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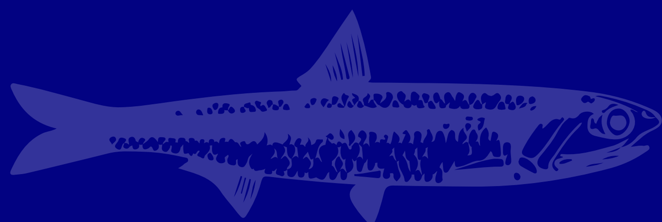
Commission

April 2026

eDNA Expeditions 2026-2028

Implementation plan



ednaexpeditions.org

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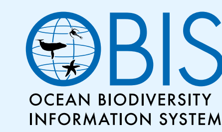
eDNA Expeditions 2026-2028 mobilizes environmental DNA observations to advance the development of a global biomolecular observatory for marine life, an essential step to better monitor and understand ocean biodiversity. For three years, the project will carry out repeated sampling campaigns conducted by local teams at 25 marine sites worldwide.

Powered by the Intergovernmental Oceanographic Commission (IOC) of UNESCO's Ocean Biodiversity Information System (OBIS) and supported by Minderoo Foundation in collaboration with Wilderlab, eDNA Expeditions is local by impact and global by design: Data from samples flows back to sites as decision-ready insights via co-designed pipelines that take into account local context. These insights can support local marine management and conservation, as well as advance our global knowledge of the ocean.



Students of the Catherine Ségurane high school, taking part in a sampling event at UNOC-3 in Nice, in June 2025.

Photo: RR3-Films / Minderoo Foundation

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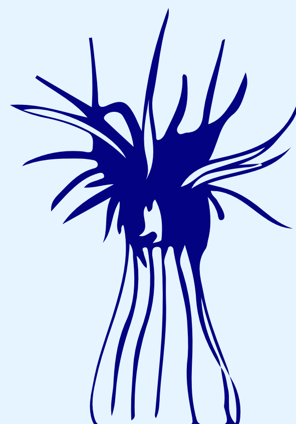
1. Introduction



Global commitments such as the Kunming-Montreal Global Biodiversity Framework (KMGBF) highlight the urgent need to halt biodiversity loss and improve ecosystem protection, including through the "30 by 30" target (KMGBF Target 3). However, achieving these goals requires not only expanding protected areas but also strengthening biodiversity monitoring, which remains limited in many regions due to resource, capacity, and technology constraints. This is particularly true in developing countries and small island nations, where ecosystems are both highly valuable and vulnerable. Traditional monitoring methods are often costly and inaccessible, leaving significant data gaps and limiting informed decision-making.

To address to this need, in January 2026, the Intergovernmental Oceanographic Commission (IOC) of UNESCO's Ocean Biodiversity Information System (OBIS) launched eDNA Expeditions, **a global environmental DNA (eDNA) project in marine sites** to study marine biodiversity at the participating sites, and their changes across the project timeframe. The project has a duration of three years (1 January 2026 – 31 December 2028) and foresees the collection of eDNA in 25 marine sites, totalling about 3000 water samples obtained with easy-to-use sampling kits (15 samples available per participating marine site for each sampling event).

The focus of the project is to facilitate repeated sampling of eDNA, collecting data to understand trends in biodiversity and develop scientific analyses to answer local questions. Samples will be analyzed with a tree-of-life approach, aiming to collect information from all branches of life. Samples will be analyzed rapidly, and data will be provided directly to the site team to allow a continuous feedback loop to local management. All data will be made available free-of-charge to the site management and be processed and published on **OBIS**, the world's largest open-access data system on the distribution and diversity of marine species.

Environmental DNA is DNA that is collected from a variety of environmental samples such as (sea) water, soil or air, rather than directly sampled from an individual organism. With a metabarcoding approach, the collected DNA can then inform on the diversity of life forms inhabiting or passing through the sampled environment. Environmental DNA sampling consists of collecting and filtering a water volume on site with minimal equipment, and is thus user friendly, cost effective, and non-invasive. This approach allows the participating marine sites to invite members of the local community as young as 10 years old to participate in the sampling, without higher scientific training. The project is coordinated by experienced eDNA scientists and is guided by an international advisory board that brings together some of the world's leading science and expertise in molecular ecology, eDNA, bioinformatics, metabarcoding and ocean science.

By providing expert guidance throughout the project, using standardized sampling protocols, working with a specialized laboratory for the eDNA sample processing, and applying rigorous taxonomic validation which combines automated pipelines with local biodiversity knowledge, the eDNA expeditions will provide high-quality biodiversity data to marine sites.

→ The project aims to achieve the following results:

- Measure species diversity through eDNA metabarcoding and develop an understanding of the trends in marine biodiversity at the sites
- Inspire and educate the next generation of ocean scientists through the participation of local youth and community members in the collection of eDNA, thereby increasing awareness of the importance of the protection of the marine environment;
- Showcase how eDNA can improve marine biodiversity monitoring and facilitate the work of local managers, by identifying local concerns and co-developing indicators from the collected eDNA data;
- Promote the standardization of eDNA sampling, analysis protocols and data sharing supporting the principle of open science. Environmental DNA sampling is already being used around the world, but remains difficult to compare and consolidate. Importantly, much of the data is not yet openly accessible.

→ For more information, visit the project website **ednaexpeditions.org**

2. Overall project implementation

Selected sites will receive full technical support to run repeated eDNA surveys over the three years of the project. OBIS will provide the sampling kits and online training to guide participants step-by-step. Sampling follows a protocol that allows anyone to collect scientifically valid material.

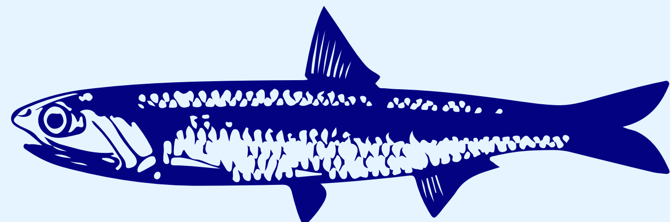
All samples will be processed at a specialized biomolecular laboratory. Analyses use a "tree-of-life" workflow capable of detecting biodiversity across all domains. Together with the site teams and local scientists, the project team will review the resulting species lists, ensuring that wrong assignments are minimized. From these outputs, the OBIS team will build an interactive dashboard, to help sites explore their results. Beyond the dashboard, OBIS will work jointly with participating sites to identify monitoring priorities and co-develop indicators that respond to local management and policy needs. All resulting data will be openly shared through OBIS in accordance with FAIR principles[1], contributing to advancing science and supporting policy at multiple scales.

→ Participating sites will receive:

> Training and guidance for eDNA sampling and data interpretation
> eDNA sampling kits with prepaid biomolecular analyses
> Access to the results and decision-ready insights via a co-designed dashboard
> Visibility as part of a global biomolecular observatory
> Connection with the eDNA Expeditions community

→ Participating sites commit to

> Participate for the duration of the project (3 years)
> Organize six to nine sampling campaigns
> Follow the project's sampling protocol
> Organize at least one sampling event involving local community members per year
> Support the selection of scientific contacts and the review of species annotations from collected eDNA data



[1] Wilkinson, M., Dumontier, M., Aalbersberg, I. et al. The FAIR Guiding Principles for scientific data management and stewardship. Sci Data 3, 160018 (2016). https://doi.org/10.1038/sdata.2016.18

2.1 Timeline for participation

Sites will join the project in a staggered manner, based on their availability, permitting and planning of the sampling campaigns. The first and most important step to successfully join the project is ensuring that the project consent form is signed by the relevant country authorities and returned to the project team (see section 6.1).

The first sampling event will be a test event, where all sampling kits will be used at one site. With this test sampling event, the amount of water filtered and the number of replicates will be defined based on conditions at each site, allowing the optimization of the sampling protocol to local conditions, and improving the acquired data for future sampling events.

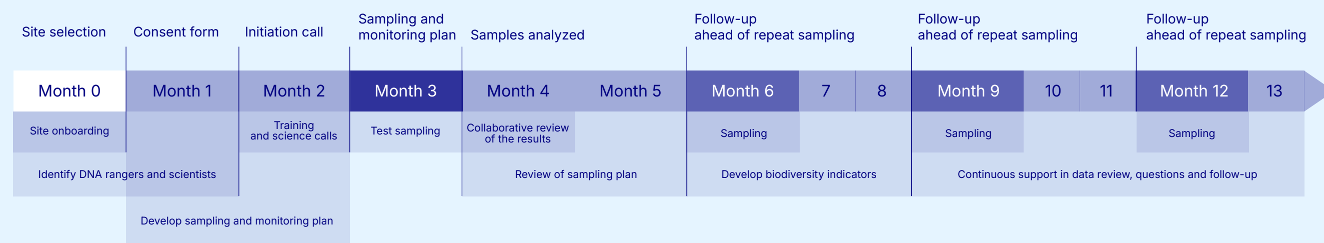


Figure 1. The timeline of anual activities and communication milestones for each site taking part in eDNA expeditions (2026-2028).

At each sampling event 15 kits will be provided: 12 for samples, two for negative controls and one as an extra back-up kit. The negative controls will be taken both before and after the sampling event by filtering clean, bottled drinking water at the site. The negative controls will ensure that common contaminants and cross-contamination are detected, ensuring that the results can be treated accordingly to reflect the local biodiversity as best as possible.

After the sites are selected based on their interest and capability, each of the sites will go through a structured process with pre-defined requirements and steps.

Throughout the participation in the project there are the following milestones for connecting with the project team and support.

1- The **consent form** for data sharing and sampling is signed by the relevant authorities of the site. This will be a prerequisite for joining in this edition of eDNA Expeditions.

2- An **initiation call is held with each site team**. In this call the possibilities for eDNA monitoring will be discussed. An explanation of what eDNA can provide in terms of the current issues that the site is facing, and a plan for sampling sites based on this will be defined. In addition, for each site it will be evaluated if the sampling will be done in conjunction with existing sampling campaigns, with local schools or with park rangers, whether the locations are easily accessible and what other requirements there may be for sampling.

3- Scientists related to the site will be identified, and an initial **science call** will be held to identify important questions as well as the level of prior knowledge on site biodiversity.

4- Based on this call, a simple **sampling and monitoring plan** will be drafted by each site with the support of the scientific coordinator, based on predefined questions and objectives.

5- After the plan is in place, the **sampling instructions** will be shared with the rangers, and a **training call** will be held, where the education officer will go through the sampling plan, and take any potential questions from the sites.

6- When the sampling is finished the samples will be analyzed within one or two months of sampling. The data will be immediately shared with the sites, initially through the staging page of the biomolecular laboratory (Wilderlab) for rapid review, and finally through the eDNA Expeditions dashboard. A **feedback call** will be held to understand the issues and needs for the following sampling campaign.

7- Simultaneously, the scientific coordinator will communicate with the scientists identified for the site, to perform a comprehensive **species review** and evaluate the results from the sampling campaigns. In this phase, the scientific coordinator may also hold a call to interpret the data and answer any questions or doubts that the sites may have.

After the initial round of sampling, the sites will be contacted to confirm whether subsequent sampling campaigns are going forward as expected, approximately one month before the next scheduled sampling campaign. In addition, sites will be asked to conduct further review of the identified species.

2.2 Developing your sampling plan

A sampling plan will be developed with each site individually, and will become the overall standard operating protocol (SOP), for each site. The plan makes practicalities of the sampling clear for anyone involved, allowing for switching responsibilities or sampling teams during the project duration. This sampling plan will already include detailed information on the selected sampling locations (coordinates), the sampling dates that the team is aiming for, the list of practical steps required for sample collection, and the responsible officers to contact with sampling information.

1. Objectives and Rationale

a. What are the main challenges facing the site? (i.e. invasive species, threatened species, keystone species.)
b. What seasonal changes does the site have?
c. Will/Can this project be a part of other regular monitoring activities?
d. The research question

2. Sampling frequency

a. Seasonal timing and/or justification for sample collection timing
b. Approximate dates

3. Sampling locations

a. Coordinates
b. Description of the sampling locations

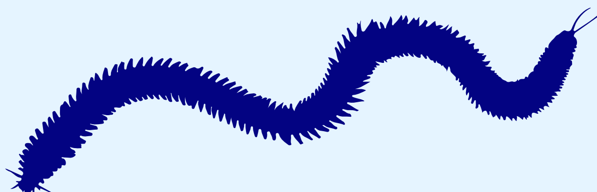
4. Sampling protocol

a. Location of sampling equipment
b. Preparation for sampling: cleaning of sampling equipment
c. Packing list for sampling
d. Special considerations for the sampling campaign

5. Responsible rangers

a. List of 2-3 responsible contacts for the sampling campaigns
b. Will sampling be organized with local communities?

3. Choosing the sampling location



Selected eDNA expeditions marine sites will be responsible for choosing the final sampling locations to ensure that the obtained data is as useful as possible for conservation and management. The following guidance for the choice of sampling locations apply:

For each selected marine site, the aim should be to choose two to four locations and take three to six replicate samples at each location. Replicate samples are samples taken with the same methodology at the same time and in close proximity. It is recommended to take replicate samples within five to ten meters from each other, but this can vary depending on the size of the habitat being sampled.

The amount of locations and replicates per location will depend on the monitoring question as well as the representativeness of eDNA replicates for the selected site. This will be determined during the first sampling event where all samples will be taken from a single location to determine species accumulation across replicate samples.

Since the samples will be taken in surface water, the total water depth of the sampling locations should be shallower than 15 m (to ensure that the collected eDNA is representative for the sample location).

Each sample will provide a snapshot of marine biodiversity. The objective is to take all samples during one day across the selected marine site. If this is not possible, there is flexibility to choose two consecutive dates for sampling.

The objective of this campaign is to sample time-series (repeated sampling at the same location). Choose locations that can be returned to every three months (when possible in terms of seasons and weather phenomena).

3.1 Scientific question development

The project's main target is to deliver actionable data to marine sites while engaging local communities in the collection of this data. The repeated sampling will allow us to observe within- and cross-yearly patterns of biodiversity. The panel of PCR primer sets (table 1) will allow to survey a wide set of taxonomic groups, covering both conspicuous, routinely surveyed species and lesser surveyed species groups across the tree of life. The sampling locations at each participating site will be chosen carefully together with the local management- and scientific team to tailor the monitoring efforts to their needs.

> These needs could cover baseline and temporal observations across different habitats, comparison of biodiversity across different levels of protection or management, following of biodiversity patterns at restoration sites, or monitoring certain habitats for the arrival of non-indigenous and potentially invasive species.

Finally, the temporal sampling across locations will contribute towards developing local eDNA-based indicators of biodiversity change. The project team will aid the site in developing a short sampling plan. A few example cases can guide the development of the monitoring question and associated sampling plan:

→ Example case 1: Baseline monitoring of habitats and species

- Even spread of four locations within the same habitat type
- Three replicate samples within each of these locations
- Analyses will focus on the species inventories and their presence and associations across (micro) habitats, as well as the potential detection of non-indigenous species

→ **Example case 2: Targeted monitoring of MPA management categories**
- Ideally two sampling locations in each considered management category (e.g. two in the MPA core area, two in the selected non-protected site) with three replicates in each location
- Alternatively, three locations could be chosen representing a core MPA area, a buffer zone and a comparative non-protected site. In this case, four replicate samples can be taken in each location
- The chosen locations should cover similar habitats and geomorphological and oceanographic conditions
- Analyses will focus on elucidating differences in species communities between the management categories

→ **Example case 3: Targeted monitoring of restoration action**
- Equal representation of restoration site and control site. E.g. Two locations within the restoration site, and two locations within the control site
- The chosen locations should cover similar habitats and geomorphological and oceanographic conditions
- Minimum three replicate samples in each location
- Analyses will focus on following the trajectory of species communities in time with restoration activities

3.2 Known target species distribution

After selection of the research question, sampling sites should also be chosen based on the known densities of the target species. The project works with a tree-of-life approach, where a wide panel of species groups are included in the analysis.

Table 1: Assays included in the Wilderlab marine panel for analysis (Assays with * are in testing phase at Wilderlab)

Assay	Original name	Target organisms	Reference
WV	16S_FishSyn_Short	Vertebrate 16S	Based on Nester et al. 2020
RV	12S-V5	Vertebrate 12S ecoprimers	Riaz et al. 2011
LM	MarVer1	Vertebrate 12S MarVer1	Valsecchi et al. 2020
CI	mlCOlintF/ClR	COI for metazoans/aquatic insects	Leray et al. 2013 (modified)/ Wilkinson et al. 2024
HD	MiDeca	Crustaceans 16S	Komai et al. 2019
BU	1389F/1510R	General eukaryotes and prokaryotes 18S V9	Amaral-Zettler et al. 2009
UM	U785F/U907R	Microbes 16S V5	Morey et al. 2006/ Lane et al. 1985
BX	18S_uni_1F/400R	General eukaryote 18S	Pochon et al. 2013
GF	Fung02/ITS5	Fungi ITS1	White et al. 1990/ Taberlet et al. 2018
GD	SCLER5.8SForw/28SRev/Acro874_Rev	Coral ITS2	Brian et al. 2019/ Alexander et al. 2019
WG	Vene01	Venerid clams 16S	Prié et al. 2021
MC	28S POR 100 FDG/ LSU 300RV	Marine sponge 28S	Martineau et al. 2024/ Redmond et al. 2011
LX*	MiFish-U/E (modified)	Fish and sharks 12S	Modified from Miya et al. 2015
MA*	Anth-28S-eDNA	Antipatharia and Octocorallia	McCartin et al. 2024

Further information on the assays including the primer sequences can be found in Annex 1. Before targeting a specific group or species, the project team can aid in checking if the species falls into the scope of the analysis assays.

eDNA sampling is opportunistic, so it will not catch all species that are present at the site. By carefully selecting the sampling locations based on the research question, we aim to showcase how eDNA can be used for monitoring purposes. First and foremost, sampling locations should therefore be selected based on where the target species is likely to be detected. It is not likely that large marine mammals, for example, would be detected from near-shore sampling sites.

3.3 Season/sampling time

When choosing the sampling sites, take into account the accessibility of the site throughout the project duration, as well as what are the expected changes in the target species distribution across seasons. As the objective is for repeated sampling campaigns, the sampling sites should be accessible every three months throughout the project duration (unless otherwise defined and discussed with the site team). There might also be other considerations which are important for choosing the best sampling time, such as logistics, weather, etc.

Above all, safety comes first.

3.4 Sources of contamination

The choice of sampling locations should take into account any sources of contamination that could interfere with the biodiversity signal. The following areas should be strictly avoided: sewage runoff (either from underwater pipes or from rivers), bilge pumps of vessels used for sampling as well as other vessels, and other places of human activities. Special attention should be given to avoid sampling in or near fish cleaning stations if local communities in your marine site are known to use fish cleaning stations to discard garbage or unwanted catches caught far away. Otherwise, a sample taken at a fish cleaning station could find deep water fish in a coral reef, for example.

It is highly recommended to avoid taking samples inside or close to a port or river plume, because contamination might have a huge impact (because of sewage pipes, more invasive species, contamination from boats, people, etc.). If it is completely unavoidable to take samples near a port, it is recommended to take samples on the outer side of the port, not inside the port.

4. Choosing your sampling community

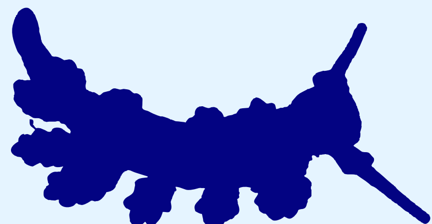
Students of the Catherine Ségurane high school, taking part in a sampling event at UNOC-3 in Nice, in June 2025.

Photo: RR3-Films / Minderoo Foundation

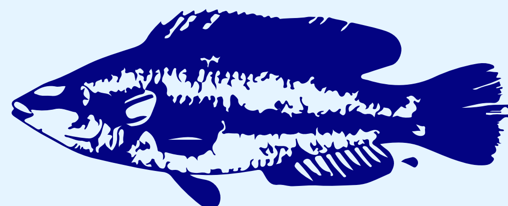
As an initial step after the required permits are in place and the time of the sampling has been chosen, the marine site should identify an appropriate group of local community members to undertake the sampling. It is encouraged to hold information sessions before the sampling campaign on the importance of protecting the marine site, and on what is eDNA. The project team can assist in organizing such information sessions.

At least two or three people are required for the collection of each sample, but each pair can collect multiple samples during the sampling day if so required.

The sampling campaigns will constitute a crucial element of the global communication campaign. Marine sites are invited to contact the project team to prepare the communication well in advance (photography, video, potential participation of journalists, etc.).



5. Required agreements



5.1 Permits for sampling

Each marine site will ensure that all required sampling permits are in place. These might include local permits (e.g. a permit for scientific research in your site or marine protected area), and/or permits in the context of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (or "The Nagoya Protocol"), a supplementary agreement to the Convention on Biological Diversity (CBD). The Nagoya Protocol aims to avoid that an entity from country X collects a DNA sample in country Y, and then earns benefits from the knowledge gained (for example through a patent, or by using it in a medicine), without sharing those benefits with the local communities from country Y. **Samples which have been collected without the required national permits can not be taken into account by the eDNA Expeditions project.**

eDNA Expeditions do not have a purpose linked to monetary benefits. Environmental DNA will be used only as a tool for the taxonomic identification of species through the analysis of short DNA sequences. No specific genetic and/or biochemical properties of the function of genes will be analyzed, therefore it is considered that the project does not constitute utilization of genetic resources and falls outside the scope of the Nagoya Protocol. However, the Project Team advises that the marine site consult with the national focal point and confirm agreement with the interpretation of the Project Team, (i.e. that metabarcoding of genetic biomarkers does not fall under the Nagoya Protocol), or, if your country is not a signatory to the Nagoya Protocol, if there is any specific legislation under your national law regarding marine genetic resources. It is also recommended to collect advice from scientists who have already collected genetic material in your country, and who will typically also have this information.

The data collected during the project will be used to build a snapshot of baseline information on marine biodiversity at the marine sites. Further benefits to the marine sites include:

- Increased knowledge about the marine biodiversity of the marine site which is useful for conservation and management
- Increased capacity of local site management to use eDNA for biodiversity monitoring
- Outreach to citizen scientists, young people and other stakeholders on the importance of conservation and ocean science education

→ Country focal points for the Nagoya Protocol are available here: https://absch.cbd.int/en/countries

IOC will not use the eDNA samples for purposes other than this project. The sample processing will be undertaken by Wilderlab, which has been contracted by the funder, Minderoo Foundation, for this work. The contract between the laboratory and Minderoo Foundation ensures that the samples cannot be used for purposes other than this project.

→ Before sampling begins, each marine site is requested to:

- Complete the project's **Collaboration Consent Form** (Annex 2) with IOC and agree on the open sharing of data resulting from the project as detailed in the project's **data policy** (Annex 4).

- If legally required by your country, also complete a Material Transfer Agreement. A Material Transfer Agreement is a legal contract between the entity sending the sample abroad, and the entity receiving the sample (in this case, Wilderlab). Please note that drawing up a Material Transfer Agreement might require a substantial amount of time and approvals. It should only be undertaken if strictly necessary by country regulations.

Saara Suominen, eDNA Expeditions project coordinator, collecting water from the Nice harbour for a sampling event at UNOC-3 in Nice, in June 2025.

Photo: RR3-Films / Minderoo Foundation

5.2 Biobanking of samples

Recognizing that samples provide a unique genetic snapshot that might be useful for future research, acknowledging the substantial investment made in their collection, marine sites are encouraged to provide the samples for permanent archiving at a biobank. As owners of the samples, this decision is up to the respective marine sites.

→ Benefits of adding the samples to a biobank include:

- Preserving the ‘genetic heritage’ of a marine site for future generations
- Future research can do more comprehensive analysis, or time series analyses
- The future discovery of a new biomarker or improved sequencing methods might reveal different species

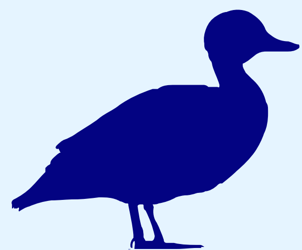
The University of Western Australia (working with the funder of this project, Minderoo Foundation), can provide biobanking facilities for this project. For this purpose, a biobanking agreement between the marine site and the facility will be drawn up, ensuring that each party is aware of their responsibilities in the sample storage.

If the site does not want their samples to be stored, they can be returned to the site (with additional costs), or discarded.

5.3 Image authorisations

Participants are encouraged to take photos and videos from the sampling events, develop blog posts and outreach materials related to the project’s activities. For any media a grant of rights is required (Annex 3) and for any participants in these images, image authorisations will need to be provided (Annex 5).

6. Materials for sampling



The project team will provide 15 sampling kits to each participating marine site for each sampling event. Depending on when the site conducts their first sampling, it is expected that the sampling will be conducted siz to nine times throughout the project duration. The kits will contain all material required for sampling. 12 of these kits will be for filtering marine water for the analysis of marine biodiversity. Two kits will be provided to be used as a negative control. One extra kit is provided in case of issues with the sampling. The kits will contain the individual filter and syringe which are required for clean sampling (see section 5.1 for details). Additionally, sampling bottles and a rod will be provided by the project team as part of the package and will need to be reused between the sampling locations and samples.
The following sections provide further details on sampling material.

6.1 Sampling kit contents

The project team will provide 15 sampling kits to each participating marine site for each sampling event (repeated every three months) with the following contents:
- Gloves for clean sampling, size M
- 1 sterile 60 ml syringe
- 1.2 µm disc filter
- 0.5 ml of preservation liquid (DNA/RNA shield) in a small 5 ml syringe, with a cap[2]

In addition, a pump adaptor kit will be included for each sampling kit, which contains:
- The tubing to run through the peristaltic pump
- A prefilter on one end of the tubing, and a connector for the eDNA filter on the other

6.2 Additional equipment for sampling

In addition to the sampling kits, the project team will provide the following material for sampling:
- A telescopic sampling rod (95-280 cm) with a bottle holder attachment
- 4 x 1000 ml bottles for sampling
- 1 x 1000 ml graduated beaker for measuring volume
- 2 clipboards as a support for writing
- Pencils for writing down sampling information
- 2 safety glasses, for use when adding the preservation liquid
- 5 OBIS-branded hats
- 5 copies of the Field Sampling Booklet with detailed written instructions
- 1 handheld pump[3]

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[2] Safety data sheet for the preservation liquid: https://files.zymoresearch.com/sds/dna-rna_shield_products_(us).pdf
[3] More information on the SC600/YZ1515x peristaltic pump https://www.crpumpshop.com/SC600-Portable-Battery-Powered-Peristaltic-Pump-Water-Sampling-Outdoor-Long-Flow-8-Meter-p5693260.html

6.3 Materials to be acquired by the local sampling team

In addition, the local sampling team is kindly asked to bring:
- 0.5 litre of 10 % household bleach for rinsing the sampling bottles
- 5 litres of sealed, bottled drinking water:
    - 2 litres of bottled water required for the negative control
    - 3 litres for rinsing the bottles after bleach cleaning
- A cooler or box to store the collected samples with the filters and shield them from direct sunlight once they are completed (UV radiation will degrade the DNA). There is no need for cold storage, but in places with very high temperatures, some cooling of the samples can be beneficial, as the preservation liquid is designed to preserve samples at room temperature (21°C)
- If easily available, extra gloves can be brought for clean sampling
- A mobile phone with the sample registration application installed

6.4 Available training information and instructions

The project team will provide several training materials to show how to carry out eDNA collection with the sampling kits, as well as the following training materials in English, French and Spanish:
- Field Sampling Booklet with detailed instructions on how to use the sampling kits in the field (five copies will be part of the package sent by the project team)
- Sampling instruction video (will be available on the project website, as well as on the sample registration application)
- Infographic with the nine key sampling steps
- Prior to undertaking the sampling, a video call with the project team will be organized to go over the sampling plan and the sampling kits in case any steps are unclear

6.5 Shipping and return of samples

All materials (listed in sections 6.1 and 6.2) will be sent by the project team to each of the participating marine sites after receipt of the Collaboration Consent Form. The handheld pump will be delivered separately. In addition to the above, the box will contain shipping materials required for sending the completed eDNA samples back to Wilderlab. After sampling, only the completed samples should be returned to Wilderlab, no other materials of the sampling kits need to be returned.

→ The small package which will be shipped back to Wilderlab should contain 14 bags (one for each sample), each containing:

- One collected eDNA filter (preserved and capped) connected to the syringe with the preservation liquid
- One completed sample information sheet

Each marine site is expected to return 12 filters with eDNA from marine samples, and two negative control filters for each sampling event. For more details on the sampling protocol see section 7.

It is not necessary to return other elements from the sampling kit (rod, clipboards, safety glasses, OBIS-branded caps, etc.): you can reuse this equipment during the following sampling events. The return shipping label will be paid in advance and will cover the costs of the shipment of the 14 samples.

The shipping labels need to include the shipping address below to return the samples:

Wilderlab
PO Box 15059
Miramar
Wellington 6243
New Zealand

In addition, for the import of samples into New Zealand, a Manufacturer's Declaration form is necessary. This form needs to be attached to the outside of your sample package before it is sent to Wilderlab. Without this form, the shipment will not pass through customs smoothly. You will receive this form from the project team when the samples are ready to be sent.

◀ **Students taking part in a sampling event in the Wadden Sea during eDNA Expeditions 2022-2024.**
Photo: Rolf Salomonsen / UNESCO

7. Sampling protocol

Sampling consists of filtering marine water collected at each designated location. The goal is to collect 12 samples from each marine site, as well as two negative control samples (clean bottled drinking water): one negative control sample should be filtered at the start and one should be filtered at the end of each sampling campaign. The objective will be to filter one litre of water with each sampling kit, and to preserve the DNA in the filters using the provided preservation buffer. The Field Sampling Booklet provided to the marine sites will contain more elaborate instructions, with images for each step to help with the explanation. Each sampling should be performed by at least two or three people, and the same people can collect multiple samples. While one person is performing the sampling, the others should be writing down the collected information, and making sure that all sampling materials are collected in a clean way back into the sampling kit bag.

Before and after the 12 marine water samples are filtered, at the beginning and end of the sampling day, one team of people should filter one litre of bottled water with the 13th sampling kit, and one one litre of bottled water with the 14th sampling kit. These two samples will be the negative controls, and will be used to rule out common contaminants (non-target organisms like farm animals and contaminants from food) and cross-contaminants. Therefore, this water should also be filtered at the sampling location, to get an accurate signal of possible contaminants. The sampling protocol with images of each step will be provided together with the sampling kits (five paper copies of the Field Sampling Booklet for each marine site). The instructions will also be available through the sample registration application.

7.1 Step 0: Cleaning the sampling bottles

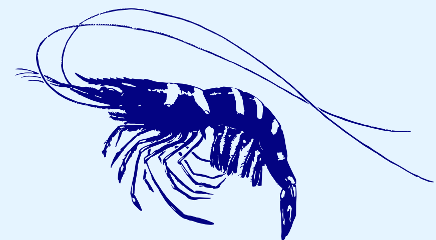
→ **Note:** if you are sampling with the pump, before heading out, remember to fully charge both batteries that are provided with the pump kit.

Before heading out to the sampling location, the sampling bottles should all be cleaned following the strict decontamination procedure and packed accordingly. At the sampling location, each time a sampling bottle is re-used in between locations or replicates, the bottle should first be cleaned again following the decontamination procedure.

The project will provide four plastic bottles of one litre to the sites. These bottles should be reused between locations and sampling events. To avoid contamination between sampling locations, the bottles should be rinsed with household bleach and clean water before sampling and after each location.

→ **Before sampling on land:**

1- Pour 10% bleach across the four bottles.
2- Close the bottle caps and shake each bottle for about one minute, so that all surfaces of the bottle have come in contact with the bleach. If you have time, the bottles can be left to soak with bleach for 24 hours
3- Collect the bleach.
4- Rinse the bottles with abundant clean water.
5- Close the bottle caps and shake each bottle for about one minute, so that all the surfaces of the bottle have come in contact with the water.
6- Collect the water and bleach mixture and dispose of it safely through an appropriate collection system where it can be properly treated. Do not release the mixture into the environment
7- Repeat steps 4. and 5. another two times; rinse the bottles in total three times.
8- Pack at least **0.5 litres of 10% household bleach securely with you to the sampling event**
9- Before sampling, rinse with sample water (marine water or a small amount of the drinking water) three times before filtering.



→ At the site:

1- Distribute the bleach across the four used bottles (one negative control, three samples).
2- Close the bottle caps and shake each bottle for about one minute, so that all surfaces of the bottle have come in contact with the bleach.
3- Return the bleach to the bleach bottle. This bleach can be reused for the next cleaning.
4- Take clean water and distribute about half a litre across the four sample bottles.
5- Close the bottle caps and shake each bottle for about one minute, so that all the surfaces of the bottle have come in contact with the water.
6- Collect the mix of water and bleach (this mix should not be used again) and dispose of it safely through an appropriate collection system where it can be properly treated. Do not release the mixture into the environment.
7- Repeat steps 4 and 5 another two times; rinse the bottles in total three times.
8- Before sampling, rinse with sample water (marine water or a small amount of the drinking water) three times before filtering.

7.2 Step 1: Sample information

When you have arrived at the sampling location, open the sample registration application and take the sample information sheet.

1- Write down the name and email of the person(s) sampling.
2- Record the **sample ID** (the code located on kit, e.g. "558447").
3- Record the **sampling time and location** by tapping on the application and write down the same information on the sample information sheet.
4- Write down any notes on the sampling site: what is the biome, are there any visible blooms of algae, or any other organisms. What is the total water depth, weather conditions and water temperature (if you have the information).
5- Remember you can also add photos of the site and of the sampling, to the application: This will be helpful in determining how the conditions were at the site, and in communication about the project.

7.3 Step 2: First negative control

→ Warning: Wear gloves at all times during sampling. Do not touch anything other than the sampling equipment to avoid contamination. Also, consider where to store the sampling equipment as it should be kept as clean as possible. Two negative controls will be collected with each sampling event. One before the collection of the first sample, and one after the collection of the 12th (and last) sample. In each case the protocol should be followed exactly the same as with a real sample. This protocol is given here in brief but detailed below. The first negative control will be taken from a pre-cleaned bottle. The second negative control will be taken from an on-site cleaned bottle (see section 7.1).

1- Start by recording the sample ID, and indicating on the sample information sheet and in the notes in the app, that this is the first negative control. There is no need to collect environmental metadata.
2- Rinse the sample bottle with half of the bottled water of the first negative control.
3- Pour the remaining half of the bottled water in a sample bottle, and proceed with filtering as detailed below.
4- Proceed with the sample preservation steps as detailed below, and seal the sampling bag.
5- Proceed with step 3: (seawater) eDNA sampling.

7.4 Step 3 / alternative A: Manual eDNA sampling

→ Warning: Wear gloves at all times during sampling. Do not touch anything other than the sampling equipment to avoid contamination. Also, consider where to store the sampling equipment as it should be kept as clean as possible.

1- Rinse the sampling bottle with the rod three times with water from the sampling location.
2- If you are close to the shore, aim to take samples as far away from land as possible with the rod, to avoid runoff from land.
3- Collect the sample by immersing the bottle approximately 30 cm below the surface.
→ Note: If you are in an area with a sandy bottom, let the sample water stand for a few minutes before filtering, to allow the sand to settle at the bottom of the bag.
4- Fill the syringe with sample water.
5- Attach the filter to the syringe.
6- Be careful, proceed slowly, the next step will require some effort and patience. Take turns filtering if needed. **Filter the content of the syringe by pushing it through the filter.**
7- Carefully remove the filter from the empty syringe, without touching the inlet and outlet of the filter.
8- Aim to **repeat the steps 4 to 6 for 20 times to filter one litre.**
9- Count the number of times the syringe is filled and/or the amount of water that you have filtered, in the sample registration application and on the sample information sheet.
10- When the filter is full, the filtering will be slower.
11- The final amount of water that can be filtered will depend on local conditions. Stop if the filtering is too slow, or is taking too long (for example more than 30 minutes). The filter is then clogged.
→ Warning: Avoid air bubbles in the filter, this will cause the filter to malfunction. To prevent air from being sucked in, keep the syringe above the filter at all times, and do not fully empty the syringe (leave a bit of water at the end of each syringeful). If air bubbles appear in the filter while filtering, they can easily be removed. For example, the filtering becomes difficult after <10 times, you may have an air bubble that is clogging the filter. **See 7.6 Troubleshooting: In case of an air bubble**.

7.5 Step 3 / alternative B: eDNA sampling using the pump

→ Warning: Wear gloves at all times during sampling. Do not touch anything other than the sampling equipment to avoid contamination. Also, consider where to store the sampling equipment as it should be kept as clean as possible.

1- Before sampling, **fully charge both drill batteries.** Ensure that the pump is running in the right direction by pushing the red arrow button.
2- Open one of the Wilderlab kit bags and put the gloves on. Open one of the drill pump adapter kits and take out the tubing.
3- Move the top lever to the left and push the switches on both sides of the peristaltic pump up to the open position. Insert the tubing into the open pathway with the prefilter (larger disk end) on the left and luer connector (smaller end) on the right.
4- The luer lock end of the tubing should be flush with the side of the pump. Push both switches down to the closed position and move the lever to the right to lock tubing in place.
5- Using gloves, take the filter out of the kit bag and screw onto the luer lock connector (smaller end)
6- Place bucket or plastic measuring jug so that outflowing water will be captured and measured.
7- To sample, hold the prefilter just below the surface of the water, gently press the trigger of the drill to start pumping at a low speed. Continue pumping until ~1L has been sampled into the bucket (flow rate at full speed: about 0.75 L per min).
8- Once finished, move to section 7.7. Sample preservation.

7.6 Troubleshooting: In case of an air bubble

→ Warning: Only perform these steps if you suspect you have an air bubble clogging the filter!

Air bubbles block the filter membrane and have a negative effect on the filter capacity. These air bubbles are easy to remove from the capsule, by pulling them out with the syringe upright, or by briefly reversing the flow of the pump.

1- Turn the syringe with the filter attached upright, so that the filter is below the syringe and there is still some water in the syringe.
2- Gently pull back on the plunger and allow any air bubbles to travel upwards.
3- Try this a few times to dislodge possible air bubbles from the filter.
4- Always leave a little bit of water in your syringe to avoid any more air bubbles when filtering

7.7 Step 4: Sample preservation

→ Warning: This step should be performed by an adult wearing gloves and safety glasses.

1- Empty the water from the filter by pushing through air with the syringe.
2- Remove the filter from the syringe.
3- Wear the safety glasses, be very careful with the preservation liquid, do not swallow, avoid contact with skin and eyes, and do not discard in the environment.
4- Close the top end of the filter with the cap from the small syringe.
5- Add the preservation liquid to the filter from the provided small syringe.
6- Keep the small syringe attached to the filter, and return as such to the sample bag.
7- Take a picture of the sample information sheet with the app, and upload it.
8- Close all materials including the filter with the small syringe attached and the sample information sheet in the provided sample bag.
9- Save the sample information collected in the app.

→ Warning: Keep the filters sheltered from direct sunlight at all times, as UV radiation will degrade the collected DNA

→ Note: When finished with the sampling kit, remember to also save sample information on the application by pressing save.

Dispose of any other waste in a sustainable and clean manner.

7.8 Step 5: Filtering the negative control

Two negative controls will be collected with each sampling event. One before the start of sampling, and one after the last sample. In each case the protocol should be followed exactly the same as with a real sample.

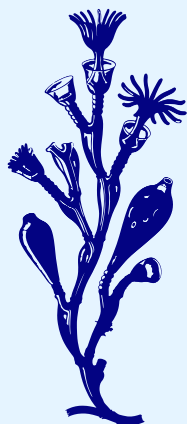
1- Start by recording the sample ID, and indicating on the sample information sheet and in the notes in the app, that this is the negative control. There is no need to collect environmental metadata.
2- Rinse the sample bottle with a small amount of bottled water.
3- Pour one litre of bottled water in a sample bottle, and proceed with filtering as before.
4- Proceed with the sample preservation steps as before, and seal the sampling bag.
5- As soon as all 12 samples and two negative controls are collected, the samples should be sent to Wilderlab.

All samples should be collected and stored together in a dark place at room temperature (21°C). For example the organizers can bring a cooler to the sites, and once the filters are brought back to land, store them in an air-conditioned room. The most important thing is to keep the filters away from direct sunlight.

Students of the Catherine Ségurane high school, taking part in a sampling event at UNOC-3 in Nice, in June 2025.

Photo: RR3-Films / Minderoo Foundation

8. Sample processing and analysis



The eDNA laboratory will be responsible for the processing of the samples as well as the sequencing of the DNA for marine biodiversity information. A metabarcoding approach will be taken to describe the taxonomic diversity found in the collected DNA. It will be possible to check on the **project website** when samples have been received by the lab, and when processing has been completed. The four stages of the sample processing workflow are detailed below.

Students of the Catherine Ségurane high school, taking part in a sampling event at UNOC-3 in Nice, in June 2025.
Photo: RR3-Films / Minderoo Foundation

8.1 DNA extraction

DNA is extracted from the collected eDNA filters in a clean laboratory. The DNA will be extracted ensuring maximum recovery of eDNA, and minimizing contamination during the process.

8.2 Amplification of target genes

Defined target genes chosen for their differentiation across species will be amplified using the polymerase chain reaction (PCR). Twelve to fourteen different target genes will be amplified (see Annex 1 for details). The short target regions (e.g. < 600 base pairs long) are part of the mitochondrial genome or ribosomal genes, are very well known, and serve no purpose other than determining the taxonomy of the species in the sample. Each of the target genes will target a different subset of animals. The tree-of-life approach aims at describing a wide range of biodiversity at the site - from bacteria to whales. However, all target gene regions and primers have biases, which means that not all organisms will be detected. Whether a species is detected or not depends on many factors during sampling as well as processing, and therefore cannot be guaranteed.

8.3 Sequencing

Sequencing will be performed by the contracted eDNA laboratory. Sequencing of the amplified target genes will be done using an Illumina MiSeq platform. Approximately 100,000 sequencing reads will be generated for each sample.

8.4 Bioinformatic analysis

The DNA sequence reads obtained from the sample metabarcoding will be further processed by the project team. The taxonomic assignment of the DNA sequences will be done with a bioinformatic pipeline that will be publicly available (e.g. https://github.com/iobis/PacMAN-pipeline), and based on publicly available genetic reference databases.

The bioinformatic analysis is done by cleaning and trimming DNA sequences based on quality, clustering all identical DNA sequences into amplicon sequence variants (ASVs), and assigning taxonomy of these DNA sequences by comparing them to reference databases. The reference databases contain known, named DNA sequences of the target gene. The target gene regions (the sequenced regions) are chosen because of their power in taxonomic differentiation. Small differences in the short DNA sequences indicate different species. By comparing the DNA sequences to the reference databases, the DNA sequences are named to the closest available known DNA sequence. The level of similarity will determine how precisely the taxonomy will be defined, e.g. if the DNA sequence is only 80% similar to a known (named) DNA sequence, it might be assigned a name at the Family or Class taxonomic rank.

A quality control workflow will check how probable the taxonomic assignments are, based on known geographic distribution and habitat temperatures of the species. Species will be given a probability score, which can be used to filter out the most unlikely species assignments (false positives).

Local scientists working on the project will be asked to check the taxonomic assignments, to identify any possible cases of mislabelled DNA sequences.

Taxonomic assignments will be published on the **Ocean Biodiversity Information System** (OBIS). Due to the incompleteness of genetic reference databases many DNA sequences are expected to remain unclassified, or classified only to a higher taxonomic category. However, these sequences will also be included in the datasets, and will give an idea of the remaining gaps in the reference databases.

◀ **Ludovic Perrochia, teacher at the Catherine Ségurane high school explaining the tree of life wheel to his students during a sampling event at UNOC-3 in Nice, in June 2025.**

Photo: Ward Appeltans / IOC

9. Data management

All sample metadata and data artefacts will be managed in the eDNA Expeditions data management platform. This platform will:

- Keep track of all samples collected in the project, but potentially also for samples from future projects and ad-hoc sampling campaigns.
- Synchronize with other systems holding sample metadata, for example at the sequencing facility.
- Automate data processing and publishing as much as possible, but with the necessary breakpoints to ensure control over the data flow as well as quality assurance.
- Provide flexibility in terms of the types of data that can be processed by allowing multiple pipelines to be plugged into the system.
- Allow for data submission with minimal human intervention.
- Allow for easy reanalysis and republishing of existing samples.
- Provide access to raw reads as well as pipeline results (such as quality profile, ASVs, taxonomic annotations) by sample.

9.1 Sample metadata collection

Sample metadata will be collected using the sampling sheets as well as the mobile application, and synchronized between the eDNA Expeditions platform and the eDNA laboratory using the laboratory's web services.

→ The information collected includes:

- Kit identifier
- Collector name and email
- Sampling date and time (including location uncertainty)
- Volume filtered
- Description of the environment
- Other comments

The mobile application will be web-based, but it will support offline use and data synchronization when the connection has been restored.

9.2 Raw sequence data

According to good scientific practice, raw (unanalyzed) DNA sequence data will be submitted to public sequence databases that are part of the International Nucleotide Sequence Database Collaboration (INSDC) like NCBI or ENA. The INSDC is committed to the open sharing of DNA sequence data, and thereby enabling scientific work, and has been one of the most celebrated global initiatives in the open sharing of data. Most scientific publications have required the open sharing of DNA sequences since the 1990s, a practice that has ensured the development of molecular sciences. The sharing of sequence data ensures that derived scientific results can be confirmed and checked by other scientists working in the field.

9.3 Reference databases

Reference databases will be created for each assay using publicly available reference sequences from NCBI and BOLD. The reference databases will be versioned and will receive a DOI so they can easily be cited.

Students of the Catherine Ségurane high school, being interviewed during a sampling event at UNOC-3 in Nice, in June 2025.

Photo: Ward Appeltans / IOC

9.4 Data quality control

Metabarcoding datasets tend to include a certain amount of false positives due to inaccuracies or incompleteness of the reference database as well as insufficient marker resolution, and therefore need thorough quality control. OBIS is working on a general purpose quality control tool and a quality annotations database which will be integrated into the eDNA Expeditions workflow. These tools will be standalone, so that annotations created outside the project can be used for quality control of project data, and vice versa. The data management platform will allow submitting datasets to the quality control tool for data cleaning before publishing.

9.5 Data sharing

A fundamental part of the project is the open sharing of project protocols and data. It is a prerequisite for participation to agree to open data sharing (Section 3.1). All protocols and methods will be published as a guide on the **Ocean Best Practices System** (OBPS) and **GitHub**, allowing full transparency and reproducibility of the scientific results.

Species lists resulting from the analysis of the DNA sequence data will be shared through the IOC's Ocean Biodiversity Information System (OBIS) portal using the Darwin Core Data Package structure (DwC-DP). Data will be summarized on the **project website** and dashboard, where community participants will be able to track their samples, receive notifications when results are available, and explore the data for their sample or site. Through the portal it will be possible to discover sampling sites on a map, and explore species lists, detections of charismatic megavertebrates, and potential biodiversity indicators for each marine site. A special emphasis will be put on showcasing the number of species categorized to global, local, endemic and threatened categories, as well as unknown species. To ensure data traceability, the following Persistent Identifiers (PID) will be generated along the workflow:

Type of data	Identifier	Description
Participating marine site	Site PID	A code unambiguously identifying each participating site
Raw sequence data	ENA accession number	A unique identifier of the European Nucleotide Archive for each sequence file submitted to their database
Processed biodiversity data	OBIS DOI	A DOI identifying each site-specific occurrence dataset submitted to OBIS

10. Scientific analysis

After the bioinformatic analysis, taxonomic assignment and validation, the data will be accompanied by a set of scientific analyses for each site. The aim of these analyses is to provide each site with a basic overview of their biodiversity results. With the overall aim of the project of providing actionable data for participating sites, these analyses will be the starting block for a more tailored approach as the project progresses.

The first sampling event of each site will serve as a test sampling event to calibrate the volume and replication requirements, evaluate the quality of negative controls, as well as evaluate the detectability of (local) species of interest. Sites will filter two negative controls (one at the beginning, one at the end of the sampling event), and three samples until clogging of the filter instead of a fixed 1L volume.
The nine remaining samples will be taken at the same location to evaluate the replication coverage (Figure 2) as well as the coverage of detected species. Additionally, we aim to develop a prior species detectability evaluation based on their availability in reference databases, the barcode resolution and potential mismatches of the primer annealing sites.

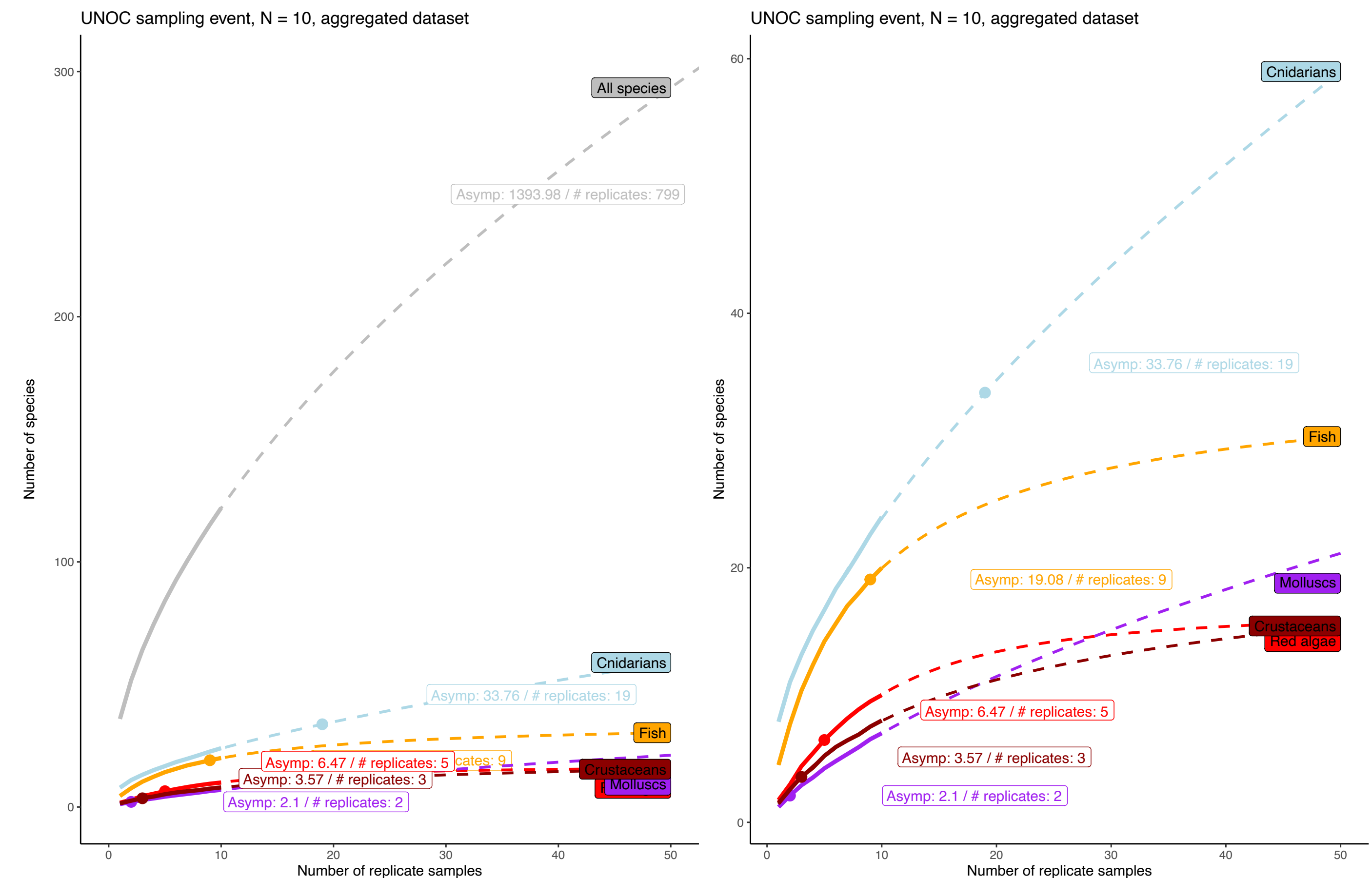
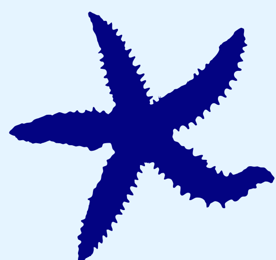


Figure 2. Example analysis for the data obtained by a class of school children sampling harbour seawater during the project signing event at UNOC 2025. Species accumulation curves (solid lines), and from that the extrapolated species richness by incremental replicates (dashed lines), as well as their asymptotes (number of species and number of samples) where the increase in number of species per sample < 1 are shown.



Next, the samples obtained during the rest of the “standard” sampling events will be analyzed with a panel of standard analyses. These analyses will measure both within- and between-site diversity metrics across the different sampling locations and conditions. As results are obtained after each sampling event, these analyses will be updated in time to provide a temporal perspective of biodiversity patterns. Metabarcoding data does not linearly correlate to species abundances or biomass. Analyses will thus focus on species’ presence and presumed absence and their relative sequence read abundance. We will calculate species richness, weighted species diversity and (differences in) species composition. These will be compared across locations depending on the sites’ research or monitoring question. We will also highlight the presence of charismatic megavertebrates (sharks, cetaceans, turtles) as well as rare, endemic or endangered species (IUCN Red List of Threatened Species). The selected assay panel/pcr primer sets cover a diverse group of species (see 2.2 tree-of-life approach). The analyses will thus integrate both the entire set of detected species as well as separate out different target groups depending on the sites’ interest.

In time, the objective of the project is to co-develop indicators of biodiversity change that can be applied to each site. These could focus on select indicator species, or integrate detections and relative abundances across species groups covered by the tree-of-life approach. As the detected species will represent different trophic levels, we will look into co-occurrence analyses of different trophic levels across sites to detect changes in trophic organization.

A young participant filtering water at sampling event during eDNA Expeditions 2022-2024, in Corsica, in December 2024.
Photo: UNESCO

10.1 Existing research information on biodiversity already available at the marine sites

If the marine sites or partnering scientists or scientific institutions already have relevant datasets available, they are invited to share this information with the project team. These datasets will allow to not only complete the data already available on OBIS, but also to compare the obtained eDNA results against previously available and complementary knowledge.

Such datasets could include, for example:

- Results from earlier eDNA analysis in UNESCO World Heritage marine sites
- Any existing data on biodiversity in the marine sites. If available, it is recommended to include spatial and temporal information, as well as references to related reports, research papers, online datasets, etc.
- Maps or other spatial datasets on the distribution of habitat types within the marine site
- Reference datasets of local species, if available.

While species lists can be useful for the project, it is recommended to include the following fields when sharing biodiversity datasets:

→ Highly recommended:

- Species name or full taxonomy
- Time
- Location (including uncertainty if available)

→ Nice to have:

- Quantitative information (abundance, biomass)
- Sampling methodology, protocol, SOPs
- Taxonomic scope of the study or dataset
- Links to voucher specimens
- Links to papers or reports

Some examples of biodiversity papers, reports, and datasets

> Seabed biodiversity on the continental shelf of the Great Barrier Reef World Heritage Area

> Fish diversity patterns along coastal habitats of the southeastern Galapagos archipelago and their relationship with environmental variables

> Wadden Sea Quality Status Report

> Marine Habitats of Western Australia

11. Global communication

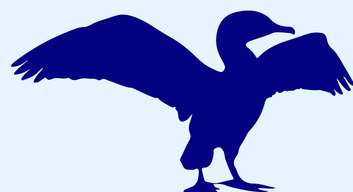
Communication and outreach play a major role in this project through a global media campaign coordinated by the Intergovernmental Oceanographic Commission (IOC) of UNESCO. The communication campaign will largely focus on the people involved in the project at selected sites, on the impact generated from the data mobilized by the project, and on the behind-the-scenes infrastructure needed to run the project, including highlighting its scalability and reproducibility. Communication will be built around three major waves:

- The announcement of the 25 selected sites, with localized content to engage with national and international media (main narrative: "what we hope to achieve at our site")
- Mid-project sampling events, with a strong focus on community engagement (main narrative: "eDNA Expeditions as a tool to generate local engagement and concrete action for the ocean")
- Result implementation and local impact (main narrative "impact-driven success stories, scalability and reproducibility of the project)

The marine sites and citizen scientists will be encouraged to document sampling efforts through images, candid videos, and reactions that will be shared through the project's website and social media, as well as taking part in interviews and contributing to articles. Beyond informing on the progress of the project, this material aims to inspire and effectively engage the next generation of ocean scientists and conservationists. The project's communication campaign is a unique opportunity for the participating sites and for IOC, to highlight the role of local communities in the protection and management of the ocean, especially youth and local communities.

All participating sites are encouraged to film, photograph and interview local citizens during their eDNA sampling activities in an effort to bring global attention to the project's ambitions and expected outcomes. The project team will provide guidance and support for generating this communication material, including image consent forms. Local teams conducting the eDNA sampling will be provided with branding material to facilitate communicating the core ambitions of the project, the critical importance of protecting the marine sites for future generations, and more generally the mission of IOC in the field of science and ocean conservation.

The marine sites are invited to help identify inspiring youth voices (young scientists, indigenous communities, young citizen scientists) whose interviews could be integrated in the global communication campaign.



12. Annexes

Annex 1 List of primers and PCR conditions used in the project

Assay	Primer	Sequence	Annealing temp.	Extension time	Cycles	Target	Reference
WV	WVF WVR	GACGAGAAGACCCTWTGGAGC CCRYGGTCGCCCCAAC	58	20	38	Vertebrate 16S	Based on Nester et al. 2020
RV	RVF RVR	TTAGATACCCCACTATGC TAGAACAGGCTCCTCTAG	58	20	38	Vertebrate 12S ecoprimers	Riaz et al. 2011
LM	LMF LMR	CGTGCCAGCCACCGCG GGGTATCTAATCCYAGTTTG	55	20	38	Vertebrate 12S MarVer1	Valsecchi et al. 2020
CI	CIF CIR	DACWGGWTGAACWGTWTAYCCHCC GTTGTAATAAAATTAAYDGCYCCTARAATDGA	45	20	38	COI based on mlCOlintF COI for aquatic insects	Leray et al. 2013 (modified) Wilkinson et al. 2024
HD	HDF HDR	GGACGATAAGACCCTATAAA ACGCTGTTATCCCTAAAGT	55	20	38	Crustaceans 16S	Komai et al. 2019
BU	BUF BUR	TTGTACACACCGCCC CCTTCYGCAGGTTCACCTAC	52	25	38	General eukaryotes and prokaryotes 18S V9	Amaral-Zettler et al. 2009
UM	UMF UMR	GGATTAGATACCCTGGTA CCGTCAATTCMTTTRAGTTT	52	25	38	Microbes 16S V5 Microbes 16S V5	Morey et al. 2006 Lane et al. 1985
BX	BXF BXR	GCCAGTAGTCATATGCTTGTCT GCCTGCTGCCTTCCTT	52	25	38	General eukaryote 18S	Pochon et al. 2013
GF	GFF GFR	GGAAGTAAAAGTCGTAACAAGG CAAGAGATCCGTTGYTGAAAGTK	52	25	38	Fungi ITS1 Fungi ITS1	White et al. 1990 Taberlet et al. 2018
GD	GDF GCR GDR	GARTCTTTGAACGCAAATGGC GCTTATTAATATGCTTAAATTCAGCG TCGCCGTTACTGAGGGAATC	52	25	38	Coral ITS2 Coral ITS2 Coral ITS2	Brian et al. 2019 Brian et al. 2019 Alexander et al. 2019
WG	WGF WGR	TTDTAAAAGMCGAGAAGACCC CSCTGTTATCCCYRCGGTA	52	25	38	Venerid clams 16S	Prié et al. 2021
MC	MCF MCR	TTCCCTCAGTAACGGCGAGY CAACTTTCCCTCACGGTACTT	52	25	38	Marine sponge 28S Marine sponge 28S	Martineau et al. 2024 Redmond et al. 2011
LX	LXF LXR	GGTAAAWCTCGTGCCAGC CATAGTGGGGTATCTAATCCYAGTTTG	55	20	38	Fish and sharks 12S	Modified from Miya et al. 2015
MA	MAF MAR	CGTGAAACCGYTRRAAGGG TTGGTCCGTGTTTCAAGACG	52	25	38	Antipatharia and Octocorallia	McCartin et al. 2024

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Annex 2 Template of the eDNA expeditions collaboration consent form

eDNA Expeditions (2026-2028) Collaboration Consent Form

1. [name of Partner] hereafter called the "Partner" joins the Project "Environmental DNA Expeditions 2026-2028" led by the Intergovernmental Oceanographic Commission (IOC) hereafter called the "The Project", to be implemented in [name of the Site] and agrees with the objectives, activities, and terms listed below.

2. The Project aims to provide information on marine biodiversity across 25 marine sites, and to support capacity development and environmental management activities. The Project will analyze the presence of all organisms across the tree of life based on metabarcoding markers (i.e., short genetic fragments), for taxonomic assignment.

3. By joining this Project, the Partner will benefit from:
   a. Increased knowledge about the marine biodiversity at the marine site,
   b. Increased knowledge on key indicators of the marine site's biodiversity,
   c. Increased capacity to use environmental DNA for biodiversity monitoring,
   d. Increased awareness of the importance of the marine environment to local and indigenous communities, young people, and stakeholders
   e. Training and education in ocean science, innovation, and best practices,
   f. A unique opportunity to engage youth in the protection of the marine site.

4. The Partner agrees to coordinate the collection of environmental DNA samples inside the marine site three or four times within a year. IOC will ship the sampling kits to the Partner. Note that sampling kits will be sent once this present Collaboration Consent Form is signed and returned to IOC. The receipt by IOC of this consent form confirms to the Project team that the Partner has obtained all necessary permissions for environmental DNA sampling and shipping of samples at the local and national levels[4].

5. The project team will provide the necessary training material (video, Field Sampling Booklet) to undertake the sampling, in English, French, and Spanish.

6. The Partner will choose the sampling locations within the marine site, taking into account the recommendations provided in the Project's Implementation Plan.

7. During the sampling, the Partner will ensure the collection of the necessary information via the sample information sheets and the sample registration application as indicated in the Field Sampling Booklet. The information includes the unique sample ID provided in each sampling kit, name and email addresses of the contact person or person(s) sampling, date and time of sampling, coordinates of the sampling location, and total amount of water filtered, as well as further notes on the sampling location.

8. The Partner commits to return the samples to the address specified on the shipping label. The return shipping label is prepaid by IOC and covers the costs of the shipment of the samples. The Partner may keep the remaining sampling equipment. Maintenance of the material for other uses beyond the scope of this project is the responsibility of the Partner.

9. For this project, IOC is working with the environmental DNA company Wilderlab, hereafter called "the Lab". For each sample, the Lab will extract DNA, amplify short target regions of DNA for the analysis of marine biodiversity, and sequence the amplified DNA regions. Samples will be analyzed for marine biodiversity, targeting well-known short DNA sequences (genetic biomarkers) with several primer sets. The target regions will be chosen based on their differentiation across different species, including target groups such as Actinopterygii, Chondrichthyes, and vertebrates. The Lab has no rights for further analysis of samples or use of data, including all communication and publishing of data and the analysis thereof, beyond the explicit scope of The Project.

10. The Project will use environmental DNA only as a tool for the taxonomic identification of species through the analysis of short, well-known DNA sequences (genetic biomarkers). No specific functional and/or biochemical properties of the collected genetic material will be analyzed. Therefore, the Project considers that this does not constitute utilization of genetic resources. More details are specified in the Implementation Plan.

11. The Project complies with (i) the FAIR Guiding Principles (which means data, metadata and products should be findable, accessible, interoperable and reproducible) and (ii) the IOC Recommendation on Open Science.

    a. All steps undertaken during sample and data analysis will be documented and published in open-access repositories such as IOC's Ocean Best Practices system (OBPS) and the OBIS GitHub channel.
    b. The data and information resulting from The Project will only be used for scientific purposes, i.e., publishing in the IOC's Ocean Biodiversity Information System (OBIS), the world's largest open-access data system on the distribution and diversity of marine species, and publishing the raw DNA sequences in public repositories (e.g., NCBI or ENA). IOC will ensure that the Partner is invited to nominate co-authors and/or contributing authors for a general publication, and indicate the agencies that should be mentioned in the acknowledgements.
    c. All data and information will be licensed with an open-access Creative Commons (**CC BY 4.0**) license, which allows anyone to copy, redistribute, and make use of the data, while ensuring proper attribution to the data creators (the project and its partners).
    d. If, for legitimate reasons, such as for the protection of endangered species, the location information of species will be generalized, rather than providing the exact location. Such data will be shared free of charge to the local management of the respective marine site.
    e. All data resulting from the sampling campaign will be published as a stand-alone dataset in the IOC's Ocean Biodiversity Information System (OBIS) and will hold a unique and persistent Digital Object Identifier (DOI) and a dataset citation, which includes the names of the people responsible for the expedition, including the local Partners. The dataset citation should provide proper credit to those who are involved in the Project.
    f. With the permission of the Partner, IOC will commit the samples for long-term storage called a 'biobank', but the samples will remain in the ownership of the Partner. With the explicit consent of the Partner, the samples can be analyzed further in the future for biodiversity research that will provide more data on important indicators for the sites. A separate agreement will be made between the Partner and the storage facility for the storage of the DNA material in a biobank.
    g. The Partner commits to engage with indigenous peoples conform with the United Nations Declaration on the Rights of Indigenous Peoples (UNDRIP) and the UNESCO policy on engaging with indigenous peoples.

By signing this form, the Partner confirms that it has obtained all necessary national approvals or permits to join this Project with the terms stipulated above.

Signature and stamp:	Signed by: (name of person and position)	Date:

[4] Some countries apply a strict policy for the sampling and shipping of genetic resources. The Partner commits to handle, process and ship samples conforming to relevant legislation and permits.

Annex 3 Template of the eDNA expeditions grant of rights

eDNA Expeditions (2026-2028) Project grant of rights

Whereas IOC intends to publish a volume and/or communication materials for print and/or for web or social media distribution, provisionally entitled

“Promotion of the work of IOC and the marine sites participating in the project eDNA Expeditions 2026-2028, via brochures, printed publications, online, and social media”
(hereinafter called the ‘Publication’) including

[Pictures and video footage from [SITE NAME] [site type, e.g. World Heritage site/marine protected area]]
(hereinafter called the ‘Work’)

by

[NAME OF CONTRIBUTOR]
the copyright to which is owned by
[NAME OF COPYRIGHT HOLDER]
(hereinafter called the ‘Owner’),

And whereas, in accordance with IOC publishing policy, all IOC publications and communications materials are to be made available in Open Access under the Creative Commons or any other open licensing system,

1. The Owner of the Work grants to IOC:
    a. The worldwide non-exclusive right for the whole term of copyright, to reproduce, translate, adapt, publish, perform, broadcast and communicate to the public, in any language and for all future editions and revisions, in printed and electronic format, the whole or any part of the Work in the Publication, and to authorize other publishers or co-publishers to exercise any or all of these rights.
    b. The worldwide irrevocable right to deposit the whole or any part of the Work in its multilingual Open Access Repository or other official archives in electronic form. This entails the right of access to copy, usage, distribution, and adaptation, for lawful purposes, within specified constraints.
    c. The right to use the Work in other IOC and UNESCO communications, promotional materials, and institutional publications related to ocean biodiversity, marine conservation, and IOC’s broader mission, beyond the scope of the Publication defined above.

2. The granting of rights as specified in Paragraph 1 bears no impact upon the moral rights vested with the author, save as otherwise expressly provided.

3. Permission is granted to IOC free of charge.

4. The following credit shall be given (Credit line/Form of acknowledgment):
   [CONTRIBUTOR NAME] / eDNA Expeditions 2026-2028 / IOC

5. The permission is granted on the understanding that the Publication or communication material, including its electronic version as maintained in the Open Access Repository, or other official archives, may be distributed free of charge by IOC or its publishing partners or commercialized within IOC’s own distribution channels or those of its publishing partners.

6. The Owner of the Work certifies that:
    a. They are the sole copyright-holder of the Work and have full power to make this Agreement and to authorize the use of the Work as set forth in Paragraph 1 above.
    b. The Work is original to them.
    c. The Work is in no way a violation or an infringement of any existing copyright or any other right granted to any other publisher and contains nothing otherwise unlawful.

7. The Owner of the Work shall:
    a. For all instances, categorically and clearly identify material(s) in the Work (e.g. texts, illustrations, tables, charts et cetera) where they do not own the copyright, and will:
        i. Expressly state in writing their inability, if any, to authorize use of such materials in the context of the Work as set forth in Paragraph 1 above; or
        ii. Provide IOC, free of charge, with written permissions (using this permission agreement form) secured from rights holders of such material(s) specifically authorizing its usage, as set forth above.
    b. For all instances of inclusion of third-party material(s), provide IOC with a copy of the written permissions obtained from the respective copyright holders.

8. The Owner agrees to indemnify IOC and hold it harmless against all loss, injury or damage (including any legal costs and/or other expenses properly incurred) occasioned to IOC in consequence of any breach of the above warranty.

9. Any dispute, controversy, or claim arising out of or in connection with the granting of the rights foreseen herein shall, unless it is settled amicably, be settled by arbitration in accordance with the United Nations Commission on International Trade Law (UNCITRAL) Arbitration Rules in force.

The Owner: Name:

Date: Institution / Affiliation:

Signature: Address: Email:

Annex 4 Project data policy

eDNA Expeditions (2026-2028) Project data policy

Preamble

The project partners underline the importance of timely, free and unrestricted international sharing of biodiversity data and metadata for the preservation of life, as well as for the advancement of scientific understanding that makes this possible.

The project partners agree with the FAIR Guiding Principles, which means data, metadata and products should be findable, accessible, interoperable and reproducible.

The project partners agree that the data and information resulting from the environmental DNA expeditions will be used for scientific purposes.

Purpose

The purpose of this data policy is to outline the requirements with respect to data sharing, access, preservation, and attribution to facilitate the broad use and reuse of data and information.

Conditions of use

To comply with the FAIR Guiding Principles the data and metadata will be:

Quality controlled, standardized following Darwin Core, archived in and published via the IOC's Ocean Biodiversity Information System (OBIS) before the end of the project (i.e. 31 December 2028).
Licenced under the CC BY 4.0 license, ensures proper attribution and allows others to copy, distribute and make use of the data.
Each dataset will hold a Digital Object Identifier (DOI) and a dataset citation.
All data and metadata will be made available with minimal restrictions unless for legitimate reasons such as for the protection of endangered species. In that case the location information could be generalized, rather than providing the exact location.
Raw DNA sequences will be submitted to public repositories (e.g. INSDC).

Definitions

Project partners: IOC, the environmental DNA lab and the marine sites or their partners which have signed the Collaboration Consent Form.

Annex 5 Template of Project Image and video consent form

eDNA Expeditions (2026-2028) Image and video consent form

About this form

eDNA Expeditions 2026-2028 is a project powered by the Intergovernmental Oceanographic Commission (IOC) of UNESCO's Ocean Biodiversity Information System (OBIS), and supported by Minderoo Foundation, in collaboration with Wilderlab. As part of the project, photos and video footage may be taken during sampling activities, community events, field work, and other project-related activities. These materials may be used to promote the project and the work of participating sites.

Consent

I, the undersigned, consent to the use of photographs and/or video footage in which I appear, taken in the context of eDNA Expeditions 2026-2028, by IOC for the following purposes:
Promotion of eDNA Expeditions 2026-2028 via printed publications, brochures, online platforms, and social media
Educational and institutional communications by IOC and UNESCO related to ocean biodiversity monitoring
Archiving in UNESCO's Open Access Repository or other official UNESCO archives

I understand that:
My image may be reproduced, adapted, and distributed worldwide, in printed and electronic format, free of charge

My name and/or affiliation may be included as a caption or credit if relevant.
I may withdraw this consent at any time by written request, with effect from the date of withdrawal, but this will not affect uses already made of the material.
No financial compensation is provided for this consent.

For minors (under 18)
If the person depicted is under 18, this form must be signed by a parent or legal guardian.

Name of minor:

Relationship to minor:

Signature: Date:

Name: Institution / Affiliation (if applicable):

Email (if you wish to receive a copy):

Contact
edna@ioc-unesco.org

ednaexpeditions.org