Mystery #1: Is there an endangered species in this estuary?

Pre-Activity Engagement: Have your students read this article before introducing the topic. **https://www.ioes.ucla.edu/article/tiny-endangered-shrimp-may-get-big-hand-from-environmental-dna-testing/**

Objective: Students will learn about genetic analysis in the marine environment and how eDNA can be used to detect the presence of certain species. Here is a short video of one of our biologists sampling for eDNA at the South Carolina Department of Natural Resources Marine Resources Research Institute!

Materials

- eDNA sampling video
- Animal Cards and eDNA Sequences
    - DNA sample cards (short sequence of base pairs, no photo)
    - Species cards (photo and name)
- Species ID chart
- PCR Primer cards
- Data sheet
- ACE Basin map
- 6 Gallon Ziplock bags

Teacher Preparation (These materials are enough for 6 groups)

1. Print 6 Species ID Charts and 6 Data Sheets, 1 set for each group
2. Print the Primer Cards pdf, cut out boxes and give one to each group
3. Print the Animal Cards and eDNA Sequence pdf.
4. Cut out and laminate all animal cards and all individual eDNA sequences.
5. Make 6 piles of the species cards so that each pile has one of each species.
6. Combine all eDNA sequences into a bag and shake to shuffle. These sequences represent eDNA found in the water samples taken from various locations.
7. Label 6 gallon size Ziploc bags; Location 1: Combahee River, Location 2: Ashepoo River, Location 3: St. Helena Sound, Location 4: North Edisto River, Location 5: South Edisto River, Location 6: Coosaw River.
8. Divide eDNA sequences into the 6 Ziplock bags. This is a ‘water sample’ so they do not have to be equal, just roughly even amounts.

Engagement question: What are some applications of DNA and genetic analysis that you know of? (medicine: vaccines and insulin, forensics: identify individuals, and agriculture: selective breeding dogs, modify food)

Student Worksheet

Question: What are some applications of DNA and genetic analysis that you know of?

Pre-Activity Engagement: Read this article first.
https://www.ioes.ucla.edu/article/tiny-endangered-shrimp-may-get-big-hand-from-environmental-dna-testing/

You need a knowledge of DNA and PCR prior to this activity. Here are two options:
DNA from Khan Academy: https://www.khanacademy.org/science/high-school-biology/hs-molecular-genetics/hs-discovery-and-structure-of-dna/v/dna-deoxyribonucleic-acid
PCR from Khan Academy: https://www.youtube.com/watch?v=aUBJtHwHASA

eDNA in Natural Resources: We will learn about genetic analysis in the marine environment and how eDNA can be used to detect the presence of certain species. Here is a short video of one of our biologists sampling for eDNA at the South Carolina Department of Natural Resources Marine Resources Research Institute!

Background:
Genetic analysis is also an important aspect of wildlife conservation and can give scientists a better idea of how species are doing in an area. Every plant and animal on earth has a unique genetic code stored in their DNA. Just like humans shed hair and skin cells, plants and animals also leave traces of DNA in the wild as they move and grow. These fragments of genetic information are called eDNA or environmental DNA because they are present in small pieces throughout the environment. eDNA is analyzed by scientists using complex techniques like metabarcoding and Polymerase Chain Reaction (PCR). These techniques amplify (make copies) the small fragments of eDNA so they can be matched to a specific species.

DNA strands are made up of a series of four nucleotide bases; Adenine, Thymine, Guanine, and Cytosine. The DNA sequence of a species can be written like this: Periwinkle Snail (AGTCCAG) although in reality they are millions or billions of bases long. The bases pair up with each other to create two connected strands held together in the middle like a ladder. The complementary base pairs are A-T and G-C. Knowing the sequence of one strand automatically tells you the sequence of the other!

Biologists at South Carolina Department of Natural Resources use genetic analysis for multiple reasons. They use it to assess population health and genetic diversity, to know where species are and how many remain, to confirm genetic identification, and to understand where one population starts and another ends. Here we are going to see how these tools can be used to identify the presence or absence of an endangered species without having to find and capture one! Scientists call this technique minimally invasive because no organisms, especially endangered ones, are harmed when sampling.

Round 1: A friend of yours, another scientist, is studying an endangered species, shortnose sturgeon, in the estuary and needs your help. Since they are endangered, they do not get collected often through other fisheries sampling methods. They give you the PCR primer sequence and water samples from different estuaries in the state. Using the eDNA from the water samples, your group will attempt to identify which estuaries are important for the shortnose sturgeon's survival.

1. Each group should have a Ziplock bag containing eDNA sequence cards. This is your water sample from a specific location.
2. Spread out the eDNA sequences on the table.
3. Group cards that have the same exact sequence. These represent the same species. Are there more cards of a certain sequence? (eDNA prevalence is not directly correlated to biomass but it can give an indication of whether or not some species are more present than others).
4. Record the eDNA sequences and number of occurrences on the data sheet.
5. Use the Species ID sheet to match up the eDNA sequences to the correct species. Letters should **match up** exactly to identify the species. Record species name on Data Sheet and place the species photo above the correct eDNA sequences (ex. eDNA card says ATGGCT, species ID list says Blue Crab: ATGGCT).
6. The primer is specific to the target species and will be used in PCR to amplify specific eDNA segments. Sometimes the primer sequence is long and may amplify more than one sequence of eDNA. Primer sequences contain the **complementary sequence** to the eDNA segment.
7. Record the complementary sequence for each species on your data sheet. Remember, A-T and G-C.
8. Now, look at the PCR primer sequence card. If you have the endangered species in your sample, the PCR primer sequence should match one of the complementary eDNA sequences!
9. Have each group share what they think the endangered species is and where it was found.

Discussion:

10. What endangered species are the scientists looking for?

11. Was it present in every group's eDNA sample?

12. Why did some groups not discover the endangered species?

13. How could knowing if the endangered species is present help that species?

Reset for Round 2:

1. Place eDNA sequences back in the Ziplock bags.
2. Give your bag to another group

Round 2: Invasive species detection

Background: eDNA can also be used to identify the presence of invasive species. This information can help scientists identify when and where the species are introduced. Then efforts can be made to contain the species before spreading further.

In this next round, you will receive another water sample taken from a creek or river which flows into the estuary. It is your job to identify the presence or absence of the invasive Red Swamp Crayfish. There are several invasive species that we can track when and where they're introduced and hope to contain them if we catch them early enough. Probably more important than their presence is that these crayfish often carry White Spot Shrimp Virus which decimates our native populations and aquaculture populations. We can use eDNA to detect the invasive species itself OR the DISEASE itself. Some water samples might not contain eDNA from the invasive crayfish - this is good news! On the other hand, some samples may contain high percentages of the crayfish's eDNA. What does this tell us?

Red Swamp Crayfish PCR primer sequence: CGATGT

1. Group the same eDNA sequences together on the table like you did in Round 1.
2. Use the red swamp crayfish PCR primer sequence to determine if it is present in your water sample.
3. As a class, use the map to determine where the crayfish is found and how far it has spread.

ACE Basin — Site #9C

SCMaps

"SCMaps" (South Carolina Maps and Aerial Photography System) is an interdisciplinary educational program designed for the cooperative learning classroom environment of the 8th grade. "SCMaps" employs a portfolio collection of aerial prints, satellite images and special-purpose maps as geographical resources to enhance students understanding of South Carolina's natural and cultural landscape. The program focuses on the geology and natural environment of the state and how historical events and cultural development is influenced. In addition to the map products portfolio, the program is accompanied by a "Teaching Manual" providing student activities and background information. The ultimate goal of "SCMaps" is to provide students an opportunity to heighten their appreciation for the state's landscape diversity.

SCALE 1:100 000

KILOMETERS 0 1 2 3 4 5 6 7 8 9 10

MILES 0 1 2 3 4 5

FEET 5000 0 5000 10000 15000 20000 25000

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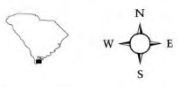
ACE Basin

Site # 9C
Data Source: "Beaufort" & "Walterboro" USGS Quadrangle
Date: 1978 & 1990



Mystery 1: eDNA
Data Sheet

Sample location: _____

Primer sequence: _____

Number of occurrences	eDNA sequence (these are the sequences found in your water sample)	Species name	Complementary sequence

Mystery 1: eDNA
Species ID Chart

Species Name	eDNA Sequence
Sheepshead	ATTGCA
Spotted Seatrout	GATCCT
Shortnose Sturgeon (endangered)	ATGCAT
Bottlenose Dolphin	GGTACT
Loggerhead Sea Turtle (endangered)	TGCATG
Eastern Oyster	AATGCT
West Indian Manatee (endangered)	TCCGAA
Striped Burrfish	CACTGG
Blue Crab	ATGCTA
Bald Eagle (endangered)	CTAGCT
Red Drum	TAGCTT
American Oystercatcher	TGCCAG
Cobia	GTCATT
Wilson’s Plover (endangered)	CGTGAT
Bonnethead Shark	TGAACG
Diamondback Terrapin	AGCAAT
Carolina Hammerhead	CCATTG
Right Whale (endangered)	GTAGCA
Red Swamp Crayfish (invasive)	GCTACA
Fiddler Crab	ACATGG
Acorn Barnacle	TCCGGT
Atlantic Sharpnose Shark	CAGTAA
Southern Flounder	AAGCTA
Cannonball Jellyfish	GTCGCC

Round 1
PCR Primer Sequence:

TACGTA

Round 1
PCR Primer Sequence:

TACGTA

Round 1
PCR Primer Sequence:

TACGTA

Round 1
PCR Primer Sequence:

TACGTA

Round 1
PCR Primer Sequence:

TACGTA

Round 1
PCR Primer Sequence:

TACGTA

Round 2
Red swamp crayfish
PCR Primer Sequence:

CGATGT

Round 2
Red swamp crayfish
PCR Primer Sequence:

CGATGT

Round 2
Red swamp crayfish
PCR Primer Sequence:

CGATGT

Round 2
Red swamp crayfish
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