Monitoring Biodiversity with Environmental DNA

Instructor Background:
The primary purpose of this lesson bundle is to demonstrate methods for measuring biodiversity. Answering biology's most fundamental questions, "Who is there and what are they doing?" be it in the woods, river, or prairie. Throughout these lessons, students will appreciate DNA's role in understanding species richness and measuring species' presence or absence. Secondarily, but no less critically, students will also engage in thinking about evidence, how it is gathered and measured, and how both the quality and relevance of evidence allow scientists to draw conclusions emphasizing the importance of sampling. Students will be expected to consider the importance of sampling, multiple lines of evidence, and understanding types of evidence beyond direct in-person observation.

The Lessons:

The Teaching Tool consists of six separate lessons that can be taught independently or combined over six 50-minute class periods.

Next Generation Science Standards:

3-Dimensional lesson to support performance expectations HS-LS1-1, HS-LS2-2

- Disciplinary Core Ideas
  - LS1.A
  - LS2.C
- Cross-Cutting Concepts
  - Scale, Proportion, and Quality
  - Cause and Effect
  - Structure and Function
- Science & Engineering Practices
  - Using Mathematics and Computational Thinking
  - Constructing Explanations and Designing Solutions
  - Obtaining, Evaluating, and Communicating Information
- Nature of Science
  - Scientific knowledge is open to revision in light of new evidence
  - Scientific knowledge is based on empirical evidence
  - Science is a human endeavor

Activity 1: Collecting Evidence

Tool Type: Phenomenal Image
Time: 50 minutes or 1 class period

Materials:
Collecting Evidence

Objective:
Students will apply their knowledge of DNA collection and analysis in crime scene forensics to using DNA sampling to monitor the presence or absence of organisms in a natural environment.

Overview:
Crime forensics is familiar and exciting for many students. This 2-part introductory activity activates prior knowledge and primes students to think about evidence collection and interpretation.

Part 1: Crime Scene
During the first activity, students will observe photos of investigators processing a crime for evidence. During class discussion, draw students' attention to the protective gear worn by investigators to prevent contaminating the crime scene (only minorly to protect themselves). Remind students that sources of contamination are mainly invisible to the naked eye, such as hairs, skin cells, dust, soil, pollen, AND DNA. Encourage students to consider what can be observed directly on the scene (blood, furnishings, prints, objects) and evidence that is gathered and then analyzed in a lab using chemistry, microscopy, and molecular biology after students begin to recognize that DNA is all around us, constantly being shed into the environment transition to the second activity.

Part 2: Forensics in Nature
This activity connects student thinking about forensic evidence to methods for studying nature, particularly detecting what species live in a particular location. The questions scaffold student thinking toward understanding how DNA sampling and analysis, like that used in crime scene investigation, can be applied to the natural environment and help answer questions about biodiversity and ecology. Forensic approaches can be used in natural settings to understand aspects of the natural environment, such as:

- What organisms live in the area?
- How are different species interacting?
- What makes up the diet of the organism?
- What are the traits of individual animals, such as gender, age, and health?

During class and group discussion, reinforce the major categories of observation: (1) what is seen and measured directly on-site/in the field and (2) samples collected to be analyzed in a laboratory. By the end of this activity, students should be awakened to the potential power of DNA in ecological studies and motivated by the idea of environmental DNA (eDNA), which will be covered in the final section.

Activity 2: What is Environmental DNA

Tool Type: Graphic Reading
Time: 50 minutes or 1 class period

Materials:

What is Environmental DNA, When a Bear Poops in the Woods

Objective:
The lesson introduces students to the concept of eDNA. They will be able to identify various sources of DNA in the environment that scientists can collect, analyze, and identify to know what organism is living in an area.

Overview:
In the previous activities, students thought about how forensic DNA analysis can be used in ecological studies, in particular, to identify the presence of an animal, similar to the way DNA can be used to identify a person. Scientists extract DNA from scats and can identify the animals that have deposited it. Still, the scat also contains DNA from organisms it ate, along with large quantities of bacterial DNA that reside in the digestive tracks of all animals. The presence of that DNA can complicate DNA extraction and analysis, but other DNA can also provide helpful information. In this activity, the goal is to extend student thinking beyond using DNA to identify a person (the suspect) or one species (a brown bear) to consider how DNA can be used to identify all the organisms occupying a given area. This activity is designed to get students thinking about the challenges involved in getting useful information from eDNA by featuring a graphic depiction of DNA being shed into the environment, zooming out to demonstrate that every living thing is continuously releasing DNA into the environment and because DNA is stable it will remain in the environment for a very long time. eDNA is traditionally obtained from soil, water, ice, or air and then returned to a lab. It is sorted, amplified, and matched. The sorting process separates the DNA from one species to another. Then, through barcoding, the DNA is compared to a vast bank of previously identified sequences until it is matched, revealing the species from which the DNA came. The purpose of this activity is not to get into the laboratory process's details but to provide some context to the practices scientists use to identify species from trace amounts of DNA in the environment.

Part 1: Small Group.
Distribute copies of the graphic reading, the allow students to discuss the contents in small groups for about 5 minutes while creating a list of things they notice and wonder.

Part 2: Whole Group.

Project the graphic for the whole class. Ask students to share what they discussed in their groups. When student groups share something, they wonder. Allow other groups to offer suggestions and be mindful to address misconceptions after the class discussion. Students should have a solid understanding of how and why DNA is in the environment and how scientists could use this information to determine if a species is present or absent in an area.
Part 3: Partners or Individuals:
Provide students with the graphic reading to complete the three-question worksheet.

Activity 3: Who Pooped?

Tool Type: Guided Inquiry
Time: 50 – 100 minutes or 1-2 class periods

Materials:
Who Pooped?
Printed Cards Who Pooped, Teacher's Guide
Scissors
Tape
Hunt for the Oldest DNA

Objective:
Students will describe how eDNA can be used to monitor and assess an area's biodiversity while recognizing potential problems with the method.

Overview:
This hands-on activity was designed to simulate the collection, analysis, and identification of eDNA to determine species' presence or absence in a given area. Students will first try to identify feces without genetic information, then again after being provided with the genetic bar codes. After completing the activity, students are introduced to using eDNA to monitor biodiversity and asked to consider any problems arising from using this method. As an extension, students can view the NOVA documentary Hunt for the Oldest DNA, where they will follow scientists as they recover DNA millions of years old and reveal the genes of long-extinct organisms that once lived in the warm Arctic.

Teacher Preparation Before Class:

1. Print the images on the following pages.
    - Depending on groupings and class size, you may have to print multiple copies of pages 6-10.
2. Cut out each box
    - Do not separate the genetic information from the image for the samples on pages 2-8.
3. Tape the species cards around the room; they can be on desks or walls.
    - Carefully fold the card in half so that the genetic information behind the photo is not easily seen.
4. Place them so students can easily move around the room to observe the species and determine the source of the fecal sample.

Part 1: Without genetic information

1. Organize students into pairs (one group may have 3)
2. Distribute the student handouts
3. Distribute one fecal sample **without genetic information** to each student pair **(**pages 9-10).

    - It will be necessary to distribute duplicate samples. Depending on class size, two or more groups may receive the same fecal sample - this duplication contributes to class discussion.
4. Allow students to move around the room to “observe” the animals in the area (approximately 5 minutes).
    - After five minutes, instruct each pair to stand beside the animal they believe deposited the fecal sample.
5. Ask students to raise their hands if they are certain their sample belongs to the animal they selected.
6. Ask for volunteers (starting with those who raised their hands) to explain their decisions and facilitate discussions between student groups.
7. Give students 5-10 minutes to complete page 1 of their student handout.
8. Ask students what information could make the identification easier.
9. When students suggest genetic information (may require prompting), ask them to return to their seats, where they can exchange their fecal samples for the **same fecal sample** but with the genetic information included.

Part 2: With genetic information

1. Inform students that the DNA from the fecal sample was collected and amplified, generating a bar code found on their new fecal sample.
2. Inform students that the genomes of organisms known to be in the area have been collected and barcoded. The section of DNA and a barcode can be found on the back of animal cards.
3. Allow students to move around the room to compare the DNA collected from the fecal sample to the known bar codes of the animals in the area (approximately 5 minutes).
    - After five minutes, instruct each student group to stand beside the animal they believe deposited the fecal sample.
4. Ask students to raise their hands if they are certain their fecal sample belongs to the animal they selected.
5. Ask for volunteers (starting with those who raised their hands) to explain their decisions and facilitate discussions between student groups.
6. As groups share their results, students may fill in the photographs on their handouts to identify the sources of all nine samples.
7. Give students 5-10 minutes to complete page 2 of their student handout.

Activity 4: Collecting Environmental DNA

Tool Type: Guided Inquiry
Time: 50 – 100 minutes or 1-2 class periods

Materials:
Collecting Environmental DNA
Environmental DNA Is Everywhere. Scientists Are Gathering It

Collaborative Slide Deck

Objective:
Students will consider how eDNA can be used to answer one of the most fundamental questions in biology: Who is there, and what are they doing there?
Overview:
This collaborative reading activity allows students to work through the article in three parts, where they will read, summarize, and share it in short group presentations. The article explores environmental DNA (eDNA) use in scientific research, its revolutionary impact on various fields, and the ability to gather genetic material from soil, water, air, and other environments.

Part 1:
The teacher reads as students follow along the article's first section (8 paragraphs) describing the use of environmental DNA (eDNA) as a revolutionary tool in science for detecting and studying organisms in various environments. Students then work in pairs to complete the student worksheet's first page (3 questions).

Part 2:
Assign students to four groups, each responsible for one of the remaining four sections of the article. Groups should either read the article individually or aloud as members follow along. Then, working together, groups complete the graphic organizer by identifying three critical points in their assigned section and noting the supporting details from the text.

Part 3:
Each group creates a slide in a collaborative presentation to share the contents of their section. The teacher then presents the slideshow as a class, with a spokesperson from each group sharing their three key points.
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Activity 5: Could Spiderwebs Measure Biodiversity?

Tool Type: Phenomenal Image, Audio Recording
Time: 30 minutes or ½ class period

Materials
Could Spiderwebs
Need to track animals around the world? Tap into the ‘spider-verse,’ scientists say

Objective:
Students will recognize how spider webs' physical properties capture airborne DNA from nearby organisms, making them a potential tool for obtaining eDNA for monitoring biodiversity.

Overview:

Students apply what they have learned about biodiversity monitoring and eDNA to infer whether spider webs would be a good sampling source to monitor biodiversity. Then, students listen to a 4-minute NPR episode that describes how researchers have discovered a new, noninvasive method for tracking animal biodiversity using spiderwebs. By analyzing environmental DNA (eDNA) collected from webs, they can detect the presence of various species without disturbing them. This method, tested in natural habitats and zoos, successfully identified DNA from numerous mammals, birds, reptiles, and amphibians. The technique offers a promising, low-cost tool for monitoring biodiversity and aiding conservation efforts by providing insight into local and invasive species.

Activity 6: Consider the Research

Tool Type: Data Play
Time 30 minutes or ½ class

Materials
Consider the Research
Spider webs capture environmental DNA from terrestrial vertebrates (Newton et al., 2024)

Objective:
Using the summary and data from the original study mentioned in the NPR story, students determine that species type, biomass, and proximity to a spiderweb influence the likelihood it will capture airborne DNA and its effectiveness as a tool for measuring biodiversity.

Overview:
Students first read the abstract from the original scientific study (Newton et al., 2024) to identify the question being asked, “Can spiderwebs serve as natural passive biofilters for airborne DNA?” and determine that biomass and proximity are the greatest influence on the efficacy of spiderwebs as a biofilter and concluded that spiderwebs hold great potential as a non-invasive method for monitoring terrestrial vertebrates. Students interpret two graphs from the study to predict the likelihood an organism’s DNA will be captured on the web. The study is outlined below to provide additional context for teachers.

I. Introduction
    A. Background on environmental DNA (eDNA) and its applications in biodiversity monitoring
        1. Only trace amounts needed
        2. Improves detection and monitoring of rare, cryptic, or protected species
        3. Increases taxonomic resolution of biodiversity surveys
        4. Allows for the sampling of difficult environments
        5. Offers early detection of invasive species
    B. Need for additional eDNA substrates

1. Most terrestrial eDNA substrates require direct contact with species, leading to variability in species diversity and detection rates between substrate types due to
    a. Animal interaction
    b. Substrate accumulation and preservation properties
2. Airborne DNA collection for vertebrate monitoring
    a. Highlight movement of airborne DNA and potential for passive collection.
    b. Examples:
        1. Powered air samplers
        2. Open containers of water
        3. Dust collectors

C. Introduction of spider webs as passive biofilters for capturing eDNA
1. Can collect bioaerosols and invertebrate-derived DNA
2. Present in natural and human environments
3. Act as sticky traps

D. Previous Research
1. Used to obtain DNA from host spiders and prey and genetic traces of invertebrates, fungi, and bacteria.

E. Operational benefits - Convenience
1. No initial setup
2. Easy collection
3. Rapid sampling

II. Experiment:

A. Collection of 49 spider webs across two locations
1. Karakamia Wildlife Sanctuary
    a. To explore the effectiveness of spider webs for eDNA monitoring in a natural environment
2. Perth Zoo
    a. To examine the impact of biomass and proximity on detectability

B. Analysis
1. eDNA metabarcoding was used to identify vertebrate species
2. DNA extraction using the Qiagen PowerLyzer PowerSoil DNA Isolation Kit
3. Amplification of vertebrate DNA using three assays targeting different gene regions
4. Bioinformatics pipeline for sequence data processing and taxonomic assignment

II. Results

C. Detection of 85 non-human vertebrate DNA across both sample sites
1. Karakamina Wildlife Sanctuary (KWS)
    a. 32 vertebrates identified
    b. 26 out of 177 species known to be on the site (15%)
        1. 16 mammals (11 of 20 known, 55%)

2. 14 birds (13 of 188 known, 11%)
3. 1 reptile (1 of 20 known, 3%)
4. 1 amphibian (1 of 9 known, 11%)

c. Additional DNA came from nearby livestock
d. 66% of samples were only found at KWS

2. Perth Zoo
   a. 61 vertebrates identified
   b. 34 of the 98 animals housed outdoors at the zoo detected (37%)
      1. 23 mammals of 47 (49%)
      2. 11 birds of 34 (32%)
      3. No outdoor reptiles were detected
   c. Indoor spider webs collected inside the reptile room detected 4 of 4 reptiles housed in the reptile room and individual enclosures.
   d. Additional DNA came from feed species or common non-zoo animals in and around the zoo.
   e. 84% of species detected were only found at the zoo

D. Major influences on the likeliness of species detection in eDNA obtained from spiderwebs
   1. Biomass - organisms comprising greater biomass of the area more likely detected
   2. Proximity - organisms closest to the webs were more likely to be detected

II. Discussion

E. Spider webs as effective biofilters for capturing vertebrate eDNA
   1. Highly localized: eDNA metabarcoding of spider webs can detect vertebrate species living in the immediate vicinity of the webs
      a. This may be due to the dilution of the eDNA pool when moving away from the source
   2. Potential to travel from great distances in natural ecosystems but specifics are unknown
   3. Biomass increases detection

F. Spider webs monitor a wide range of terrestrial vertebrates
   1. Organisms with varying behaviors were detected at both sites
      a. Arboreal
      b. Ground dwelling
      c. Nocturnal
      d. Diurnal
   2. Organisms with varying external characteristics were detected
      a. Fur
      b. Scales - observed at a lower rate
         1. Shedding hypothesis - organisms with hard exteriors may shed eDNA at lower rates, reducing detectability
      c. Feathers
      d. Naked skin

II. More research needed to understand:

7. The dynamics of airborne eDNA transfer
8. The quality and quantity of DNA shed from various tissue types and taxa
9. How airborne DNA interacts with wind and air currents
10. Degree of mobility
11. Influence of species richness
12. Influence of spider species, their web types, and locations

II. Limitations of the Study

M. Unknown factors influencing the ability of spider webs to act as eDNA substrates

1. Web location
2. Web architecture
3. Suspension time
4. Silk type
5. Adhesive substance
6. Composition
7. Other trapped material
8. Enzymes secreted by the spider
9. Environmental conditions of the area

Activity 6: Mapping a Plan to Monitor Biodiversity

Tool Type: Mapping
Time: 50 minutes or 1 class period

Materials
Mapping a Plan to Monitor Biodiversity
Navigating the Half-Earth Project Map, Navigating the Map of Life

Objective:
Students will apply their knowledge of eDNA and the use of spiderwebs as genetic biofilters to identify a species known to exist in a protected area that would be a good candidate for spiderweb sampling and monitoring.

Overview:
This activity can be used as a summative assessment, as students are expected to apply the knowledge acquired during the previous five lessons to complete the activity. Students begin by reading two paragraphs selected from Newton et al. (2024). After reading the paragraphs, they will reflect upon the contents to determine the characteristics of the organisms and locations most likely to be successfully monitored using eDNA sampling from spiderwebs. Students use the Half-Earth Project map to identify a protected area that meets the characteristics. Students are expected to collect data about the protected area. Next, using the Map of Life, students will identify a species living in that area whose conservation may benefit from spiderweb eDNA sampling and monitoring. Finally, students identify locations in the protected area to collect

spiderwebs for sampling, explaining why they chose those locations. A tutorial for using the Half-Earth Project Map and Map of Life is provided.

To cite this lesson:

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