

ENVIRONMENTAL DNA STUDIES HAVE THE POTENTIAL TO ADVANCE DEEP-SEA BIODIVERSITY KNOWLEDGE

Introduction

In addition to regulating and controlling activities in the international seabed area (the Area), the International Seabed Authority (ISA) is mandated by the United Nations Convention on the Law of the Sea (UNCLOS) to promote the conduct of marine scientific research and coordinate and disseminate the results of such research (UNCLOS, Article 143). The importance of this mandate is highlighted in the Strategic Plan of ISA for the period 2019-2025.¹

In 2020, the ISA Assembly adopted the Action Plan for Marine Scientific Research in support of the United Nations Decade of Ocean Science for Sustainable Development (MSR Action Plan) to fulfil these mandates under UNCLOS.² One of the six strategic research priorities identified in the MSR Action Plan is focused on standardizing and innovating methodologies for deep-sea biodiversity assessment in the Area, including taxonomic identification and description.

Recent scientific reports have highlighted advances towards significant environmental DNA (eDNA) as a noncost-effective destructive. and easily replicable methodology for studying deepsea biodiversity (Barnes and Turner 2015; Govindarajan et al. 2022; Laroche et al. 2020). Nonetheless, many concerns have been raised regarding consistency across surveys and the absence of comprehensive genetic reference catalogues, which are critical in identifying organisms and matching the genetic sequences.

This policy brief aims to (1) describe eDNA methods, highlighting some of the main challenges and opportunities for effectively using eDNA approaches in deep-sea biodiversity assessments and (2) identify the role of ISA in addressing scientific/technical challenges in deep-sea eDNA research.

What is environmental DNA?

Environmental DNA (eDNA) is the genetic material recovered from environmental samples (water, sediment, soil, rocks, plants, etc.) without the need for prior isolation of the individual organisms (Stewart, 2019). When studying microorganisms, eDNA can represent the biodiversity inhabiting an environmental sample. Or, it can be used to assess the DNA "left behind" by larger organisms because DNA particles are released in the marine environment from skin, mucous, sperm, eggs, excretes and decomposing bodies, forming the bulk eDNA (Bohmann et al. 2014). In eDNA approaches, environmental samples containing DNA particles from various organisms are processed to extract the "DNA soup."

Using DNA sequencing technologies and cutting-edge bioinformatic methods, scientists can assess the genetic information present in eDNA samples (Figure 1). By targeting regions in the genomes with unique sequences for each organism (DNA barcodes) and comparing with information available in public databases, it is possible to assign taxonomy (i.e. organism's name and classification) to the DNA particles found in the environmental sample and identify

 $^{^1}$ ISA. 2018. Decision of the Assembly of the International Seabed Authority relating to the strategic plan of the Authority for the period 2019–2023 (ISBA/24/A/10). Available at: https://www.isa.org.jm/wp-content/uploads/2022/06/isba24_a10-en.pdf.

² International Seabed Authority. 2021. Action Plan for Marine Scientific Research in support of the UN Decade of Ocean Science for Sustainable Development. Available at: https://www.isa.org.jm/wp-content/uploads/2022/07/ISA_MSR_Action_Plan.pdf.

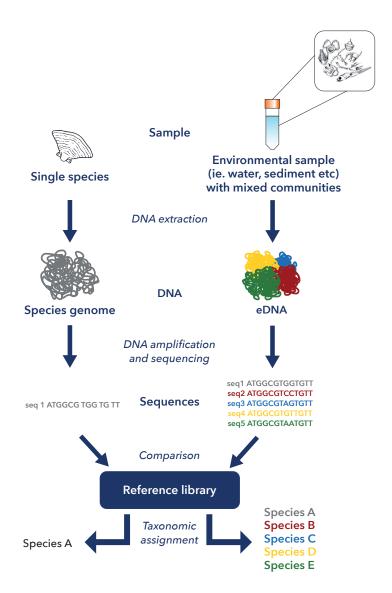


Figure 1. DNA barcoding (left) uses a short and standardized region of the genomic DNA (DNA barcode) to identify an organism. DNA metabarcoding (right) uses DNA barcodes to assess the biodiversity in eDNA samples

Source: adapted from Corell and Rodríguez-Ezpelata (2014)

multiple species in parallel. This approach is called DNA metabarcoding (Pawlowski et al. 2022).

Challenges and opportunities in using eDNA tools in deep-sea biodiversity assessments

Considering the challenges in sampling marine fauna, particularly from the deep sea (>200 m depth), using eDNA presents new opportunities for marine biodiversity research. This non-invasive approach is particularly valuable to conduct large-scale biodiversity assessments with greater

efficiency than traditional methods, identify new species, including those that are rare, elusive or difficult to observe directly and monitor changes in marine populations over time to understand their vulnerability to natural and human disturbances. With the recent joint efforts of the scientific community, the use of eDNA for marine faunal research is becoming more feasible and it is contributing to assessing biodiversity even in the most remote areas of the deep sea (Adams et al. 2023; Le et al. 2022; Liu et al. 2022). However, several challenges need to be addressed to use eDNA in deep-sea biodiversity assessments efficiently.

The low representation of deep-sea species in public DNA databases (Laroche et al. 2020) is one of the main challenges in eDNA-focused research for the deep sea. The identification of species using eDNA relies on comparing the DNA particles sampled with public databases containing reference DNA sequences of known taxonomic origin. Box 1 shows more information on sampling and data flow in deep-sea biodiversity assessments using eDNA. The biodiversity coverage of the database is crucial in this process, as the use of incomplete databases may lead to failure in the identification task. Reliable biodiversity assessments using eDNA demand curated reference libraries that associate barcodes with voucher specimens (individuals of a species collected and preserved) morphologically identified by an expert (Gold et al. 2022).

Using eDNA in deep-sea research also demands standardization of methods and benchmarks. DNA studies often comprise several steps with an array of methodologies available, including different sampling approaches, DNA extraction protocols, target genes, sequencing platforms and bioinformatic pipelines. Definitions of these methodologies affect the range of biodiversity captured and data interoperability. However, optimizations in the eDNA approach and comparisons with conventional methodologies are needed to ensure they can efficiently assess and quantify deep-sea biodiversity (Aylagas et al. 2020).

Environmental DNA has also been suggested as a promising tool for marine environmental management and monitoring programmes (Gold et al. 2022). While characterizing ecosystems during baseline studies, collecting specimens for morphological and genetic identification and long-term archives in biobanks is critical. In parallel, adopting eDNA tools during baseline studies can contribute to an improved characterization of biodiversity. Moreover, the eDNA data collected during baseline studies can be used for setting standards for monitoring efforts. In long-term monitoring programmes, eDNA could be used as a routine tool to track changes in biodiversity and monitor indicator species. Together with other methods, eDNA could contribute to enhancing our knowledge of deep-sea biodiversity unprecedentedly and provide valuable scientific evidence to support the development and implementation of effective policies and regulations.

In addition to the application in biodiversity assessments and taxonomy surveys, eDNA can further be used to investigate genetic information related to functional traits harboured by the organism inhabiting certain areas. Such an approach can provide insights into the ecological roles of species and ecosystem functioning (Alneberg et al. 2020), as well as potential marine genetic resources (Rabone et al. 2019).

Box 1. Sampling and data flow in deep-sea biodiversity assessments using eDNA

During deep-seasampling expeditions, pelagic and benthic samples for eDNA analysis can be obtained using different methodologies. The figure below illustrates sampling procedures for specimens and eDNA sample acquisition and processing (solid lines).

Taxa coverage in the databases is crucial to successfully assigning taxonomy to DNA sequences derived from eDNA samples (dotted lines).

In microbial surveys, eDNA tools have undergone extensive improvement and are widely applied to tackle different research questions. Increasing application and standardization are seen in micro- and meiofauna research (Hug et al. 2016). The use of eDNA to assess larger faunal groups is less developed and requires high investments in research and standardization. These differences in current use, applicability and standardization across the major groups of organisms are illustrated in the gradient bar.

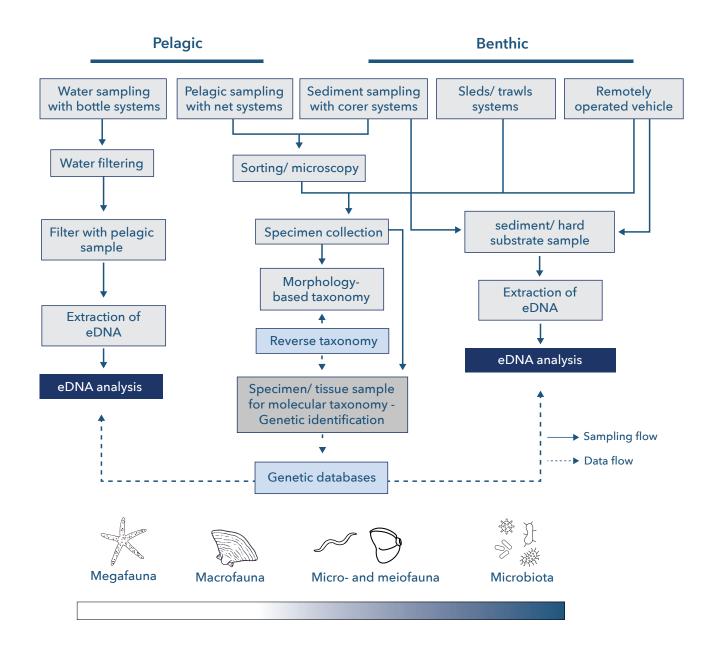


Figure 2. Schematic workflow for sample acquisition and taxonomy assignment in eDNA-based deep-sea research

The importance of eDNA studies for effective management

eDNA could represent an important source of data on occurrences, abundance and distribution of species in the Area, allowing an evidence-based approach to the development, implementation and update of regional environmental management plans in regions under mineral exploration. Genetic data is also essential to decipher the connectivity between populations, a key

concept to applying area-based management tools in the context of regional environmental management plans and modelling the impacts of deep-sea mineral exploration and future exploitation. Robust methodologies and innovative approaches are also demanded for environmental impact assessments in the Area. In addition, eDNA could provide cost-effective, easily replicable and scalable means to monitor harmful effects that may arise from deep-seabed-related activities in the Area in environmental monitoring plans.

How can ISA contribute to addressing challenges in eDNA deep-sea research as part of its contribution to the MSR Action Plan?

Supporting knowledge enhancement

In 2022, ISA launched the Sustainable Seabed Knowledge Initiative (SSKI) as a collaborative platform to advance the knowledge of deepsea biodiversity.3 SSKI aims to promote access to scientific knowledge in line with ISA's mandate. The first of five SSKI's components, aims to describe a minimum of 1,000 deepsea species new to science by 2030. SSKI will support advancing public knowledge on deep-sea biodiversity through different activities, including funding taxonomy projects, convening expert workshops and creating a registry of taxonomic expertise and biocollections. SSKI will enable a significant enhancement in the number of species described with their associated genetic information (DNA barcode). This will expand the coverage of deep-sea species in public genetic databases, enabling more efficient use of eDNA tools.

Promoting data standardization and sharing

Several initiatives carried out under ISA's mandate contribute to promoting the standardization of deep-sea data obtained in areas under exploration for mineral resources, including workshops and national and regional scientific research programmes. Such initiatives are valuable for compiling the best available scientific information and techniques to inform the work of the ISA Legal and Technical Commission, including the development and review of recommendations to ISA contractors for the collection of

environmental baseline data.⁴ In this regard, ISA has convened a workshop called "Enhancing genetic approaches to advance deep-sea taxonomy" in Seocheon, Republic of Korea, in November 2022.⁵

ISA can also facilitate contractors' access to best practices (e.g. standardized operational procedures) for sampling, processing and reporting biological data. Integrating data generated for contract areas with other deepsea data can be facilitated by applying standard operational procedures and following the FAIR data principles.

In 2019, ISA launched DeepData, a global open-access ocean data management system designed to share biological and non-confidential environmental collected in the Area to promote effective data management and sharing.6 ISA also became the first United Nations System organization to become a node of the Ocean Biodiversity Information System to support the free exchange of the information generated for deep-sea biodiversity in the Area. As of December 2023, scientific surveys carried out as part of baseline studies and environmental assessment in areas under mineral exploration have led to over 137,000 records of species occurrence in the ISA DeepData database. Regarding genetic data, it is of great interest to create mechanisms to integrate the information contained in DeepData with the existing DNA data repositories. This would allow easier access to the taxonomic identification of organisms in the Area.

Supporting capacity development

Using eDNA in deep-sea research and exploration demands high investments in human and technological capacity. The fast-paced developments in DNA research, from

³ ISA. Sustainable Seabed Knowledge Initiative. Available at: https://www.isa.org.jm/sski.

⁴ ISA. 2020. Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area (ISBA/25/LTC/6/Rev.1). Available at: https://www.isa.org.jm/wp-content/uploads/2022/06/25ltc-6-rev1-en.pdf.

⁵ ISA. 2022. Workshop on Enhancing Genetic Approaches to Advance Deep-Sea Taxonomy. Available at: https://www.isa.org.jm/events/workshop-on-enhancing-genetic-approaches-to-advance-deep-sea-taxonomy.

⁶ ISA. DeepData Database. Available at: https://www.isa.org.jm/deepdata-database.

⁷ ISA. 2022. Capacity development strategy of the International Seabed Authority (ISBA/27/A/5). Available at: https://www.isa.org.jm/wp-content/uploads/2022/12/ISBA_27_A_5-2209799E.pdf.

sample acquisition to laboratory analysis and data processing, pose numerous challenges to less-equipped institutions, mainly in developing States. ISA can contribute strengthening capacity-building development, promoting access to and transfer of technology and fostering joint technology development in developing States and ioint scientific research programmes for the conservation and sustainable use of marine biodiversity. The ISA Capacity Development Strategy comprises individual development, institutional development and development that results from the enabling environment at the national level.⁷ In this regard, the SSKI platform will establish training/mentoring programmes in taxonomy, bioinformatics and artificial intelligence tools. It will also equip centres with appropriate facilities to support sample processing for DNA analysis, which will bring together deep-sea regional researchers to share knowledge and tools and build scientific partnerships. These efforts are in line with the second (establishing and furthering strategic partnerships in support of capacity development) and third (strengthening institutional capacities through technology transfer and technical assistance) key areas of the ISA's Capacity Development Strategy.

Conclusions

DNA tools are transforming marine science. eDNA is at the forefront of marine scientific and technological advances.

Collaboration between scientists, policymakers and the private sector is essential to ensure that eDNA research is used effectively to protect the marine environment and promote sustainable ocean governance. At the centre of the global deep-sea research agenda, ISA plays a critical role in coordinating and promoting synergies between exploration and scientific programmes through the inclusive and transparent implementation of the MSR Action Plan.

Continued research and development of eDNA tools and other innovative remote

sensing technologies would allow an enhanced understanding of the deep sea and contribute to ensuring its resources are managed responsibly for the benefit of all.

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ABOUT THE INTERNATIONAL SEABED AUTHORITY

Made up of 168 Member States, and the European Union, ISA is mandated under the UN Convention on the Law of the Sea to organize, regulate and control all mineral-related activities in the international seabed area for the benefit of mankind as a whole. In so doing, ISA has the duty to ensure the effective protection of the marine environment from harmful effects that may arise from deep-seabed related activities.