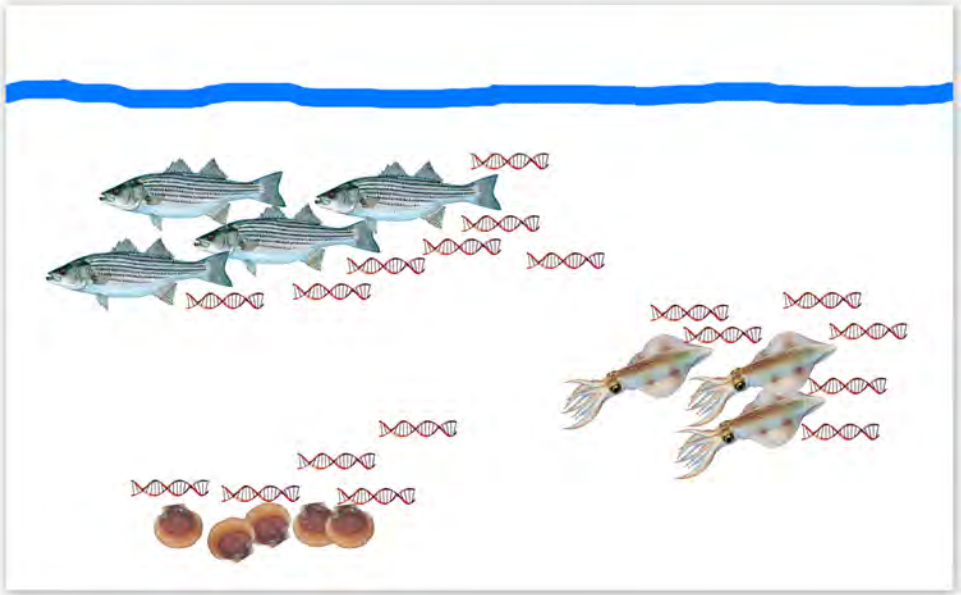
Marine eDNA 101



Marine environmental DNA (eDNA) offers a relatively low-cost, harmless tool for tracking presence and abundance of marine animals in space and time.

Marine eDNA will help monitor impacts of human activities and natural events in our changing oceans, and aid research and exploration in ocean, estuary, and coastal habitats.

Prepared by Mark Stoeckle, Jesse Ausubel, and Paul Gaffney for
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What are potential marine eDNA applications?

Monitoring impacts

Fishing | Aquaculture | Extraction | Energy | Shipping

Weather/Climate | Conservation | Restoration

Research/Exploration

Marine eDNA FAQs

1. What is environmental DNA (eDNA)?

eDNA is DNA shed by macroscopic organisms into the environment. Some consider aquatic eDNA to refer to all DNA in a water sample, including from bacteria, viruses, and planktonic organisms.

2. Where does animal eDNA come from?

Sources include cells shed from body surfaces, body wastes, and tissue remnants following predation, death, or injury. Most aquatic eDNA is in particulate form--probably cells or fragments of cells. This particulate fraction can be captured with a small-pore size (0.2 -10 micron) filter.

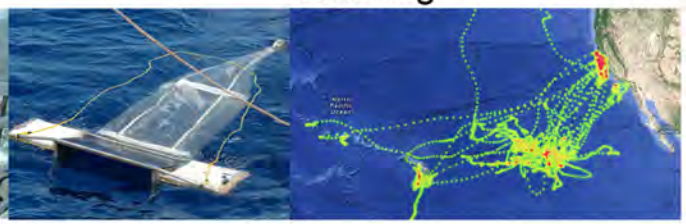
3. How long does aquatic eDNA last?

Degradation and dispersal typically limit detection to a few days after animals leave the local environment. More abundant eDNAs are likely detectable for longer and throughout a greater volume than rarer eDNAs.

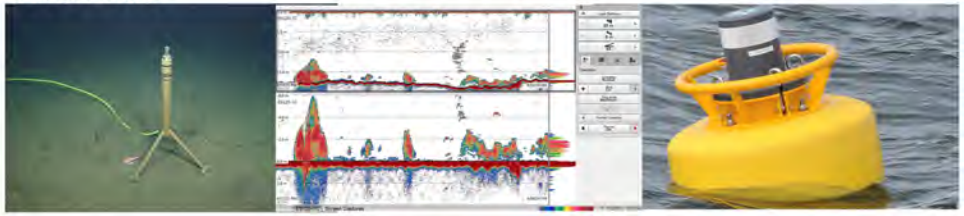
Existing marine surveys offer multiple possibilities for integrating eDNA study.

Capture

Tracking



Acoustic



Visual

4. How much water is needed?

One liter is typical target collection volume. With near-shore samples, clogging due to suspended sediments and microrganisms limits filtration volume. In environments where sediments and planktonic organisms are sparse, much larger volumes could be filtered.

5. How long does it take to analyze eDNA?

Typical work-flow for metabarcoding is one month, for single-species assays a few days. If needed, both approaches could be performed in 24 hours. Same-day single-species assays are available.

6. How much does it cost?

Non-labor costs are about $20-$50 per sample, depending on assay, replicates, other factors.

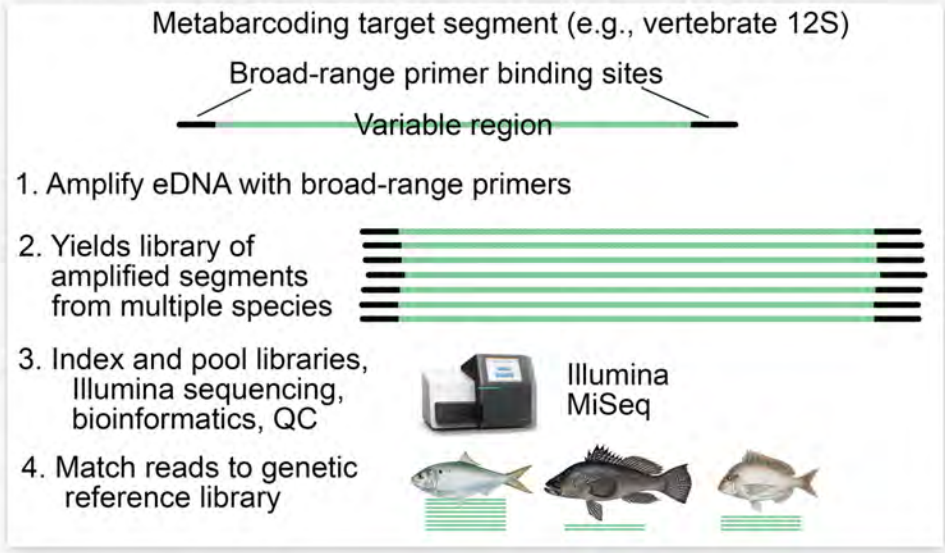
How do you analyze marine eDNA?

1. Collect water

2. Filter

3. Extract DNA

4. Option 1--analyze DNA by metabarcoding (targets all species in a taxonomic group, e.g., vertebrates)



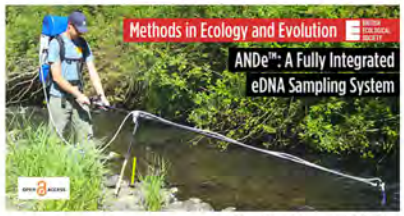
OR

4. Option 2--analyze DNA with single-species assays

Assays include quantitative PCR (qPCR), real-time PCR, droplet digital PCR (ddPCR), and GoFish nested PCR.

Relevant commercial technologies--examples

Water collection



Smith-Root ANDe

MBARI AUV

DNA sequencing

Illumina MiSeq

Oxford Nanopore MinION

Single-species DNA detection

Biomeme real-time PCR

QuantStudio 3 qPCR

BioRad QX200 ddPCR

Diverse U.S. ocean, coastal, estuary sites promise diverse insights with eDNA.

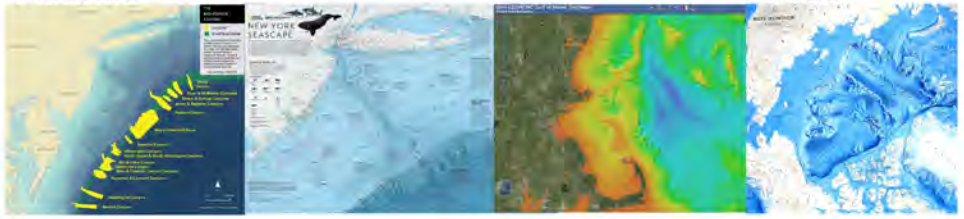
Pacific

Gulf of Mexico

Caribbean

Atlantic

Arctic



More marine eDNA FAQs

7. What are eDNA advantages compared to other marine survey tools?

-Relative ease and low cost of collecting water facilitates sampling by a broad-range of interested persons, including in otherwise hard-to-survey environments.

-Potentially detects all nearby species, as compared to capture and visual methods, which are limited by equipment, visibility, and avoidance.

-Non-destructive sampling with minimal environmental impact.

-Taxonomic expertise not needed, assuming genetic reference library is available.

-Metabarcoding output (DNA sequences, reads) suitable for data aggregation, and opens the door to automated monitoring.

8. What are some limitations to eDNA?

-Partial understanding of how eDNA levels relate to presence and abundance of organisms, including potential differences among species, seasons, and environments.

-Genetic reference libraries are incomplete.

-eDNA provides no information on organism size, age, or sex, or health, and typical assays do not distinguish individuals.

-DNA in wastewater and laboratory contaminants can generate false positives.

9. Is eDNA a stand-alone method?

Given relative ease and low cost of collecting water and potential to identify all nearby species, eDNA promises to be a primary technology in marine monitoring and exploration. Integrating eDNA with other methods will speed and multiply insight.

Three eDNA challenges

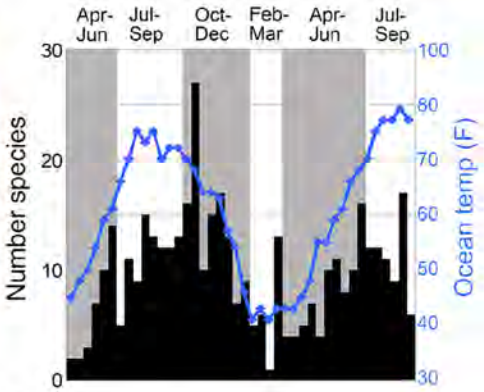
Real-time, in situ analysis
We should not wait a month for results or have to send the cup of water ashore.The tech challenges are speed of analysis, doing it on-board at sea and even eventually in situ by the collecting instrument, so that in near real-time the collection pattern can be adjusted in direction or depth as something unexpected is found.

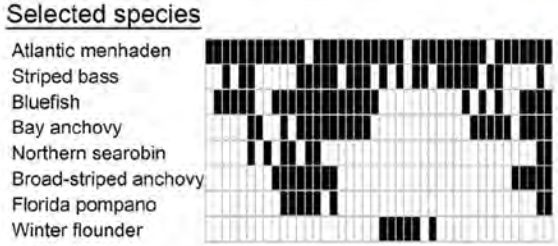
Sampling strategies
At what depths and ocean features is sampling most appropriate? These might include sites where you take other samples (Nansen casts), a set of discrete depths, or a significant level (surface, mid mixed layer depth, thermocline break, current or eddy deep boundary, other).

Data archiving and access
A challenge is easy, fast, instant access to and submission of samples to a global database that also aggregates records to allow discovery of larger patterns.

A marine eDNA time series

eDNA tracks seasonal abundance of NJ coastal fishes. 18-month time series of surface water samples analyzed by metabarcoding is shown.





Source: M. Stoeckle
http://phe.rockefeller.edu/

References, links

National Conference on Marine Environmental DNA
November 29-30, 2018, The Rockefeller University, New York, NY
https://phe.rockefeller.edu/eDNAmarine2018/

Methods

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Shaw et al., Using environmental (e)DNA sequencing for aquatic biodiversity surveys: a beginner's guide. Marine Freshwater Res 2016, 68: 20-33. http://www.publish.csiro.au/mf/MF15361

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