

## Engaging Communities to Safeguard Ocean Life

### UNESCO Environmental DNA Expeditions

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### SHORT SUMMARY

# Citizens can help protect ocean biodiversity

As climate change heats the ocean, marine conservation is hindered by how little is known about the effects of warming on biodiversity. There is simply not enough data to act on.

To begin addressing this, UNESCO's Environmental DNA (eDNA) Expeditions inventoried marine biodiversity at 21 of UNESCO's 51 World Heritage marine sites. These are good starting points—the 51 sites host over one third of the world's vulnerable and endangered marine species, a fifth of the world's blue carbon, and at least 15% of the global surface area of coral reefs. Yet most of the sites are threatened by climate change.

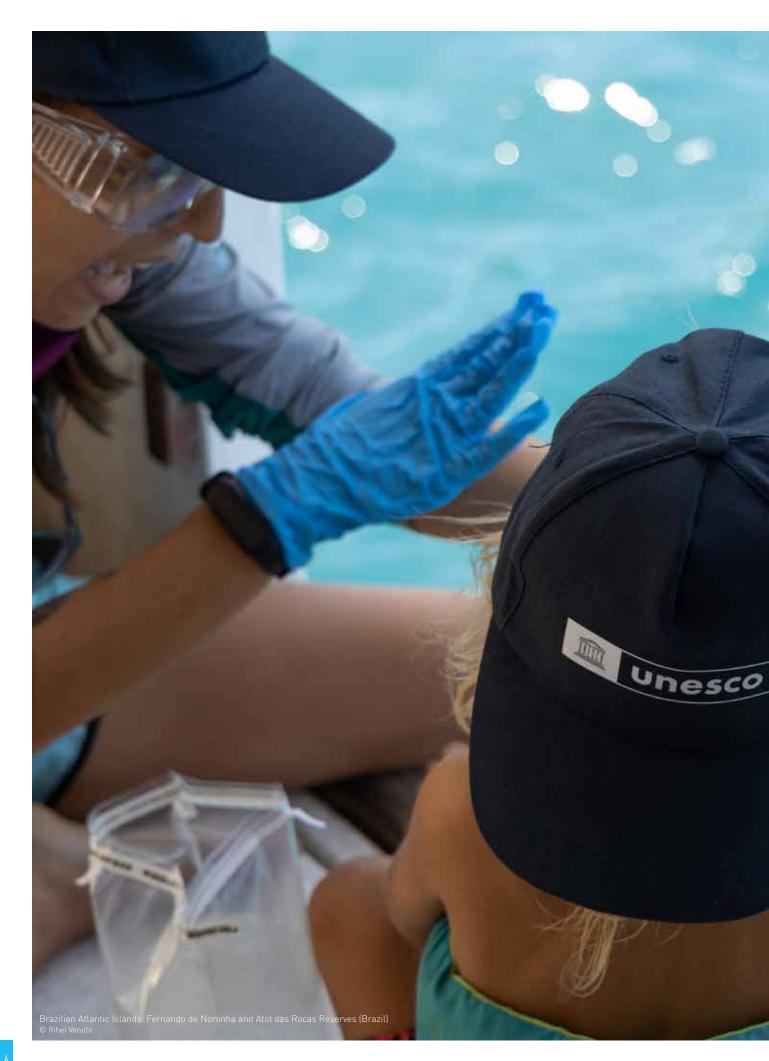


eDNA Expeditions' 250 volunteers – ages 6 and up, from 19 countries – identified over 4,000 species. Their data suggest that warming oceans risk pushing many species outside of their known thermal ranges, raising key questions about how to protect them.

eDNA monitoring is part of the solution. As a powerful, cost-effective method that can be conducted by thousands of citizen scientists, eDNA monitoring can provide the data needed to support science-based decision making. It can also revolutionize the world's understanding of marine biodiversity – and inspire the next generation of ocean researchers.



"Since wars begin in the minds of men and women it is in the minds of men and women that the defences of peace must be constructed"



# Marine Sentinels in a Changing Ocean

Given their broad distribution across the world, the UNESCO World Heritage marine sites act as proxies for the global network of over 18,000 marine protected areas.

UNESCO World Heritage marine sites are critical refuges for global biodiversity.

They host about 35% of all IUCN Red Listed threatened marine species<sup>1</sup> and are important for their globally unique ecosystems, such as seagrasses and coral reefs, which contribute to the overall health of the planet's ocean, landmasses, and atmosphere. They are inscribed on UNESCO's World Heritage List because of their irreplaceable biodiversity, ecosystem functioning, exceptional natural beauty, or for representing major stages of the Earth's history. They also cover over one fifth of the world's blue carbon ecosystems and host at least 15% of the world's coral reef ecosystems by surface area. But over 70% of these sites are currently threatened by climate change, according to the 2020 International Union for the Conservation of Nature (IUCN) World Heritage Outlook<sup>2</sup>.

Given their broad distribution across the world, the UNESCO World Heritage marine sites act as proxies for the global network of over 18,000 marine protected areas (MPAs). MPAs are essential refuges for fish and other marine life. In fact, although MPAs account for just 9% of the world's ocean, 50% of all marine species and 72% of all threatened marine species have been detected within MPAs, according to the Intergovernmental Oceanographic Commission (IOC) State of the Ocean Report<sup>3</sup>.

Still, only a small fraction of these species' home ranges falls within MPAs. A key question therefore is whether the current MPA boundaries can successfully protect these species from extinction. MPAs may well need to be enlarged or shifted –perhaps significantly– to accommodate increased pressures such as shifting habitats as the ocean continues to warm.

UNESCO World Heritage marine sites are sentinels of the changes that are affecting marine ecosystems worldwide. By monitoring and studying biodiversity in UNESCO World Heritage marine sites, data can be gathered and insights drawn to help nations predict, adapt to, and mitigate the impact of climate change on marine life worldwide. Such research can also inform conservation strategies and policies to help nations preserve marine biodiversity.

- https://worldheritageoutlook.iucn.org/
- https://unesdoc.unesco.org/ark:/48223/pf0000390054.locale=en

<sup>&</sup>quot;Threatened species" are defined as all species on the IUCN Red List, which includes threatened, endangered, and critically endangered species.

## UNESCO eDNA Expeditions

By deploying this innovative approach, UNESCO eDNA Expeditions aims to contribute to a baseline of marine biodiversity that can be revisited to track biodiversity changes over time.

From September 2022 to July 2023, eDNA sampling campaigns were conducted across 21 UNESCO World Heritage marine sites. These sites were carefully selected to represent a diverse range of marine environments and geographic locations across the globe (Figure 1).

The goals of UNESCO eDNA Expeditions were as follows:

- Provide an initial snapshot of fish biodiversity (and of other priority species) in UNESCO World Heritage marine sites, using a shared eDNA methodology.
- Test and refine a simple eDNA sampling protocol for citizen scientists and demonstrate its use in biodiversity monitoring across various environmental and socioeconomic settings.

- Promote open science by making all data, tools, and protocols freely accessible for global use.
- Estimate climate change impacts by identifying species' thermal limits and assessing their vulnerability to rising temperatures.

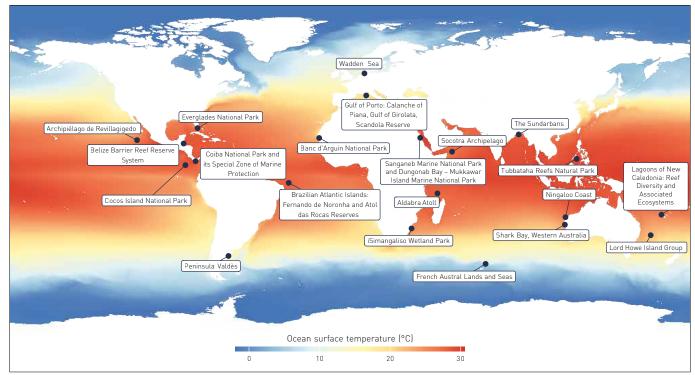
By deploying this innovative approach, UNESCO eDNA Expeditions aims to contribute to a baseline of marine biodiversity that can be revisited to track biodiversity changes over time. Through this citizen science initiative, over 250 young volunteers from 19 countries were trained and involved to collect samples, providing hands-on learning in marine conservation science.

The initiative is the world's first global use case for detecting ocean biodiversity with citizen-science using shared eDNA collection approaches.



Learn more about eDNA Expeditions

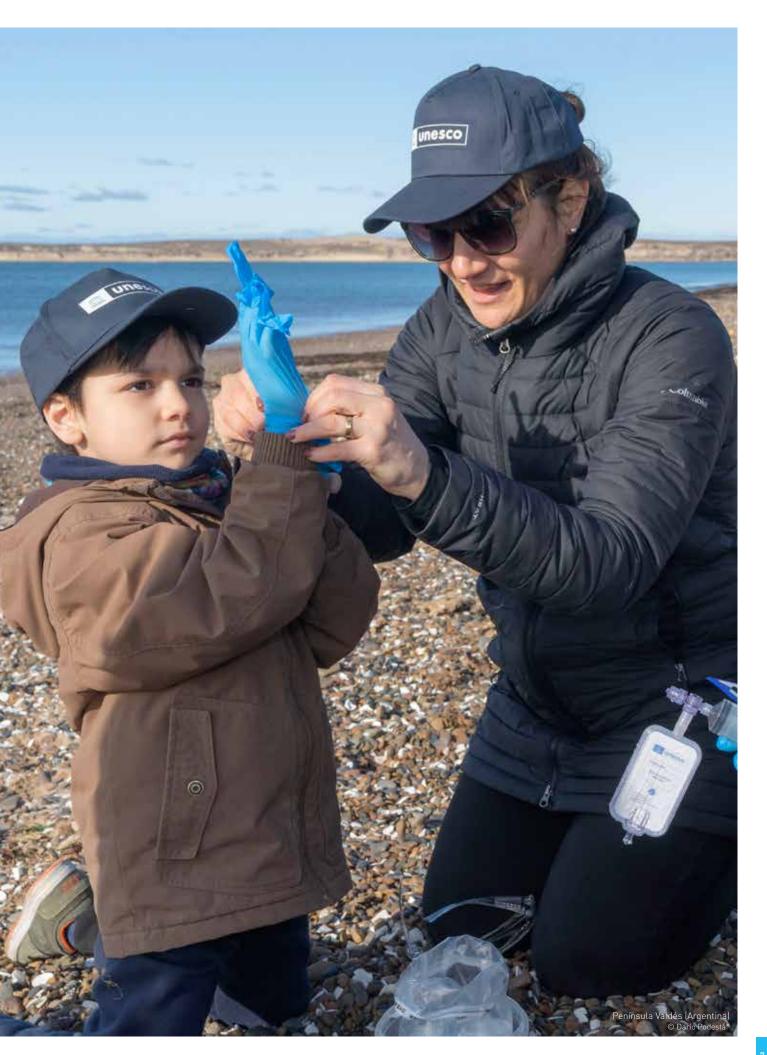
https://www.unesco.org/en/edna-expeditions?hub=66910



#### Figure 1. Locations of the 21 UNESCO World Heritage marine sites participating in UNESCO eDNA Expeditions.

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### **eDNA Sampling:** Standardized Biodiversity Surveys That Can Be Done by Anyone

#### The method is non-invasive and can provide a close to real-time snapshot of biodiversity.

All organisms naturally leave DNA traces in the environment, through skin cells, saliva, and excrement. Filtering and extracting DNA from environmental samples—for example, filtered seawater— enables the identification of species that inhabit the area without having to directly observe, collect, or harm them.

Environmental DNA (eDNA) sampling can provide a quick, easy, and comparatively cost-effective picture of biodiversity from across the tree of life, making it feasible to sample with higher taxonomic resolution more frequently in more remote areas. It can trace even transient organisms passing through an area, or species at life stages that are hard to visually identify, without the need for direct human observation. The method is non-invasive and can provide a close to real-time snapshot of biodiversity in a given area, making it an invaluable tool for monitoring marine life in a rapidly changing world.

eDNA methodology cannot replace critical traditional survey methods, which provide invaluable information on species abundances, sizes, life stages, and disease status, but eDNA sampling requires less effort in the sampling and has less impact on the environment. While eDNA analysis still requires specialized lab work, the sampling process is simple enough for volunteers and citizen scientists to participate in, contributing valuable data on a global scale and enabling sampling in more marine areas, and with higher resolution.





https://www.unesco.org/en/edna-training-materials/

#### From water to data–eDNA sampling essentials

UNESCO eDNA Expeditions relied on a sampling technique simple enough for children as young as six years of age to use with support from adults. Over 250 young students were engaged at 21 World Heritage marine sites across 19 countries. Sampling was conducted under the supervision of local science teams trained by UNESCO. Training was conducted with only video calls, a training video explaining the sampling protocol and a training booklet and infographic that were provided in the kits to bring along in the sampling campaigns. Sampling manuals were provided in six languages for the local sampling campaigns.

Complete eDNA sampling kits—including gloves, safety goggles, syringe, filter, waterproof labels, Longmire's buffer (preservation liquid), thermometer, beaker, telescopic rod, and sterile sample bags—were sent to each participating UNESCO World Heritage marine site. Each sampling kit contained all required materials for sampling. Cross-contamination between samples is thus avoided by utilizing clean materials for each sample.

Surface seawater was collected in a bottle immersed about 30 cm below the surface, using the rod, as far from land as possible but in water no deeper than 15 m. Each kit was used to filter a 1.5 L water sample. The water sample was left to stand, allowing any sediment to settle, before being pumped through the filter using the syringe. To avoid contamination from human DNA, protective gear was worn, and filtering was done within a closed system, minimizing any contact with potential sources of contamination. Finally, the remaining water in the filter was removed and replaced by a preservation buffer to minimize the degradation of DNA. Likewise to avoid the degradation of DNA molecules, samples were kept at room temperature and shielded from sunlight at all times. The preserved filters were then sent to a central laboratory for analysis.

At the lab, eDNA was extracted, purified, and amplified using polymerase chain reaction (PCR), a technique that creates many copies of specific DNA segments. These amplified DNA fragments were sequenced, analysed with bioinformatics, and then compared to public reference databases for species identification. To help confirm the results, the identified species were cross-checked with previous species records from the sites, which are available in two global biodiversity databases, Ocean Biodiversity Information System (OBIS) and Global Biodiversity Information Facility (GBIF).

## A Revelation of Fascinating Marine Creatures

#### Among the 4,000 different marine species identified, 120 were threatened marine species featured on the IUCN Red List.

UNESCO eDNA Expeditions provides a snapshot of biodiversity on a global scale, focusing on fishes, mammals and other marine life, many of which are on the IUCN Red List of Threatened Species.

Not less than 432 samples from 20 Heritage marine sites<sup>4</sup> were shipped back. Of these, 396<sup>5</sup> samples were successfully sequenced and analyzed, resulting in the identification of over 4,000 marine species, nearly half of them fishes.

Species were identified by sequencing and analyzing four different chromosome locations (known as loci) that can be used for taxonomic identification from each eDNA sample. These yielded over 600 million sequences in total—an average of 1.5 million sequences per sample. Among these, nearly 450,000 sequences were unique, differing by at least one nucleotide<sup>6</sup>. The number of unique sequences per sample averaged nearly 6,000, although this varied widely, ranging from fewer than 100 to over 10,000 unique sequences per sample. These sequences could be matched to unique species if their genetic information for the marker was present in public reference databases.

Among the 4,000 different marine species identified, 120 were threatened marine species featured on the IUCN Red List, of which 75 marine species were classified as vulnerable, 29 as endangered, and 16 as critically endangered. The initiative also detected rare species: for example, the Whitespotted Guitarfish (*Rhynchobatus australiae* Whitley, 1939) in Australia's Ningaloo Coast and Shark Bay, Western Australia World Heritage sites; the Giant Guitarfish (*Rhynchobatus djiddensis* (Forsskål, 1775)) in iSimangaliso Wetland Park World Heritage site in South Africa; and Commerson's dolphin (*Cephalorhynchus commersonii* (Lacépède, 1804)) in France's French Austral Lands and Seas World Heritage site. This single-campaign sampling initiative detected approximately 10% to 20% of the local fauna, as recorded in public databases such as UNESCO-IOC's Ocean Biodiversity Information System (OBIS) and the Global Biodiversity Information Facility (GBIF). This is an astounding achievement, since obtaining this amount of information could take many years of traditional surveying, and may cost millions of dollars (U.S.). It is all the more remarkable an achievement given the limited number of samples, short timeframe, and brief training given to the sampling communities.

#### Iconic species across World Heritage marine sites

The initiative discovered exceptional species across the World Heritage marine sites, including those critical to the sites' Oustanding Universal Value (OUV)<sup>7</sup> status. Among them are:

- The Multibarred triplefin (*Axoclinus multicinctus* Allen & Robertson, 1992) and the Socorro wrasse (*Halichoeres insularis* Allen & Robertson, 1992), two fish species endemic to the Archipiélago de Revillagigedo World Heritage site (Mexico) region. These isolated and relatively pristine islands have supported the evolution of a great number of land and marine species that are unique to the area (endemic). The waters surrounding these islands contain large congregations of sharks, rays, cetaceans, turtles, and fishes, many of which are endemic or near-endemic.
- The southern right whale (*Eubalaena australis* (Desmoulins, 1822)) was detected in the Península Valdés World Heritage site in Argentina. More than 1,500 southern right whales visit these critical breeding grounds. Conservation efforts in Península Valdés are playing a critical role in this whale's recovery from the severe depredations of commercial whaling—an encouraging success story in preservation.
- The Irrawady dolphin (Orcaella brevirostris Owen in Gray, 1866) was detected in The Sundarbans World Heritage Site in Bangladesh, which is part of the largest mangrove ecosystem in the world and crucial for the conservation of this endangered species. The Sundarbans is home to a subpopulation of around 451 individuals, representing one of the last strongholds for the species. Despite severe declines in other areas of its traditional habitat, the Irrawady dolphin thrives in this unique ecosystem, where it continues to be preserved.

<sup>4</sup> Samples from Sanganeb Atoll and Dungonab Bay - Mukkawar Islands National Park World Heritage site in Sudan did not arrive at the laboratory because of the war that broke out a few days after sampling.

<sup>5</sup> Of the 432 total samples, 36 were either controls or did not contain amplifiable DNA.

<sup>6</sup> There are two concepts in sequencing: sequences' - the genetic code of the fragment - and 'reads' - the number of times each genetic code is detected from the sample. Many unique sequences are detected from the sample more than once; these duplications are recorded in the read count, and are why -450,000 of the 1.5 million sequences were unique.

<sup>7 &</sup>quot;Outstanding Universal Value" means cultural and/or natural significance which is so exceptional as to transcend national boundaries and to be of common importance for present and future generations of all humanity." Operational Guidelines for the Implementation of the World Heritage Convention (WHC.23/01 - 24 September 2023).



#### "In the future, environmental DNA might help us to better detect invasive lionfish and prevent them from devastating our World Heritage site."

**Ms. Carla Cristina de Castro Guaitanele,** Chief, Fernando de Noronha Marine National Park (Brazil)

eDNA sampling has many different applications, including early warning of the presence of invasive species, allowing local management teams to take decisive action before they become overabundant and irreversibly damage existing ecosystems.

As an example, UNESCO eDNA Expeditions sampling in Belize successfully confirmed the presence of invasive lionfish at the Belize Barrier Reef Reserve System World Heritage site. Although lionfish are already well established in the Caribbean Sea, this documented sensitivity of eDNA monitoring makes it a promising tool for tracking their spread. Indeed, this potential was recognized by local management at the Brazilian Atlantic Islands: Fernando de Noronha and Atol das Rocas World Heritage site.

Recognizing the limitations of eDNA sampling (see separate discussion, on page 14), the project's design included cross-checking all identified species with local management and science teams to filter out potential errors.

Despite the challenges inherent in any global study of this kind, the project clearly demonstrated the potential of eDNA as a powerful tool for global-scale biodiversity monitoring. With increased sampling effort—either by collecting more samples or by sampling across many points in time—eDNA can provide comprehensive biodiversity assessments that can be repeated over time to establish baselines and time series, including in remote locations and areas with very few resources.

#### eDNA's Promise in Protecting Red List Species

Because they are non-invasive, eDNA-based observations are highly suited to conservation science. eDNA sampling appears equally effective for threatened and non-threatened species. Indeed, preliminary analysis reveals similar ratios between eDNA observations and occurrence records in OBIS and GBIF for all the IUCN Red List categories. And, as stated above, the UNESCO eDNA initiative detected a remarkable 10% to 20% of local marine fauna in one set of samples.

But eDNA's success in detection varies considerably between taxonomic groups. Among its systematic biases, the current eDNA analyses did not detect members of the family Carcharhinidae (Requiem sharks) known to be found at the sites. This includes the oceanic whitetip shark (*Carcharhinus longimanus (Poey, 1861)*), the Pondicherry shark (*Carcharhinus hemiodon* (Müller & Henle, 1839)), and the Ganges shark (*Glyphis gangeticus* (Müller & Henle, 1839)). The oceanic whitetip shark's preference for offshore habitat and the extreme rarity of the other two species likely explains their low eDNA detection rate.

Most new and confirmatory<sup>8</sup> eDNA observations of endangered species across the UNESCO World Heritage marine sites belong to three families: giant guitarfishes (Glaucostegidae), wedgefishes (Rhinidae), and hammerhead sharks (Sphyrnidae). The frequent detection of wedgefishes and hammerhead sharks with eDNA reflects their occurrence at many UNESCO World Heritage marine sites, and their detection usually confirmed earlier visual observations of the same species. In contrast, eDNA provided new observations and showed a high confirmation rate for giant guitarfishes.

These mesopredators play a crucial role in their ecosystems, consuming a significant portion of benthic biomass production and serving as prey for apex predators such as large sharks<sup>o</sup>.

Unfortunately, their fins and meat are commercially valuable, exceeding the average price of shark fins in some cases<sup>10</sup>. Their preference for shallow habitats makes them susceptible to overexploitation by bottom trawling, gillnetting, and longlining<sup>11</sup>. In recent decades, unsustainable fishing practices have also contributed to their population decline<sup>12</sup>. Identifying the exact species within the family of giant guitarfishes and related families can be challenging<sup>13</sup>, which may also contribute to this observed decline. Both tendencies complicate efforts to monitor these species.

The findings of this study suggest that eDNA-based observations hold promise for the conservation of giant guitarfishes and potentially other rhino ray (Rhinopristiformes) species. By enabling more high-resolution monitoring, eDNA sampling may be an effective tool in detecting rare and highly endangered species.

<sup>8</sup> Confirmatory species detections indicate detections of endangered species that have existing records in the OBIS and GBIF databases.

<sup>9</sup> Moore 2017.

<sup>10</sup> Ibid.

<sup>11</sup> White et al. 2013.

<sup>12</sup> Moore 2017.

<sup>13</sup> Jabado 2019





Discover the species identified in each World Heritage marine site https://dashboard.ednaexpeditions.org

#### A Pioneering Citizen-Science Portrait of Ocean Biodiversity

| <b>Q</b>   |                                      |   |
|--|--------------------------------------|---|
| Site name  | Country                              | Species identified  |
| Aldabra Atoll  | Seychelles                           | 836 species identified, including 390 fish species and 19 threatened species. |
| Archipelago de Revillagigedo   | Mexico                               | 402 species identified, including 182 fish species and 14 threatened species. |
| Banc d'Arguin National Park  | Mauritania                           | 266 species identified, including 112 fish species and 12 threatened species. |
| Belize Barrier Reef Reserve System   | Belize                               | 479 species identified, including 235 fish species and 19 threatened species. |
| Brazilian Atlantic Islands - Fernando<br>de Noronha and Atol das Rocas<br>Reserves | Brazil                               | 319 species identified, including 95 fish species and 6 threatened species.   |
| Cocos Islands National Park  | Costa Rica                           | 347 species identified, including 164 fish species and 14 threatened species. |
| Coiba National Park and its Special<br>Zone of Marine Protection                   | Panama                               | 546 species identified, including 257 fish species and 12 threatened species. |
| Everglades National Park   | USA                                  | 243 species identified, including 110 fish species and 10 threatened species. |
| French Austral Lands and Seas  | France                               | 203 species identified, including 41 fish species and 5 threatened species.   |
| Gulf of Porto: Calanche of Piana,<br>Gulf of Girolata, Scandola Reserve            | France                               | 149 species identified, including 57 fish species and 2 threatened species.   |
| iSimangaliso Wetland Park  | South Africa                         | 671 species identified, including 324 fish species and 19 threatened species. |
| Lagoons of New Caledonia: Reef<br>Diversity and Associated Ecosystems              | France                               | 847 species identified, including 418 fish species and 16 threatened species. |
| Lord Howe Island Group   | Australia                            | 522 species identified, including 163 fish species and 2 threatened species.  |
| Ningaloo Coast   | Australia                            | 787 species identified, including 340 fish species and 24 threatened species. |
| Península Valdés   | Argentina                            | 180 species identified, including 38 fish species and 6 threatened species.   |
| Shark Bay, Western Australia   | Australia                            | 286 species identified, including 148 fish species and 19 threatened species. |
| Socotra Archipelago  | Yemen                                | 260 species identified, including 139 fish species and 3 threatened species.  |
| The Sundarbans   | Bangladesh                           | 165 species identified, including 114 fish species and 8 threatened species.  |
| Tubbataha Reefs Natural Park   | The Philippines                      | 621 species identified, including 343 fish species and 10 threatened species. |
| Wadden Sea   | Denmark, Germany,<br>the Netherlands | 311 species identified, including 58 fish species and 1 threatened species.   |

# A High-Resolution Snapshot of **Biodiversity Change**

#### eDNA sampling is among the most sustainable, least harmful methods of monitoring biodiversity.

As a sampling method that is quick, easy, and sensitive to target species, eDNA is a powerful method for regularly monitoring biodiversity, yielding information on up to hundreds of species in a single small (1.5L) sample. Although eDNA is not a replacement for other monitoring methods, it complements and supports more targeted surveys using conventional methods.

The UNESCO eDNA Expeditions initiative confirms advantages of eDNA monitoring that are considerable for the future of marine biodiversity science, conservation, and monitoring:

**Large scale.** eDNA sampling allows for geographically broad surveys that seek an overview of current biodiversity.

**Powerful.** Just one liter of water can contain the DNA of hundreds of species, including common and hard-to-observe species, specifically:

- Small, elusive species that hide under rocks or corals or that bury themselves in sand, or only appear at night
- Juveniles of any species that are too small to see or difficult to identify by sight
- Open-ocean species passing overhead or just beyond an observer's or drone's view
- Benthic species, including deep-sea animals, that feed in surface waters at night

**Broader reach.** eDNA sampling can be done regardless of water conditions, from remote locations to cold, turbid environments with limited visibility. For example, eDNA sampling allowed UNESCO to survey such hard-to-reach World Heritage marine sites as the Austral Lands and Seas in the French subantarctic region, Socotra Archipelago in Yemen, and The Sundarbans in Bangladesh, all in a short timeframe.

**Cutting-edge technology:** Collection can be automated for remote collections and for more easily conducting time series samples. Further, as a relatively new research field there are ample possibilities for innovation and development, all of which can improve methodologies of future eDNA campaigns.

**Less biased.** eDNA monitoring may also reduce the bias implicit in other sampling methods, including species bias (e.g., undercounting elusive species; overlooking new or

lesser-known species) and spatial bias (e.g., excluding inadequately resourced areas).

**Repeatable.** eDNA sampling can be repeated at any frequency and any desired scale, with limited harmful impact.

**Reanalyzable.** The "dark" sequences that lack a taxonomic identification at the date of analysis can be re-analyzed years later, when the DNA reference libraries for the target region or ecosystem are more complete. This allows continual improvement of baseline studies and increased accuracy of biodiversity estimates and inferences over time.

**Accessible.** eDNA sampling equipment is easy to use, relatively easy to ship and distribute, and can thus be readily placed into the hands of citizen volunteers anywhere on the globe, at low cost.

**Cost-effective (in part).** By itself, eDNA sampling doesn't require expensive field equipment and can be performed from a small boat or from shore, making it cost-effective. It should be noted, however, that the full analysis of samples, including laboratory work, remains expensive.

**Ethical.** eDNA sampling is one of the most sustainable methods of monitoring biodiversity. In contrast, bottom trawling—a common method for surveying fish population over large areas, often used in fisheries management—is highly effective but very environmentally destructive. eDNA sampling, however, is environmentally harmless and is among the most comprehensive survey techniques. In one study, species diversity information from DNA in one liter of water was comparable to information produced from 66 million liters trawled by a net<sup>14</sup> — equivalent to 26 Olympic swimming pools.

Further, by empowering local citizens and science communities across the globe, the UNESCO eDNA Expeditions project had a remarkably low carbon footprint considering the amount of information collected across 21 sites in 19 countries.

**Complementary.** The large-scale, standardized baseline information provided by eDNA sampling also augments and strengthens other techniques such as visual, drone, and other survey methods.

**Engaging.** eDNA sampling's simplicity makes it generally well-suited for citizen scientists. And by directly engaging the public, eDNA monitoring grows public awareness and support for biodiversity.

<sup>14</sup> Stoeckle et al. 2021.

### **No Perfect Method:** The Limitations of eDNA Sampling

#### eDNA analysis records only the presence of species. eDNA does not capture the number, size, sex, or life stage of detected organisms.

Like all monitoring methods, eDNA sampling has its limitations. The reference data used for taxonomic (i.e., species) assignment of eDNA results remains incomplete. eDNA sampling is also subject to biases in detection and amplification. While sampling is easy, it can be prone to contamination. Additionally, eDNA analysis labs may not be nearby or easily accessible. Species may be incorrectly identified because of incomplete molecular barcode reference databases or limited resolution of the marker genes, making local species knowledge essential for proper validation of the eDNA results.

Furthermore, eDNA analysis records only the presence of species. eDNA does not capture the number, size, sex, or life stage of detected organisms. Certain techniques can be used to count the number of target DNA molecules in the sample, which can estimate species prevalence, but these methods are also highly dependent on local conditions.

The detection of eDNA can also be highly dependent on currents and DNA degradation rates in the water body sampled. Since eDNA is carried with the current, it is important to investigate those prior to sampling to ensure an accurate signal.

UNESCO eDNA Expeditions had several specific limitations. The number of sequences and species identified was highly dependent on local site conditions. Tropical sites with clear water tended to yield more species than those located outside the tropics, possibly reflecting real differences in overall species numbers. Turbid waters posed additional issues, resulting in the need to repeat the sampling at some locations such as the Wadden Sea World Heritage site (Denmark, Germany, Kingdom of the Netherlands). Improved sampling techniques—for example, adjusting the volume of water filtered or using pre-filtration based on local conditions—would likely further enhance results. Although the citizen scientists collected high quality samples, there was still substantial contamination from human DNA. Nevertheless, the measures taken during sampling were sufficient to ensure the eDNA signal from wildlife was not overwhelmed by human DNA contamination. Limiting the direct handling of sampled water—by directly attaching the filtering hose to the water collection instrument, for example—will help minimize such contamination in future citizen science campaigns.

A major limitation to eDNA studies is the absence of many species in DNA reference databases. Without these references, some sequences simply cannot be identified, as there is no reference DNA data to match the eDNA against. With a lack of comprehensive, global, open-science reference databases misidentifications can still happen to geographically distant but taxonomically closely related species.

As World Heritage marine sites contain high degrees of endemic species, it is critical that eDNA results are checked to ensure matches are to local species. Therefore, after an initial automated screening using known species distributions and thermal affinities, as well as the robustness of the molecular evidence, all species that resulted from the UNESCO eDNA Expeditions project were cross-checked with local management and science teams to filter out potential errors.

While eDNA sampling is often efficient and can be done frequently across different locations and times, its analysis is most useful for specific purposes in biodiversity monitoring.

#### A major limitation to eDNA studies is the absence of many species in DNA reference databases.

eDNA sampling is most useful for broad-scale surveys that provide an overview and—as intended by UNESCO eDNA Expeditions—for a biodiversity snapshot that identifies areas and issues requiring more focused monitoring. eDNA monitoring is one method in the analytical toolbox, and ideally is complemented by more traditional methods such as visual surveys.



## Promoting **Open Science**

The resulting data from eDNA studies are difficult to access, and the sampling and analysis protocols are often not widely shared. Both reduce the benefits to people and nations globally.

A longstanding challenge in biodiversity research, including eDNA studies, is the limited public availability of resulting data. Not only is much of the resulting data from eDNA studies difficult to access, but the sampling and analysis protocols are often not widely shared. Both reduce the benefits to people and nations globally.

As a firm proponent of open science for the benefit of all nations, UNESCO designed the eDNA Expeditions' initiative to be fully accessible and on-line, including:

- Sampling and analysis methodologies
- Sampling kits
- Training materials, videos, and instructions
- Site selection criteria
- All resulting survey data
- Bioinformatics pipeline<sup>15</sup> and reference libraries

UNESCO eDNA Expeditions referenced its survey data with the open-access Ocean Biodiversity Information System (OBIS), a programme component under the International Oceanographic Data and Information Exchange (IODE) of the IOC of UNESCO. Also, all resulting data is publicly available in OBIS (see sidebar).

A dedicated online platform (https://dashboard. ednaexpeditions.org) enables researchers, managers and all citizens of the planet to access the data for each participating site and jointly strengthen ocean protection for future generations.

#### **OBIS: A Gateway to the Ocean's Biodiversity**

OBIS is the world's largest open-access database of marine life observations. With contributions from 33 OBIS nodes worldwide, the network unites 1,000 institutions across 99 countries, providing more than 132 million records of nearly 200,000 marine species—from bacteria to whales. OBIS enables users to explore global marine biodiversity data from the ocean's surface to deep reaches, from the tropics to the poles.

OBIS is a global community collaborating with scientists to deliver open access to critical biodiversity data, supporting marine conservation and informed decision-making. Through maps and tools, OBIS allows researchers, policymakers, and the public to freely explore marine biodiversity data by species, geographic area, depth, timeframe, and more.

OBIS emanated from the Census of Marine Life (2000-2010) and was adopted by UNESCO's IOC in 2009. It is now a programme component of the IODE programme, furthering its mission to advance ocean knowledge and foster the protection of marine life for future generations.

OBIS serves as a vital and comprehensive platform for species detection, integrating sequence data with other biodiversity records for seamless comparisons across data types. By offering a complete workflow—from managing sampling campaign data to processing sequences through bioinformatics-OBIS transforms raw data into accessible insights. Dashboards developed by OBIS enable intuitive data discovery, helping researchers to explore and utilize biodiversity information. With eDNA Expeditions data, OBIS ensured robust quality control, using species distribution models to validate findings and improve accuracy of species assignments together with the local communities. Through these tools and processes, OBIS elevates the impact of biodiversity data, empowering researchers to deepen understanding of species distributions and respond to environmental challenges more effectively.

For eDNA Expeditions, OBIS not only served as the data publication platform but also developed tools for the bioinformatic processing, quality control, analysis and dissemination of eDNA data. Additionally, OBIS provided support in designing the field sampling and laboratory work.





https://www.unesco.org/en/edna-training-materials/



Discover all the results of our expeditions per site via our dashboard

https://dashboard.ednaexpeditions.org

# A Carefully Designed eDNA Sampling Methodology

UNESCO eDNA Expeditions was meticulously designed to enhance the world's understanding of global marine biodiversity. By using experimental protocols that are accessible to citizen scientists, including schoolchildren and indigenous peoples, UNESCO ensured that data collection was widespread and accessible, while also being scientifically consistent across all surveyed locations.

The sampling methods and protocols were developed collaboratively by UNESCO and an international scientific advisory board. This process helped ensure that the methods were both scientifically robust and adaptable to various skill levels and field conditions.

Sampling locations were chosen in collaboration with the managers of each site to reflect the characteristics of the OUV for which the site is inscribed on the UNESCO World Heritage List and protected by the global community for future generations. These OUVs often include rare species, unique habitats, and ecosystem functions that are globally significant. These carefully chosen locations therefore represent key areas for biodiversity within each site, allowing for consistent monitoring over time. To increase the potential for species detection, at each location four replicate samples were collected, resulting in a total of 20 samples per site. Considering the large geographical area and ecosystems covered by each site, the sampling campaigns were small and were not intended to capture the full biodiversity at each site. Instead, each campaign focused on critical areas for biodiversity and citizen engagement.

More than 250 young citizen scientists, mainly local schoolchildren, participated in the project across various marine sites. Under the guidance of local scientists, these young volunteers collected samples that were filtered and preserved on-site before being sent to a central lab for analysis (see eDNA sampling sidebar on page 9 for further information). Depending on the local conditions and available resources, samples were taken both from boats or from the shore. This hands-on approach provided students with an educational experience in conservation science while gathering valuable data on marine biodiversity.

Local scientific teams played a crucial role in verification by cross-referencing eDNA results with their knowledge of site-specific species diversity. This step was essential for identifying any potential misidentifications that could arise from incomplete reference databases or limitations in genetic marker resolution.



#### eDNA sampling in Aldabra Atoll World Heritage Site (Seychelles)

Located in the Indian Ocean, the remote and hard-to-access Aldabra Atoll has remained largely untouched by humans for most of its existence. The atoll was inscribed on the UNESCO World Heritage List in 1982 and hosts globally important breeding populations of endangered green turtles, the secondlargest frigatebird colony in the world, and an oceanic flamingo population (one of only two in the world).

From 31st March to 4th April 2023, four teachers and 11 Seychellois students aged 11 to 17 visited the Aldabra Atoll World Heritage site as part of their yearly eco-school programme, run by the Seychelles Ministry of Education and sponsored by the Seychelles Islands Foundation. Students collected water samples with guidance from local scientists at the Aldabra research station.

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"The application of ground-breaking technology in the Seychelles, involving local school kids, is an amazing opportunity that will show them the importance and potential of technology and science in protecting our natural World Heritage for the future."

**Dr Frauke Fleischer-Dogley,** CEO, Seychelles Island Foundation

The group collected water samples at five different stations across the site and filtered genetic material using the eDNA citizen-science sampling protocols and equipment provided by UNESCO. The samples were then preserved and shipped to a specialized lab for analysis.

UNESCO's eDNA sampling methodology was well-suited to this location, and its results reflected the site's exceptional biodiversity. The 20 samples produced approximately 30 million DNA sequences. Analysis yielded a dataset of almost 40,000 unique DNA sequences, ranging from 100 to 10,000 unique sequences in each sample. Over 800 marine species could be identified from these sequences, including almost 400 fish species and 13 shark species. One sample (1.5 L water) contained DNA from eight different shark species.



### **The Effects of Climate Change** on Biodiversity in World Heritage marine sites

As nations continue to struggle with carbon emissions and as ocean warming accelerates, marine biodiversity faces increasingly severe challenges. According to UNESCO's June 2024 State of the Ocean Report, "the ocean is now warming at twice the rate it was twenty years ago, with 2023 seeing among the highest increases since the 1950s."

The current trend for marine biodiversity is concerning. Sites in the tropical and subtropical ocean are expected to be most affected by the impact of climate change, while the temperate ocean will experience a range of different impacts. Combining the eDNA results from the project with data records available on OBIS and GBIF, UNESCO evaluated the proportion of species at each site that could soon face temperatures beyond their known tolerance limits. These limits are defined as the temperature range across all locations where the species were recorded, excluding the top 5% of higher temperatures. Under the warmest future climate scenario (SSP5-8.5, corresponding to a best estimate projected warming of 4.4°C by the end of the century)<sup>16</sup>, up to 100% of fish species in tropical and subtropical sites would be beyond their current thermal ranges and potentially at risk, while up to 25% of fish

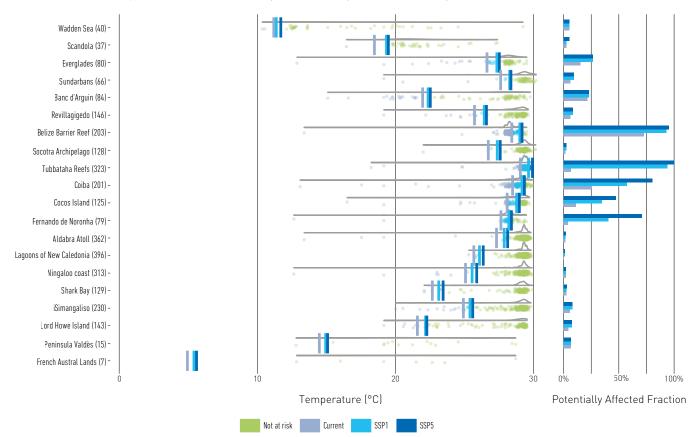
species in temperate ocean waters would exceed their current thermal ranges.

It is not clearly understood today how well species will adapt to warming. As ocean temperatures rise, species that cannot adapt or migrate face the risk of extinction, leading to a potential loss of marine biodiversity, or shifts in biodiversity, in affected areas.

This risk of biodiversity shift is greatest in the tropical and subtropical regions, where species already live at the upper thermal limits of the ocean's temperature range. As shown in Figure 2, World Heritage marine sites in warmer waters have a much greater proportion of potentially vulnerable species than sites in temperate regions. Predicting how these species will respond to unfamiliar temperature extremes is challenging, as none have ever experienced similar conditions. Although some species may have higher thermal tolerances and could potentially adapt, others may not be able to adjust quickly enough. Even those with some adaptive capacity may experience increased stress, which can affect their survival and reproductive success.

<sup>16</sup> Shared Socio-economic Pathways (SSPs), which are climate projections developed by the Intergovernmental Panel on Climate Change (IPCC). IPCC, 2023: Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, H. Lee and J. Romero (eds.]]. IPCC, Geneva, Switzerland, 184 pp., doi: 10.59327/IPCC/AR6-9789291691647.

**Figure 2:** Distribution of upper temperature limits for species detected by eDNA sampling across UNESCO World Heritage marine sites, sorted by latitude (Northern Hemisphere at the top, Southern Hemisphere at the bottom).



#### © UNESCO

Upper edge temperatures for each species (equivalent to 95% of its global thermal range) are shown by circles: colored blue for those species where local average sea surface temperatures exceed upper edge temperatures, and thereby more vulnerable to warming, and green for those whose upper edge temperatures are higher than local averages, and thereby not at risk of warming. Effects on the proportion of species vulnerable to warming under the increased temperatures of future climate scenarios SSP1-1.9 and SSP5-8.5 are shown by the shift to the right of average temperatures of sites (vertical bars). Horizontal bars on the right show the Potentially Vulnerable Fraction of species under each future warming scenario. The numbers of species evaluated for each site are included in brackets after each site name.





## Implications for Marine Protected Areas

As ocean temperatures continue to rise, two critical questions emerge:

(1) where should new MPAs be established to protect crucial areas of ocean biodiversity?

(2) How should new and existing MPAs be monitored and protected under such fastchanging conditions?

Ocean managers must be prepared to rethink MPA boundaries not once but continually, as long as ocean temperatures continue to vary. Actions may include:

- **Establishing better biodiversity baselines**, to monitor changes over time and detect potential risks to species diversity.
- Enlarging existing MPAs to create refuges for species that are widespread, or will be relocated because of changing temperatures: in other words, accommodate "climate refugees." Distribution maps of current and future species ranges based on biodiversity baselines and niche models could provide guidance for the design of MPAs.
- Connecting MPAs, to form networks that facilitate safe migration, help maintain genetic diversity, and support species under climate stress. Connectivity modeling is an actionable analysis for assessing such connections and can be a powerful decision-making tool in marine conservation and management.
- **Frequent and adaptive monitoring** of MPAs to provide timely data for decision-making as local and regional conditions evolve.
- Embracing adaptive management by using data to make more informed decisions and establishing monitoring and feedback loops to continually improve management actions and arrangements.
- **Enhancing ecosystem resilience** by reducing local pressures such as overfishing, pollution, and habitat destruction, offering the best chance to maintain biodiversity in this era of rapid environmental change.

As UNESCO eDNA Expeditions has demonstrated, eDNA monitoring provides a powerful, cost-effective approach to tracking biodiversity. By engaging volunteers worldwide, eDNA offers real-time insights that can guide conservation planning and adaptive management, ultimately helping to protect marine biodiversity in an era of rapid environmental change.

# The Critical Role of eDNA in a **Rapidly Changing Ocean**

The expected transformation in ocean biodiversity over the coming decades will require proactive monitoring and management. As species migrate and habitats shift, it will be essential to adjust MPAs to ensure they continue to provide safe havens for marine life.

Meeting the Kunming-Montreal Global Biodiversity Framework goal of protecting 30% of the ocean by 2030 will require selecting key areas for new MPAs and continually monitoring existing ones. Well-managed MPAs enhance the resilience of marine ecosystems, helping to mitigate the impacts of further warming.

eDNA monitoring can play many important roles in this highly active form of management. First, by quickly identifying critical habitats and tracking biodiversity changes, eDNA enables science-based decisions on where to establish MPAs, supporting resilient marine ecosystems in a warming world.

Second, eDNA sampling can enable near-real-time monitoring of biodiversity, providing crucial data for adaptive management in response to environmental changes. These may include changing existing MPA boundaries, rapidly address invasive species or create connected MPAs. eDNA is a cost-effective and efficient way to gather data on a large scale, enabling timely and informed decisions.

eDNA analysis offers a unique opportunity to gain detailed insights into biodiversity, helping to understand ecosystems as a whole by using biodiversity indexes based on ecosystem management. Both the GRI 101: Biodiversity 2024 and the EU Corporate Sustainability Reporting Directive (CSRD)<sup>17</sup> have called for more corporate biodiversity reporting—and both eDNA data and these indexes can support sites in monitoring their biodiversity impact, enabling better tracking of anthropogenic activities on biodiversity.

Open science practices provide the best guarantee that eDNA data is accessible and comparable across different projects and borders, facilitating global collaboration in biodiversity conservation. The sharing of open-source data will lead to a comprehensive understanding of marine ecosystems and will support efforts to protect them effectively. By enhancing transparency and enabling collaboration, open data can also drive scientific innovation.



### The Sundarbans World Heritage Site (Bangladesh)

The Sundarbans was inscribed on the UNESCO World Heritage List in 1997 for its immense tidal mangrove forests—the world's largest—and its exceptional biodiversity. It is the only mangrove habitat in the world for the Bengal tiger (*Panthera tigris tigris*).

In January 2023, an eDNA Expeditions team from Bangladesh visited five locations—each reflecting comparative ecosystems—in the Sundarbans West Wildlife Sanctuary, one of three wildlife sanctuaries in The Sundarbans UNESCO World Heritage area. After a brief training session, this team of primary school students ages 8 to 12, university-level students, and young teachers took water samples and preserved the eDNA using sampling kits provided by UNESCO.

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"eDNA could transform the way we monitor and protect marine biodiversity in the Sundarbans. UNESCO's initiative is critical to help ensure developing nations have access to the latest innovation and technologies."

**Prof. Dr. Kazi Ahsan Habib,** Sher-e-Bangla Agricultural University in Bangladesh

Almost 20 million sequences were produced from 20 collected samples, of which almost 15,000 unique sequences were identified, for a per-sample average of 3,500 unique sequences. From these sequences, 165 unique species were identified (a per-sample average of approximately 60 species). Of these species, a total of 114 were categorized as fishes. Several interesting species were identified, including the guitarfish (see sidebar on page 11) and the Irrawaddy dolphin, an endangered river dolphin.

When combined with ocean warming data, these results will provide insights on how climate change may influence the distribution of fish species in the Sundarbans.

<sup>17</sup> GRI 101: Biodiversity 2024 is a standard by the Global Reporting Initiative (GRI) that sets guidelines for companies around the world to report on their biodiversity impacts, while the EU Corporate Sustainability Reporting Directive is an EU regulation requiring businesses to disclose more detailed environmental, social, and governance information, including biodiversity-related data, to enhance transparency and alignment with EU sustainability objectives.



## **Calling All Citizens:** Everyone Has a Stake and Everyone Can Take Action

The threat of climate change to ocean biodiversity is a defining challenge of today's generations. The consequences of a warming ocean affect everyone. The task of monitoring the ocean and bringing together the science that is needed to make sound decisions is as large as the ocean itself. And the changes are happening rapidly.

Everyone can play a part in addressing it, from policymakers to citizens. Engaging the public in citizen science projects like eDNA sampling fosters a sense of responsibility and involvement in conservation efforts. It can shift the world's mindset from passive concern to confident agency. eDNA monitoring won't stop ocean warming. But it can help us raise awareness and manage our pristine sites for the best possible outcome for ocean biodiversity-and for the global well-being that ocean biodiversity provides by providing unprecedented levels of information.

#### lt's Your Turn

UNESCO eDNA Expeditions has set a baseline of marine biodiversity data. But the real potential of eDNA monitoring lies in repeated sampling. That is where you can help.

To find out more about how your community, organization, or institution can assist in eDNA monitoring, contact your nearest UNESCO World Heritage marine site.

### Engaging Communities to Safeguard Ocean Life

### UNESCO Environmental DNA Expeditions

Ocean warming is reducing marine biodiversity, with changes observed all over the world. What does this mean for life on our planet, and what can we do to protect it? To protect ocean biodiversity, decisions must be based on sound science. That requires inventorying and monitoring the ocean. And because 70% of the planet's surface and 95% of the biosphere are ocean, we need all hands-on deck.

UNESCO's citizen science eDNA Expeditions span 21 World Heritage marine sites around the world. This groundbreaking initiative demonstrates that people of every nation and of all ages can help document the ocean's rich biodiversity and the changes already underway.

This report describes UNESCO's first global eDNA initiative, methods, and findings, including the many fascinating species identified. It includes on-the-ground stories of citizen science, inspiring readers to get involved in science and contribute to safeguarding our planet. Regular eDNA monitoring as described in this report can provide the critical information needed to actively manage and protect marine biodiversity—and help reach the global target of protecting 30% of the ocean by 2030.

For more information: https://www.unesco.org/en/edna-expeditions



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