Environmental DNA Expeditions in UNESCO World Heritage marine sites

Frequently Asked Questions

What is environmental DNA or eDNA?

Just as the DNA in a string of hair can tell lots about you as a person, environmental DNA allows measuring the marine biodiversity that is present in a given area. The reason for this is that DNA carries the genetic information which can be found in the cells of all living organisms. Marine organisms leave material such as skin cells, saliva, or excrements, in the water and by collecting this and extracting the DNA we can study the environment and list the species that live in it. This is called 'environmental DNA' or eDNA, and is similar to forensic DNA analyses. Just one sample of soil, water or even air contains traces of DNA from organisms which have passed through the area and can reveal the different species that live in a certain place, without the need to extract, track or physically count those species.

What are the benefits of eDNA?

eDNA has multiple benefits. First, eDNA allows monitoring, observing and researching the marine environment and the species that live in it without the need to actually collect species. There is no need to disturb the environment which makes eDNA an ethical research method. Just a few liters of water can contain the DNA of hundreds of species.

Second, the sampling methods used for eDNA can be simple. It does not require bringing expensive equipment to the field. The sampling can be performed easily from onboard a zodiac and, provided contamination is kept to a minimum, it can be done from shore. It does not require diving for visual detection. As a result, eDNA can be a very cost effective method to monitor and track changes in marine biodiversity and it can be efficient in repeat monitoring efforts over the long term.

Third, due to the ease of sampling, the method is also exceptionally well suited for citizen scientists and can, with the supervision of adults, be undertaken also by young children. The sampling methods require little training. The simplicity of the methods allows everyone to take part in furthering the scientific understanding of biodiversity. Both the cost effectiveness and the citizen science suitability make it possible to also research large, and remote areas that are otherwise difficult to monitor, especially over the long-term.

How is eDNA sampled for the UNESCO eDNA initiative?

The UNESCO eDNA initiative focuses on sampling 25 marine World Heritage sites between September 2022 and April 2023 and concentrates primarily on the detection of fish species. eDNA is collected by taking a 1,5 liter water sample from the surface of the ocean (max. 15 meter total water depth) at 5 different locations at each World Heritage site. A total of 500 samples will be collected. The sampling locations are chosen in collaboration with the local World Heritage site management and reflect the sites' Outstanding Universal Value for which it is listed as UNESCO World Heritage.

The eDNA samples are taken by citizen scientists, mainly schoolchildren, under the supervision of local scientists, who follow a sampling protocol that was specially designed for the UNESCO eDNA initiative. The water is filtered and by adding a liquid to the filter the eDNA that remains in the filter is preserved immediately at the location of the sampling. The samples are shipped to a central lab that is contracted by UNESCO. The sampling methods and protocols were developed by UNESCO in collaboration with an international scientific advisory board.

How is eDNA analysed?

eDNA is analysed by first extracting and purifying all DNA from the environmental sample. From this DNA, a specific (usually short) region of the DNA is copied using PCR (Polymerase chain reaction is a laboratory technique for rapidly producing millions of copies of DNA) to facilitate analysis. This sequence of this short DNA region varies slightly across all animal species, and therefore can be used as a barcode to identify the species from. Very small amounts of DNA of animals is found in environmental samples, therefore selection and amplification of target DNA regions is crucial for the scientists to be able to read the unique DNA sequences of each animal from the sample. The amplified DNA is then sequenced to obtain the sequence of each of the target DNA copies. The sequences are then analysed on the computer to identify unique good quality sequences. The unique sequences are finally compared to a reference database to assign species names. This whole process is called eDNA metabarcoding. The DNA analysis protocol used by the UNESCO eDNA initiative was developed by UNESCO in collaboration with the project's scientific advisory board.

What exactly can be detected in each sample?

DNA metabarcoding (the analysis of multiple species from one eDNA sample) is based on the use of broad or targeted PCR primers (short DNA fragments that are complementary to the DNA of taxonomic groups). The choice of primers defines which species' DNA is targeted and amplified in the analysis. In addition to this, what species or how many species are detected depends on the situation at the sampling site. The UNESCO eDNA Expeditions' targets primarily megafauna like fish, elasmobranch (sharks and rays), mammals, and vertebrates. The samples are analysed using multiple broad-scale PCR primers.

A primer targeting all animals has been added to allow a more general view on biodiversity at the site. Most DNA found in the water is of small organisms; bacteria, algae and invertebrates. Therefore, to be able to detect larger animals (less of the DNA in the environmental sample) primers targeting these taxonomic groups are required. For example, the primers are designed to target all fish, but no primers are perfect. Some taxonomic groups are missed, while others are detected more efficiently. The selection of primers was done by UNESCO in consultation with the project's scientific advisory board and reflects the latest understanding from across the eDNA community.

Can you tell from the sample the abundance of populations?

The number of sequences detected in metabarcoding of eDNA depends on many factors, including the sampling method and place, the PCR protocol and its efficiency, the size and DNA shedding rate of the organisms, and DNA degradation rates. Therefore, the UNESCO eDNA initiative does not consider that eDNA metabarcoding can provide abundance information. eDNA is an innovative method and an active field of research and some studies have found good correlation between the number of sequences recorded and the abundance or biomass of species. However, abundance analyses still require careful testing on a site-by-site basis and for the particular species of concern and comparison to surveys made with traditional methods like trawling studies made for fisheries analysis. Quantitative PCR is a method where the amount of DNA molecules of a targeted species in a sample can be calculated, and therefore get a more accurate idea of the abundance of these molecules. However, these are also highly dependent on the conditions at the sampling site, and therefore require careful consideration, as well as optimization for each different species. The scientific

community is working to develop more abundance-based estimates also from eDNA analysis, but there is still a lot of work to be done until this is possible. The UNESCO eDNA initiative considers the latest learnings from the scientific community while taking into consideration that this is a rapidly evolving field of research globally.

What information will the eDNA samples provide?

eDNA metabarcoding gives us information on the diversity of species that is present at the given location. It is most useful for detecting species not previously observed in the area (like introduced or invasive species), rare species, species that are difficult to visually detect, species at different life stages, species that are transient (present only for a short time), nocturnal (active only at night), or otherwise hide efficiently. The UNESCO eDNA initiative considers that, while eDNA cannot provide all the information that is required for monitoring a marine ecosystem, the ease of sampling means that it is a very powerful method for regular monitoring, and can provide information on hundreds of species in one simple water sample. This considerably increases the ability to assess and monitor species composition. While it does not replace all other monitoring methods, it is an effective way to complement and support targeted monitoring surveys done with traditional visual methods.

How long does it take to get results from a sample?

The UNESCO eDNA expeditions are undertaken between September 2022 and April 2023. Once all samples are received in the lab, analysis of the samples will start as of May 2023. Initial results are expected to be public in the second half of 2023. It generally takes 1-3 months between the eDNA sampling in the field and the resulting list of species, however in the UNESCO eDNA Expeditions, the PCR step will only happen once all samples have arrived at the lab. The final results of this campaign are expected in early 2024.

How will the vulnerability identified species to climate change be determined?

A key objective of the UNESCO eDNA initiative is to improve understanding of the vulnerability of marine species across marine World Heritage sites to the effects of climate change. Vulnerability to climate change will be estimated using climate envelope models. Climate envelope models are a particular type of species distribution models which delineate areas of climate suitability around species distributions. Using different climate scenarios, climate envelope models can be extrapolated to future climate conditions at the sites. For more information see Use and Interpretation of Climate Envelope Models: A Practical Guide.

Why is it important that we follow exactly the same protocol at each site, and why take 4 samples each at 5 different locations?

Because eDNA analysis requires sample processing in the laboratory as well as on the computer, the resulting lists of species are affected by choices at multiple steps of the protocols. Following the same protocol across all eDNA sampling locations ensures that the final results can be compared across sites, without confounding factors. While internationally accepted eDNA standards do not exist yet, they are being discussed and explored in multiple countries and fora. The UNESCO eDNA initiative uses the latest practices in consultation with the project scientific advisory board.

Replication is an important part of eDNA sampling to research the efficiency and reduce error margins. Replicate samples are compared, and depending on the amount of overlap in the species detected in each sample, the UNESCO eDNA initiative can determine the importance of small scale effects and estimate the amount of water that would be sufficient for getting a full overview of one sampling location with this sampling method. Four replicate samples are being collected at each sampling location. It allows for the analysis of a sufficient volume of water and helps define the importance and level of replication for future eDNA monitoring.

Can we expect a solid snapshot of species diversity by a single eDNA campaign?

The UNESCO eDNA initiative is conceived as a pilot initiative, with the objective to test sampling methods and approaches that are most effective and cost-efficient for citizen science use and broad application across many different geographic and socio-economic contexts. The resulting data will provide a first snapshot of fish species across 25 marine World Heritage sites and allow establishing a first baseline for future sampling and monitoring.

The number of eDNA samples required to obtain a full biodiversity survey depends on the size and complexity of the respective World Heritage sites. The amount of samples used in this pilot is low (500 samples) and is not intended to give a holistic view of all biodiversity at the sites. At each location, the eDNA sampling results will be ground truthed and validated with local science teams and will be complemented with existing datasets, collected by eDNA methods or traditional monitoring.