

## FLARE Lesson



# Detecting AIS with eDNA

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

### ◆ Grade Level(s)

Middle School

### ◆ Subject Areas

Life Science, Environmental Science, Ecology, and Human Impacts

### ◆ Key Topics

Aquatic Invasive Species, Monitoring, Methodology

### ◆ Duration

Preparation Time: 25 min

Activity Time: 45 min

### ◆ Setting

Classroom (divided into 4 groups)

### ◆ Skills

Analyzing data, interpreting information

### ◆ Standards

#### **NGSS & MT Science Std.:**

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

#### CORE IDEA:

ETS1.B: Developing Possible Solutions

#### CROSSCUTTING CONCEPT(S):

Science Addresses Questions About the Natural and Material World

#### SCIENTIFIC & ENGINEERING

#### PRACTICE

Engaging in Argument from Evidence

## Overview

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.

## 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)

## 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 found on the FLBS website.

## 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



Zebra Mussel

Quagga Mussel

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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.

### Lesson 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)** – Method 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)**
- 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 I: Searching for AIS (10 minutes)**
- 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.
- 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.

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- 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 II: 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

## Detecting AIS with eDNA

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)**
- 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.

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- 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. You may need to right click on the video and select “Preview” to play. If the video doesn’t work, you can access it at this link <https://flbs.umt.edu/newflbs/outreach/k-12-education/teaching-material/ais-materials/> and it’s located under the “AIS Mussel Monitoring Video” tab.

### Teacher Resources

#### Assessment Options

Have students:

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

#### Modifications

- The 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:

- **complete** 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

#### Online Resources

FLBS Website on Invasive Mussels:

<https://flbs.umt.edu/newflbs/flathead-lake/aisinvasive-mussels/>

Journal review of AIS detection with eDNA:

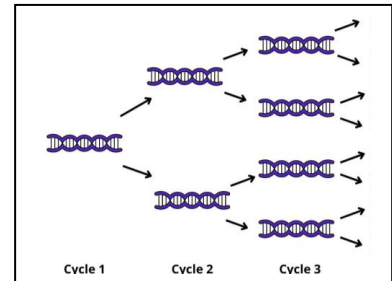
<https://besjournals.onlinelibrary.wiley.com/doi/10.1111/1365-2664.12306>

National Park Service eDNA article:

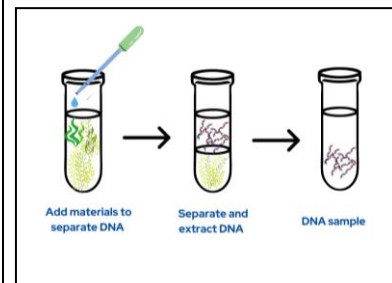
<https://www.nps.gov/articles/aps-19-1-6.htm>

#### Acknowledgements

FLBS intern Sophia Richter designed and created this eDNA lesson and associated materials and illustrations. Thanks to Dr. Matt Church and Miranda Seixas for reviewing the lesson. Thanks to the 2022-23 FWTTs



*PCR DNA amplification*



*DNA filtering and extraction*

## Detecting AIS with eDNA

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## Detecting AIS with eDNA

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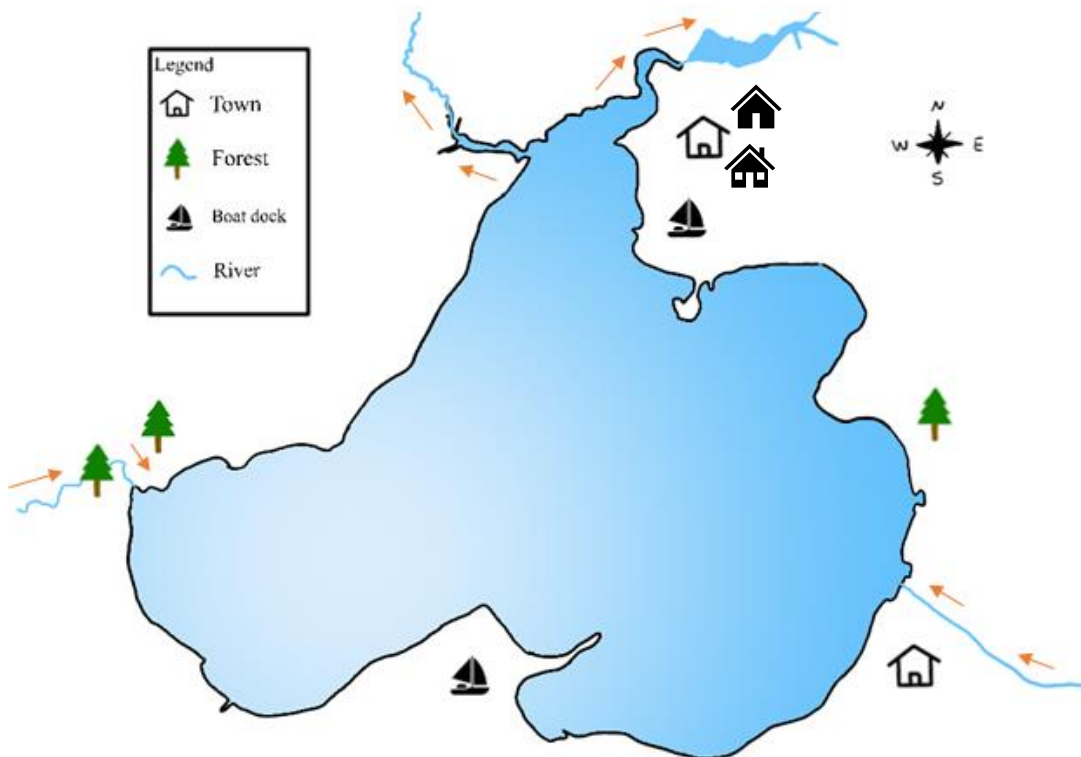


## Warm Up

1. What are AIS?
2. What is eDNA?
3. Brainstorm materials left by an organism that may leave eDNA in the environment.

## Part I: Searching for AIS

4. **Mark all four sample locations** on the map. Put a **circle** around the site number your group will focus on.
5. Examine the map and the key. Look for tributaries (inlets) and outlets. Is this an open or closed system? \_\_\_\_\_ **Add an arrow** to show the direction water is flowing in Lake Lago.



## Detecting AIS with eDNA

Student Worksheet (2 of 5)

6. List abiotic/biotic factors that may impact what species live at your sample location.

### Detecting AIS: Visual Search & eDNA Sampling

Using the image and species key, identify as many species as you can see within the time limit. Record your data in the table below. Be ready to share results when the time ends.

Data Table of species found during a visual inspection at site # \_\_\_\_\_.

Type	Species	Count	Native? Non-Native? Invasive?
Fish	Largemouth Bass		
	Walleye		
	Brook Stickleback		
	Yellow Perch		
Insects	Damselfly		
	Mayfly		
	Crane fly		
Mussels	Zebra Mussel		
	Quagga Mussel		
Total			

1. Complete the count column first. Use tally marks.
2. Calculate the totals and record in the bottom row
3. Classify each species as native, non-native, or invasive.
4. Do you think using eDNA will give the same or different results? Why or why not?

Record the data (total organisms, species counts, if mussels are found) that your classmates share below. \*After the results are reported, mark the location with the highest biodiversity with a star on the map on page 1.

## Part II: Using eDNA to monitor AIS

Follow along with the presentation to complete the tasks below.

### COLLECTING AND INTERPRETING AN eDNA SAMPLE

#### A. FILTERING THE WATER SAMPLE

1. Why do scientists filter a water sample before they send it to the lab?

#### B. IDENTIFY DESIRED DNA

To identify DNA, scientists use primers that will bind to DNA strands with the complementary sequence. We will be using three primers to identify our species. One for fish, one for insects, and another for invasive mussels, written below.

**Write out the corresponding DNA sequence above each primer. Then send the DNA strips 4 groups.**

Fish primer

→					
	A	T	C	G	G

Present? Absent?

Insect primer

→					
	G	A	T	C	G

Present? Absent?

Mussel primer

→					
	C	G	A	T	A

Present? Absent?

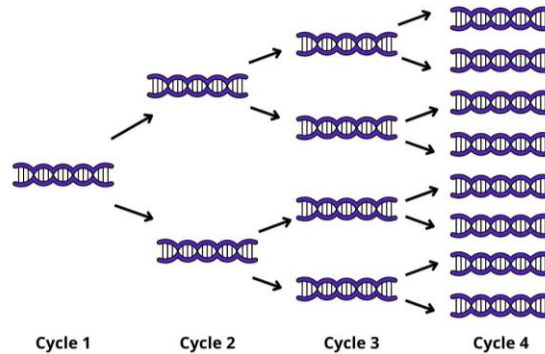
2. What kinds of organisms did the DNA strands that did not correspond to a primer sequence come from? List at least 5 kinds of organisms.

**Set the DNA strips that do not match the above primers off to the side. You will not be doing any further analysis of these.**

## Detecting AIS with eDNA

### C. PERFORM PCR

Instead of physically copying the DNA strips, the following is a quick exercise to imagine how the DNA is replicated in PCR. Fill in the table below to see how many DNA strands PCR creates in each round via the process below.



Round	Beginning # DNA Strands	Multiplied by	=	# Strands after PCR Round
1	1	2	=	
2		2	=	
3		2	=	
4		2	=	
5		2	=	

### D. DNA SEQUENCING

Open the identified DNA strips by carefully opening the tape. Using the provided color key, first write out the DNA sequence in the space below. Please do not write on the DNA strips. Then, using the DNA sequence key, identify the species of each strip and write it here.

## Detecting AIS with eDNA

Student Worksheet (5 of 5)

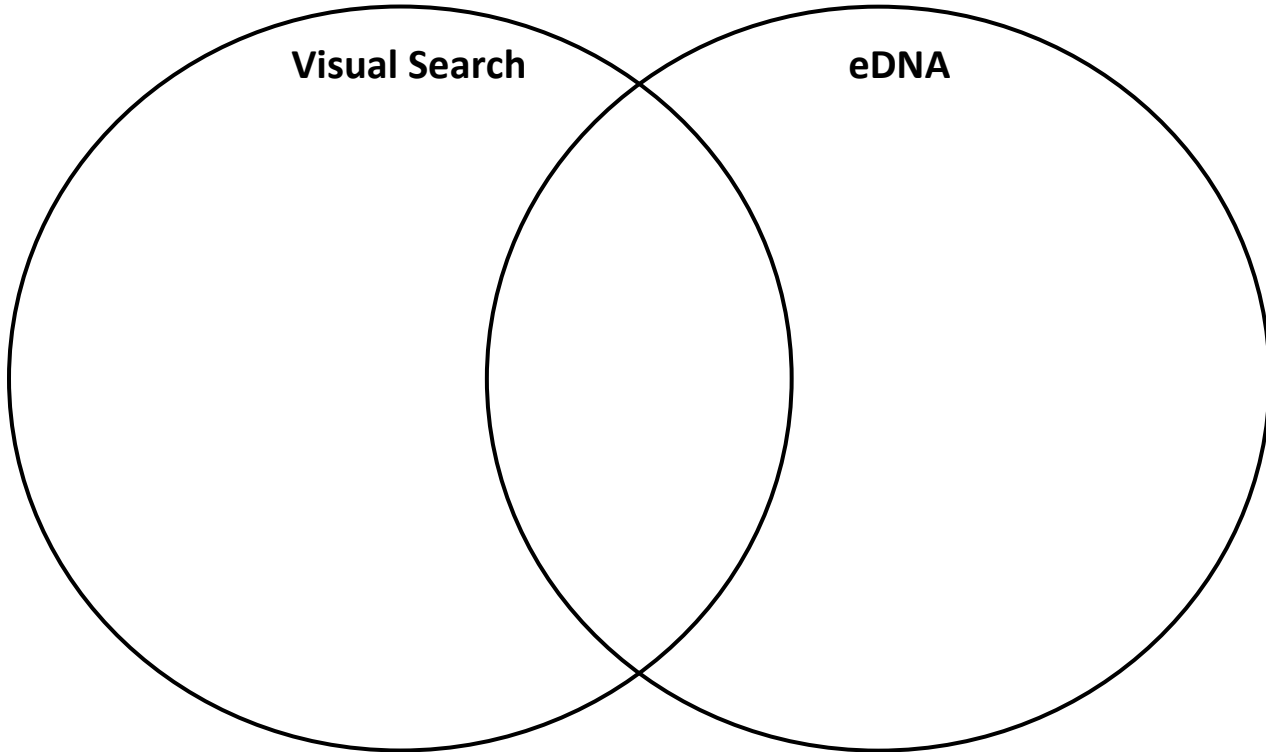
Data Table summarizing results of eDNA analysis at site # \_\_\_\_\_.

Type	Species	Presence/Absence
Fish	Largemouth Bass	
	Walleye	
	Brook Stickleback	
	Yellow Perch	
Insects	Damselfly	
	Mayfly	
	Crane fly	
Mussels	Zebra Mussel	
	Quagga Mussel	
Total		

1. How many species did you identify?
2. Were any of the species invasive? If yes, which ones?
2. How do the species identified in your eDNA sample compare to the species identified by the visual search? If they are different, explain why?
4. If invasive mussels were found in Lake Lago, what are some steps managers could take to help stop the spread? List at least two ideas.

Wrap Up

1. Compare and contrast the visual search method and eDNA sampling for AIS monitoring:



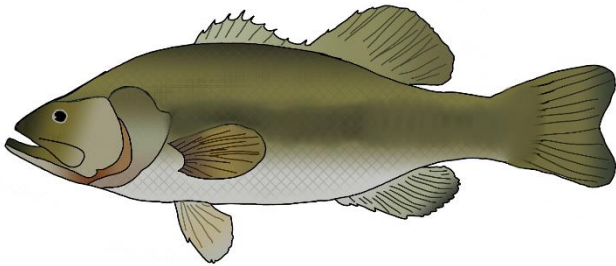
2. Why do scientists at FLBS and FWP use eDNA to monitor Flathead Lake for AIS?

## Detecting AIS with eDNA

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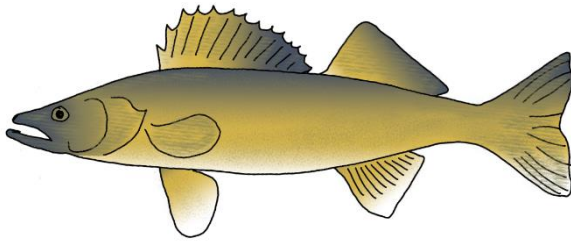
# Search and Find Species Key

## Fish



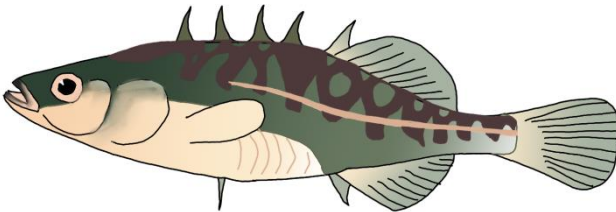
### Largemouth Bass

- Introduced
- Native to southeastern US
- Prefers warmer, shallower waters



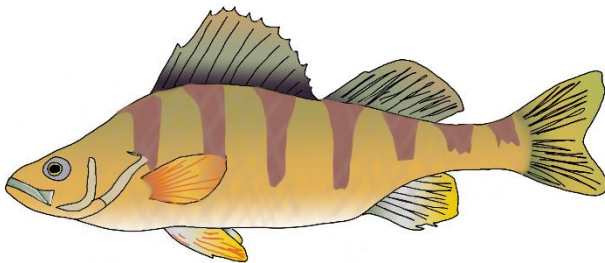
### Walleye

- Introduced
- More active at night
- Popular gamefish
- Found in larger lakes



### Brook Stickleback

- Native
- Lives in lakes with submerged plants
- Lives in groups during the non-breeding season



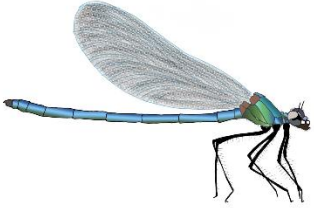
### Yellow Perch

- Introduced
- Popular for fishing
- Prefers warm to cool lakes with vegetation



## Insects

### Adult



#### Damselfly

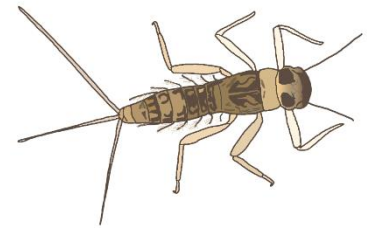
- Native
- Found in most MT lakes
- Nymphs move to shallows before emerging

### Nymph



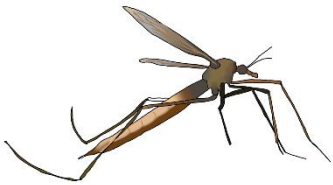
#### Mayfly

- Native
- Found in moving water and edges of lakes
- Adults fly to shore to mate



#### Crane fly

- Native
- Prefer still water
- Spread out across the water surface



## Mussels



#### Zebra Mussel

- Invasive to the United States
- Not currently found in MT
- Attaches to surfaces with byssal threads

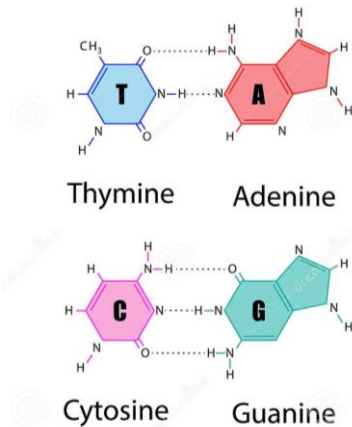


#### Quagga Mussel

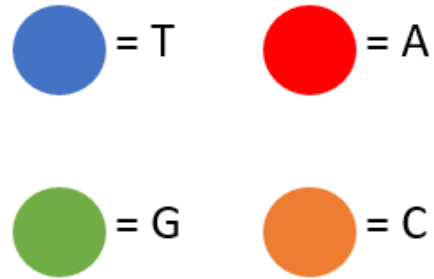
- Invasive to the United States
- Not currently detected in MT
- Attaches to surfaces with byssal threads

# AIS Activity Handout

## Nucleotide Base Pairs



## DNA Color Sequence Key



## DNA Sequence Code

Type	Species	DNA Sequence
Fish	Largemouth Bass	TAGCCATCGT
	Walleye	TAGCCGCAGC
	Brook Stickleback	TAGCCTCGAG
	Yellow Perch	TAGCCTAGCT
Insects	Damselfly	CTAGCGGCTA
	Mayfly	CTAGCTTAGA
	Crane fly	CTAGCCTAGT
Mussels	Zebra Mussel	GCTATGTGTA
	Quagga Mussel	GCTATTTAGC

# Sample Locations

