeat (Figure 1).

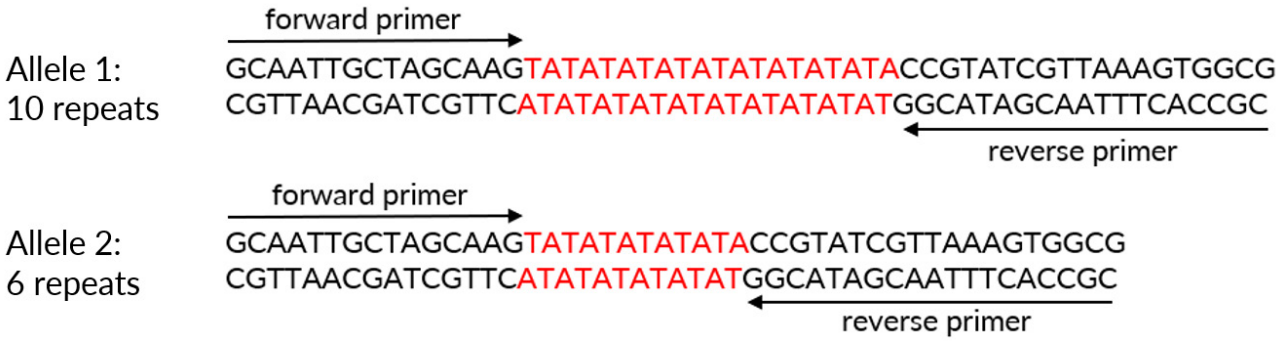

Figure 1. DNA fragments with different numbers of repeats.

In addition, an individual may have two copies of the region with the same number of microsatellite repeats (e.g., allele a=34 bp and allele b=34 bp, meaning it is homozygous), or it may have copies of the region with different numbers of repeats (e.g., allele a=34 bp and allele b=58 bp, meaning it is heterozygous). Figures 2 and 3 show the output of fragment analysis using capillary electrophoresis. The x-axis represents the size of the PCR product (how many repeats) and the height of each peak shows the amount of PCR product. In Figure 2, the individual is homozygous at this locus, with allele a=58 bp and allele b=58 bp. The individual in Figure 3 however, is heterozygous at this locus, with allele a=34 bp and allele b=58 bp.

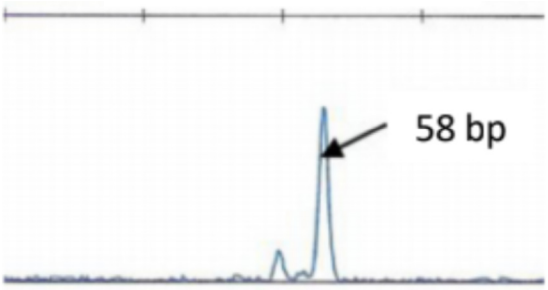

Figure 2. Output of fragment analysis of an individual that is homozygous at this locus. Adapted from Arif et al. 2010.

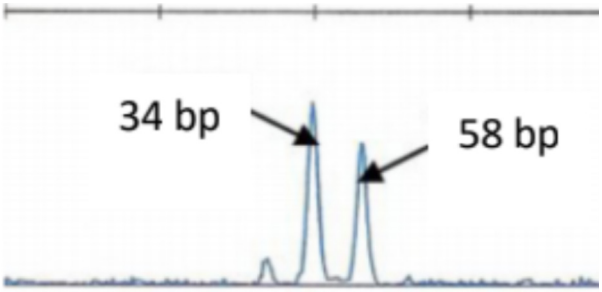

Figure 3. Output of fragment analysis of an individual that is heterozygous at this locus. Adapted from Arif et al. 2010.

Using these microsatellite data, scientists can determine genotypic diversity, genetic relatedness, gene flow, and genetic structure within and among populations.

This activity will explore how wasting disease presents itself in eelgrass, how to use microsatellites to identify different genotypes, and if different genotypes are affected differently by the disease.

Materials

For each student:
- 'Seagrass Wasting Disease' student worksheet
- Writing utensil

Teacher Preparation

1. Students should have a basic understanding of PCR (polymerase chain reaction) and gel/capillary electrophoresis before completing this activity
    a. Can conduct this activity after a lesson about PCR and gel electrophoresis or have a brief introduction before this activity
2. Gather all materials
    a. Print out seagrass wasting disease student worksheet for each student

Procedure

Step 1. Introduce focus question to the class (5 minutes)
Pose the focus question, *"How does genetic identity/diversity of a host affect disease outbreaks?"*, to the class and begin the lesson with a short discussion. Do any students have predictions or educated guesses about why genetic diversity is important? How would this impact disease outbreaks?

Step 2. Provide the context for the activity topic (5 - 10 minutes)
Once the short discussion finishes, begin introducing the topic of the activity: the impact of seagrass wasting disease on different genotypes of eelgrass.
Information about this topic can be found in the 'background' section earlier in this packet.

Explain that seagrasses provide many ecosystem services, such as carbon sequestration, nutrient cycling, sediment stabilization, and enhanced biodiversity. This is why it is important to protect seagrasses from seagrass wasting disease, which is caused by a protist called Labyrinthula zosterae. Wasting disease causes lesions to occur on the leaves of eelgrass, a species of seagrass, and is spread via direct contact. In this activity, students will examine infected eelgrass and determine whether various genotypes are affected by the disease differently. Genotypes will be determined by examining microsatellite data generated by PCR and capillary electrophoresis.

If needed, briefly explain what PCR and gel/capillary electrophoresis are and how they are conducted.

Step 3. Set up the activity (2 minutes)
Provide the printed worksheet to each student.

Step 4. Conduct the activity (15-20 minutes)

1. Introduce PCR and gel electrophoresis if necessary.
2. Explain that in their packet, they have one page for recording data, five pages of seagrass images, and one page of microsatellite data. There are a total of 10 different seagrass samples that students need to analyze. They will be assessing the percent cover of wasting disease and determining the genotype of each seagrass sample.
    a. For the seagrass wasting disease section, there are five leaves of seagrass per sample. Use all five leaves to estimate the percent cover of wasting disease. Students could find the percent cover for each leaf and then average the percents together, or decide that the five leaves are a whole and take the percent cover of that whole.
    b. For the genotyping section, students must look at the microsatellite data, find where the peaks are for each sample, and determine which samples have the same or different genotype.
    c. Record their findings on the datasheet.
    d. Answer the questions at the bottom of the datatable

Step 5. Discuss main takeaways and evaluate understanding of the topic (5 minutes)
To evaluate understanding, go over the students' datasheets. The first question asked how many different genotypes there were and which genotype was the least affected by seagrass wasting disease. Allow students to answer how many genotypes they discovered and then ask why students thought that some genotypes were more resistant to the disease than others. After they answer, explain that the genotypes that had the least percent cover of seagrass wasting disease likely had a genome that was more resistant to the disease. This may be because an advantageous mutation occurred in a specific place in the genome that allowed for this resistance.

The last question on the students' worksheet states: as ocean temperatures rise, what do you think may be the potential impact on seagrass beds and risk of wasting disease outbreaks? Will disease percent cover increase or decrease? Allow students to discuss what they put for their answers. Then, explain that because of increased ocean temperature, seagrasses may be more stressed and therefore their fitness and resistance can decrease. An increase in temperature also could be beneficial to the seagrass wasting disease fungus by promoting transmission. Scientists are still studying what these effects may be. If rising temperatures are indeed bad for seagrasses but good for the disease, then in the future, percent coverage of seagrass wasting disease may increase.

Return back to the focus question mentioned at the beginning of the lesson, *"How does genetic identity/ diversity of a host affect disease outbreaks?"* Ask students to use the knowledge that they have gained from the activity to answer this question either in groups or as a class. Genetic diversity allows natural selection to increase or decrease the frequency of alleles in the population. It is incredibly important because some individuals with a specific beneficial allele are able to adapt to a changing environment while maintaining the survival of the population.

Conclude with a statement saying that genetic diversity is important in managing seagrass wasting disease because certain genotypes are more resistant. These genotypes will then be able to persist in the environment because they will survive the disease.

Vocabulary

foundation species: a species that has large, community-wide effects by virtue of its size or abundance, its strong competitive ability, or its provision of habitat or food for other species

gel electrophoresis: a laboratory method used to separate mixtures of DNA, RNA, or proteins according to molecular size

genetic diversity: the variation in the amount of genetic information within and among individuals of a population, a species, and assemblage, or a community

genotype: the genetic makeup of an organism

intraspecific variation: differences that occur within a species

microsatellite: repetitive segments of DNA scattered throughout the genome in noncoding regions between genes or within genes

phenotype: the observable characteristics of an organism as a consequence of genetic traits and environmental influences

polymerase chain reaction: a laboratory technique used to amplify DNA sequences

primer: a short single-stranded nucleic acid sequence that provides a starting point for

References

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Student Worksheet Solutions

Datatable

Sample ID	Wasting Disease Percent Cover	Genotype (if same as #1, then A; if different than #1, then B, C, etc.)	Notes/Observations
1	**35 - 45%**	**A**	
2	**0 - 2%**	**B**	
3	**0 - 1%**	**C**	
4	**35 - 45%**	**A**	
5	**1 - 2%**	**B**	
6	**5 - 10%**	**D**	
7	**0 - 2%**	**C**	
8	**5 - 10%**	**E**	
9	**5 - 10%**	**F**	
10	**2 - 5%**	**G**	

Total number of genotypes: **seven (7)**

Which seagrass genotype is least affected by wasting disease? Why is it the least affected?

Solution: Genotype C (also could be B) because it has the least percent cover of seagrass wasting disease. It could be the least affected because its genotype is better suited to deal with the wasting disease. It has more advantageous traits than some other seagrass genotypes.

As ocean temperatures rise, what do you think may be the potential impact on seagrass beds and risk of wasting disease outbreaks? Will disease percent cover increase or decrease?

Solution: Any answer is acceptable if students explain their thinking. However, it is found that with increased temperatures, seagrasses may be more stressed, which lowers their fitness and resistance. An increase in temperature could be beneficial to diseases by promoting transmission. If rising temperatures are indeed bad for seagrass but good for disease, then in the future, percent coverage of seagrass wasting disease may increase.

Name: _____ **Date:** _____

Seagrass Wasting Disease

Activity Worksheet

Focus Question

How does genetic identity/diversity of a host affect disease outbreaks?

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Activity

This packet contains the materials needed to complete this activity. Included are a datatable, 5 pages of seagrass samples, and 1 page of microsatellite data.

1. Examine each sample of seagrass and estimate percent cover of seagrass wasting disease. Remember, the disease appears as black or brown lesions on the leaf. The seagrass may vary in green coloration (from light to dark), but this does not necessarily mean the plant is infected. Look for the characteristic black or brown lesions. Record percent cover in the datatable.

2. Interpret the microsatellite data and determine which seagrass samples have different genotypes. Record genotype in the datatable.

3. If you have any notes or observations about the seagrass samples, write them in the notes/observations column.

4. Answer the questions below the datatable.

Name: _____ **Date:** _____

Datatable

Sample ID	Wasting Disease Percent Cover	Genotype (if same as #1, then A; if different than #1, then B, C, etc.)	Notes/Observations
1		**A**	
2			
3			
4			
5			
6			
7			
8			
9			
10			

Total number of genotypes:_____

Which seagrass genotype is least affected by wasting disease? Why do you think it is the least affected?

_____

_____

_____

As ocean temperatures rise, what do you think may be the potential impact on seagrass beds and risk of wasting disease outbreaks? Will disease percent cover increase or decrease?

_____

_____

_____

Vocabulary

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About the Scientist

Image by Ruby Wallau, Northeastern University

Behind this activity is a scientist working on valuable research in the marine science field. **Dr. Torrance Hanley** is an associate research scientist at the Northeastern University Marine Science Center. She is interested in examining the role of diversity in trophic interactions using seagrass and salt marsh communities. To learn more about Dr. Hanley's research, please visit the links below or contact her with the information below.

Email: t.hanley@northeastern.edu
Google Scholar: https://tinyurl.com/THanleyGoogleScholar

#1

Image by T. C. Hanley and F. R. Schenck

#2

Image by T. C. Hanley and F. R. Schenck

#3

Image by T. C. Hanley and F. R. Schenck

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Image by T. C. Hanley and F. R. Schenck

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Image by T. C. Hanley and F. R. Schenck

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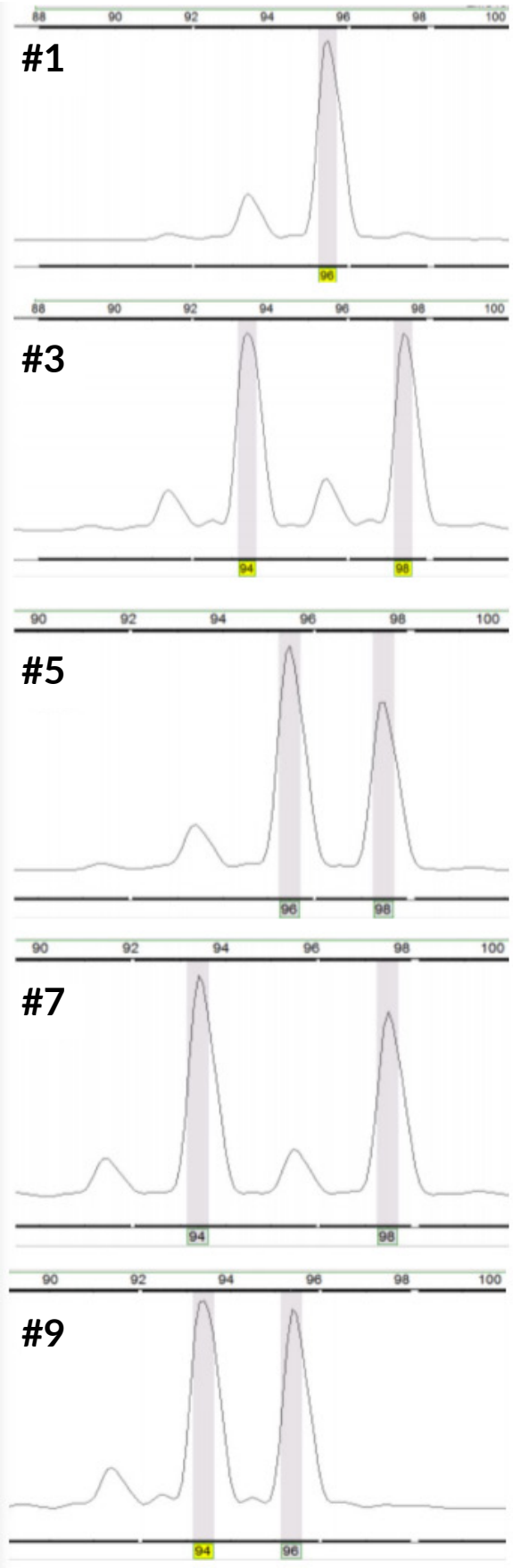

Images by T. C. Hanley