Biodiversity in the Ocean Twilight Zone

Knowledge for action on the new Agreement for Biodiversity Beyond National Jurisdiction

WOODS HOLE OCEANOGRAPHIC INSTITUTION

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The ocean twilight zone is a vast and dynamic ecosystem harboring unique biodiversity.

EXECUTIVE SUMMARY

Effective governance of the global ocean under accelerating environmental and social changes depends on scientific knowledge to unravel the complexity of dynamic ecosystems. In the case of the vast and remote ocean twilight zone—which circles the globe from 200 to 1,000 meters deep—resource management is particularly challenged by the lack of data. Traditional biodiversity monitoring methods, such as those based on net tows or trawls, are unable to scale efficiently or effectively to address these knowledge gaps and new technologies are needed to inform policy efforts. Environmental DNA (eDNA) analysis has quickly gained traction in recent years as an enabling technology for marine conservation that will play a transformative role in twilight zone biodiversity conservation and ocean governance, broadly. An integrated science-policy approach based on transdisciplinary eDNA research is essential for achieving sustainability in the twilight zone and the 30x30 target for conserving ocean biodiversity.

Illustration by Eric Taylor, ©WHOI Creative

KEY TAKEAWAYS

- The ocean twilight zone is a vast and dynamic ecosystem harboring **unique biodiversity** that is difficult to monitor across spatial and temporal scales.

- Biodiversity in the ocean twilight zone is **critical to ocean health** and ecosystem resilience and supports ecosystem services that benefit all of society.

- **Significant knowledge gaps** in marine biodiversity hinder effective decision-making for conservation and resource management.

- Environmental DNA, or eDNA, analysis is an **enabling technology for biodiversity monitoring** that is scalable and cost-effective.

- With the 30x30 conservation goal fast approaching, new tools and an accelerated research agenda are needed to **generate the necessary knowledge** that will inform conservation and sustainable management of ocean resources.

NEED FOR SCIENTIFIC KNOWLEDGE

Biodiversity, encompassing the composition and functionality of life on Earth, is foundational for preserving ecosystem services in the face of anthropogenic change. Marine organisms are increasingly vulnerable to human activities including climate change, overfishing, invasive species introductions, and pollution (IPBES, 2019). Below the ocean's surface, the twilight zone hosts a unique and diverse community of organisms from microscopic bacteria to top predators. This community creates a vital ecosystem link between the sunlit ocean and the deep sea, sustaining complex food webs and functioning as a critical pathway for carbon sequestration (Hoagland et al., 2019).

In recent years, growing interest in exploiting twilight zone fish biomass as a potential source of protein to support aquaculture growth and an increasing demand for nutraceuticals (such as fish oil and krill oil) have placed a target on midwater ecosystems (St. John et al., 2016). While scientists have made significant headway in understanding twilight zone biodiversity, much of this ecosystem remains unexplored and significant gaps in our knowledge present a challenge for policy makers to sustainably manage its resources.

The world has set a target of conserving 30% of the Earth's waters by the year 2030, an effort known as "30x30" (Kunming-Montreal Global Biodiversity Framework). With this target approaching fast, new tools are needed to unravel the mysteries of the vast twilight zone. In this report, we review the current state of twilight zone biodiversity knowledge and explore the application of new molecular tools towards the conservation and sustainable management of twilight zone biodiversity. Now more than ever, it is crucial we understand what lives in this remote ocean region to conserve biodiversity and reap its many co-benefits for society.

Nina Yang, WHOI postdoctoral investigator, prepares a multi-eDNA sample collector for deployment on the underwater vehicle *Mesobot*. *Mesobot* is a hybrid robot, specifically to study life in the ocean twilight zone. Photo by Nova West, courtesy of NOAA Ocean Exploration Cooperative Institute

WHAT IS THE OCEAN TWILIGHT ZONE?

The ocean's mesopelagic region, also known as the twilight zone, encompasses the ocean's midwater depths ranging from approximately 200 to 1,000 meters. The region is characterized by faint levels of sunlight too low to support photosynthesis, and so this life is reliant on biological production from the surface waters. As part of the biological carbon pump, life in the twilight zone is critically important in transferring organic material to the deep ocean.

The twilight zone extends across the global ocean beyond shallow continental shelves. Most of the twilight zone is located on the High Seas, but some mesopelagic regions may also be found within the 200-nautical-mile exclusive economic zones (EEZs) of many nations, including those of Small Island Developing States (below).

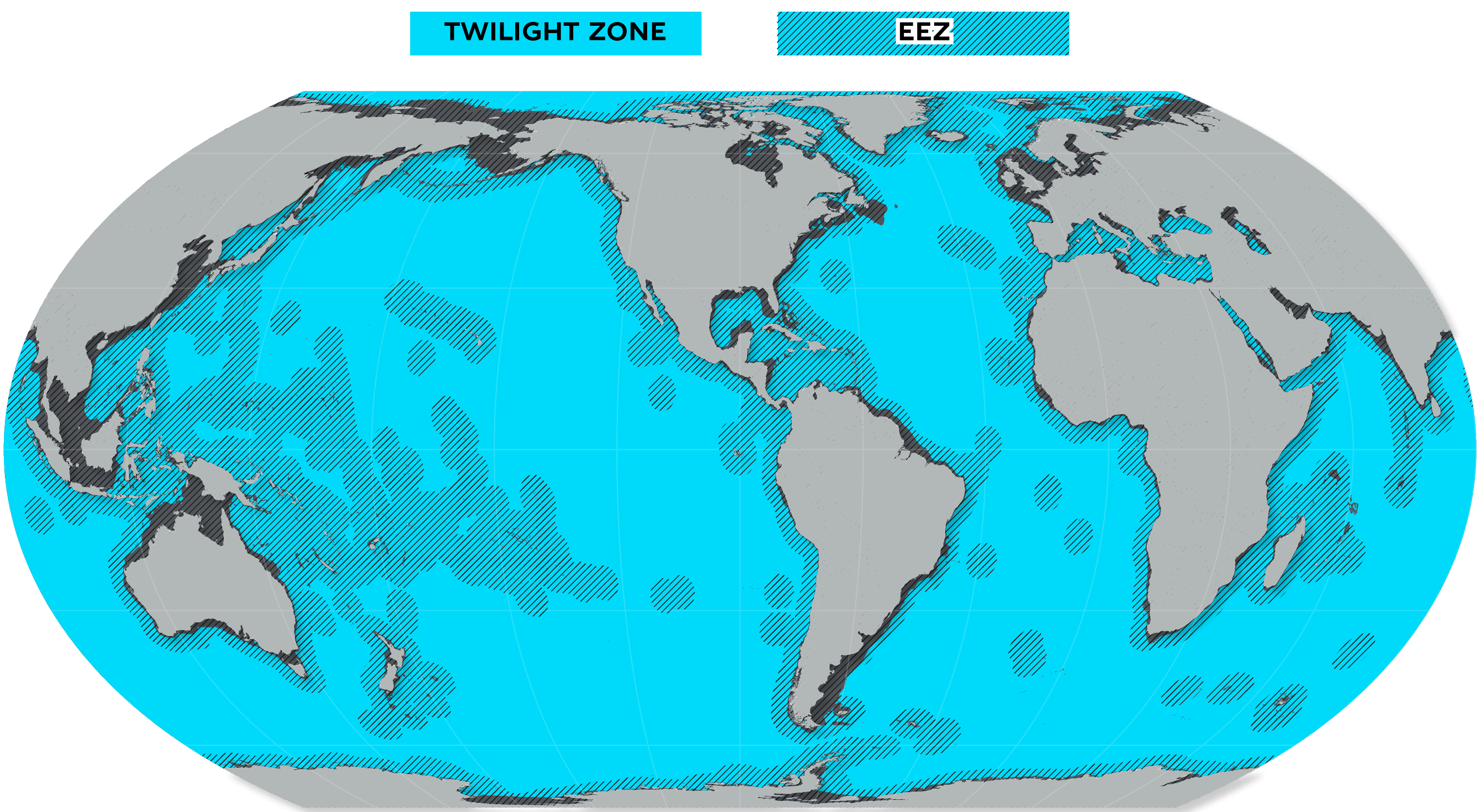


Illustration by Natalie Renier ©WHOI Creative

SIGNIFICANCE OF BIODIVERSITY IN THE OCEAN TWILIGHT ZONE

It is a crucial part of earth's climate system

Phytoplankton near the ocean's surface convert carbon dioxide originating in the atmosphere into organic matter through the process of photosynthesis. As animals across the ocean graze on phytoplankton and are consumed in turn, they produce organic carbon particles that descend into the deep ocean, thereby sequestering a significant fraction of carbon emissions each year.

Its animals undertake the largest migration on earth

Many fish and zooplankton move to surface waters to feed at night, and spend the day in deeper waters. This diel vertical migration helps to expedite the transfer of carbon to the deep sea by fish and zooplankton.

It provides critical links to surface species and ecosystems

Many apex predators, including marine mammals, seabirds, and commercially-important finfish rely on twilight zone animals for prey. Some predators take advantage of migrating twilight zone biomass, targeting them when they are present in shallower depths, and others dive deep into the depths during the day for their food. It's been shown that as much as 2/3 of the diet of tuna is from the twilight zone.

It is a source of untapped natural resources

The immense biomass of twilight zone fish has piqued interest in establishing mesopelagic fisheries to meet increasing demand for aquaculture feed and nutraceuticals. The genetic resources associated with twilight zone biodiversity may also lead to the discovery of commercially valuable natural products.

It is an indicator of ecosystem health and resilience

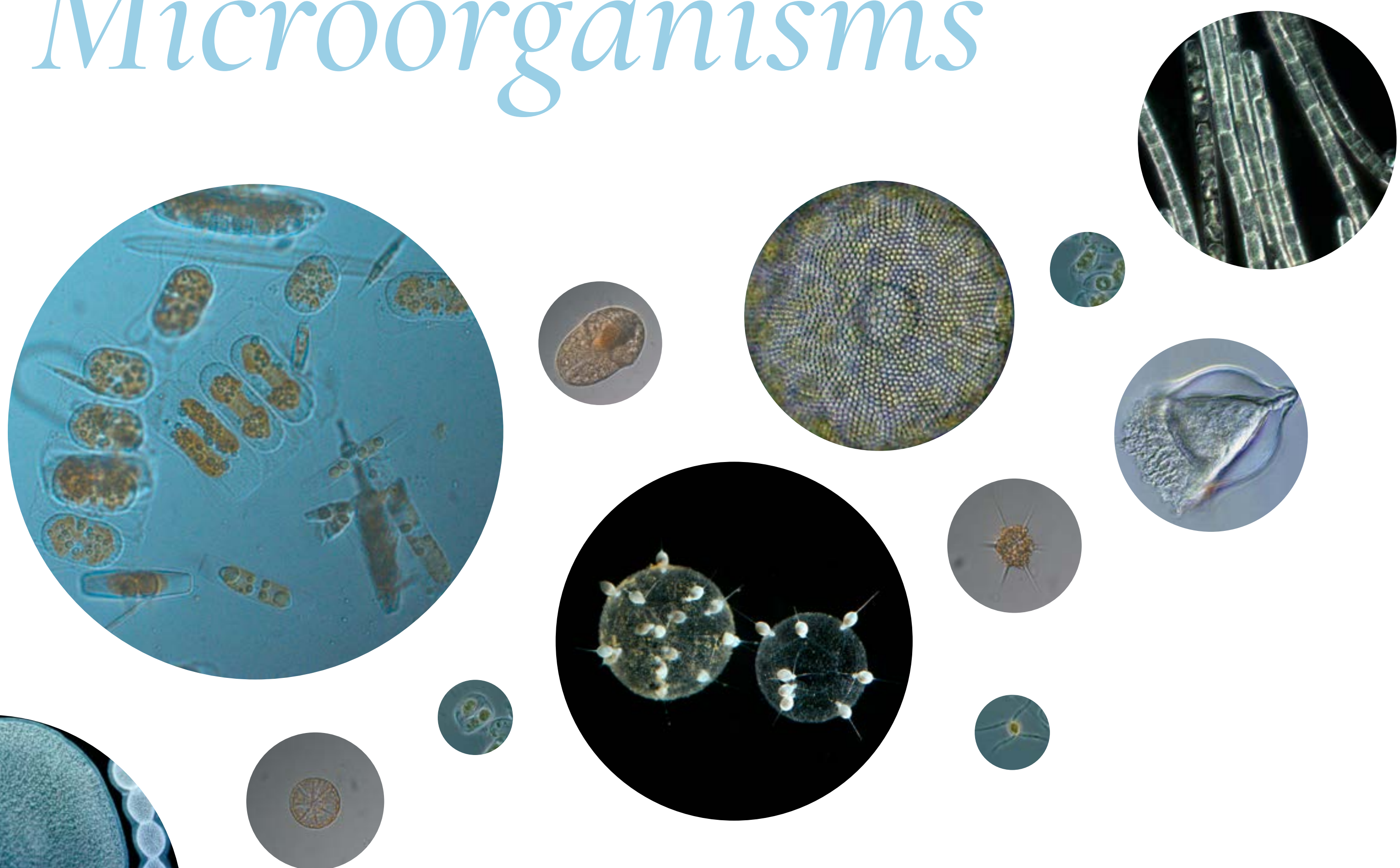
Biodiversity is linked to ecosystem resilience—the ability of the ecosystem to maintain its functioning in the face of environmental disturbances. The richness and breadth of biodiversity in the twilight zone is an indicator of how well it can perform services such as carbon transport and food web functioning despite the impacts of human activities related to climate change and resource extraction.

Lanternfish Collage by Paul Caiger, ©Woods Hole Oceanographic Instittituion

REPRESENTATIVE twilight zone *life*

The twilight zone is known for harboring a broad range of life forms that are adapted to the physical environment of the mesopelagic. Some organisms are permanent residents of the twilight zone while others visit periodically as part of their migrations or to hunt prey.

Microorganisms



The foundation of the food chain

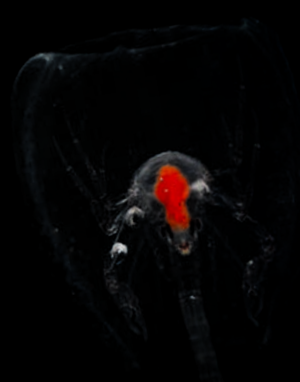
A single drop of water near the ocean's surface contains about a million microscopic organisms: phytoplankton, bacteria, and diatoms. Phytoplankton are the primary producers of the open ocean. They turn sunlight into energy, serving as both the base of the food chain and the fuel that powers the majority of ocean life—including that in the twilight zone. Other microscopic organisms, including bacteria and protists such as foraminifera and dinoflagellates around found beyond sunlight's depths and make up much of the microscopic life in the twilight zone. Microscopic foraminifera have been found as deep as the Mariana Trench.

Grazers

Heroes of carbon transport

Krill, copepods, salps and other grazers migrate from the twilight zone up to the surface each night to feast on plankton and then swim back down to the deep during the day. They are critical for transporting carbon from the surface waters deep into the ocean by creating fast sinking fecal pellets and migrating deep in the ocean. Salps are one of the fastest-growing multicellular animals on Earth and can quickly bloom in areas of high productivity, moving huge amounts of carbon deep as they migrate, poop, and die off. Grazers are found throughout the world ocean, and play an essential role in the biological carbon pump.

Photos by Paul Caiger and Larry Madin, ©Woods Hole Oceanographic Institution



Gelatinous animals

Flourishing under pressure

Much of the life in the twilight zone is gelatinous. These organisms do well in the deep ocean because their bodies are mostly made of water, which enable them to withstand crushing pressures. A variety of gelatinous organisms inhabit the twilight zone, including salps, jellyfish, and siphonophores. Deep water species are often dark orange or red in color, and are bioluminescent—glowing green, blue, and sometimes red—a function that likely attracts prey.





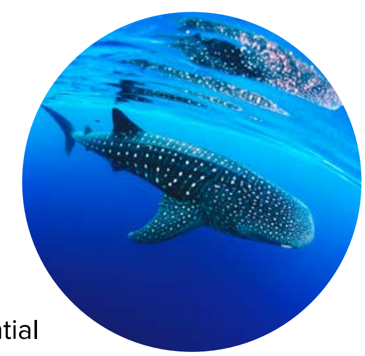

Active swimmers

The most abundant vertebrate on Earth

Most mesopelagic fish are only a few inches long. But their size does not keep twilight zone animals from being a powerful force in the ocean. The bristlemouth—a small non-migrating fish only a few inches long with a large jaw full of spiny teeth—is the most abundant vertebrate on Earth, possibly numbering in the quadrillions (1,000,000,000,000,000). Lanternfish are a group of migrating twilight zone swimmers encompassing more than 245 individual species. These glowing blue-green lights embedded all over their bodies, the aptly-named lanternfish are well equipped for seeing and signaling in the dark. Cephalopods, the group of animals that includes octopus, squid, cuttlefish, and nautilus, are prolific hunters within the twilight zone.

Photos by Paul Caiger, ©Woods Hole Oceanographic Institution

Diving predators



Feasting in the twilight zone

Apex predators, the hunters at the top of ocean food webs, are an essential part of the ocean twilight zone. They form a bridge between the surface, where they spend most of their time, to deep waters of the mesopelagic, where they feed on a buffet of sea life. These predators include a diverse range of animals: sharks, whales, northern elephant seals, and commercially-important species like tuna and swordfish, to name a few. Some species of tuna get as much as 60% of their diet from mesopelagic fish.

Swordfish photo by Steve Dougherty © Steve Dougherty Photography. Hammerhead and whaleshark by Dr. Tom Burns, DVM, penguins by Peter Kimball, basking shark by Sean Patrick Whelan, and elephant seal by Peter Wiebe, ©Woods Hole Oceanographic Institution

OBSERVING TWILIGHT ZONE BIODIVERSITY

Despite its global importance, twilight zone biodiversity is poorly explored due to the immense size of the habitat and the many logistical and technical challenges in accessing it. Scientists rely on a variety of observational tools to measure twilight zone biodiversity, but are also developing new approaches such as environmental DNA (eDNA) analysis to fill in critical knowledge gaps. It is important to note that all approaches have strengths and weaknesses, and applying them together will yield the most comprehensive picture. Some key biodiversity observing tools include the below.

Deploying a midwater trawl net. Photo by Daniel Hentz, ©Woods Hole Oceanographic Institution

Nets and trawls

BRIEF DESCRIPTION: Net-based sampling involves towing nets through the water to collect animals, which collect into a container or "cod end" at the back of the net. Some net tows are integrative through the water column and others can open and close to target discrete depth intervals.

BEST FOR: Collecting a wide variety of organisms for assessing diversity and abundance and provides physical specimens for assessing life histories and ecology.

LIMITATIONS: Nets are unable to sample the microbial community and miss out on many animals including fragile gelatinous animals that can break and fish that avoid capture. Expert taxonomists may be required for identifying the collected animals. DNA barcoding, which refers to the sequencing and analysis of diagnostic genetic sequences, can facilitate identifications. However, accurate reference sequences which are necessary for identifications, are often lacking.

Video and imaging

BRIEF DESCRIPTION: Underwater cameras deployed on remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) capture footage of animals in their natural environment.

BEST FOR: Observing marine life in real- or near real-time in a non-invasive manner to assess biodiversity, behaviors, and ecological interactions.

LIMITATIONS: Identifying species from images requires taxonomic expertise. Once visual data is acquired, specialized training and resources to develop machine learning techniques are needed for post-processing of large datasets. These methods may also miss organisms that are too small, too fast, or hidden (e.g. camouflaged).

Environmental DNA analysis

BRIEF DESCRIPTION: Environmental DNA, commonly known as eDNA, analysis use genetic material present in an environment to detect and identify organisms in said environment. Environmental DNA approaches were first developed to assess the microbial community. More recently, they have been adapted to study animals in a type of forensic approach based on genetic traces that they leave behind in the water. This allows scientists to detect the presence of animals in an environment without the need to collect animals themselves. Detection is based on DNA barcodes from bulk environmental samples, rather than from individual specimens, and hundreds of thousands of DNA barcodes can be obtained from a single sample, representing a snapshot of the community (below).

BEST FOR: Detecting the presence of organisms spanning all the domains of life, ranging from single-celled microorganisms to large predators, from the same samples. These methods are non-invasive and may be more cost-effective and scalable compared to traditional sampling methods.

LIMITATIONS: The application of eDNA approaches to animal diversity assessments, particularly in the twilight zone and deep sea, is still in its infancy and methods are still being developed and refined. Without physical specimens, animal eDNA cannot provide ecological information such as the life history stages of the detected organisms. Environmental DNA analyses also may not yield quantitative information on abundance and biomass.

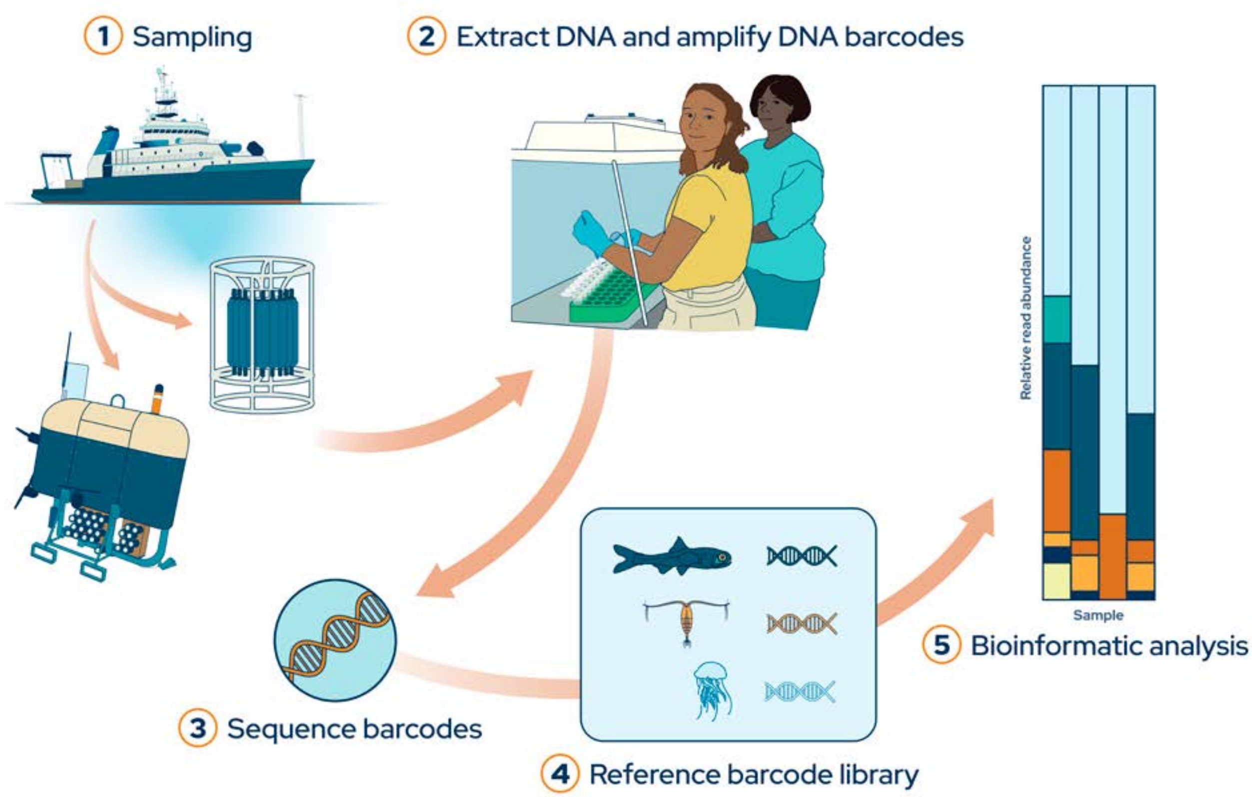


Illustration by Charin Park ©WHOI Creative

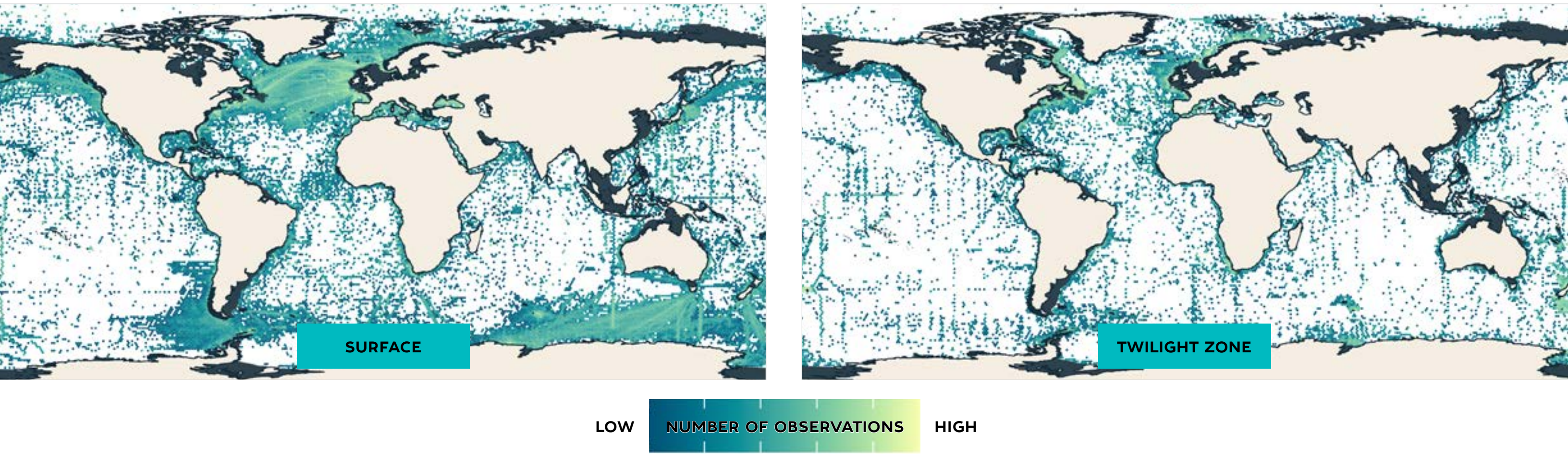
KNOWLEDGE GAPS AND REGIONAL BIASES IN TWILIGHT ZONE BIODIVERSITY

Scientific research is illuminating many unknowns of twilight zone biodiversity including:

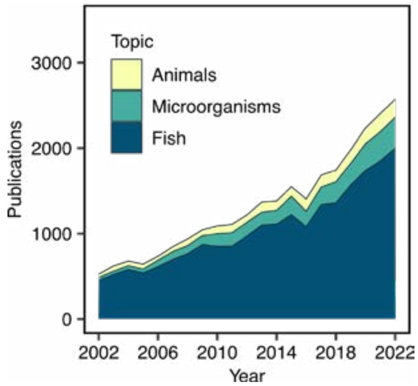
- Who lives in the twilight zone and how are these inhabitants distributed?
- What are the predator-prey relationships in the twilight zone?
- Which species undertake diel vertical migration?
- Which organisms play a role in carbon export and sequestration?

While existing knowledge provides a strong baseline understanding for twilight zone biodiversity, there are significant knowledge gaps. *In general, most areas of the ocean remain unexplored. Where data is available, there is a glaring discrepancy between the amount of data we have for the surface ocean (0 m – 200 m) and the twilight zone (200 m – 1000 m).* The number of biodiversity observations available for the top 200 meters of the ocean is ~1.5 times greater than the number of observations for the whole of the twilight zone.

These knowledge gaps are largely due to the difficulty of accessing and studying the twilight zone. Ocean exploration activities are typically limited to only a handful of nations with both deep-sea technology and expertise to conduct scientific research and exploit twilight zone resources (Amon et al., 2022). *Uneven regional knowledge has created a biased understanding of biodiversity across the global ocean* with more data available for the North Atlantic, Arctic, and Southern Ocean surface waters and little to no data available anywhere else. This is especially evident for ocean basins in the Global South (map below).



In addition to depth and spatial biases, biodiversity data is also skewed at the taxonomic level. Historically, marine biodiversity research has focused on commercially important species of fish and some marine mammals while ignoring other taxonomic groups of ecological relevance including microorganisms and invertebrates (right). This narrow focus misses the various interactions between the different taxonomic groups that underpin important marine food web relationships. *Understanding these ecological dynamics and the connectivity of biodiversity across all ocean basins is necessary* for developing ecosystem-based management strategies that recognize the importance of a diverse and resilient ecosystem for sustainable and adaptive resource management in the face of ongoing climate change.



WHOI scientists address delegates at the BBNJ meeting negotiations. ©Woods Hole Oceanographic Institution

BIODIVERSITY CONSERVATION AS A POLICY GOAL

Currently, less than 10% of the ocean is protected with just 1% of that designated as "highly protected" Marine Protected Areas (MPAs) (Marine Conservation Institute). In December 2022 at the UN Convention on Biological Diversity (COP15), the global community converged on the 30x30 goal to conserve and sustainably manage at least 30% of the world's waters by 2030 as well as prioritize regions of importance for biodiversity and ecosystem services (Kunming-Montreal Global Biodiversity Framework).

With the ambitious 30x30 conservation deadline fast approaching, we must develop a global baseline of twilight zone biodiversity to inform future biodiversity monitoring and management efforts. Closing existing knowledge gaps enables the effective assessment of human impacts to develop policy and strategies towards sustainable ocean management.

How does biodiversity inform ocean governance?

Effective ocean governance is crucial to achieve sustainability under climate change and growing ocean use pressures. Effective governance will allow the preservation of marine ecosystem health and resilience and improve livelihoods and jobs, balancing protection and prosperity (UN SDG 14 and 8).

Knowledge on the diversity, abundance, and distribution of organisms within and across ecosystems provides insight on how organisms interact with each other and how they respond to environmental changes. This information is crucial, allowing us to assess the impacts of ocean uses such as twilight zone fishing and seabed mining and to make robust projections to inform policy and decision making, including climate adaptation.

A thorough understanding of biodiversity improves ecosystem-based ocean management strategies, ecosystem valuation efforts, financing of conservation measures, and natural capital accounting to ensure sustainability (Yang et al., in review)

CONCLUSION

Conserving biodiversity in the ocean twilight zone is crucial for maintaining its ecosystem health and services (see appendix 1) which benefit humanity. However, effective governance of twilight zone is extremely challenging due to scientific uncertainty.

Obtaining sufficient biodiversity data to achieve effective conservation and policy goals is simply not possible with traditional biodiversity assessments. eDNA approaches can fill this critical need.

In the last decade, eDNA analyses have quickly gained traction as an approach for informing marine conservation, that can play a transformative role in High Seas governance and in particular, twilight zone biodiversity conservation. Since the approaches are efficient and cost-effective, eDNA can complement existing monitoring approaches and expand assessment capabilities to ensure the full scope of biodiversity is considered when developing a global ocean governance framework.

Ongoing research and technological development to facilitate the application and interpretation of eDNA datasets and increase sampling scale and automation are on the forefront of scientific advancement towards wide-spread biodiversity monitoring of the High Seas.

An integrated science-policy approach based on transdisciplinary eDNA research is essential for achieving sustainability in the twilight zone and the 30x30 target for marine biodiversity conservation.

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APPENDIX I.

Ocean twilight zone ecosystem services

SERVICE TYPE	DEFINITION	TWILIGHT ZONE ECOSYSTEM SERVICES
REGULATORY	Those that help govern natural ecosystems and planetary processes	The biological carbon pump (BCP) moves carbon from surface ocean through twilight zone into deep ocean, helping sequester 2 to 6 billion metric tons of carbon per year. These regulating services are valued at hundreds of billions of dollars (Hoagland et al., 2019)
CULTURAL	Non-material benefits people obtain from natural ecosystem functions	Knowledge systems and educational values are important components of twilight zone cultural services (Daniel et al., 2012). The economic value of oceanographic research undertaken to reduce the level of uncertainty about BCP carbon sequestration could be on the order of hundreds of billions of dollars (Jin et al., 2020)
SUPPORTING	Environmental functions that underpin other ecosystem services	The value of supporting services provided by twilight zone organisms may be imputed from net values arising from the commercial harvest of surface water predators and from the nonuse values arising from their importance to a healthy ecosystem (Dowd et al., 2022; Iglesias et al., 2023)
PROVISIONING	Economically valuable products obtained from ecosystems	Potentially harvesting twilight zone fish to feed for aquaculture or extracting oils for nutraceuticals and waxes for cosmetics; twilight zone genetic resources may lead to multiple novel natural products (Harden-Davies, 2020)

APPENDIX II.

Defining biodiversity

Biodiversity has been defined as: “The variability among living organisms from all sources, including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species, and of ecosystems” (Convention on Biological Diversity, 1993).

FOUR FUNDAMENTAL FACETS OF BIODIVERSITY CAN BE DISTINGUISHED (MAESTRE ANDRÉS ET AL. 2012):

- Taxonomic diversity refers to diversity at different taxonomic levels (e.g., class, order, family, genus, species). Species diversity or the richness and abundance of species within a community is commonly referenced.
- Genetic (or phylogenetic) diversity refers to the genetic variation within and between species which is the fundamental level of diversity underpinning the other types.
- Functional diversity refers to the range of functions performed by organisms within an ecosystem, and thus reflects the diversity of morphological, physiological, and ecological traits within biological communities and their interactions.
- Ecosystem diversity refers to the diversity of assemblages and their environments over a defined landscape, ecological zone, or at global scale.

DIVERSITY CAN ALSO BE CLASSIFIED BASED ON SPATIAL CHARACTERISTICS (WHITTAKER 1972):

- Alpha diversity refers to local diversity or diversity within a habitat.
- Beta diversity refers to the variation in diversity between different locations or habitats and reflects the number of different habitats within a region.
- Gamma diversity refers to the total diversity across a region. Gamma diversity can be considered a function of both alpha and beta diversity.

The above definitions are complimentary, and multiple dimensions of biodiversity can be combined to more fully understand ecosystem structure and functioning. Biodiversity studies and assessments should consider all facets of biodiversity to generate a holistic understanding of ecological interactions underpinning healthy ecosystems for biodiversity conservation and sustainable management of ecosystem resources and services.

APPENDIX III.

Twilight Zone Forensics: revealing hidden animal diversity with eDNA metabarcoding

Animals release their genetic "fingerprints" into the surrounding seawater in many ways, such as by shedding sloughed cells, fecal pellets, and gametes, and through their feeding activities. Scientists can filter the water to collect and analyze these genetic traces, without having to collect the animals themselves. DNA is extracted from the filters in the laboratory and in eDNA metabarcoding analysis. Genetic barcodes are sequenced from a range of organisms in the community. Many thousands of barcode sequences can be obtained from a single sample, which can be analyzed for microbial diversity in addition to animal diversity. Barcodes are compared to a database that contains reference sequences from specimens that have already been identified, and the barcodes can then be assigned a species name or classified to a higher-level taxonomic category. Because eDNA analyses are cost-effective, scalable, and can detect species that are rare or that are missed by other methods, they are transforming how life in the ocean is studied.

Like all biodiversity sensing approaches, eDNA metabarcoding analyses have biases as well as advantages and it is imperative to understand the different factors that may influence the interpretation of results (below). Environmental factors such as the rates of eDNA shedding and decay and how eDNA is transported and dispersed from the location where it is shed, sampling factors such as the volume of water filtered and how

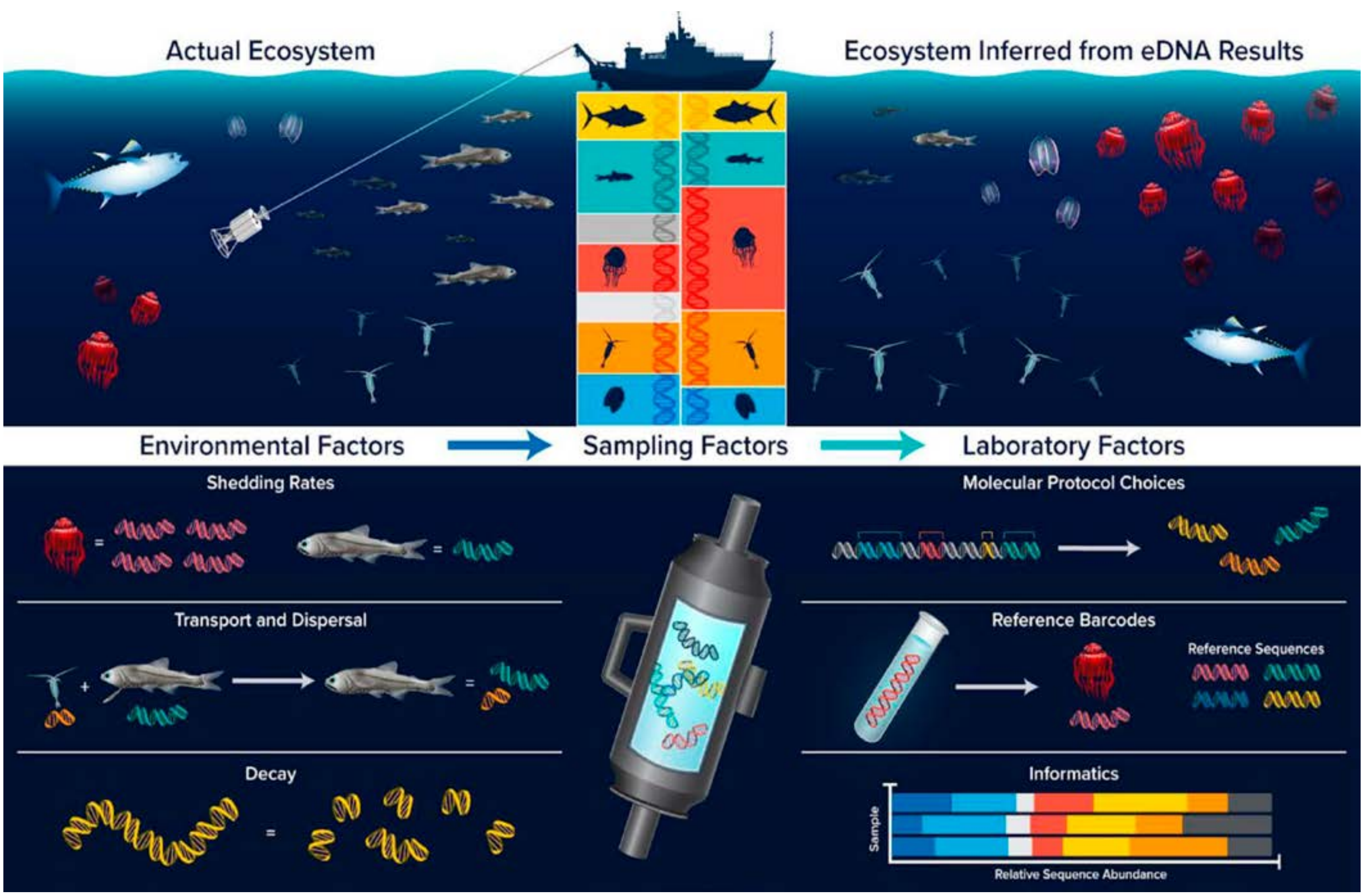

Illustration by Natalie Renier ©WHOI Creative

samples are integrated over space and time, and laboratory factors such as molecular and bioinformatic protocol choices all may lead to an interpretation of results that differs from the actual biodiversity landscape. Thus, studying the "ecology of eDNA", development of new sampling technologies and strategies, augmenting reference libraries, and standardization of protocols are imperative for large-scale implementation of eDNA analyses in ocean observing efforts. Another vital area of research is developing eDNA approaches that are quantitative and can shed light on organism abundances and biomass.

There are many complementary efforts involving autonomous samplers which filter water in situ, and these are being integrated on diverse ocean exploration platforms ranging from AUVs, ROVs, landers, towed instruments, autonomous surface vehicles (ASVs), and moorings. Environmental DNA collection is becoming routine and will be an integral component of global ocean observing efforts to understand the impacts of climate change and potential mitigation efforts and to sustainably manage the twilight zone's natural resources. As the technology is rapidly advancing, biodiversity information will soon be attainable with far greater spatial and temporal resolution than is currently available, enabling effective global twilight zone stewardship as well as new scientific discoveries.

Appendix IV. A short list of eDNA-based programs for marine biodiversity monitoring

PROGRAM	REGION	BRIEF DESCRIPTION AND WEBSITE
OCEAN BIOMOLECULAR OBSERVING NETWORK (OBON)	Global	A global coordination effort to advance the development and use of biological molecules for ocean observing, research, and management (Leinen et al., 2022).
NORTH AMERICAN PACIFIC EDNA COASTAL OBSERVATORY (PECO)	North America, Pacific	A North American Pacific coast program that focuses on seagrass-associated fish biogeography (https://peco-project.weebly.com/).
OMICS BIODIVERSITY OBSERVATION NETWORK (EMO BON)	European Union	A marine observation network that connects existing observation efforts across the European Union in support of coordinated efforts to survey biodiversity (Santi et al., 2023).
OCEANOMICS (MINDEROO FOUNDATION)	Australia	Currently focused on Australian waters, this program seeks to advance marine biodiversity monitoring through eDNA approaches (minderoo.org/oceanomics).
ALL NIPPON EDNA MONITORING NETWORK (ANEMONE)	Japan	An eDNA monitoring network in Japan focuses on fish biodiversity and provides publicly available data in the ANEMONE database (https://db.anemone.bio/).
ENVIRONMENTAL DNA EXPEDITIONS	Global (UNESCO World Heritage Marine Sites)	A global citizen science effort to monitor marine biodiversity across 25 World Heritage sites (unesco.org/en/edna-expeditions).

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THE OCEAN TWILIGHT ZONE PROJECT

Combining science, innovative technology, and broad engagement to turn knowledge into action

Questions or feedback? Contact:
Kathryn Baltes, OTZ Project Manager
kbaltes@whoi.edu or (508) 289-4910
twilightzone.whoi.edu

Authors: Nina Yang, Di Jin, Annette Govindarajan
Editors: Emily Greenhalgh, Kathryn Baltes, Ken Kostel
Graphic Design and Illustration: Hannah Piecuch, Natalie Renier, Eric Taylor, and Charin Park