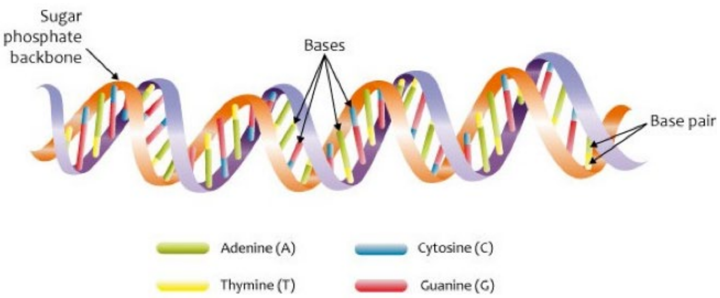
Environmental DNA (eDNA)

As organisms interact with their environment, they shed DNA into their surroundings.

Environmental DNA (eDNA) are tiny bits of genetic material that organisms leave behind in the water, soil, and even air. Sources of eDNA include secreted matter such as urine, feces and mucus, as well as sloughed off materials like skin, scales, and hair. In aquatic environments, these DNA fragments can be collected in water samples, however, eDNA only lasts in the water for about 7 to 21 days. Exposure to ultraviolet radiation (sun), acidity, heat, and microbial activity can all break down eDNA.

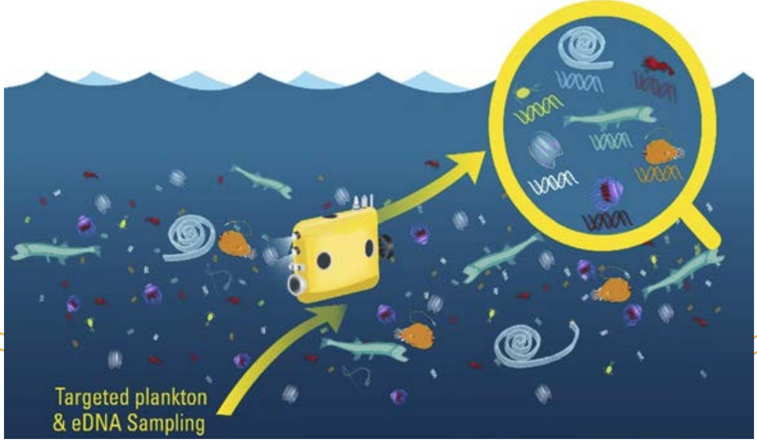
Excreted or sloughed eDNA offers a unique, non-invasive opportunity find out what organisms have been in an area using only environmental samples (specimens do not need to be sampled directly). Think of a fingerprint left behind after a person has exited an area- eDNA is a trace, genetic signature left behind by an organism.



Deoxyribonucleic acid (DNA) is an organism's unique genetic code. DNA is made of two, linked strands, each of which has four bases – adenine, guanine, cytosine, and thymine, known by their first letter for short – A, G, C, or T. All organisms have a unique DNA sequence (length and pattern) of these bases. Similarities in DNA can help scientists understand how organisms are related to each other - if they are an offspring, part of the same species, or distant members of the same phyla. *Image courtesy of Wikimedia.*

Collecting eDNA

Environmental DNA can be collected from the ocean in a variety of ways. What sampling tools and methods are used depends on the research question to be answered.



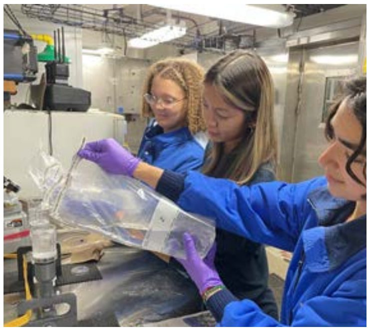
A conceptual image of eDNA sampling on the AUV *Mesobot*.
Illustration courtesy of Govindarajan and Renier, © Woods Hole Oceanographic Institution.

CTD Rosette	ROVs	AUVs	Autonomous Samplers
			
A conductivity, temperature, and depth **(CTD) rosette** carries 8-24 collection cylinders called **Niskin bottles.** These bottles are open when the rosette is deployed, and triggered to close at certain depths. By analyzing and comparing eDNA samples collected at different locations and depths, scientists can investigate how eDNA travels through the ocean. *Image courtesy of Erin Frates, © Woods Hole Oceanographic Institution*	**Niskin bottles** can be integrated into **remotely operated vehicles (ROVs)** to collect samples to determine what organisms have been present at sites of interest (e.g. seeps and vents). *Image of ROV Hercules with Niskin bottles mounted on the side courtesy of Ocean Exploration Trust*	Some **autonomous underwater vehicles (AUVs)** that are deployed for multiple weeks or months, traveling long distances, can be equipped with samplers that capture, filter, and preserve a large number of eDNA samples in-situ (on site at that depth and moment in time). Distinct from Niskin bottles, which "gulp" water, these samplers "sip" water over time as the vehicle moves through the water. eDNA collected allows scientists to better understand the broad, geographic range of organisms. *Image of REMUS 600 AUV courtesy of B. Eakins, CU Boulder and NOAA NCEI, Image of AUV Sentry courtesy of Schmidt Ocean Institute.*	Similar to AUVs, **autonomous samplers,** which can sit on the ocean bottom for extended periods of time, collect, filter, and preserve samples in-situ. Multiple autonomous samplers can be deployed in one area to collect many samples at the same time. In this way, scientists can see how biological communities may change over time. *Image of sub-surface eDNA sampler courtesy of NOAA AOML*

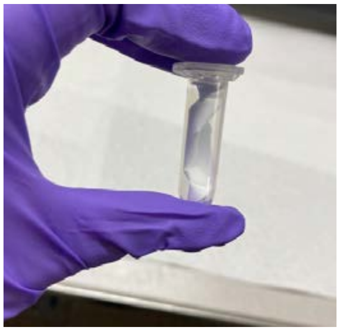
Analyzing eDNA

Once a water sample is recovered it is passed through a very fine filter and cleaned/purified. The filters with the captured eDNA are then frozen or preserved in a solution, and stored for analysis after the expedition. Each sample is labeled with key identifiers like location latitude, longitude, and depth, salinity, and temperature.

Once preserved, eDNA samples are usually sent to a lab for extraction (removing the eDNA from the filter) and genetic sequencing. **DNA sequencing** is a process used to determine the order in which the four bases appear on a DNA strand. The sequence tells scientists the kind of genetic information that is carried in a particular DNA segment.

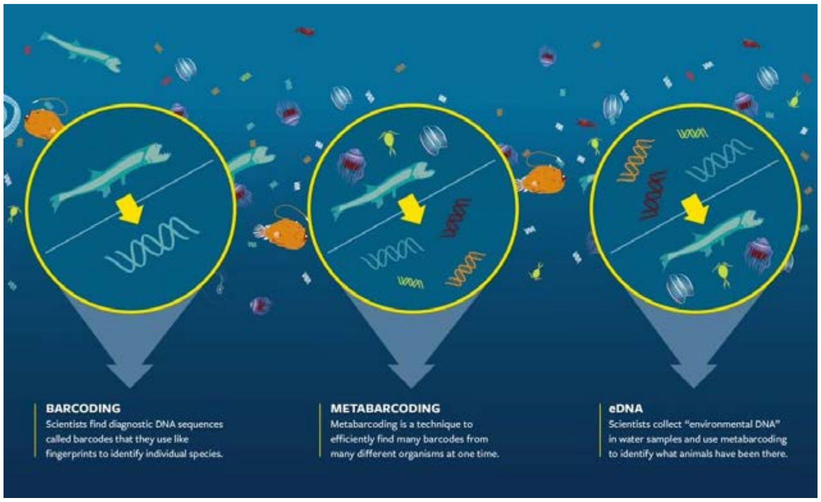


Explorers-in-Training filter water samples for eDNA collection during the Seascape Alaska 2 expedition. *Image courtesy of NOAA Ocean Exploration.*



Once all of the water has run through the filtration system, the filters are preserved for DNA extraction and analysis after an expedition. *Image courtesy of NOAA Fisheries.*

Genetic markers, or **"barcodes"**, are short segments of DNA that have unique sequences for almost all organisms, allowing scientists to identify one family, genus, or species. A more advanced method, called **"metabarcoding"** allows researchers to identify *many* organisms in one eDNA sample, with millions of barcodes being sequenced at the same time. These barcodes are then compared to others in a reference database of all known DNA sequences, allowing an organism to be identified down to its family, genus, or even species. Scientists can also look at older samples in these genetic reference libraries to determine community changes in an ecosystem over time.



A variety of genetic techniques are advancing ocean scientists' ability to identify which organisms live where in the vast ocean twilight zone and to find previously unknown species. *Illustration courtesy of Govindarajan, Renier and Taylor, © WHOI Creative.*

Environmental DNA sampling and analysis has become an increasingly popular tool in ocean exploration and other environmental fields. It provides a relatively easy, non-invasive method to create a snapshot of a community without having to collect organism samples.

What *can* eDNA tell us?	What *can't* eDNA tell us?
• What species are present in a location. This can be used to assess biodiversity and monitor changes over time. • Estimates for how many of a species are present in a location. • The movements and habitat associations of endangered/rare species, migratory species, and cryptic/elusive species • Water quality- detecting and tracking invasive species, harmful algal blooms, pathogens, and parasites • Links between eDNA and other ocean properties, providing a deeper understanding of species distribution	• If the eDNA is from a living or dead organism • If the eDNA is from a resident species or one that was just passing through ("migratory") • The exact number of species or individuals in an area • The size, age, and life stage of a species detected • The absence of a species that had been through the area.

DNA (diagram): https://commons.wikimedia.org/wiki/File:DNA_double_helix_%2813081113544%29.jpg
eDNA (diagram): https://www2.whoi.edu/site/govindarajanlab/projects/
CTD Rosette (image): https://tos.org/oceanography/assets/images/content/ocean-observing-2023-govindarajan-f3.jpg
ROVs (image): https://nautiluslive.org/album/2021/02/08/exploring-worlds-ocean-rov-hercules#&gid=1&pid=8
AUVs (fact sheet): https://oceanexplorer.noaa.gov/edu/materials/auv-fact-sheet.pdf
Autonomous Samplers (webpage): https://www.aoml.noaa.gov/new-edna-sampling-upgrade/
Explorers-in-Training (image): https://oceanexplorer.noaa.gov/okeanos/explorations/seascape-alaska/ex2303/media/interns-edna-hires.jpg
Filters (image): https://www.fisheries.noaa.gov/s3/styles/media_500_x_750/s3/2022-06/304x-4032-Done-with-filtration-2022-nefsc.png
Unusual Suspects (webpage): https://www.whoi.edu/oceanus/feature/round-up-the-unusual-suspects/

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