eDNA - THERE'S SOMETHING IN THE WATER | EDUCATOR

Links to Next Generations Science Standards |

LS2 Ecosystems: Interactions, Energy, and Dynamics

MS-LS2-1. - Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.

HS-LS2-2. Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales.

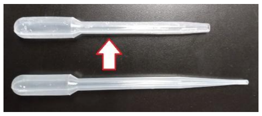
Objectives & Learning Outcomes |

- Understand and discuss the importance of ROVs in *Nautilus*' mission of ocean exploration.
- Understand and discuss why ROVs collect water samples to study marine organisms and habitats.
- Define and understand the importance of eDNA.
- Describe the basic processes involved in collecting and identifying eDNA samples.

Module Theme | Science
Contributing Authors | Cassi Weathersbee & Al Leszczynski, Science Communication Fellows
Pacing | 10+ minute interaction on a museum floor or one, 45-minute class period
Assessment | Student worksheet and participation in lab procedure
Materials/Resources | One per group of 3 to 4 students or museum guests

- Water sample in a plastic bottle.
  This activity is written with three different ecosystem water samples — seamount, abyssal plain, coral reef — you can alter the number of replicates for smaller groups. Prepare enough water samples for each group to have one. i.e. 27 students = 9 groups = 9 bottles, 3 bottles of each ecosystem.
  - bottles with caps
  - glitter (12 colors)
  - The first ecosystem of bottles [abyssal plain] contain four different glitters to represent an ecosystem with sea pigs (glitter color #1), dumbo octopus (glitter color #2), tripod fish (glitter color #3), and anglerfish (glitter color #4).
  - The second ecosystem of bottles [coral reef] contain four different glitter to represent an ecosystem with giant clams (glitter color #5), sea turtles (glitter color #6), brain coral (glitter color #7), and anemones (glitter color #8).
  - The third ecosystem of bottles [deep seamount] contain another four different glitter to represent an ecosystem with rat tail fish (glitter color #9), crinoids (glitter color #10), sea snails (glitter color #11), and bamboo coral (glitter color #12).

- Plastic pipette - cut the tips off so the opening is large enough to suck up glitter particles.



- Coffee filter or paper towel over a small cup.
- Update the ecosystem QR code chart (titled " eDNA metabarcoding" and "Teacher key") so the selected glitter colors match the correct organism. Each group will need one "eDNA metabarcoding chart."

Overview

Students or museum guests will extract eDNA from water samples containing "glitter eDNA" using pipettes and small cups with filters. After collecting the samples they will use QR codes to identify the species represented by the glitter eDNA they collected. Students or museum guests will use the identified species to determine what ecosystem the water sample was collected from.

Safety

If working with younger children, be sure they can't access the small pieces used in water samples. For example, make sure bottle tops are screwed on securely and small beads are not accessible.

More eDNA information |

eDNA in the Deep Sea
https://nautiluslive.org/blog/2018/10/26/whats-water-knowing-our-ocean-edna

eDNA & DNA barcoding
http://www.spygen.com/technologies/what-is-environmental-dna-edna/

DoD Environmental Security Tech Program
https://ednaresources.science/intro

Freshwater Habitats Trust
https://freshwaterhabitats.org.uk/projects/edna/edna/

USDA eDNA Basics https://www.usgs.gov/centers/fort/science/environmental-dna-edna?qt-science_center_objects=0#qt-science_center_objects

AAAS Collecting eDNA in the Field http://sciencenetlinks.com/science-news/science-updates/environmental-dna-collection/

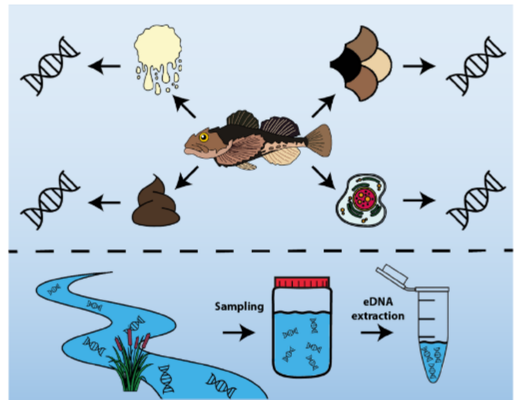
A world in a bottle of water
https://www.knowablemagazine.org/article/living-world/2019/environmental-dna-ocean-water

Guide to eDNA & Limits
https://biomeme.com/environmental-dna/

Introduction

As science advances, the methods used to explore the world around us must advance as well. DNA (deoxyribonucleic acid) is the blueprint for life within every living cell. In the past few decades, new developments have allowed the capture and processing of DNA not only from animals themselves, but from environmental samples (water, soil, air, snow, etc.) where animals have passed through. This is referred to as environmental DNA, or eDNA. In aquatic environments, eDNA is diluted and distributed through the water where it can linger for 7–21 days, depending on the conditions. However, the DNA of organisms once trapped in sediments can be preserved for thousands of years. eDNA analysis is an important new tool in assessing global biodiversity.

eDNA must be compared to a known library of genetic identifications, aka barcodes, to make a match. Libraries are built from samples obtained in different environments for many reasons including species identification, biodiversity assessment, and ecosystem health.



Aboard E/V *Nautilus*, ROV pilots collect water samples for eDNA analysis using Niskin bottles attached to ROV *Hercules*. During a dive, the ROV pilot can trigger the Niskin bottles to close, trapping water samples from various depths and environments.
Once the ROV is back on the ship, the science team removes water from the Niskin bottles, filters it, and preserves the sample for further processing comparison against DNA barcodes ashore.

Background for Educators

Niskin bottles are hollow plastic tubes with caps on the top and bottom that latch open before deployment. The caps are spring-loaded to snap closed when a trigger is pulled. Water flows through the tubes until the cap is triggered, capturing whatever water is inside. Multiple bottles within a rack or carousel are often used to capture water from different depths or areas. After a dive, water samples are processed by scientists to look for environmental DNA (eDNA). Scientific research vessels all over the world use Niskin bottles to collect water for eDNA and many other analyses.

Vocabulary

DNA - DeoxyriboNucleic Acid, the carrier of genetic information present in the cells of living organisms.

eDNA (Environmental DNA) - traces of DNA left in the environment by organisms that live there. Can be found in water, soil, snow, ice, and more. Sources of environmental DNA can be skin cells, mucus, feces, reproductive cells (eggs and sperm), etc

environment - the physical, chemical, and biotic factors (such as climate, soil, and living things) that act upon an organism or an ecological community.

ecosystem - a community of organisms and their environment functioning as an ecological unit

ecology - a branch of science concerned with the interrelationship of organisms and their environments

biodiversity - the numbers or variety of different species of plants and animals

scientific sampling - the process of selecting a number of items or individuals from a wider group or population with the goal of making statistical inferences about the population as a whole.

Engage

- Invite students or museum guests to come over and take a look at some imagery from E/V *Nautilus*.
- Ask them if they are familiar with ocean exploration.
- Let the conversation ebb and flow with the guests' interest, but push the conversation towards the idea of sampling.
- Introduce the concept of geologic and biologic samples and water samples. Discuss why would we collect those things?

Museum Interaction Procedure

- Inform guests about the existence of eDNA analysis and tell them it is a newer process of sampling. It uses traces of organisms found in water samples to help understand biodiversity of specific areas.
- Encourage them to take on the challenge of characterizing some of the 'water samples' you have prepared. Utilize the modified pipettes, cups, filters, and eDNA metabarcoding chart to collect and analyze the 'water sample'. Museum staff may want to label the chart museum explorer.
- If guests are interested, you can explore each of the ecosystems in more detail for greater background.

eDNA Articles |

Nautilus Live Expeditions exploring eDNA

Baker, Howland, & Johnston Islands https://nautl.us/2ZhkLik & Kingman & Palmyra Atolls https://nautl.us/38IhLMT

Killer Whales in Puget Sound

https://www.sciencedaily.com/releases/2018/04/180423135043.htm

eDNA as a measuring tool for historical biodiversity

https://www.sciencedirect.com/science/article/pii/S0006320714004443

Detecting rare aquatic species with eDNA

https://conbio.onlinelibrary.wiley.com/doi/full/10.1111/j.1755-263X.2010.00158.x

Estimating fish biomass

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0035868

Monitoring biodiversity

https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-294X.2011.05418.x

How long does eDNA stay in the water?

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0023398

Abyssal plain organisms |
- Sea Pig
https://nautl.us/2r99alA
- Dumbo Octopus
https://nautl.us/2Rp2DxQ
- Tripod Fish
https://nautl.us/2qp9ts1
- Anglerfish
https://nautl.us/367ZTJf

Coral reef organisms |
- Giant Clam
https://nautl.us/2DMNZYX
- Sea Turtle
https://nautl.us/2Ruowvo
- Brain Coral
https://nautl.us/33RiGad
- Anemones
https://nautl.us/2s2jtrm

Seamount organisms |
- Rat tail Fish
https://nautl.us/2PcktBm
- Crinoid
https://nautl.us/2Yl0lRD
- Sea Snail
https://nautl.us/2RyLbaj
- Bamboo Coral
https://nautl.us/33TmQhy

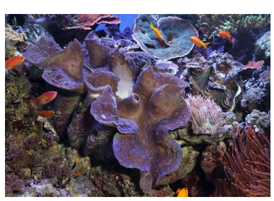
Classroom Procedure

- Create groups of three to four students.
- Pass out one student worksheet to each student. Using the information in the background section of the worksheet, discuss what eDNA is. Discuss what students think might be advantages of collecting eDNA samples instead of trapping or visually counting animals.
- Pass out one "water sample" bottle, modified pipette, small cup with filter, and an eDNA metabarcoding chart to each group.
- Task students with taking samples of the 'glitter eDNA' in their water sample following the directions on their activity worksheet. After filtering their eDNA samples, they will use a QR reader (cellphone or tablet camera) to scan the eDNA code for each of the colors they collected to determine which marine species' DNA they sampled.
- After students have identified their eDNA samples and resulting ecosystem, discuss the following:
    - How were they able to determine which ecosystem their "water sample" came from?
    - What might the amount of eDNA from different species in a single water sample tell scientists about the animals in an ecosystem?
    - Why might some groups have been unable to sample all of the different colors in a water sample during their three pipette extractions?
    - Do they think eDNA will tell researchers every organism that was in an area? Why or why not?

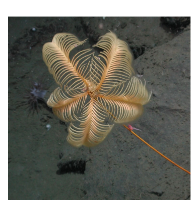
Abyssal plain organisms |

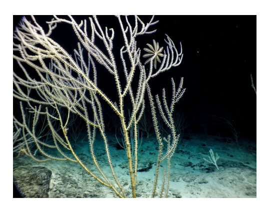



Coral reef organisms |




Seamount organisms |



eDNA metabarcoding chart

Glitter Color key	eDNA QR code (eDNA metabarcoding)	Ecosystem	Glitter Color key	eDNA QR code (eDNA metabarcoding)	Ecosystem
#1 Color:		Abyssal Plain	#7 Color:		Coral reef
#2 Color:		Abyssal Plain	#8 Color:		Coral reef
#3 Color:		Abyssal Plain	#9 Color:		Seamount
#4 Color:		Abyssal Plain	#10 Color:		Seamount
#5 Color:		Coral reef	#11 Color:		Seamount
#6 Color:		Coral reef	#12 Color:		Seamount

Teacher Key - eDNA metabarcoding chart

Glitter Color key	eDNA QR code (eDNA metabarcoding)	Animal	Ecosystem	Glitter Color key	eDNA QR code (eDNA metabarcoding)	Animal	Ecosystem
#1 Color:		Sea pig	Abyssal Plain	#7 Color:		Brain coral	Coral reef
#2 Color:		Dumbo octopus	Abyssal Plain	#8 Color:		Anemone	Coral reef
#3 Color:		Tripod fish	Abyssal Plain	#9 Color:		Rattail fish	Seamount
#4 Color:		Anglerfish	Abyssal Plain	#10 Color:		Crinoid	Seamount
#5 Color:		Giant clam	Coral reef	#11 Color:		Sea snail	Seamount
#6 Color:		Sea turtle	Coral reef	#12 Color:		Bamboo coral	Seamount

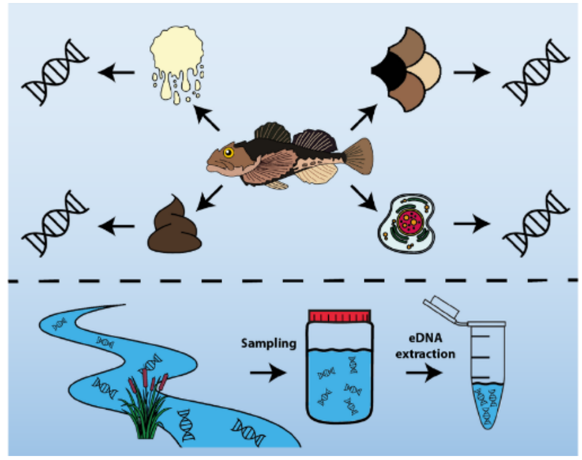
eDNA - THERE'S SOMETHING IN THE WATER | STUDENT

Name: ______________________________

Learning Goals

- Understand and discuss the importance of ROVs in *Nautilus*' mission of ocean exploration.
- Understand and discuss why ROVs collect water samples to study marine organisms and habitats.
- Define and understand the importance of eDNA.
- Describe the basic processes involved in collecting and identifying eDNA samples.

Introduction Living things leave behind lots of stuff in their environment. Cells, mucus, poop, scales, eggs, and sperm contain DNA and can all be found in aquatic environments. Scientists can use these small traces of DNA to better understand an ecosystem by understanding what lives there, even if they don't see the animal itself.



The DNA that living organisms leave behind as they move through their environment is called "environmental DNA" or eDNA (e-DNA) for short.

eDNA detection has several advantages over traditional methods of species detection. For example, traditional methods in aquatic ecology (the study of ecosystem relationships in aquatic environments) involve observation and/or trapping of the species of interest combined with extensive documentation. This can be difficult and labor-intensive, especially if the species of interest is rare or very small. eDNA is much faster than collecting actual specimens. It can also be used to determine the prevalence of a species in the environment. Without having to trap or visually count an animal[1].

The DNA collected can be identified using DNA metabarcoding. This is the barcoding of DNA or eDNA much like the barcodes we find on the products we purchase in stores

[1] A Guide To Environmental DNA (eDNA) By Biomeme, https://biomeme.com/environmental-dna/

In this module, you will extract eDNA from water samples containing "glitter eDNA" using pipettes and filters. After collecting the samples you will identify the species represented within your sample and determine what ecosystem the water sample was "collected" from.

eDNA - THERE'S SOMETHING IN THE WATER | STUDENT

Name: ___________________________________

Helpful Resources:

Background information on environmental DNA:

Detecting DNA near a volcano, updates Nautilus' mission to Loihi Seamount
https://nautl.us/2YqOO3h

Learn about the basics of sampling DNA from areas surrounding animals with this US Geological Survey article
https://nautl.us/38eVsyj

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scientific sampling - the process of selecting a number of items or individuals from a wider group or population with the goal of making statistical inferences about the population as a whole

Materials

- 1 water sample bottle
- 1 pipette
- 1 small cup with fitted filter
- eDNA metabarcoding chart
- QR reader on cellphone camera, tablet, etc

Introduction Questions

1. In your own words, describe what eDNA is.

2. What do you think is the biggest advantage of collecting eDNA samples instead of trapping or visually counting animals?

Name: ______________________________

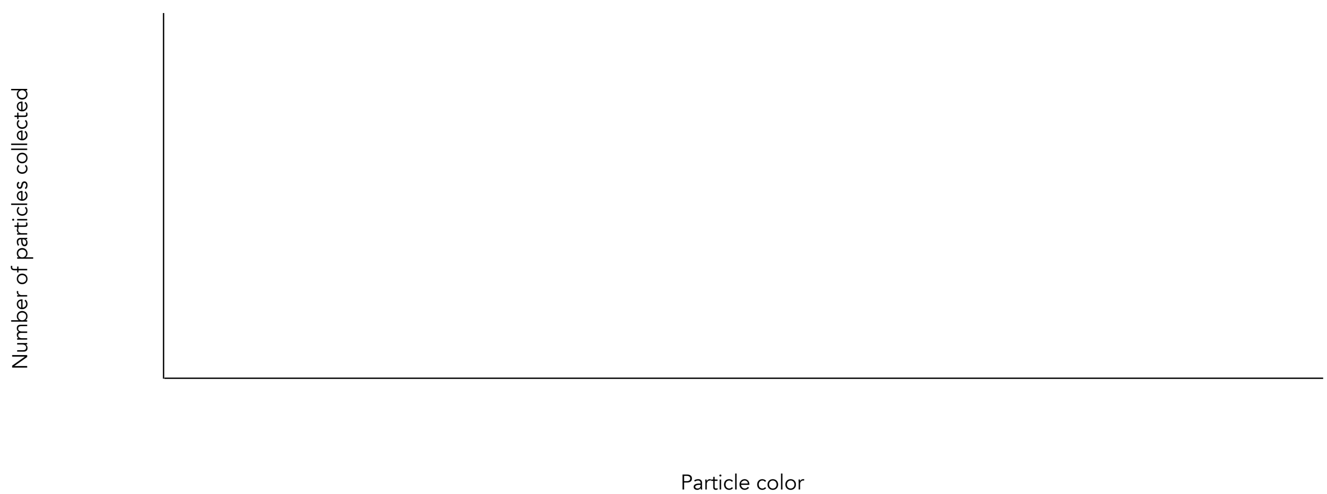
Lab Procedure

1. With the cap on, shake the bottle with the water sample provided by your teacher.
2. Place the open end of the pipette into the water sample.
3. Squeeze the top of the pipette to suck some water and glitter particles into the pipette. Turn it over to help keep the water and glitter in the pipette.
4. Squeeze the water and glitter out of the pipette on to the coffee filter over the small cup.
5. Allow the water to pass through the filter, leaving the glitter behind.
6. Repeat the above steps two more times.
7. Record your results in the observation table below.
8. Use the eDNA metabarcoding chart and a QR reader (cellphone or tablet camera) to scan the eDNA code for each of the colors you collected to determine which marine species' DNA you sampled.

Observations:

Collected glitter particle color	**Number of particles collected**	**Marine species associated with this glitter eDNA** (from the metabarcoding chart)

Graph your results:



Name: ______________________________

Analysis & Conclusions

Use the eDNA metabarcoding chart and your collected data to answer these questions about the environment your water sample was taken within.

1. Based on the glitter eDNA you sampled, which ecosystem did your "water sample" come from?

2. Did you get the same number glitter particles of each color? What do think might tell you about the animals in this ecosystem?

3. Did you get a piece of glitter eDNA for each of the species in the ecosystem? If not, why do you think this happened?

4. Do you think eDNA will tell researchers every organism that was in an area? Why or why not?

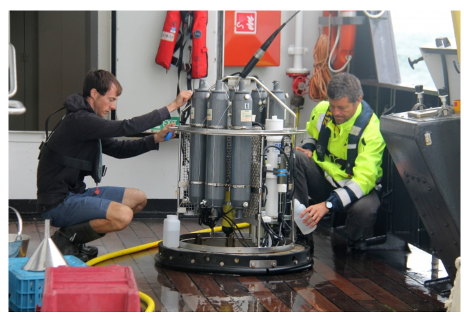
Name: ______________________________

Real-World Research Connections

Niskin bottle water sampling

eDNA sampling is one way the E/V *Nautilus* team explores the deep ocean. The team collects eDNA samples using remote operated vehicle (ROV), *Hercules*. Shown above, *Hercules* has many types of sampling gear, including gear designed to collect water samples called Niskin bottles.

Niskin bottles are the grey tubes shown above and in the photo to the right. The tubes have caps on the top and bottom that latch open before the dive and are spring-loaded to snap closed when a trigger is pulled. Water samples are trapped inside when the caps close. After a dive those water samples are processed by scientists to look for environmental DNA (eDNA). Scientific research vessels all over the world use Niskin bottles to collect water for eDNA and many other analyses.



1. One of the Niskin bottles shown in the rack in the picture is labeled with the number six. A standard Nansen bottle has a capacity of 1.25 liters. If these are six Niskin bottles are filled, how many liters of water have these scientists collected?

2. Why do you think ocean researchers and explorers would want to sample that much water?

3. Scientists often ask for Niskin bottles to be triggered to collect water at different depths. Why do you think they would want to sample water at different depths?