DETECTING AIS WITH eDNA

Presented by:

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UNIVERSITY OF MONTANA

BACKGROUND

What is DNA?

- **Deoxyribonucleic Acid**
- **Encodes genetic information through a sequence of nucleotides**
- **Macromolecule**
  - Made of nucleotide base pairs
  - Adenine (A) pairs with Thymine (T)
  - Guanine (G) pairs with Cytosine (C)
- **Found in cells of all living organisms**

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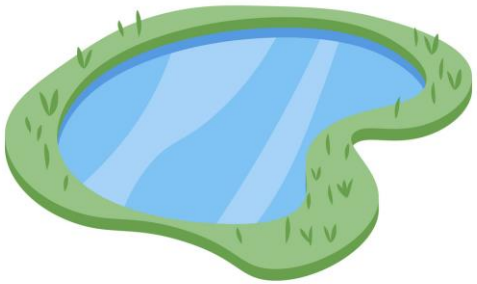
What is eDNA?

- eDNA stands for “environmental DNA”
- It is DNA obtained from sampling the environment
- DNA can be found in environments like…



How does DNA get into the environment?

- **Organisms can ‘shed’ or ‘leave’ DNA in the environment**
- **Can you think of some ways in which organisms can leave DNA in the environment?**

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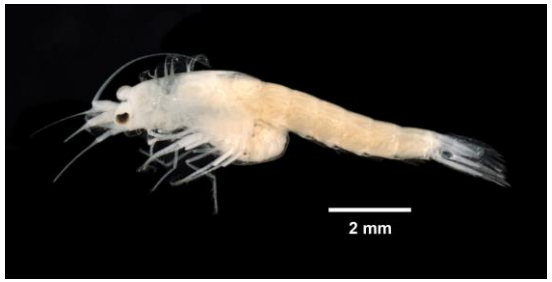
What are Aquatic Invasive Species (AIS)?

- **Non-native species that have been introduced into aquatic ecosystems**
  - Likely via humans
- **Significant negative impacts to the ecosystem and/or has a negative economic impact**
- **Some examples in lakes include:**

Zebra and Quagga mussels (not currently detected in MT)

Curly-leaf Pondweed



Mysis shrimp

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Monitoring AIS

- **Scientists look for and monitor the status of aquatic invasive species**
  - Why might scientists want to do this?
- **There are many methods used to determine if an invasive species is present in a body of water. Two examples are:**
  - searching for visual signs of the species
  - sampling for eDNA

National Park Service Rangers monitoring for AIS

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ACTIVITY PART I

Lake Lago

- In this activity, we are sampling a fictional lake in Montana called Lake Lago
- Lake Lago features wildlife, fish, insects, plants and more
- The north shore is located near a town with a public boat dock and the south shore is within forested land
- Although a nearby lake in South Dakota (less than 100 miles from the Montana border) has zebra mussels, Lake Lago is not known to have any
- Your job is to monitor the lake for zebra and quagga mussels!

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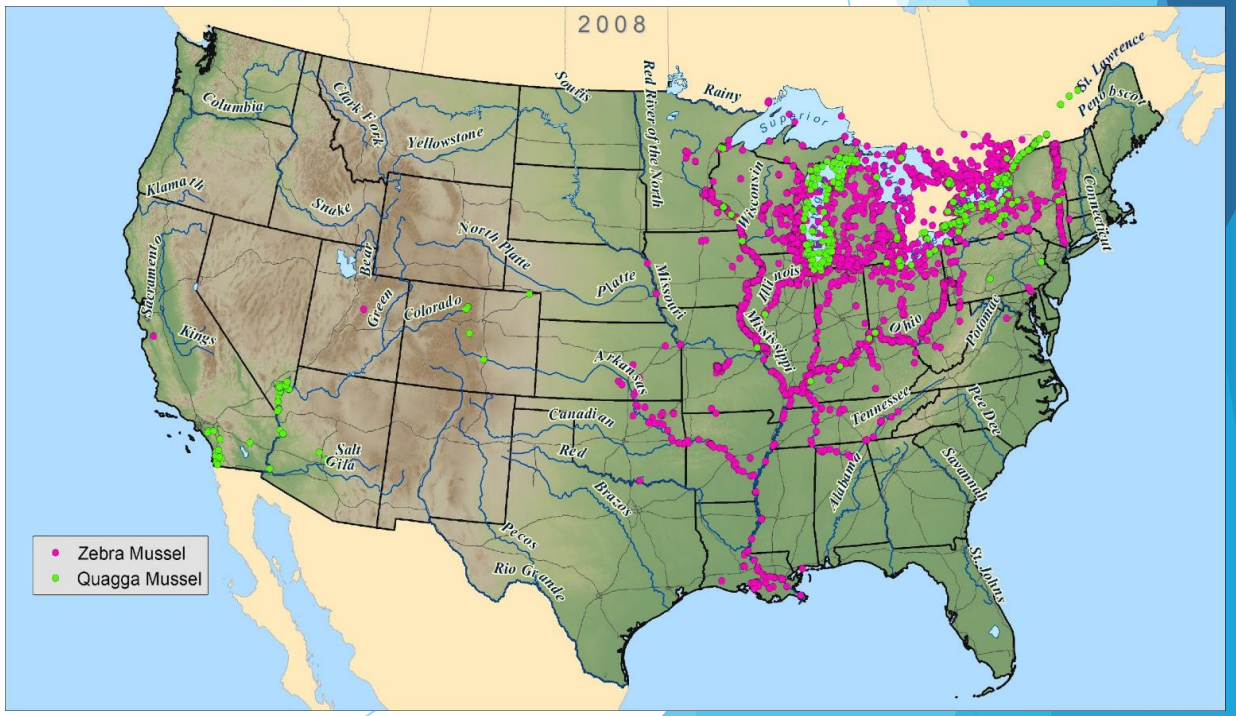
Zebra and Quagga Mussels

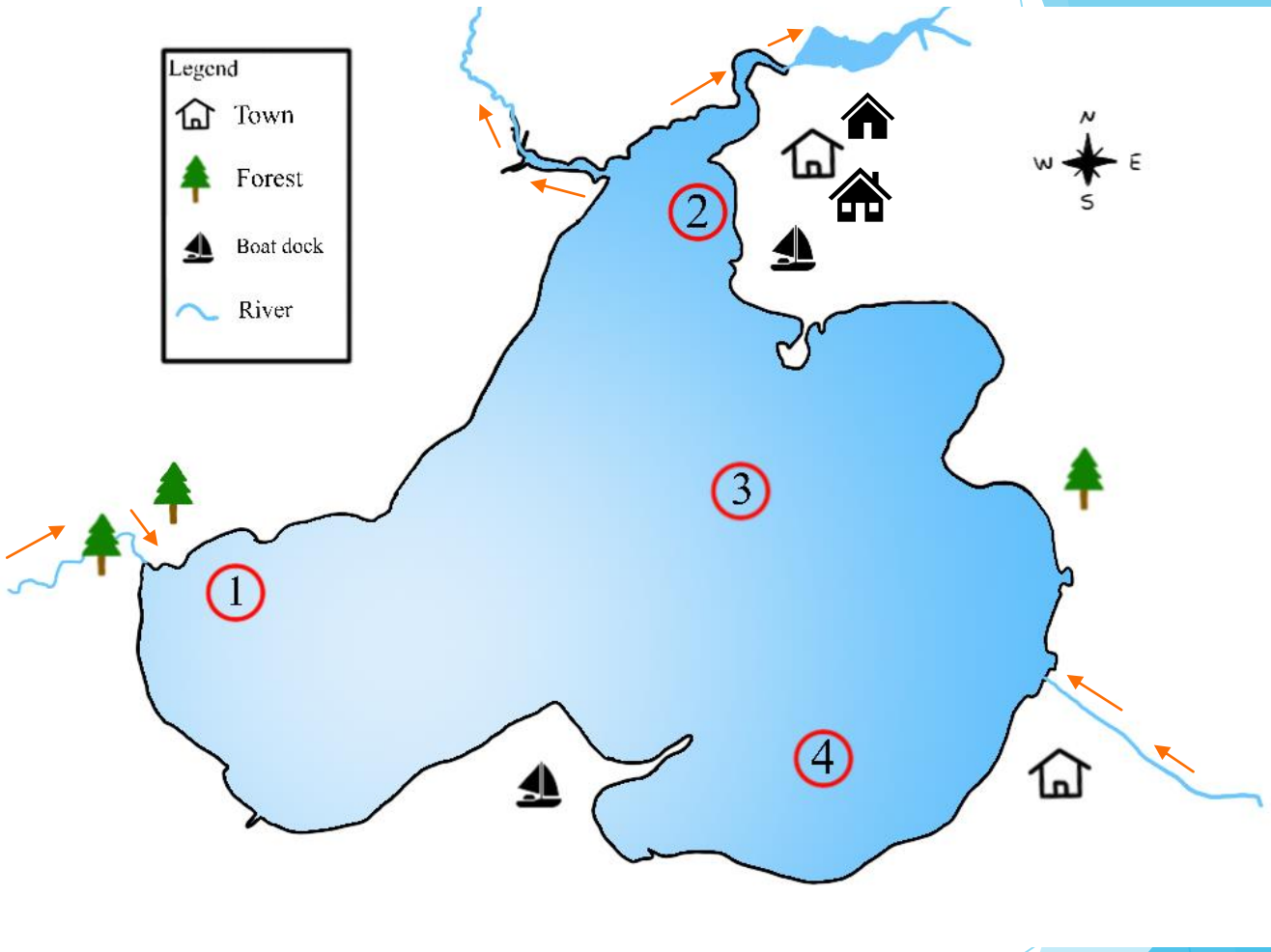
- **Non-native mussels first introduced in the Great Lakes**
- **Spread quickly through the United States by attaching to the sides of boats via their byssal threads**
- **Once introduced to a lake, they are extremely destructive and difficult to remove**
  - Clog drain pipes and destroy boat engines
  - Attach to native mussels
  - Compete for food with native species

Zebra mussels clog a boat propeller



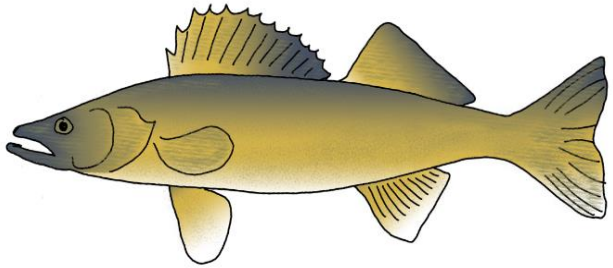
Sample Locations

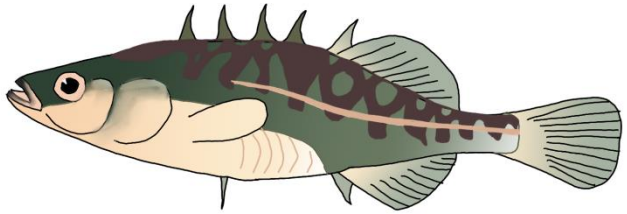
- **Each group will be assigned a sample location**

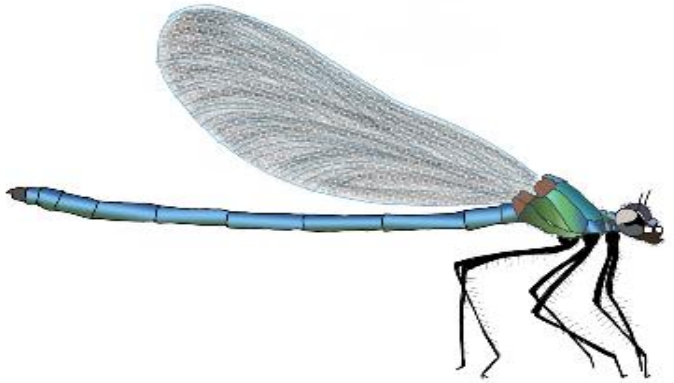


Fill out the questions about your sample location in Part I of your worksheet (questions 1-3)

Searching for AIS







Complete the search and find activity and write your species counts on the worksheet

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Searching Methodology

- **Did any groups find an invasive species with this methodology?**
- **How effective did the search and find method seem?**

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ACTIVITY PART II

Sampling eDNA

- **Now we are going to sample the lake for eDNA to see if this method can find any AIS present**
- **To sample for eDNA, scientists first use a net to catch organic materials in the lake**
  - This is because DNA sticks to organic materials like branches and leaves

Video: FLBS scientist Phil Matson sampling for mussel eDNA at the station

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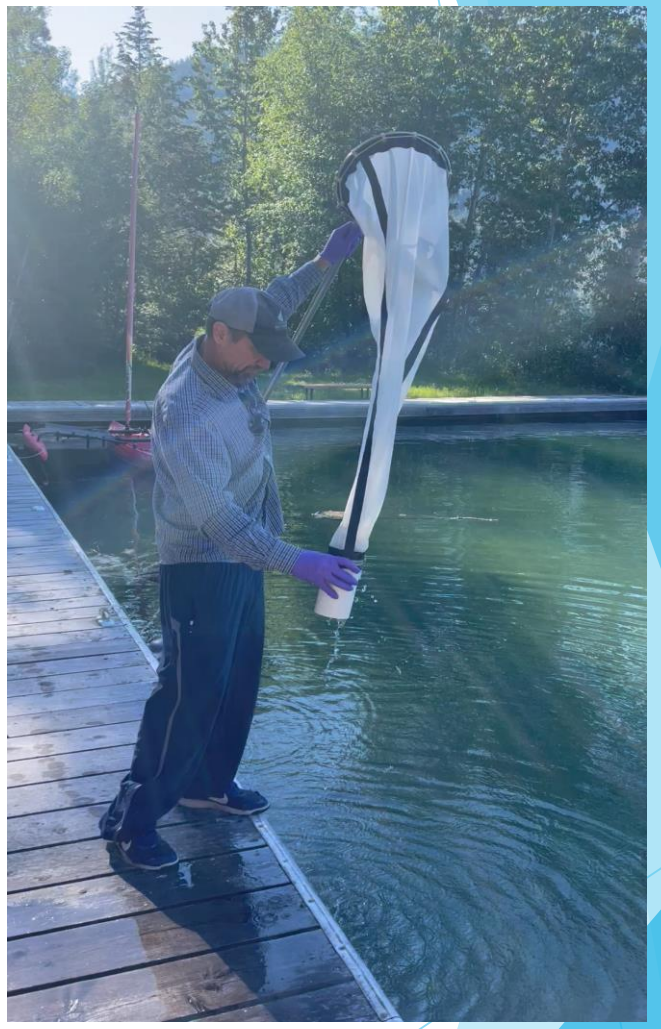
Sampling eDNA

- They then shake the water out through a filter to obtain a more concentrated sample of DNA stuck to organic materials
- The sample is poured into a tube to be sent to the lab for analysis
- All materials are sterilized before and after to prevent contamination!



Video: FLBS scientist Phil Matson filtering sample of eDNA at the station

Answer Question 1 on Part II of your worksheet

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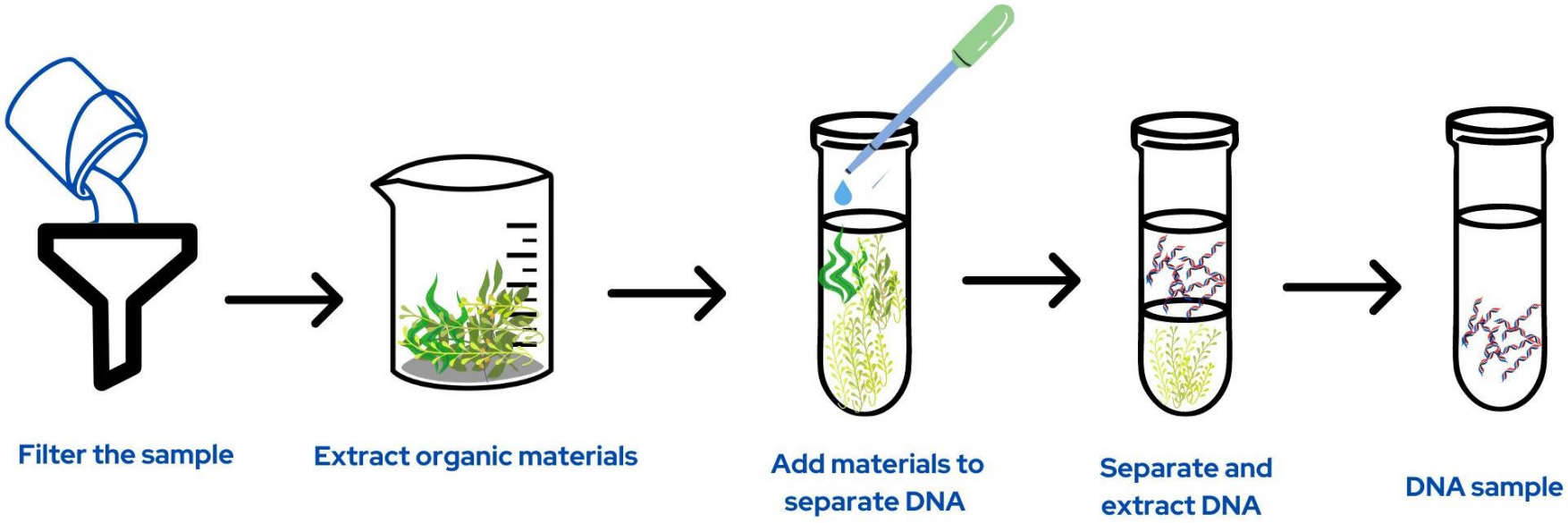
Extract the DNA from the sample

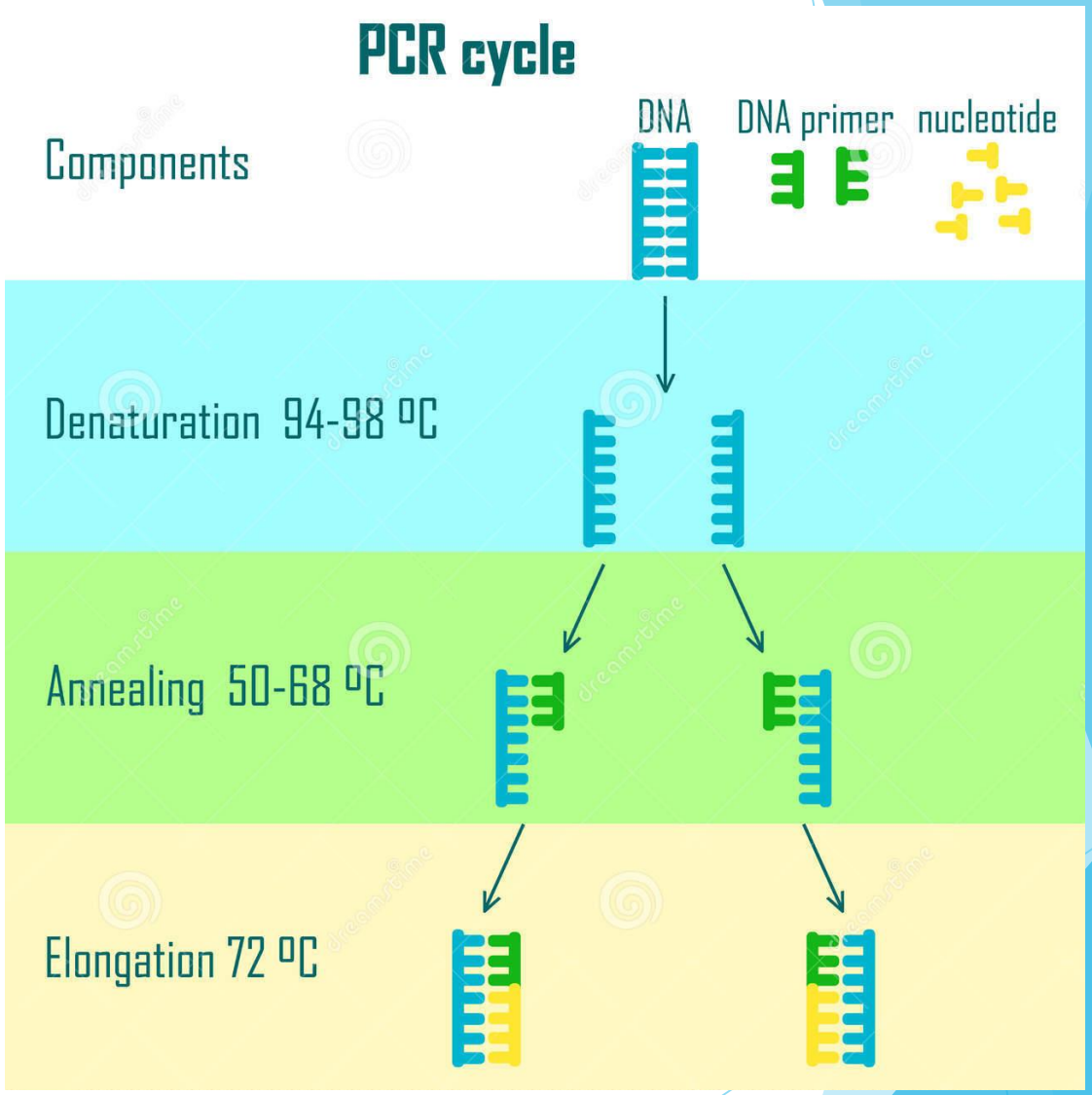
- **DNA extraction isolates the DNA strands from the organic material and proteins located in the sample**



Extracted strawberry DNA

Conducting PCR

- PCR stands for “polymerase chain reaction”
- PCR is used to rapidly make millions of copies of a target DNA sample
- Why is PCR important for eDNA detection?



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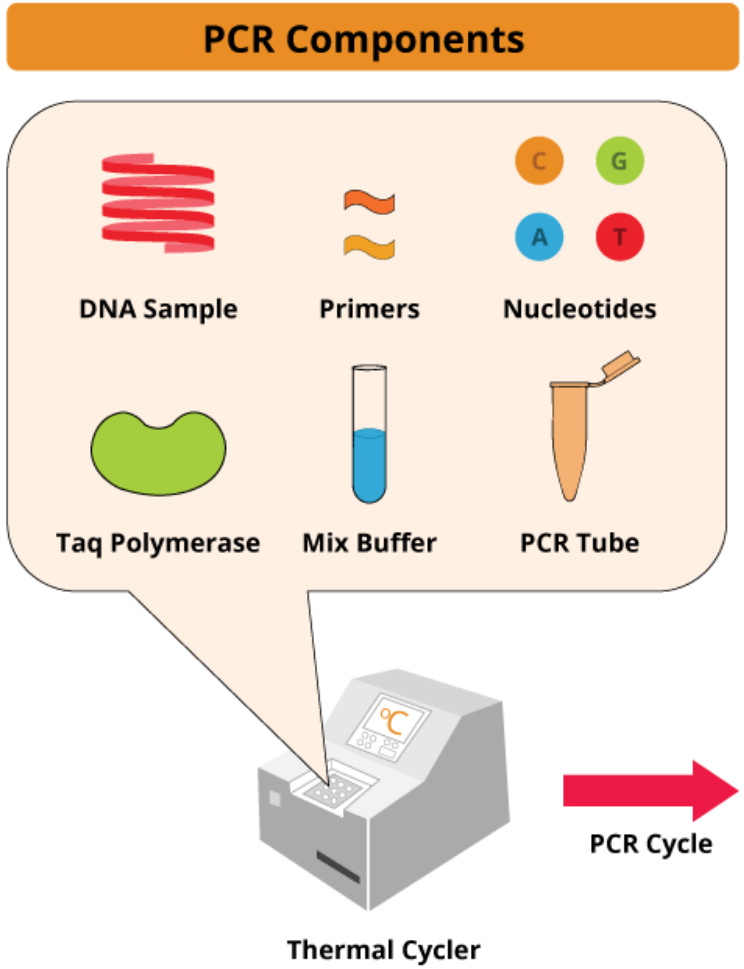
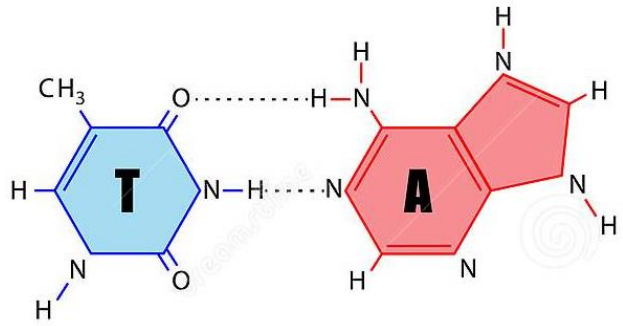
Add materials needed for PCR to the sample

- **Free nucleotides**
  - To build the new DNA strands
- **Taq Polymerase**
  - An enzyme that creates the replicated DNA strand
- **Primers**
  - Help guide Taq Polymerase to replicate the target strands
  - Pairs to a specific DNA sequence

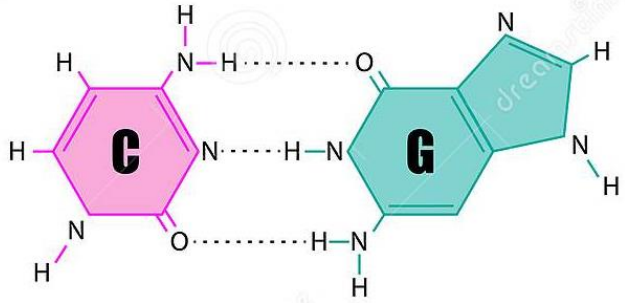


Identify Desired DNA

- We are searching for fish, mussel, and insect DNA in the lake
- First we need to pair nucleotides with the primer sequences to see what the target DNA sequences for each primer will be



Thymine Adenine



Cytosine Guanine

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Identify Desired DNA

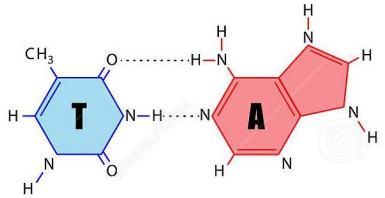
Fish Primer

A	T	C	G	G

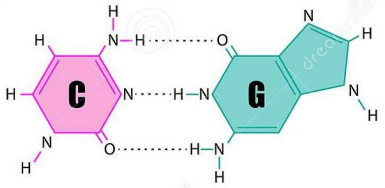
Insect Primer

G	A	T	C	G

Base Pairing Key:



Thymine Adenine



Cytosine Guanine

Mussel Primer

C	G	A	T	A

Fill out the primer tables on Part II of your worksheet

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Identify Desired DNA

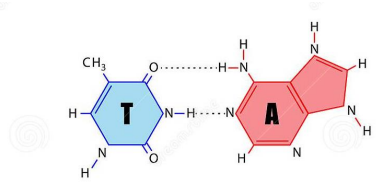
Fish Primer

T	A	G	C	C
A	T	C	G	G

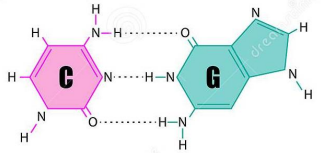
Insect Primer

C	T	A	G	C
G	A	T	C	G

Base Pairing Key:



Thymine Adenine



Cytosine Guanine

Mussel Primer

G	C	T	A	T
C	G	A	T	A

Pull all of the DNA strips that match these sequences. Set the others aside

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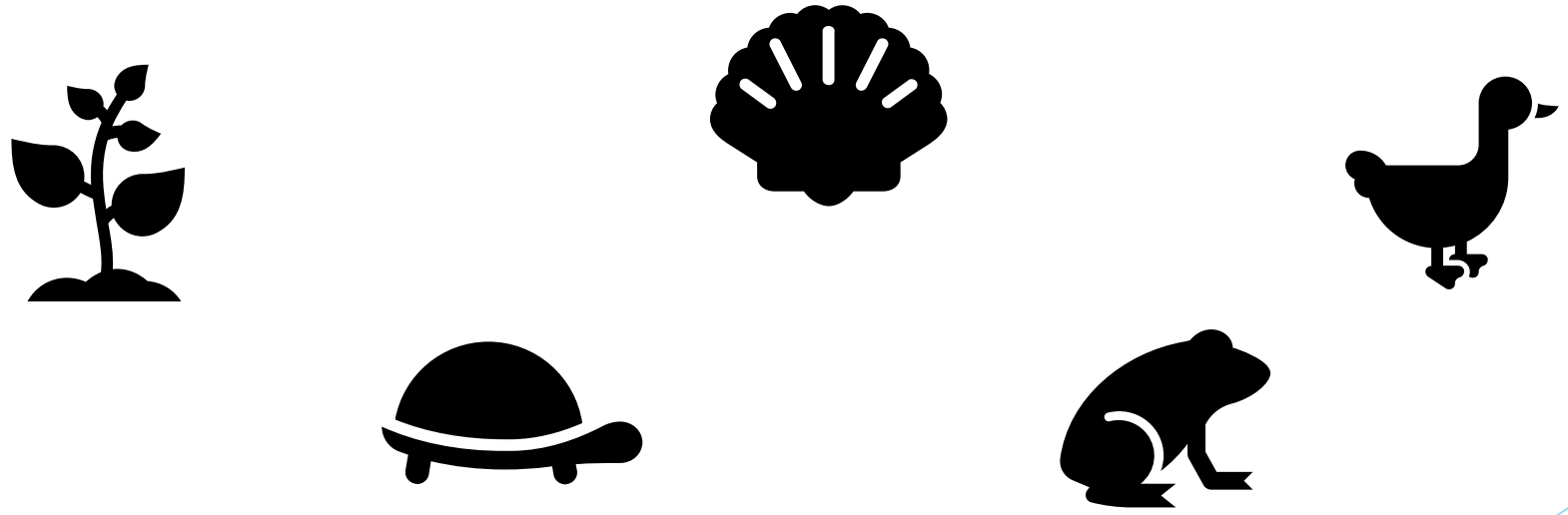
What do the other DNA strips represent?

- **DNA of organisms that did not match our primers!**
- **They could be…**



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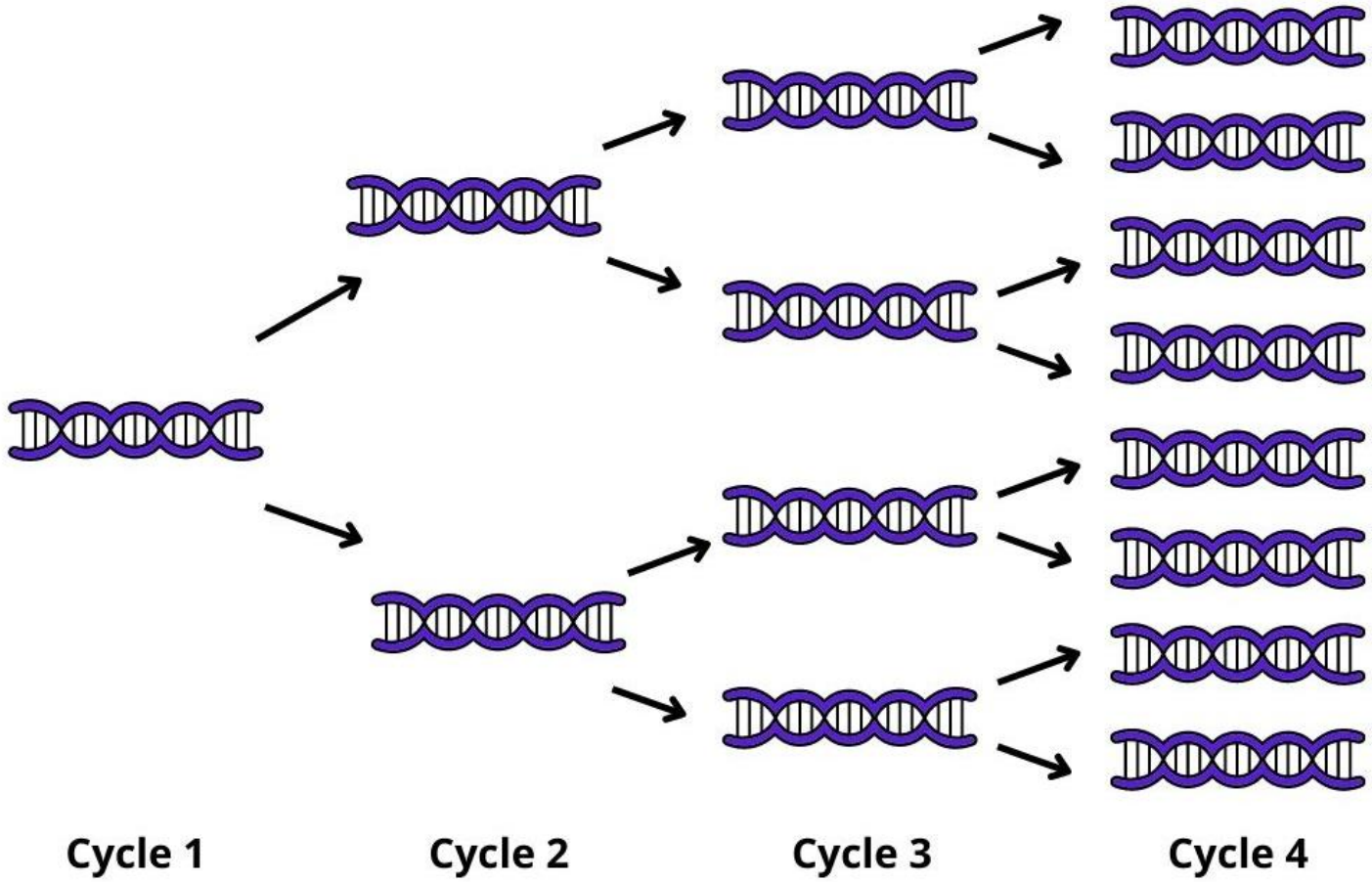
Perform PCR Rounds



Complete the PCR activity on Part II of your worksheet

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DNA Sequencing

- **DNA Sequencing allows scientists to determine the sequence of nucleotides in the DNA strands that have been replicated**

- **Steps**
  - To keep DNA in an order, DNA strands bind to a molecule on a plate
  - Nucleotides are added to the DNA strands and release a color corresponding to the nucleotide
  - A camera captures the wavelengths of the nucleotides, recording the sequence of the DNA
  - The sequence of DNA is compared to a database of known species DNA sequences

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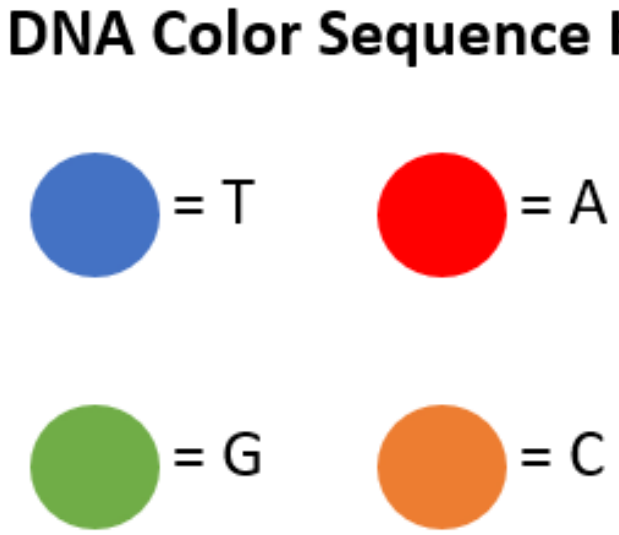
DNA Sequencing Activity

- **As a group, carefully open your target DNA strips and use the key to decode the nucleotide sequence for each strip**



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

Complete the DNA Sequencing activity and questions (#3-6) on Part II of your worksheet

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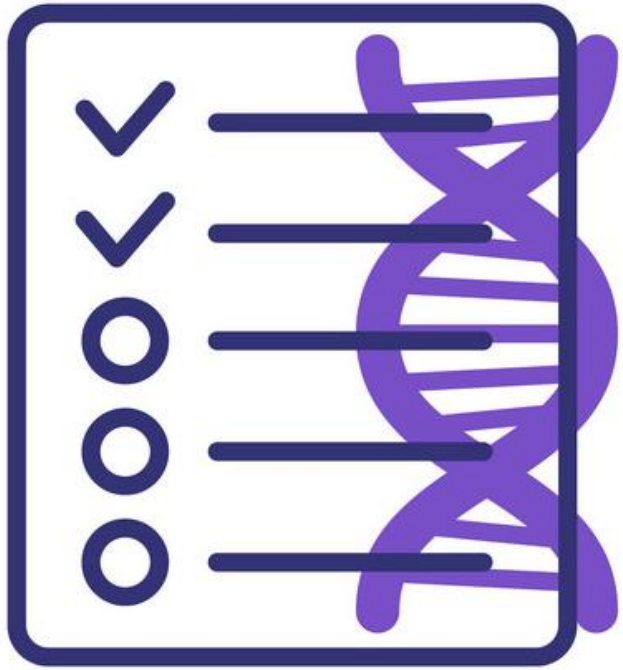
Results

- **Share with the class your group's results and what species you found in the visual search compared to the eDNA sample**
- **Did any groups discover an invasive species through eDNA analysis?**



Discuss in groups why eDNA may be more effective at finding invasive species and why the AIS was found at that specific sample sight and not others

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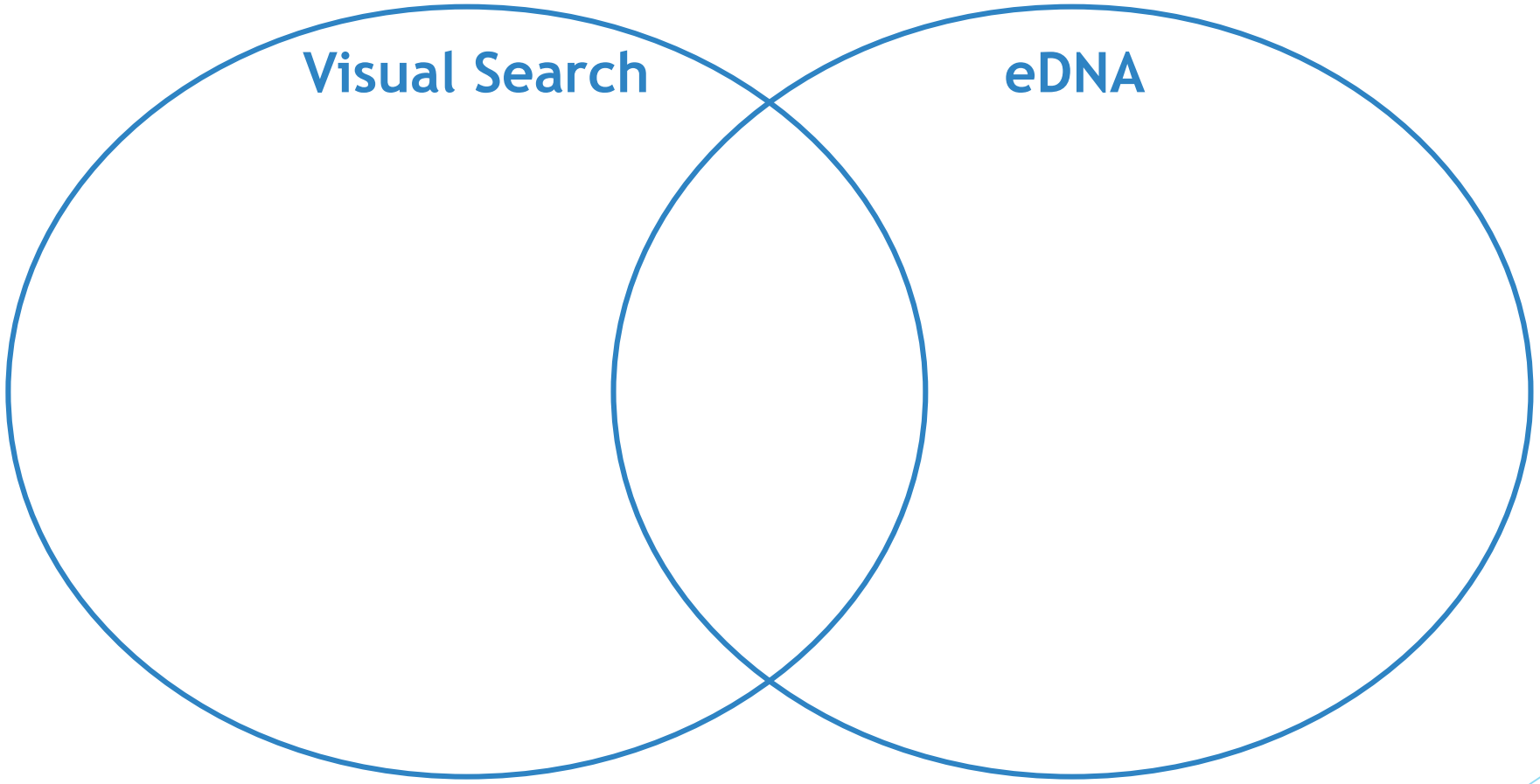
WRAP UP

Complete the Venn Diagram on the Wrap Up portion of your worksheet

Comparing visual and eDNA techniques



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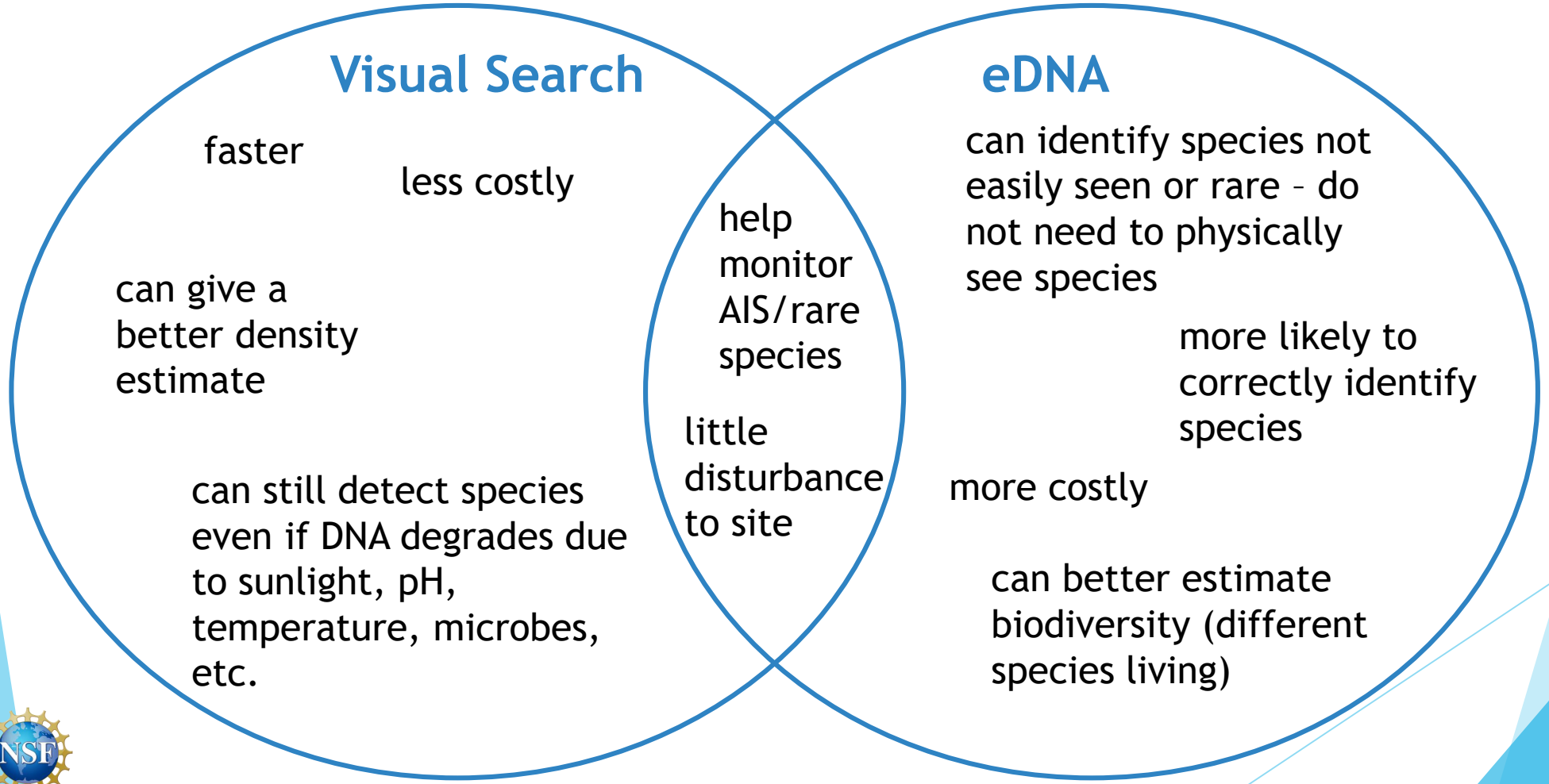
Comparing visual and eDNA techniques



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Monitoring Flathead Lake for AIS

- **Although Flathead Lake already has some AIS like Mysis Shrimp, currently there are no Zebra or Quagga Mussels**

- **At the Flathead Lake Biological Station, scientists conduct eDNA sampling four times a year to monitor the status of mussel invasion**
  - Early detection is important for managing AIS!

- **Other programs work to help prevent the introduction of non-native mussels to the lake, like…**
  - Boat inspection stations throughout the state
  - Encouraging people to clean, drain, and dry their boats between bodies of water

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Additional video on FLBS AIS monitoring

References

- https://flbs.umt.edu/newflbs/outreach/news-blog/posts/sharing-language-to-prevent-ais/
- https://flbs.umt.edu/newflbs/services/montana-conservation-genomics-laboratory-mcgl/sampling-protocol/
- MS-LS2-5 Ecosystems: Interactions, Energy, and Dynamics | Next Generation Science Standards (nextgenscience.org)
- Using environmental DNA to extend the window of early detection for dreissenid mussels (semanticscholar.org)
- Next-Generation Sequencing: Methodology and Application (acslm.ie)
- https://ep.bmj.com/content/102/5/261

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