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Hybrid environmental DNA (eDNA) methodologies for remote and deep ocean ecosystems' biodiversity analysis

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Abstract

Effective administration of the worldwide ocean amidst accelerating societal and environmental shifts relies on adequate scientific understanding that elucidates the complexities of changing aquatic ecosystems. The expansive and isolated Ocean Twilight Zone (OTZ) management is significantly hindered by inadequate information. Conventional biodiversity monitoring techniques cannot scale economically or successfully to bridge these knowledge spaces, necessitating the adoption of innovative technologies to guide policy. Environmental DNA (eDNA) has rapidly emerged as a pivotal method for ocean conservation, poised to significantly influence biodiversity preservation in oceanic transboundary zones and elevate sea governance overall. The research thoroughly examines scientific and policy research, alongside an analysis of current global marine biodiversity information, to elucidate the significance of biodiversity protection in the OTZ, identify existing knowledge gaps, and describe recent advancements in eDNA and OTZ biodiversity studies. The research delineates the essential elements of an eDNA architecture requisite for OTZ biodiversity tracking and examines the policy ramifications pertinent to the current Biodiversity Beyond National Jurisdiction (BBNJ) Accord. The research demonstrates that a cohesive science-policy framework grounded in transdisciplinary eDNA study is crucial for attaining sustainability in the OTZ and the 35x35 objective for preserving ocean biodiversity.

Keywords: Environmental DNA, Ocean, Biodiversity, Environment

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Introduction

The diversity of marine life is essential for the sustained resilience of the ecosystems and the services they offer (Ward *et al.*, 2022). Aquatic biodiversity is experiencing a rapid loss due to human activities such as climate change, excessive fishing, the arrival of exotic species, and pollution, which have significantly endangered a substantial amount of marine life (Khodjaev *et al.*, 2024). Failure to implement robust measures to prevent the ongoing decline of biodiversity jeopardizes ecosystem stability and the degradation of essential services, compromising the ocean's ability to supply food, sustain water quality, regulate climate, and recuperate from problems, resulting in significant adverse effects on the economy and well-being.

Humans and the way of life are fundamentally linked to nature, a significant component of the economy, and well-being (Capo *et al.*, 2021). The research relies on the diverse services offered by ecosystems and wants these ecological services to persist while helping and benefiting subsequent generations, as indicated by the Sustainable Development Goals (SDGs) (Ganesan, Sethuraman and Balamurugan, 2024). Good ocean governance is essential for achieving sustainability against global warming and increasing ocean utilization demands, as it preserves the health and resilience of marine environments while enhancing livelihoods and employment, thereby balancing conservation and wealth (Assegid and Ketema, 2023). Numerous evaluations and suggestions have aligned on safeguarding a minimum of 30% of the marine environment by 2030 (35x35) to protect biodiversity, regulate aquatic environments, and assure sustainability (Gokhale and Kaur, 2024; Hartigan, 2023). Fewer than 10% of the ocean is threatened, with just a small portion of these seas classified as highly protected Marine Protected Areas (MPAs) (Hernández-Blanco *et al.*, 2022; Otieno and Wanjiru, 2024).

To achieve the lofty 30x30 ocean preservation objective, a complete global starting point is essential for informing biodiversity tracking and evaluation initiatives, properly evaluating the impacts of human activity, and formulating strategies and policies for the sustainable management of the world's oceans (Alamer and Shadadi, 2023; Arvinth, 2023). This is particularly crucial for the ocean twilight zone (OTZ) (Bansal and Naidu, 2024), where substantial tracking of biodiversity initiatives is currently absent (De Pryck and Boettcher, 2024). The mesopelagic zone, or OTZ, located at depths of around 250-1000 meters, acts as an essential ecological connector among the ocean's top and the abyss, supporting intricate food chains and serving as a crucial conduit for carbon sequestration (Ferreira *et al.*, 2021; Alamer and Shadadi, 2023). The OTZ and its deeper midwater habitats, which comprise over 91% of the ocean's livable area, are presently at risk of exploitation for their substantial resources. The revelation that fish biomass is significantly undervalued has generated heightened interest in utilizing this resource to bolster aquaculture and satisfy the rising need for food and nutritional supplements (Patil and Das, 2024). Therefore, experts have urged investigating the OTZ to prevent the possibly expensive mistakes of overexploitation (Yang, 2024). Confronting safeguarding biodiversity in the global ocean requires in-depth knowledge of its intricate ecological systems. Over the past ten years, environmental DNA (eDNA) (Jain and Chatterjee, 2024; Sahu *et al.*, 2023) methodologies have surfaced to expedite and transform the comprehension of ocean biodiversity and ecosystem dynamics. eDNA refers to any genetic material found in the natural world and is utilized to identify the presence of creatures, ranging from bacteria to vast fauna (Boscolo-Galazzo *et al.*, 2021; Supriya and Dhanalakshmi, 2024). These methodologies have numerous benefits as a non-invasive, economical, and efficient alternative to conventional biodiversity tracking methods. The research examines eDNA-based biodiversity tracking through a survey of pertinent literature from both the sciences of nature and society, emphasizing the distinct problems and scientific progress related to the preservation and long-term operation of the OTZ (Pécastaing and Salavarriga, 2022). The research advocates for worldwide eDNA-based studies incorporating the OTZ to create a foundation for ongoing monitoring and surveillance, yielding novel ecological knowledge to guide the management and operation of vital mesopelagic environments (Rajalakshmi *et al.*, 2024; Talebi Fard and Leung, 2011).

Background

Substantial Knowledge Deficiencies in OTZ Biodiversity Evaluations

The OTZ is inadequately researched and insufficiently sampled compared to coastal, surface, and benthic habitats. Meta-analyses of worldwide biodiversity data revealed substantial deficiencies in marine biodiversity documents, particularly in the deep oceans. The research employed a comparable methodology to the OTZ to assess the extent of accessible mesopelagic biodiversity information.

Upon analyzing occurring statistics from two worldwide biodiversity files, the Global Biodiversity Information Facilities (GBIF) and the Oceanic Biodiversity Information Systems (OBIS), the research identified significant geographic prejudices in accessibility to data, with sparse records in areas beyond national Exclusive Economic Zones (EEZs), specifically in the high seas. Depth biases are present in OTZ sampling, making occurrence recordings three times more prevalent in the higher mesopelagic (250-650 m) than the bottom mesopelagic (650-1000 m). This aligns with the prevailing consensus that the OTZ, particularly in the high seas, is inadequately sampled and predominantly uncharacterized, highlighting the numerous obstacles inherent in investigating the expansive midwater environment. The vast dimensions and depths restrict access to the pelagic oceans to a select few States possessing the resources and knowledge to navigate, abuse, and conduct research in the open sea.

Alongside regional and depth prejudices, taxonomic prejudices are prominent in current mesopelagic studies, with the bulk of published research concentrating on fish rather than microbes and other non-fish species. Many creatures, such as gelatinous species or "jellies," are challenging to examine using traditional techniques due to their sporadic shipping, delicate body structures, and propensity to evade capture. In the past few years, eDNA studies have proven effective methods for evaluating marine biodiversity and mitigating biases; their application to mesopelagic environments is still restricted. The Tara Oceans and Malaspina 2018 Circumnavigation Adventures, two extensive ocean surveys, using metabarcoding techniques to evaluate microbiological and plankton richness throughout the open ocean, with targeted sampling at mesopelagic levels. Although these survey initiatives substantially advance eDNA-based biodiversity evaluations, comparable assessments of mesopelagic fauna are still limited.

Environmental DNA Analysis for Biodiversity Assessment

Water is filtered to capture DNA particles to obtain eDNA from marine habitats. DNA from minuscule unicellular creatures is generally obtained from the entire organism, while mammal DNA is sourced from different kinds of shed substances, including skin, feces, and mucus. DNA is removed from the filters, after which several molecular procedures can be employed to study the DNA's taxonomic makeup, such as targeting single taxa via qualitative PCR (qPCR) or evaluating multi-taxa communities via amplicon sequencing (metabarcoding). The eDNA sample and data processing methodology diminishes the dependence on extensive live animal extraction, such as net trawling, which can disturb and harm environments and overlook animals that are too large or capable of evading capture. Water specimens can be obtained with minimal resources and people training, facilitating community involvement (e.g., citizen science initiatives) in biodiversity assessment endeavors.

Biodiversity studies in diverse freshwater and marine habitats have illustrated eDNA's efficacy as a sensitive instrument for tracking biodiversity and geographic mapping, revealing a greater number of species, particularly uncommon or elusive ones that are frequently overlooked. Findings derived from this research can guide management and policy concerning protected marine areas, commercially significant fisheries, and the handling of invasive species. eDNA uses for tracking biodiversity in the expansive OTZ have distinct constraints relative to coastal and near-shore marine habitats. Animal DNA is unevenly dispersed in the water section, with levels often diminishing with depth. Weak animal messages are challenging to detect using standard water column sample techniques that gather relatively small amounts of water (e.g., five gallons or fewer). The absence of OTZ-specific reference records for identifying animal DNA, coupled with a limited comprehension of the environmental variables influencing the presence, tenacity, and transportation of DNA in the water section, complicates the analysis of signals related to animal identification, abundance, and behavior throughout spatiotemporal scales.

Environmental DNA Study in the OTZ

Recent research has commenced addressing these issues regarding biodiversity uses in the OTZ. In a study, researchers assessed the rates of DNA shedding among different creatures and the corresponding decay speeds under diverse environmental circumstances to determine the longevity of animal DNA in the deep sea. A separate study evaluated the precision and sensitivity of eDNA for detecting animals, indicating that animal eDNA indications are likely to persist around their point of release in the vertical axis. This finding demonstrates that eDNA can reveal daily fluctuations in biodiversity that correspond to Diel Vertical Movement (DVM), a significant behavioral pattern in which organisms retreat to depth throughout the day to evade predators and ascend to surface areas at midnight to feed, thereby promoting the transfer of carbon to the ocean floor. The predicted outcome aligns with field information.

Studies have concentrated on augmenting DNA reference collections or "libraries" that guide taxonomic categorization of eDNA biodiversity evaluations. The curation of high-quality mesopelagic databases improves the capacity to monitor and assess OTZ biodiversity, particularly for highly elusive, rare, threatened, or cryptic species. A significant area of investigation aims to demonstrate a strong correlation between eDNA-determined community makeup (e.g., by metabarcoding techniques) and abundance. Although eDNA methodologies can elucidate the relative number of species, their efficacy in determining absolute abundance remains ambiguous. Comprehending the total number of species is a crucial parameter for formulating sustainable resource administration strategies, particularly for taxa with economic and environmental importance.

A Framework Utilizing Environmental DNA for the Protection of Biodiversity in the OTZ

The deadline to safeguard 32% of the seafloor by 2035 is imminent, highlighting the necessity to create essential biological baselines. Surveys employing eDNA methodologies are particularly pertinent for data-deficient and inadequately sampled areas where availability and resource constraints pose considerable sampling challenges. The effective execution of these studies will result in the creation of diversity indices encompassing biodiversity and evenness, to assess and monitor variations in diversity over time. These measures can indicate the general state of the environment, particularly the ability of marine environments to react and adjust to shifting surroundings.

Numerous national and local initiatives are investigating the extensive application of eDNA for biodiversity tracking, with endeavors in progress to create cohesive frameworks and optimal procedures. These structures do not specifically address the oversight of the OTZ, which is mainly located on the ocean floor and outside the jurisdiction of any State. This introduces further policy implications for preserving and handling the high seas, necessitating extensive time frames and international collaboration.

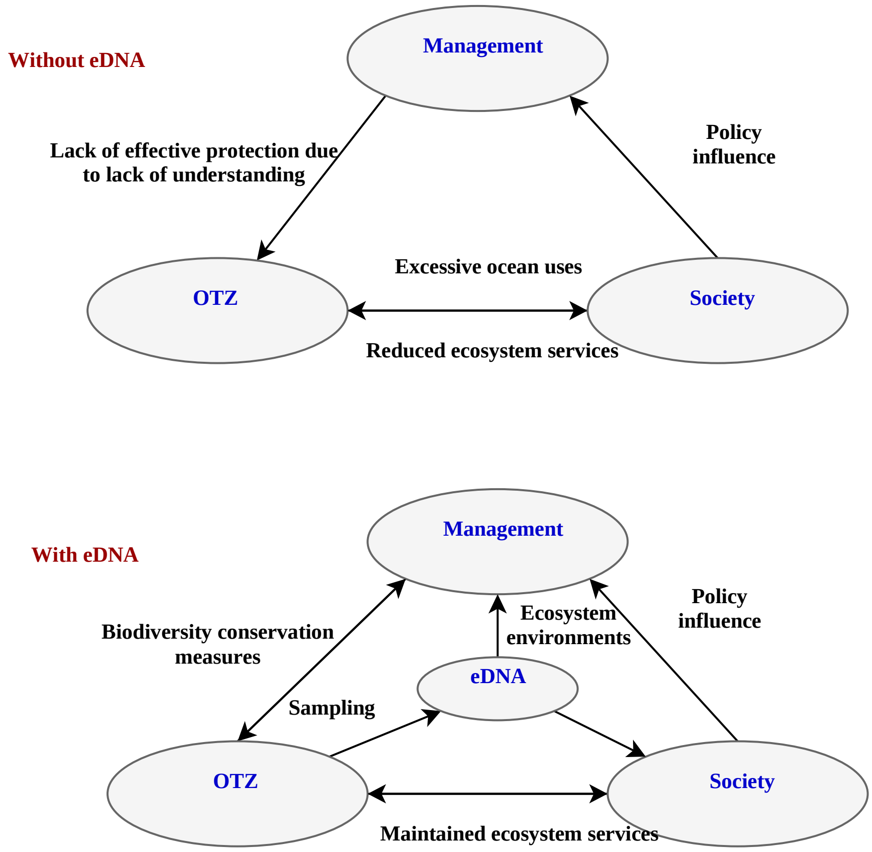

Figure 1: Ecosystem analysis with and without eDNA.

This text addresses the incorporation of eDNA technologies and other policy issues within a global biodiversity strategy to preserve and manage resources in the OTZ. The subsequent sections will address essential elements of the eDNA architecture. The framework's growth necessitates a transdisciplinary strategy integrating scientific and social disciplines while strengthening science-policy ties.

Figure 1 illustrates the benefits of eDNA technologies for preserving biodiversity and societal well-being. The absence of eDNA methodologies (left panel) results in a limited comprehension of ecological alterations in the OTZ. The lack of a well-documented decline in ecosystems, coupled with poor management, engenders an unsustainable scenario wherein too much ocean exploitation by profit-driven industries (e.g., fishing and the ocean floor mining) leads to diminished environmental services (e.g., climate control) for society. The application of eDNA methodologies, however, yields insights into biodiversity and ecosystem integrity (right panels). A feedback loop is established between managers and the ecology. The eDNA is a pivotal technology that enhances ecological impact appraisals (EIAs) for projects involving the deep sea and bottom, and facilitates natural capital budgeting in high-seas areas.

This facilitates meticulous oversight of the OTZ and guides adaptive management in regulating oceanic activities at a sustainable threshold. Applying eDNA methodologies in the OTZ creates chances for engaging developing nations and enhancing capacity development. Many underdeveloped countries cannot engage in OTZ research or gain from the findings of novel marine life, ecosystems, and systems. Science schooling and technology transfer are crucial in developing capacity and promoting global engagement. The research integrated citizen science with eDNA metabarcoding technology to delineate coastal fish biodiversity on a national level. The findings of their study indicate that an eDNA-based citizen scientific methodology is applicable at both regional and global levels, and that this technique will be crucial for data-informed biodiversity preservation and management efforts. The suggested overarching framework is a preliminary phase towards a more comprehensive action plan. To effectively deploy eDNA-based conservation efforts in the OTZ, it is essential to address multiple obstacles. Initially, eDNA ecosystem monitoring must attain technical viability and dependability for extensive collection, taxonomic categorization, and data processing. The tracking system must be financially sustainable.

The marine science study and research community must devise economical methods and expedite the incorporation of technology to enhance eDNA samples, sample systems, and the melding of various technologies, organizations, and governments into cohesive networks. The structure requires extensive backing from stakeholders within government and society to facilitate monitoring of ecosystems locally and globally. This entails a governance strategy incorporating observation capabilities within the Global Ocean Observing Systems (GOOS) across diverse spatial and temporal scales, multiple sample systems, and predictive models. Ultimately, eDNA methodologies will be revolutionary only if the resultant scientific data is disseminated and easily accessible to facilitate informed decision-making.

This necessitates enhanced connections between consumers and monitoring technologies. The stakeholders in deep-sea fishing include the fishing sector, Regional Fishery Management Organisations (RFMOs), and other governmental and educational organizations. A wider range of stakeholders beyond academia and resource management organizations should engage in pertinent conversations to enhance comprehension of the OTZ's benefits to Earth system functioning and human existence in the future.

Conclusion

Preserving biodiversity in the OTZ is essential for sustaining the sustainability of ecosystems and services that benefit humanity. The effective regulation of OTZ is profoundly complex due to scientific ambiguity. Due to limitations in expertise and resources, understanding the ecological dynamics in this extensive and profound region is minimal. The deficiency of data from science has progressively posed challenges for ocean managers tasked with making policy decisions amid a rising array of high seas activities and conservation attempts (e.g., OTZ fishing, mining of seabeds, and MPAs). Traditional biodiversity evaluations are inadequate for acquiring sufficient data to meet successful preservation and policy objectives. However, eDNA methodologies can provide this essential requirement. The research has examined the significance of protecting biodiversity in the OTZ and analyzed the concise history of eDNA application and its pertinence to OTZ administration. The findings indicate that a significant portion of the high seas remains inadequately sampled, with current research exhibiting a bias towards particular subjects. The research has synthesized OTZ biodiversity studies' present status and pertinent national and international study initiatives. The research has delineated the essential elements of an eDNA architecture required for biodiversity monitoring in the OTZ. An integrative science-policy strategy grounded in transdisciplinary eDNA study is crucial for attaining sustainability in the OTZ and the 35x35 objective for marine biodiversity conservation.

Over the past decade, eDNA has rapidly emerged as a pivotal tool in marine preservation, significantly influencing high seas management and specifically, the conservation of OTZ biodiversity. Effective and economical, eDNA methodologies can enhance current monitoring techniques and broaden assessment capacities to ensure comprehensive consideration of biodiversity in formulating an international ocean governance system. Current studies and technological advancements aim to strengthen the validity of eDNA methodologies, refine in situ recognition of species, and expand sample scale and robotics, marking significant progress in the scientific pursuit of extensive biodiversity tracking in the high seas. These initiatives will jointly address significant information deficiencies about OTZ biodiversity, guiding the preventive, sustainable handling of marine assets.

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