

Detecting Aquatic Invasive Species with eDNA

How do scientists use eDNA sampling to detect and monitor Aquatic Invasive Species (AIS)?

Overview

Subject Areas: Life Science, Environmental Science, Ecology, and Human Impacts

Key Topics: Aquatic invasive species, monitoring, methodology

Students will be introduced to how scientists detect and monitor Aquatic Invasive Species by comparing visual AIS detection with the processes of eDNA sampling, Polymerase Chain Reaction (PCR), and DNA Sequencing.

Contact the Education and Outreach team (edoutreach@flbs.umt.edu) at the Flathead Lake Biological Station to obtain the presentation and other teaching materials associated with this lesson.

Objectives

Students will be able to:

- explain the benefits and challenges of eDNA sampling for AIS detection
- describe the eDNA sampling process
- use evidence to determine the presence of AIS in a location
- compare and contrast two methods of detecting AIS (visual vs eDNA)

Grade Level

Middle School

Duration

Preparation Time: 25 minutes

Activity Time: 45 minutes

Skills

Analyzing data, interpreting information

Materials

Warm Up/Activity/Wrap Up

- Computer and projector
- Printed student worksheets (#1-6) and student resources (#1-4)
- Printed DNA strips and printed Search and Find Locations
- Scissors and tape (for preparation)
- Dry erase markers if laminating Search and Finds



- Containers for paper strips (1 for each group) labelled 1-4

Advanced Preparation

- Students should have a basic understanding of DNA
- Print the student worksheets #1-6 (double-sided) for each student.
- Print the student resources #1-4 (double-sided, in color) for each group. You can laminate for reuse.
- Print the search and find document (single-sided, landscape layout, in color), one for each group. You can laminate for reuse.
- Print the DNA strips (double-sided, in color): Cut the strips along the solid black lines and fold over the red lines (such that the colored dots are folded inwards). Tape the folded paper closed. Place the strips corresponding with each sampling location in their matching sampling location's container (1-4).
- At each station, place the student resources handouts, search and find, and DNA strip container matching each location (1-4).
- Pre-load the eDNA PowerPoint which can be obtained from the FLBS education team

Background

DNA is the basic building block of all living things. DNA is made of four **nucleotides**, which pair up into base pairs (Adenine with Thymine, Guanine with Cytosine). These base pairs are translated into proteins that perform functions within the organism – thus, the order of nucleotides encodes key differences between species and the order of unique nucleotides can be used to determine a species.

Environmental DNA (eDNA) is DNA that can be either left or shed by an organism into the environment. Sources of eDNA include dead skin cells, hair, tissues, waste, or fluids. eDNA can be sampled from a substrate where the organism is or has left DNA. The eDNA is then extracted from the sample through filtration or other methods. Scientists amplify the target DNA through many rounds of DNA replication, called a **polymerase chain reaction (PCR)**. The process of PCR first breaks apart the DNA strands, called denaturing, by increasing the temperature and breaking the nucleotide bonds. Primers, a short nucleotide sequence that bonds with the target DNA strand, binds to the DNA when the temperature is lowered. A primer will bind to a specific DNA sequence according to the nucleotide pairing rules (i.e. A with T, C with G). A specific primer is chosen to match the target species sequence, such that the strand replicated following the primer will uniquely identify which species it is. This works because primers are added in excess of the original DNA. This primer then guides the enzyme, Taq Polymerase, to synthesize a complementary DNA strand using free nucleotides, replicating the target DNA. This process is repeated many times to exponentially increase the target DNA, making the DNA easier to detect. To detect if the target DNA is present, **DNA Sequencing** is used. There are many different methods for DNA sequencing. These methods identify each individual base in a sequence of single stranded DNA by using a distinguishing factor of that base, like molecular size, ionic charge, or its complementary base pair. A commonly used method is Illumina Sequencing, which uses fluorescently tagged nucleotides to capture the DNA sequence. During this process, the temperature is raised to break the double stranded DNA into a single strand. To keep the DNA in sequential order, a molecule attached to a plate will bind to the end of each DNA strand in the sample. Nucleotides with unique emission wavelengths for

each base (A, T, G, and C) are added to the plate, along with polymerases and primers. Similarly to PCR, a complementary strand of DNA will be built alongside the original strand. Bases are added individually, and a camera with a photoreceptor detects the wavelength of each nucleotide as it is added, recording the sequence of DNA. The sequence is then compared to a database of known sequences of DNA to identify the organism.

One important use of eDNA is to detect the presence of **Aquatic Invasive Species (AIS)** within a waterway. AIS are non-native species that have been introduced to waterways, most likely by people, either intentionally or unintentionally. AIS negatively impact ecosystems. Early detection of an AIS when it is first introduced is extremely important because at this stage it is easier to manage and prevent the spread of invasive organisms. eDNA is a valuable method of early detection because it can detect the presence of a species through any form of DNA left by it, even if that species is not easily visible or at low densities. Regular monitoring of a water body through eDNA sampling provides information on whether target AIS are present. If an AIS is detected, the necessary steps can be taken to manage and prevent the spread of AIS.

Zebra and quagga mussels are a main focus for AIS monitoring because they are extremely destructive to aquatic ecosystems. In addition to competing for food, these invasive mussels attach to both native mussels and infrastructure like pipes, clogging them and causing damage. These mussels are native to Eastern Europe and were first introduced to the United States in the Great Lakes in the 1980s. They have now spread to much of Canada and the US. They can attach to the sides of boats and survive for several days, making it easy to accidentally transport these mussels to uninfected bodies of water. Additionally, because they are extremely small in the larval (**veliger**) stage they can be difficult to detect visually. eDNA monitoring is used for early detection of these mussels in lakes and rivers.

Vocabulary:

Aquatic Invasive Species: Aquatic, non-native species that cause economic or environmental harm

DNA: Deoxyribonucleic acid is a macromolecule that encodes genetic information through a sequence of nucleotide bases

DNA Sequencing: A laboratory process that determines the order of nucleotide bases on a strand of DNA

eDNA: DNA left by an organism in an environment (like soil or water)

Nucleotide: A molecule that makes up DNA and RNA. A nucleotide is made up of a phosphate group, sugar, and nitrogenous base (i.e. Adenine, Guanine, Thymine, Cytosine)

Polymerase Chain Reaction (PCR): Used to rapidly make millions of copies of a specific DNA sample

Veliger: The planktonic larval stage of dreissenid mussels (for example, Zebra or Quagga mussels)



Procedure

- **Warm Up (10 minutes)**

- Instructions Turn on the projector, display the “eDNA” presentation, and pass out the student worksheets (#1-6).
- Slide #3: Explain to students what aquatic invasive species are and have students write this definition on their worksheet. Brainstorm/view some examples of AIS and share with students.
- Slide #4: Discuss why monitoring for AIS is important. Have students brainstorm reasons why scientists might want to monitor for AIS.
 - Early detection is extremely valuable for preventing invasion and the spread of AIS!
- Slides #5-6: Review what DNA is and explain the concept of eDNA. Have students write the eDNA definition under the Warm Up section of their worksheet.
- Slide #7: Explain how organisms can “leave” DNA behind in the environment. Have the students brainstorm some ideas of how organisms leave DNA behind in the environment and have them record this on their worksheets.
 - Possible answers for materials left can include: dead skin cells, dead hair/fur strands, waste (feces/urine), etc.
- Have the students brainstorm some organisms that might leave behind eDNA in a lake.
 - Any organism living near/in a lake qualifies as a correct answer for the second question

- **Part 1: Searching for AIS (10 minutes)**

- Instructions Slides #8-9: Give background on the fictional Lake Lago. Tie in why we are monitoring for AIS in Montana and why at this lake (because mussels have been detected in a lake in South Dakota less than 100 miles from the Montana border).
- Slides #10: Show students what Zebra and Quagga mussels look like and why we care to monitor Lake Lago for them.
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- Slide #11: Show students the map of monitoring locations around Lake Lago. Point out the 4 different locations and indicate that each group will be “monitoring” a different site. Assign each group their sample location and give each group the appropriate search and find, a copy of the student resources handout, and DNA strip container corresponding to their site.
 - Students should then answer the questions on the worksheet about their location: get them to think about how protected forest vs. a boat dock/town vs. a river may impact the species living there and where an invasive species may be introduced.
- Slide #12: Explain to the students that they will be doing a visual inspection of their site to see what species may be located there and if they can detect any AIS at their site. They will have 5 minutes.
 - Direct students to their site’s search and find and pages 1 and 2 of their student resources handout. Explain to the students that these are the organisms that could potentially be living at their site. Mention to students the difference between introduced (non-native species) and invasive species (non-native species that has caused damage to the ecosystem or economy).
 - Have students circle all species that they find in their search and find. If you’ve laminated these images for reuse, provide the students with dry erase markers to

- circle the species. Once they think they've found all of the organisms, have them tally up the species they found on page 2 of their Student Worksheet. Have them fill out the chart and answer the associated questions.
- Remind students that they have **5 minutes** to complete this task.
 - Slide #13: Have students share their results. None of the groups should find an invasive mussel. Ask the class why they may not have found a mussel: either they are not there, or they were unable to see them – point out that mussel larvae are incredibly tiny and difficult to see!
 - Discuss how the search and find can be an effective way of monitoring populations, and how it may be ineffective (some species are hard to find/rare, may not be in exact location you're looking at but may be nearby).
 - **Part 2: Using eDNA to monitor AIS (15 minutes)**
 - Slide #15: Explain to the students that they will be learning about a different method to detect invasive species in a waterway.
 - Click on the image on the slide and a video bar should appear. Play the video of sample collection.
 - Slide #16: Continue explaining how the sample is collected and play the video on this slide.
 - Have students answer the first question on Part II A of their worksheet (Why do we filter our water sample?). Answers may include: to get rid of extra materials or to isolate the DNA for analysis, to concentrate the sample.
 - Slide #17: Explain how DNA is extracted from the sample by using the slide graphics
 - If you've done a strawberry DNA lab, mention that this process is very similar to what they did in that lab.
 - Slide #18: Once the DNA is extracted the next step in the process is to run PCR (Polymerase Chain Reaction). Simply put, PCR rapidly replicates DNA.
 - Ask students why PCR might be important in the process of detecting eDNA? – PCR increases the small amount of DNA in each sample so it's easier to detect.
 - Slide #19: A few different things are needed for PCR to occur. You need free nucleotides (base pairs that are unattached to DNA and are available for base pairing), Tag Polymerase (enzyme that creates the replicated DNA strand), primers (guide Tag Polymerase to replicate the target strand and pairs to a specific DNA sequence. A thermal cycler is used to run the PCR cycle.
 - Primers targeting specific regions of DNA are chosen depending upon what organism you are looking for (mussels, fish, etc.).
 - Slide #20: In our investigation of AIS in Lake Lago, we are specifically looking at fish, mussel, and insect DNA, thus using specific primers to identify these organisms. The first step is to determine what the target DNA sequences for each primer will be. To do so we need to pair nucleotides with primer sequences.
 - Remember each nucleotide has a specific pair. Adenine pairs with Thymine (abbreviated A and T) and Guanine pairs with Cytosine (abbreviated G and C).
 - Slide #21: Show students the primers for each species. These primers are for all insects, fish, and mussels in the samples.
 - Have students fill out their primer tables on Part II Section 2 of their worksheet. DO NOT advance to the next slide until students are done with this section.

- Slide #22: Show students the answers to the DNA sequences.
 - Direct students to their containers of DNA. Have students pull out all of the DNA strands that match the sequences they wrote. Set aside the DNA sequences that don't match. Use the sequence marked with a red arrow in their primer tables.
- Slide #23: Why are there some DNA sequences that don't match our primer sequences? Have students complete question # 2 and share examples of other organisms that could leave eDNA behind in the lake.
- Slide #24: The number of DNA strands rapidly increase through the cycles of PCR. Since we don't have a thermal cycler, we are going to visualize the replication of DNA through multiplication.
 - Have students complete the PCR activity (Section 3 Part II) of their worksheet. Remind students that they are starting with 1 DNA strand.
- Slide #25: The last step in PCR is DNA sequencing, which determines the order of the four base pairs. Explain how DNA sequencing works.
 - Direct students to page 3 of the student resources handout.
- Slide #26: Students will be sequencing the DNA that was collected from Lake Lago. They will open their DNA strips (that correspond to mussel, insect, and fish DNA) and complete the DNA sequencing activity (Part II Section 4 of their worksheet) and the associated questions. Tell students that these strips are for a specific species of fish, mussel, or insect (i.e. largemouth bass, damselfly, etc...).
 - Students will use page 3 of the student resources handout to decode their DNA strips. They will start reading on the side with the yellow arrow. Have them mark the presence/absence of the species in their samples on the chart on their worksheet. Once complete, have the students answer questions 3-6 below the chart.
- Slide #27: Discuss results. Have each group share with the class the difference in their results between the search and find and the eDNA activity – you can optionally make a table on the whiteboard for students to fill out with their results. Make sure the group with the mussels shares they found mussels.
 - Are the results between the two methods always the same? Why would there be differences?
 - Have students look back at their Lake Lago map (page 4 of the student resources handout) and ask them why mussels may have been found at this specific sample site and not others. – Answers may include: it's the site with a boat dock and town (more people using this area)
 - Ask what other places around Lake Lago we may want to consider monitoring in the future? Why?
- **Wrap Up (10 minutes)**
 - Ask volunteers to read their stories aloud to the class.
 - Slide #28: Explain to students that they've taken a look at two different methods to inspect a lake for AIS. They are now going to compare the two methods. Have students complete the Wrap Up worksheet questions on Part II of the worksheet.
 - Slide #29: Have students share their answers to the Venn Diagram and write them on the board. When finished, advance to Slide 30 to go over any answers the students did not come up with.

- Emphasize the point that DNA can degrade under certain conditions (organisms and bacteria can break them down, temperature, pH, etc.) and may not be detected by eDNA sampling.
- Slide #31: Discuss why FLBS and other organizations monitor Montana waters for AIS. Have students record this on their worksheet.
- Play the video on Slide 32. If the video doesn't work, you can access via this link: <https://youtu.be/bGcAegtOyMQ>

Teacher Resources

Assessment Options

Have students:

- complete the student worksheet as described.
- design a brochure advertising eDNA kits to monitor for AIS.

Modifications

- Do something he search and find activity and worksheets can be completed on a computer or iPad rather than printing (and saves paper!)
- Magnifying glasses can be used for the search and find activity.
- The worksheets can be enlarged for students in need of larger text.
- For a smaller class size, you can use one less sampling site, ensuring that one of the sampling sites with a mussel detection is used (Site 2).
- For a larger class size, you can print two copies of each sample site and have two groups per site. These duplicate groups can compare answers.

Extensions

Students can:

- completed a DNA extraction (lesson with strawberry DNA available through many sources) in conjunction with this lesson
- explore other lessons in the FLBS Be AIS Aware Teaching Trunk.
- create a project to monitor a local body of water for AIS

Standards

NGSS and Montana Science Standards:

MS-LS2-5: Evaluate competing design solutions for maintaining biodiversity and ecosystem services

CORE IDEA: ETS1.B.: Developing possible solutions

CROSSCUTTING CONCEPTS: Science addresses questions about the natural and material world

SCIENTIFIC & ENGINEERING PRACTICE: Engaging in argument from evidence



Online Resources

FLBS Website on Invasive Mussels:

<https://flbs.umt.edu/monitoring/ais/>

Journal review of AIS detection with eDNA:

<https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2664.12306>

National Park Service eDNA article:

<https://www.nps.gov/articles/aps-19-1-6.htm>

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