



OCEAN TWILIGHT ZONE

WOODS HOLE OCEANOGRAPHIC INSTITUTION

AUDACIOUS PROJECT ANNUAL REPORT
FISCAL YEAR 2023

Contents

3	INTRODUCTION
4	KEY SCIENTIFIC FINDINGS
6	KEY PERFORMANCE INDICATORS
8	SUCCESS DRIVERS
9	PROTECTING THE DEEP OCEAN
10	HOW RESILIENT IS THE TWILIGHT ZONE TO COMMERCIAL FISHING?
14	WHAT IS THE MESOPELAGIC'S ROLE IN CARBON SEQUESTRATION?
18	HOW DO WE EFFECTIVELY MONITOR THE TZ ECOSYSTEM?
19	LEGACY AND IMPACT
20	HOW ARE WE INFORMING POLICIES TO PROTECT THE TWILIGHT ZONE?
21	FUNDRAISING EFFORTS
22	RAISING PUBLIC AWARENESS
23	PUBLIC ENGAGEMENT
24	SOCIAL MEDIA AND EARNED MEDIA
25	PROJECT FINANCIAL REPORT

We have embarked on a bold new journey to explore one of our planet's final frontiers—the ocean twilight zone, a vast, remote part of the ocean teeming with life, which remains shrouded in mystery. Our goal is to rapidly explore, discover, and understand the twilight zone and to share our knowledge in ways that support sustainable use of marine resources for the health of our ocean and our planet.

Art (on front cover, back cover, and this page) from new children's book, "Where the Weird Things Are: An Ocean Twilight Zone Adventure" illustrated by Patricia Hooning, published by Earth Aware Editions Kids, distributed by Simon & Schuster.



Introduction

Dear friends,

Over the past year, WHOI's Ocean Twilight Zone project has reached many exciting milestones that have resulted in major scientific advances. In addition to our scientists' work being featured in prestigious journals (and in our long-awaited children's book), we conducted an extraordinary three-ship cruise to study the twilight zone (TZ) in detail; deployed the first moorings of our ambitious Twilight Zone Observation Network; and created comprehensive datasets in the zone that have never been seen before at this level of detail.

These datasets were produced through a number of complementary techniques, ranging from multiband acoustic surveys to high-throughput imagery, net tows, and environmental DNA analysis. When combined, these methods provide the most nuanced view yet available of the mesopelagic.

The work we're doing comes at a critical time in environmental history. In March 2023, the world celebrated the finalization of the U.N.'s Biodiversity Beyond National Jurisdiction (BBNJ) treaty, a historic piece of international policy that will protect sensitive environments—like the twilight zone—in the open seas.

The work of OTZ scientists will be crucial to the implementation of this treaty. Unlike previous studies, which have relied on static measurements that painted the TZ as a vast, homogenous area, our team's work shows that its waters change dynamically from place to place and from season to season. As a result, we're revealing that new policies and protections will have to be applied on a highly-localized basis in order to protect its waters.

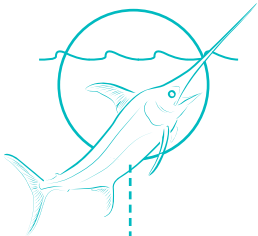
In many ways, gathering new data like ours is a means of protecting the ocean. Because of the foresight of our project's founders four years ago, we're creating unprecedented knowledge about the twilight zone's biology, ecosystem services, and physical oceanography, information that will support the decisions of 60 major nations as they meet the terms of the BBNJ treaty.



Sincerely,
Peter de Menocal
President and Director, Woods Hole Oceanographic Institution

Key Scientific Findings

Our team is protecting the twilight zone (TZ) by unraveling its central role in global climate and future fisheries.



Finding: Thanks to new methods our team developed to analyze acoustic data, we have improved how TZ biomass can be estimated. Our new tools suggest that recent acoustic-based studies overestimated the total amount of life in the TZ.

Significance: The improvements in accuracy these methods bring to acoustic analysis could allow for more informed policy decisions. Better biomass estimates will help improve our understanding of how animals move carbon through the ocean, and will inform fisheries regulation in the mid-water.

Finding: We have discovered at least three distinct food webs in the twilight zone: non-migrators, migrators, and deep migrators (a group of organisms that live in deeper water and migrate up into the TZ). Each species in these food webs plays a distinct and important role.

Significance: TZ ecosystems may not recover quickly if key species are removed through fishing or other human activities. Understanding these food web interactions gives us more insight into how carbon moves through the TZ ecosystem. Better knowledge of the structure and function of these TZ food webs will be essential for understanding human impact in the mesopelagic.



Finding: Major commercial species use the twilight zone for food, including tunas and swordfish, where they routinely forage on mesopelagic fish and invertebrates as they migrate across ocean basins. Warm-core eddies make it possible for cold-blooded species like these to enter its normally chilly waters.

Significance: Industrial fishing activities in the TZ will likely impact populations of commercially important and endangered species.

Finding: Fish respiration and excretion may be the source of up to 20% of CO₂ in the twilight zone.

Significance: We now know that life in the TZ plays a large and dynamic role in transporting and recycling of carbon in the deep ocean. Any action that impacts mesopelagic life, like commercial fishing, could have an unintended impact on global climate.

Finding: We have measured reproductive traits of four common Atlantic TZ fish species. These traits have largely been a mystery in the past.

Significance: Specific biological information on these species is key to establishing effective population models. These models can help determine when—or if—the TZ can be sustainably fished in the future.



Finding: The amount of carbon that moves through the TZ changes seasonally in the North Atlantic, peaking in the spring and dropping off in the winter.

Significance: The movement of carbon in the TZ can now be measured more accurately. This information is needed to improve global climate models and climate mitigation strategies.

Finding: The density of TZ animal life increases significantly inside warm-core eddies. We've discovered that this increase is so high that ignoring it will throw off estimates of biomass in the TZ and the ocean as a whole.

Significance: In order to write effective policy for the TZ, it will be necessary to take small and ephemeral features like eddies into account. In order to do that, we will have to observe the TZ in far higher resolution over space and time.

Finding: Biomass in the TZ changes significantly in both space and time. In different regions and seasons, the average depth of animals living in the TZ can change by hundreds of meters. Likewise, the number of those migrating animals can vary by at least 30 percent.

Significance: These changes in biological activity will impact how carbon moves into deep water in certain seasons, a phenomena not yet incorporated into coupled climate models.

Finding: Environmental DNA analyses can detect many TZ fish and invertebrates that net tows have historically missed, especially fragile gelatinous animals and fish.

Significance: Species diversity in the TZ is poorly characterized. Our work shows that eDNA sampling and analyses—which have not been widely used before in the TZ—can provide a more comprehensive accounting of animal species and their distributions.

Finding: Vertical migration habits and feeding relationships between TZ animals change depending on the species. Certain species play a larger role than others in moving carbon into deep water.

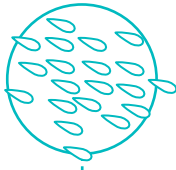
Significance: In narrowing down these roles, we are helping to understand how animal diversity, distribution, and behavior moves carbon through the TZ—information that can help to improve climate models and set future climate policy.

Finding: Our early investment in developing new technology and methods is providing more and better data on TZ than ever before. The Twilight Zone Observation Network, for instance, is recording data continually over months and even years, which has never been done before.

Significance: The TZ changes dramatically over space and time. Our new technology lets us observe those changes in multiple dimensions and high resolution, revealing critical new information about the mesopelagic. This data will be essential to helping protect the TZ and craft science-based policies governing fisheries and climate.

Finding: Mesobot has demonstrated its ability to track midwater animals, sample eDNA, map out the midwater light field and even emulate migration behaviors of mid-water animals.

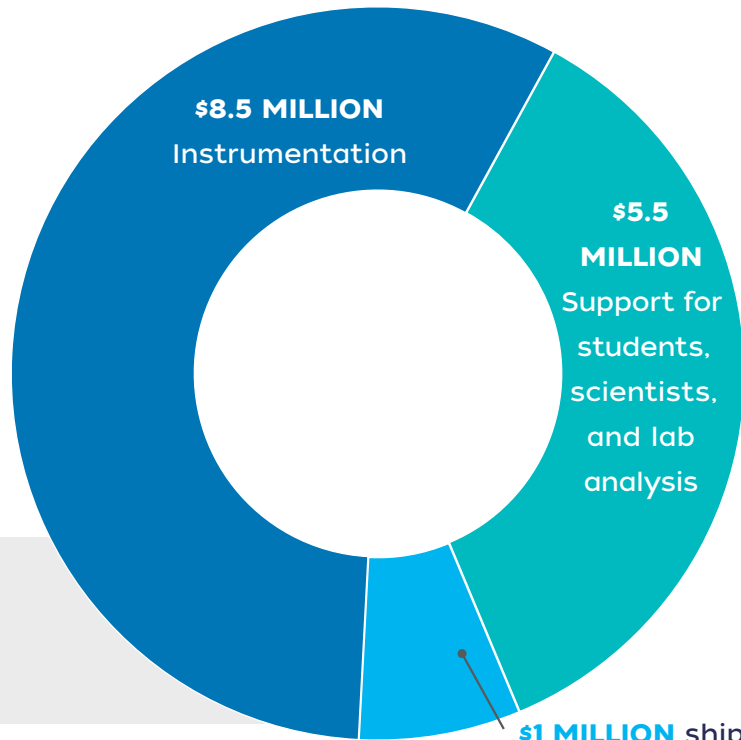
Significance: Through improved estimates of the subsea light field over the entire TZ whenever a surface irradiance estimate is available, we will be able to link all our observations, including net tows, eDNA samples, imaging from tow sleds and AUVs, and bioacoustic observations.



Key Performance Indicators

LEVERAGED FUNDING

When done collaboratively, scientific research is often the most impactful. Quantifying our leveraged funding, or funding received from outside sources, allows us to highlight how we have extended our research impact through new or existing opportunities. It is an indicator that twilight zone research is seen as critical across many fields and that our scientists are leaders in those fields.



\$15 MILLION
BROUGHT IN FROM OUTSIDE SOURCES

PUBLICATIONS

Academic publications are the standard metric used for validating impact within the scientific community, as they allow scientists to share vetted research with their peers. Our robust publication track record is a testament to the scientific advances we've accomplished.



PUBLISHED



IN PREP

NETWORK REACH

Network reach is a measure of where and how our team shares its findings and advances. Collaborations, partnerships, external outreach and engagement have all served to expand the project's overall reach.



COORDINATING WITH
13
DIFFERENT MESOPELAGIC
RESEARCH PROJECTS



45
ACADEMIC
PARTNERS

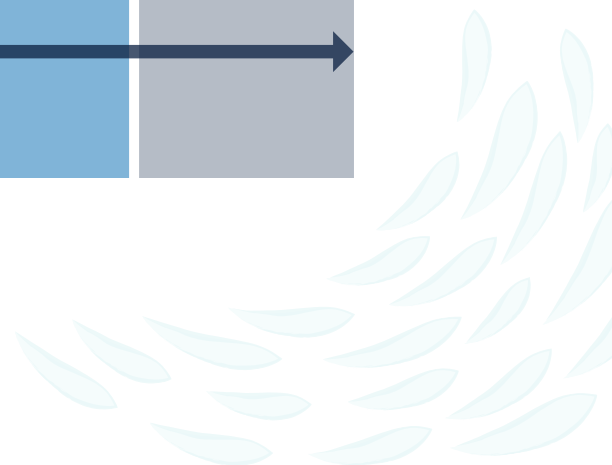
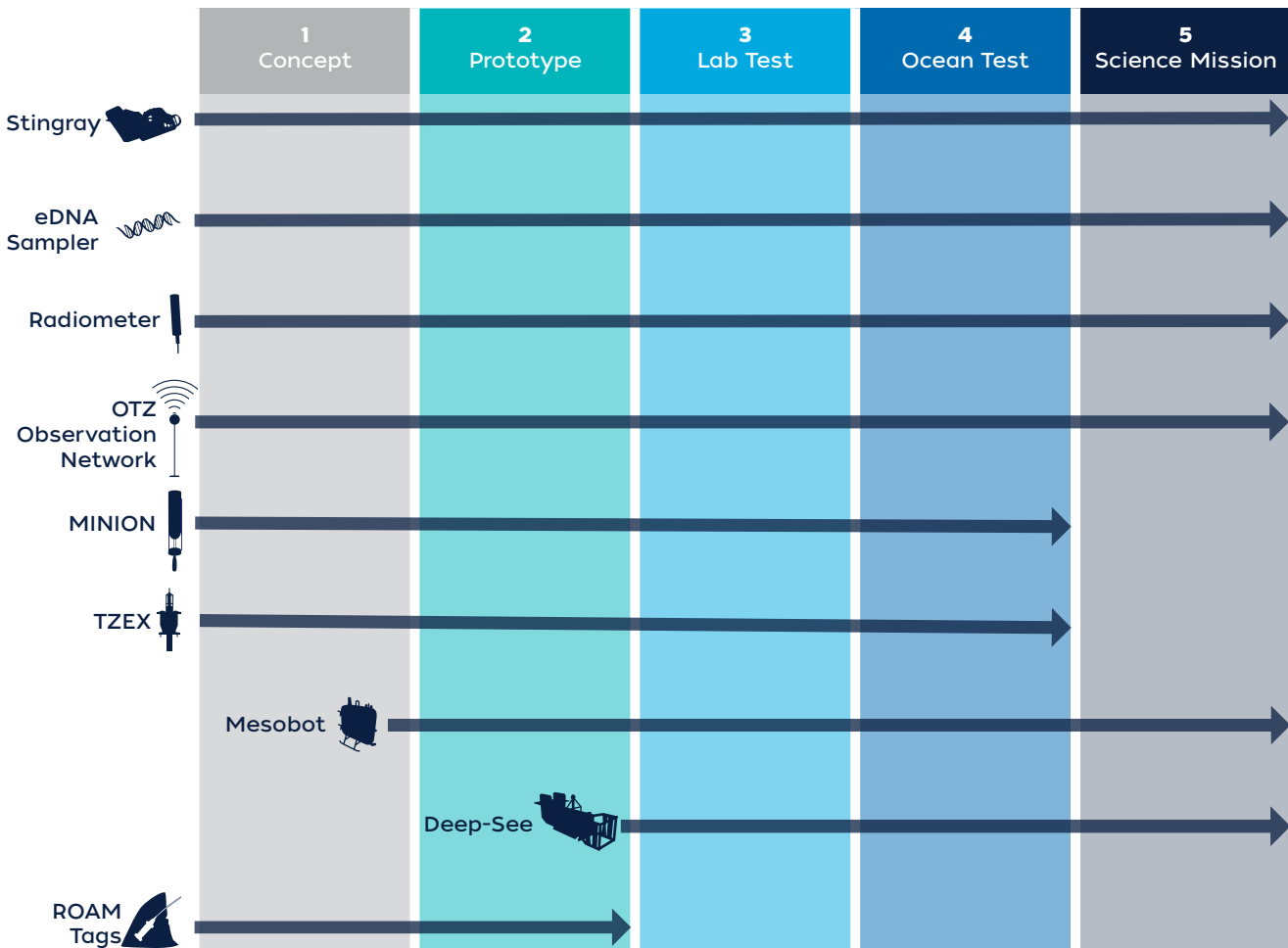
OVER
100
PRESENTATIONS TO
ACADEMIC AND
PUBLIC AUDIENCES





READINESS LEVELS AND TECHNOLOGY DEVELOPMENT

Technical innovation is a key focus of the OTZ Project. Our scientists and engineers are designing and building new technology as well as adapting existing technology to help us access and study the twilight zone.



Success Drivers

Science is iterative—it advances in countless small steps as each new discovery or insight builds on its predecessors and raises new questions.

The Ocean Twilight Zone Project has established a foundation to track progress toward our goal of achieving a baseline of scientific understanding about the mesopelagic and informing policy-makers and the public collectively to make sound decisions that ensure the long-term sustainable use of our ocean's twilight zone.



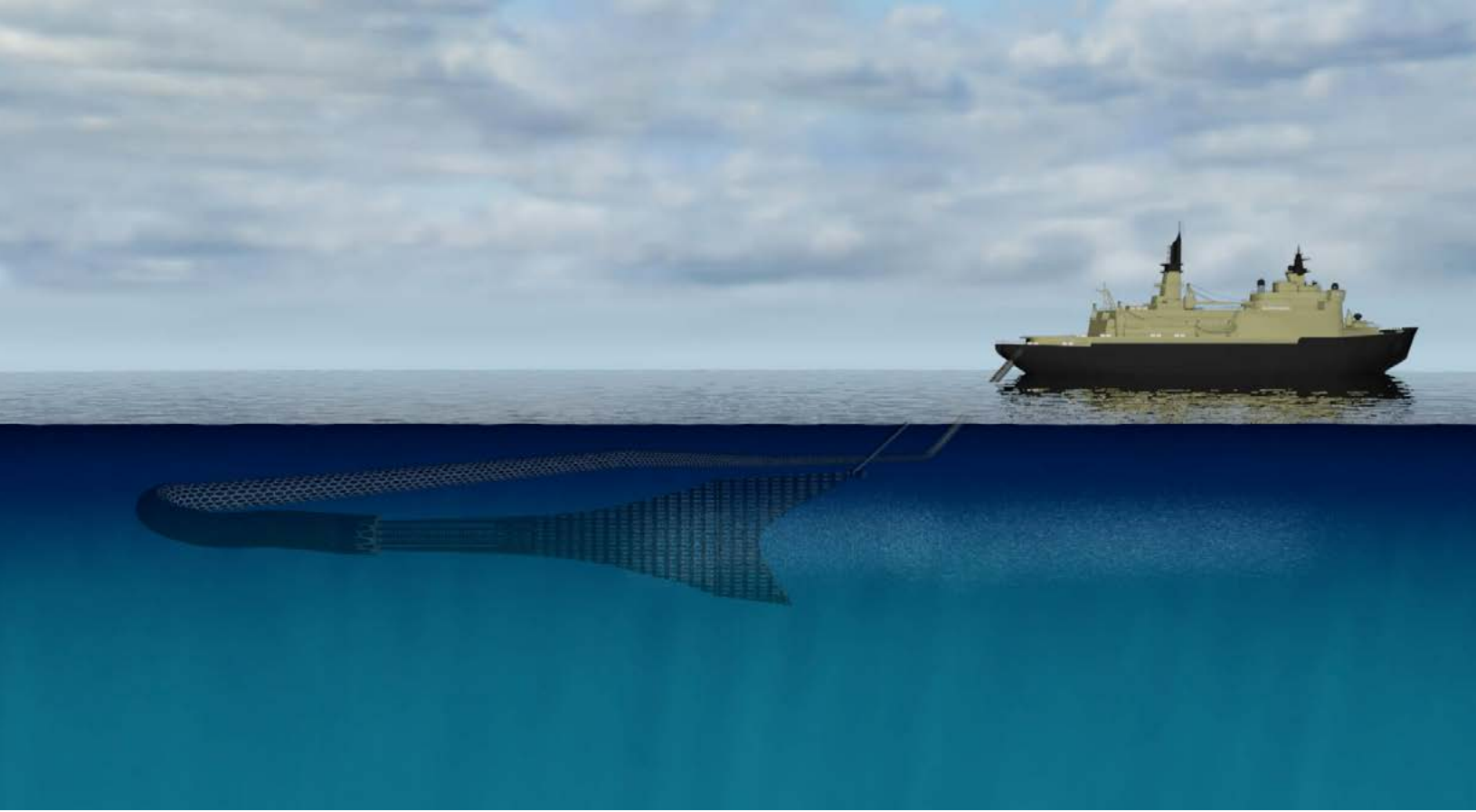


Protecting the Deep Ocean

NOW THAT THE DRAFT AGREEMENT OF THE UN'S BIODIVERSITY BEYOND NATIONAL JURISDICTION (BBNJ) TREATY HAS BEEN ADOPTED, a whopping 30 percent of the world's oceans will be regulated and protected by international law as early as 2024. This is a huge step towards preserving global marine ecosystems—and maintaining the benefits they bring to the planet as a whole—yet the way the treaty's protection will play out is not yet clear. Member states have not determined which parts of the ocean to include in that 30 percent, for instance, nor is there widespread agreement on how chosen areas will be protected.

Under the BBNJ, member states will be required to consult an international committee of scientific advisors while making these critical decisions, meaning that it is now more important than ever to have the extensive amount of fundamental science that our team has produced in the twilight zone. The work of our researchers is establishing baseline conditions in the TZ, revealing the species and behavior of the life it contains, and identifying negative consequences that might occur if those species are removed by commercial activity. In doing so, we are setting a foundation for future policy that could have a major impact on the world's oceans.

PhD student Ciara Willis, above, presents research from the OTZ project at COP27's Ocean Pavilion. Photo by Ken Kostel © Woods Hole Oceanographic Institution



How resilient is the twilight zone to commercial fishing?

ONE OF THE MAJOR QUESTIONS THAT HAS COME UP OVER THE COURSE OF THE OTZ PROJECT is whether or not sustainable commercial fisheries can exist in the twilight zone. Given the vast amount of biomass it contains, commercial fishing is looking increasingly attractive in the TZ: the species living there could be used to create feedstock for animals in aquaculture facilities, letting them produce sorely-needed protein at a time when land-based crops are faltering due to climate change.

In order to gauge the resiliency of the TZ to human activity, scientists and policymakers must take multiple factors into account, including what species it contains, how those species interact, how many individuals are present, and how and when each species reproduces. It will also be necessary to know how each of those organisms tie into the health of others in the same food web—and whether fishing one type of animal will effectively rob another of its food source. Our team has generated a number of scientific findings and datasets that will help to determine whether the twilight zone could be a viable fishery in the future, and what the impact of that fishing may be.

Illustration depicting potential TZ fishing techniques. While widespread commercial fishing of the TZ is not yet underway, several countries are currently exploring the possibility. Illustration by Tim Silva, ©WHOI Creative.

WHAT SPECIES ARE DOWN THERE?

In the decade leading up to the OTZ project, assumptions of biomass in the twilight zone were driven largely by one study. This 2014 paper, is based on a research vessel that recorded shipboard sonar data constantly during a cruise that circumnavigated the globe. Based on that information, it estimated that global mesopelagic biomass was 10-15 times the amount of historical estimates that used traditional net tows.

For nearly a decade, the 2014 study has been the best available estimate of TZ biomass. Thanks to new analysis tools developed by our team, however—an effort supported by our own scientists' deep towed acoustic surveys—we have demonstrated that single frequency sonars fail to detect many of the organisms comprising TZ biomass. Instead, our researchers are using multiple frequencies at the same time, a method that reveals a broader range of organisms. This discovery is already leading to more accurate ways of measuring and monitoring biomass in the twilight zone.

Our team also found that twilight zone biomass doesn't remain steady throughout the global ocean; instead, it can be vastly different depending on the location and time of year. Using three months of continuous acoustic data from WHOI's Twilight Zone Observation Network, the team found that oceanographic features like warm-core eddies (temporary swirls of warm water that stretch from the surface into the mesopelagic) can directly affect biomass and their interactions. The abundance of fish the team observed went up significantly when an eddy formed, but dropped off once the eddy dissipated. This exciting finding has never been documented before in the twilight zone and suggests the importance of regional ocean dynamics.

HOW MANY SPECIES ARE PRESENT IN THE TZ?

Our team is using several complementary techniques to understand the species that live in the twilight zone. When combined, tools like net tows, submersible imaging devices, isotopic analysis, and eDNA—each of which has unique strengths—provide the most comprehensive overview of life in the mesopelagic yet available.

Methods like these helped our team discover a surprising abundance of gelatinous species in the TZ. Those organisms, which have been largely missed by traditional methods like net tows, may play a large role in sending carbon into deeper water. Salps, for instance, have relatively large and dense fecal pellets, which sink to the bottom in a matter of days, whereas small, loose pellets created by fish can take weeks.

OTZ scientists confirmed the presence of these species using high-throughput camera systems, which record the detailed contents of seawater passing through them at 15 images per second. By applying AI models to classify animal types and numbers from huge image datasets, our team was able to identify the distributions of gelatinous animals in the TZ—information that would otherwise be impossible to obtain at this level of detail.

Using eDNA, our team was able to back up these findings, as well as identify traces of TZ species that cannot be sampled with nets (whales are one key example).

Taken as a whole, our scientists' findings are beginning to fill in the large gaps in our understanding of species living in the TZ. This finding alone is valuable: by identifying existing holes in our knowledge, we can focus our research accordingly. The work our team has done so far also shows that in order to have an accurate survey of TZ life, new studies will have to be done at far higher resolution than current methods, taking into account smaller geographical areas, more nuanced timing, and specific depths.



WHAT ARE THE LIFE CYCLES OF FISH IN THE TZ?

In order to set up sustainable fisheries in the TZ, policymakers will need fundamental information about their behaviors and life cycles, namely: How long do the fish live? How big do they get? When do they sexually mature? How many eggs do they produce? Much of this information comes from net tows, which collect physical samples of mesopelagic species.

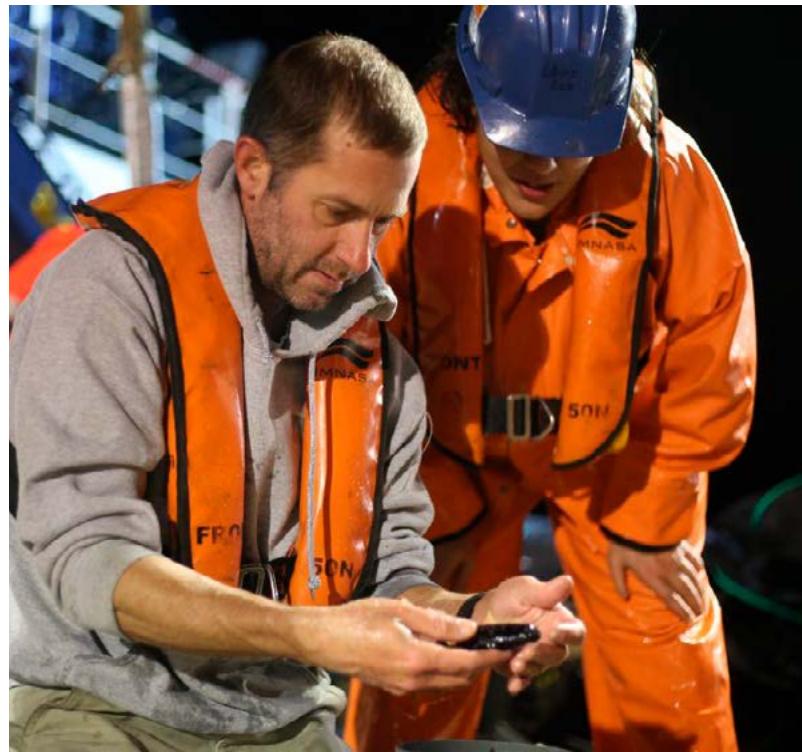
Before the OTZ project began, very little of this information was available for some of the mesopelagic's most abundant species, if it existed at all. Over the past few years, the team has selected four of these species for direct study: lanternfish, bristlemouth, bigscale, and hatchetfish. For the former two species, no data was previously available in the western Atlantic ocean; for the latter two, no data was previously available at all.

Our team's work is revealing the age at which each fish is mature and can reproduce. It also shows that all four Atlantic species are reproductively active in summer, and that bigscale and hatchetfish spawn multiple times throughout their lives (as opposed to fish like salmon, which spawn only once). These findings are the first time that any information about these species' reproductive habits has been recorded, providing crucial information for drafting informed fisheries regulation. Hard data like this helps researchers and policymakers gain insight into the population dynamics of the species to determine when—or if—these species can be sustainably harvested in the future.

WHAT ARE THE RELATIONSHIPS BETWEEN PREDATORS AND PREY IN THE TZ?

Understanding feeding relationships among mesopelagic species is a central part of deciphering the twilight zone's ecology as a whole. Before the OTZ project, almost no scientific data on the structure of mesopelagic food webs existed—yet this information is essential for modeling the potential impacts of fishing the TZ, as well as the role that those fish play in moving carbon through the mesopelagic. Regulators will need to know whether the removal of one species will deprive another species of an important food source, a consequence that could have a ripple effect throughout the entire ocean.

By analyzing the stomach contents and tissues of fish caught in research net tows and longlines, our team has identified three distinct food webs within the



OTZ researcher Joel Llopiz (left) examines a fish from the twilight zone during a net tow in 2021. Photo by Marley Parker © Woods Hole Oceanographic Institution

mesopelagic. One is made up of animals that migrate to the surface from the twilight zone each night to feed; another is comprised of animals that stay in the twilight zone at all times; and the third centers around organisms that live in the waters below the mesopelagic, yet migrate up into the twilight zone at night.

OTZ scientists also found that there are seasonal differences in those food webs. During warmer months, large blooms of phytoplankton at the surface create a plentiful food source, and animals in the twilight zone appear to eat whatever happens to come their way. As fall arrives, however, primary productivity drops off dramatically, limiting food supplies, and forcing animals in the TZ to focus on more specialized diets for survival.

These findings will dramatically improve existing ecological models of the twilight zone. Until now, most of these models have treated this region as relatively homogeneous in terms of ecosystem function. Our team's data, however, shows that when nutrient density changes, fish behavior and food web architecture change as well. This phenomenon has never been documented before.

Taken as a whole, the information our scientists have gathered about TZ food webs reveals that they change dramatically over time and space. As policymakers decide which parts of the TZ to protect, it will be essential to take these shifting norms into account.

HOW DOES TZ AFFECT EXISTING FISHERIES?

Our team's work has already confirmed that commercially important species, like tuna and swordfish, depend heavily on the twilight zone for food. Our most recent data, however, also reveals that the ways these species use the mesopelagic depends on regional oceanographic features like warm-core eddies.

These eddies are temporary columns of warm water that stretch from the surface to the TZ.

As they form, they create a highway into the twilight zone, letting predators that normally can't function in its cold waters access a bountiful food source.

In addition to offering new insights on how these animals use the twilight zone for sustenance, our team's findings underline a major point for policymakers: the impacts of commercial fishing in the TZ could be dramatically different from place to place, and new regulations will have to be written with this in mind. It's also clear that fishing in any part of the TZ will have wide-reaching effects that will be felt by species outside of the mesopelagic. Since commercially-important species forage in the twilight zone, harvesting their prey could leave them with fewer options for food. Unless done in a sustainable way, fishing in the TZ could put undue pressure on species that humans rely on for both protein and income.

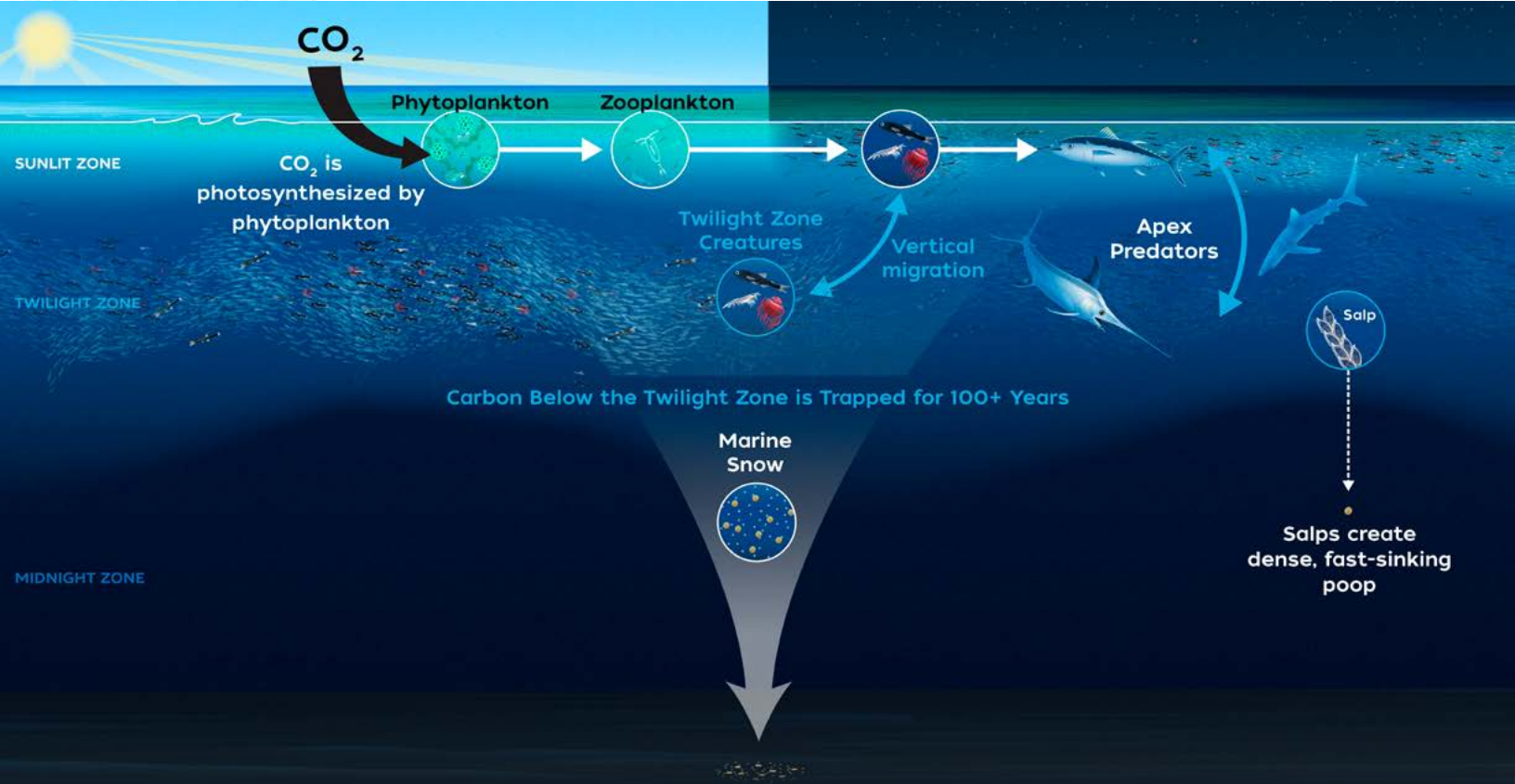


NEXT STEPS

While the OTZ team has made huge strides in understanding the mesopelagic, there is still much left to explore. Our analysis of fish stomach contents, for instance, suggests that we're missing at least two levels of predator-prey interaction using current technologies. In the stomach of a single tuna, our scientists found a snake mackerel that had swallowed a barracudina, which itself had feasted on bristle-mouths, a common TZ fish. Since snake mackerel and barracudina are too fast to be caught in net tows and can evade current imaging techniques, the way these fish fit into TZ food webs is not well understood.

Getting better information about TZ biomass and species is also an ongoing goal. To advance that knowledge, our scientists are continuing to improve techniques like eDNA analysis, and are developing new artificial intelligence tools to estimate biomass and spot different types of life using multiband acoustic data.





What is the mesopelagic's role in carbon sequestration?

GOING INTO THE OTZ PROJECT, SCIENTISTS ALREADY KNEW THAT THE TWILIGHT ZONE PLAYED AN IMPORTANT ROLE in transporting carbon from the atmosphere into deep waters, helping to regulate global climate. Our current estimates suggest that 10-12 billion tons of carbon enter the twilight zone, but only one billion tons leaves the TZ at its lower extremes. What happens in between, however, and how this varies seasonally and spatially, needs to be better quantified.

Our team is already chipping away at a number of major questions, including how carbon moves between the atmosphere and the TZ at any given time, how much of that carbon stays in the TZ, and how carbon ultimately makes it into the deep ocean. Our team is also recording unprecedented amounts of data on the amount of marine snow that sinks through the twilight zone, how fast it sinks, what it's made of, and how TZ organisms play a role in processing the carbon it contains.

In order to do so, our scientists are measuring carbon flow over large areas and long time frames at contrasting sites, bringing a level of detail and precision to this phenomenon that was simply unavailable in the past.

HOW DOES CARBON MOVE THROUGH THE TWILIGHT ZONE INTO THE DEEP OCEAN?

Understanding how carbon passes through the TZ—and what happens to it along the way—is a critical part of understanding the twilight zone’s role in regulating global climate. Our team is helping to answer that question by studying not only marine snow (the primary vehicle that moves carbon into the deep ocean), but also the biological and physical phenomenon in the TZ that affect its fate.

Our work is revealing just how deeply interrelated those phenomena may be. A recent paper by OTZ scientists showed that warm-core eddies, temporary swirling columns of warm water that reach down into the TZ, help to aggregate fish and plankton. Using data from the Twilight Zone Observation Network, we saw that over a three-month period, more

fish flocked to waters inside warm-core eddies than outside of them—an exciting finding that could have a major impact on how the TZ moves carbon into deep water. This increase in animal life suggests that more phytoplankton are growing in those relatively warm surface waters, which in turn creates more marine snow as that plankton dies or is eaten by small animals and defecated.

As a result of the increased biological activity they contain, processes like these eddies, show that short-term, localized phenomenon in the TZ can have outsized effects. In order to protect the mesopelagic as a whole, new policies and regulations in the twilight zone will likewise need to change dynamically, matching their restrictions to the needs of the TZ over a range of space and time.



Katy Baker (above), a PhD student at the University of Tasmania, examines a salp caught during a deployment of a MOCNESS tow net during a 2022 OTZ expedition. Salps play an outsized role in carbon sequestration because they produce dense, fast sinking pellets that efficiently ship carbon to the deep sea, where it is sequestered from the atmosphere. Photo by Andrea Vale © Woods Hole Oceanographic Institution

HOW FAST DOES CARBON MOVE THROUGH THE TZ?

Our team is also working to understand the rates at which carbon moves through the water, and the various routes it takes. By analyzing a radioactive isotope called Thorium-234, which is common in marine snow, OTZ scientists can trace how fast that snow falls through the water. Over the past four years, we've expanded this approach to an unprecedented number of samples, providing knowledge that will help us understand the real impact that the TZ has on regulating oceanic and atmospheric carbon—and by extension, global climate.

Using thorium, marine snow traps, and submersible cameras, our team found that the amount of marine snow sinking through the TZ can vary tremendously depending on the animals who create it. Large, dense fecal pellets from salps, for instance, can fall as fast as 1,000 meters per day, which bring the carbon they contain to the seafloor in less than a week. Smaller particles and more loosely-packed marine snow, however, can take more than 10 days to fall just 100 meters. As a result, these particles are more likely to be consumed by animals and bacteria within the TZ, which stops the carbon they contain from reaching deeper water.

Biological processes and their impacts on carbon in the TZ remains a major source of uncertainty in existing climate models. Understanding how carbon moves through the TZ more accurately and precisely will help to predict how human activity could affect that movement. As a result, scientists and policymakers will be able to more accurately gauge the global effects of new regulation in the twilight zone.

WHAT ROLE DO ORGANISMS IN THE TZ PLAY IN MOVING CARBON?

In addition to marine snow carbon content and sinking rates, our team is also factoring how the behavior of animals in the twilight zone helps to move carbon into the deep ocean. As TZ fish eat plankton and marine snow near the surface at night and migrate back down to the mesopelagic during the day, they bring a significant amount of carbon with them in their stomachs. Those animals also urinate and defecate at depth, adding additional carbon into the TZ.

Eve fish respiration (or “breathing”) adds carbon into the water as CO₂, which stays dissolved in deep seawater for long periods of time. Biological processes like these could be responsible for roughly 20 percent of CO₂ in the twilight zone.

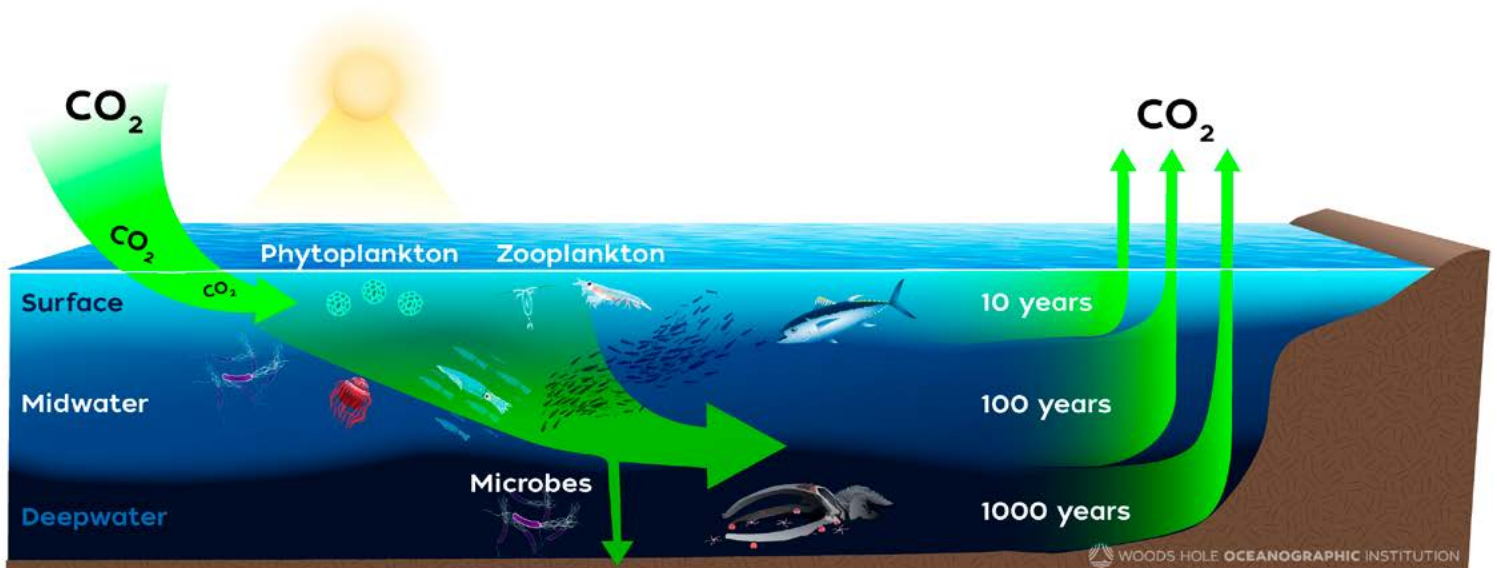
Determining the biomass of fish and other organisms in the twilight zone—and the rate at which they transport and consume carbon—remains a key element for understanding how carbon is transported by animals. Our work is developing more accurate estimates of this biomass, which will also inform where new marine protected areas may be effective, and where regulated commercial activity could safely occur without a major impact on climate.

DOES THE MOVEMENT OF CARBON THROUGH THE TZ CHANGE IN PLACE AND TIME?

Carbon in the TZ varies tremendously by location and season. Data our team collected from mooring sites in the Atlantic during spring plankton blooms, when more animals are actively eating and defecating, showed a maximum change in carbon at depths greater than 1,000m. In winter, however, productivity at the surface is far lower, and the amount of descending carbon is reduced.

OTZ scientists also found key differences in marine snow fall between the Atlantic and Pacific twilight zones. Unlike the larger, rapidly-sinking marine snow found during a bloom in the north-east Atlantic, studies in the northeast Pacific Ocean recorded mostly small particles, with high levels of recycling and consumption of marine snow in the TZ. As a result, little carbon was left to be sequestered in deeper water.

This finding shows that the amount of carbon produced at the surface may not control the amount that makes it into deep water. Rather, the primary factor may be the speed at which that carbon sinks. The faster marine snow falls through the TZ, the smaller its chances of being consumed by organisms along the way. As a result, the twilight zone may remove carbon from the ocean more efficiently in some parts of the world than others, which could alter its role in regulating climate.



NEXT STEPS

Our work shows that understanding the TZ will require persistent, high-resolution monitoring in the future. Fixed, long-term instruments like the Twilight Zone Observation Network currently provide that level of information, which is critical for gauging how much carbon moves into the deep ocean, what their sources are, and what the seasonal and spatial variability may be in these carbon flows. Moorings with acoustics, imaging systems, eDNA physical and chemical sensors, and marine snow traps will provide better data on animal behavior, as well as the amount of marine snow and its sinking rate—the “holy grail” of understanding how carbon moves through the TZ.

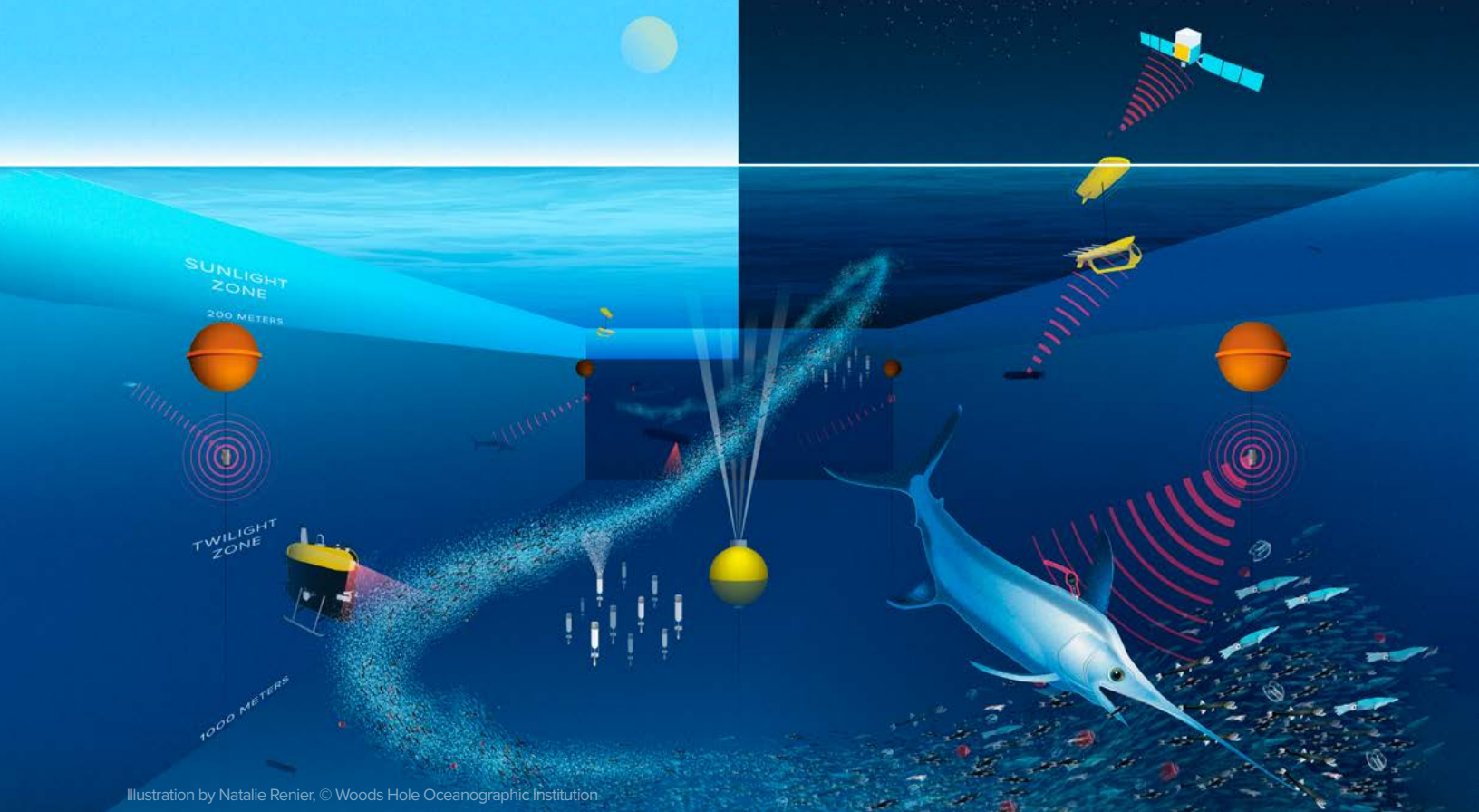


Illustration by Natalie Renier, © Woods Hole Oceanographic Institution

How do we effectively monitor the TZ ecosystem?

MONITORING THE HEALTH OF THE TWILIGHT ZONE OVER TIME WILL BE ESSENTIAL FOR FUTURE POLICYMAKING. Any changes in its ecosystem could have a massive impact on how carbon moves into the deep ocean, affecting both commercial fisheries and global climate change.

Our work has shown that environmental DNA (eDNA) is a valuable tool for monitoring which organisms exist in the twilight zone. Until now, this method has not yet been broadly applied to the mid-ocean, but thanks to the framework our scientists have developed, eDNA analyses can provide a baseline measurement of life in the TZ for future comparisons. Unlike traditional net tows, eDNA can detect species that scientists never actually see—and catalog rare or novel DNA of mesopelagic creatures. Our eDNA results reveal the presence of many species not sampled by nets, suggesting that biodiversity based on traditional net-based assessments is underestimated.

To collect eDNA, our team has created instruments that can filter seawater at depth, returning samples of biological material for study. We are currently working to make eDNA a standard measurement tool on par with other sensor data. Models built by our scientists show that eDNA remains close to its source in the water column—so genetic material sampled at particular depths will reveal individual species present in that layer of the ocean. This will be a useful tool for studying processes like diel vertical migration, in which trillions of TZ animals swim to the surface at dusk and return to deep water at dawn. Our results from the field have already identified many vertically-migrating fish species, and have revealed that animal communities do indeed change with depth.

Other key tools, like multiband acoustic data, will play a major role in monitoring the TZ as well. Our scientists are continually improving techniques for analyzing acoustic signals, and are now even building artificial intelligence systems that can identify animal types in visual images or acoustic surveys, quickly providing valuable information to the team.



Legacy and Impact

ONCE THE BBNJ TREATY IS RATIFIED, MEMBER STATES WILL START TO CRAFT NEW REGULATIONS FOR THE HIGH SEAS. As they do, the fundamental research our team is conducting will be invaluable. Our data and findings provide policymakers with real-world science on which to base their decisions—leading to regulations that preserve our oceans, fisheries, and climate systems for future generations.

Moving forward, we will need to know more about what animals are in the TZ, how they behave, and what would be affected if they are removed. To build that knowledge, we've created new techniques and tools, including a stealthy undersea robot that can follow TZ fish, highly-accurate acoustic systems, floats that can measure carbon-rich marine snow, approaches to collect and preserve eDNA, cutting-edge tags that record animals' positions in three dimensions, and so

on. Together, these technologies help us collect an unprecedented amount of information about the twilight zone, revealing never-before-seen details of life in the mesopelagic.

Moving forward, we will continue to harness these technologies in new ways. Over the past year, we've applied what we've learned to design and built the world's first Twilight Zone Observation Network in the northwest Atlantic Ocean, a system of moored instruments that can collect data on the TZ continually for years at a time. This network can monitor approximately 150,000 square miles of the ocean, offering the biggest, most comprehensive view of the twilight zone yet available. We plan to make this data available to all researchers, accelerating scientific understanding of the TZ as a whole.

How are we informing policies to protect the twilight zone?

FROM A POLICY PERSPECTIVE, THE PAST YEAR HAS BEEN IN MANY WAYS OUR MOST IMPACTFUL TO DATE. Several of our long-term efforts yielded results that will inform key policymakers—who are poised to introduce new marine regulations and protections—about the vital role that the TZ plays in the world’s oceans.

From the beginning, our team has built relationships with key stakeholders worldwide, created and distributed proprietary policy reports, and participated in international policy meetings and initiatives. In the process, we have gradually built a constituency of ocean advocates and positioned the ocean twilight zone as a central consideration in global ocean policy deliberations.

Our team’s work, for instance, is helping to inform sustainable fishing policies that will be put in place before large-scale exploitation of the twilight zone occurs. Since the TZ may contain more than 10 billion metric tons of fish—or about 95 percent of all fish in the ocean by weight—it will be an attractive target for commercial fishing operations in the decades to come.

As of June 2022, the OTZ project has been formally endorsed by the U.N. Decade of Ocean Science for Sustainable Development—a designation that will give our scientists a greater global platform and wider opportunities to collaborate, improving their ability to answer the twilight zone’s biggest mysteries. Members of the OTZ team traveled to Lisbon, Portugal for the 2022 United Nations Ocean Conference, where senior scientist Ken Buesseler presented insights on how the ocean twilight zone moves vast quantities of carbon from the atmosphere to the deep, where it can remain hidden away for hundreds to thousands of years.

In November 2022, the OTZ project was a primary driver for WHOI’s participation at COP27 and a featured element of the United Nations climate summit. OTZ team members traveled to Sharm el-Sheik, Egypt to share scientific knowledge gained about the critical climate-regulating services of the ocean twilight zone with global policymakers. A clear message that emerged from the Ocean and Climate Change Dialogue, which



Ken Buesseler (above) presents work conducted by the OTZ team at the U.N. Oceans Conference in Lisbon, Portugal. Photo by Ken Kostel © Woods Hole Oceanographic Institution

preceded COP27, is the importance of ‘blueing the Paris Agreement’—making the ocean a place for real climate innovation and action. The United Nations also declared that 2022 was ‘The Super Year of the Ocean.’

The OTZ team also recently celebrated the completion of draft text for the Biodiversity Beyond National Jurisdiction (BBNJ) treaty, a groundbreaking piece of international law that will help to protect sensitive mid-ocean environments like the twilight zone. This treaty is the culmination of two decades of negotiations, and since the OTZ project began four years ago, our scientists and policy experts have worked to inform its drafting process. Our team has educated participants, observed U.N. sessions, created informational reports, and organized panel discussions at major U.N. events. We plan to be an active part of the treaty’s future, and will provide valuable scientific data and expertise to member states as they prepare to implement the BBNJ’s terms.

Fundraising efforts

OVER THE COURSE OF THIS PROJECT'S FIVE-YEAR EXISTENCE, THE OTZ TEAM HAS BEEN ABLE TO EXPAND its work thanks to a number of generous grants from philanthropic organizations. These grants enabled the design and deployment of the Twilight Zone Observation Network, a group of scientific buoys in the northwest Atlantic that is providing year-round information about the TZ at a higher level of detail than ever before. Other philanthropic gifts and federal grants have made it possible for us to make valuable improvements to the technology we use in the field, including upgrades to the Twilight Zone Explorer (TZEX) and MINION floats, adding satellite connectivity to existing ROAM tags, and creating a small, plug-and-play version of the ISIIS shadowgraph imager that can be used on ocean gliders and other autonomous vehicles. This technology has been vital for collecting the extensive samples and data that form the bedrock of our research.

FUNDRAISING HIGHLIGHTS INCLUDE:

\$2 MILLION

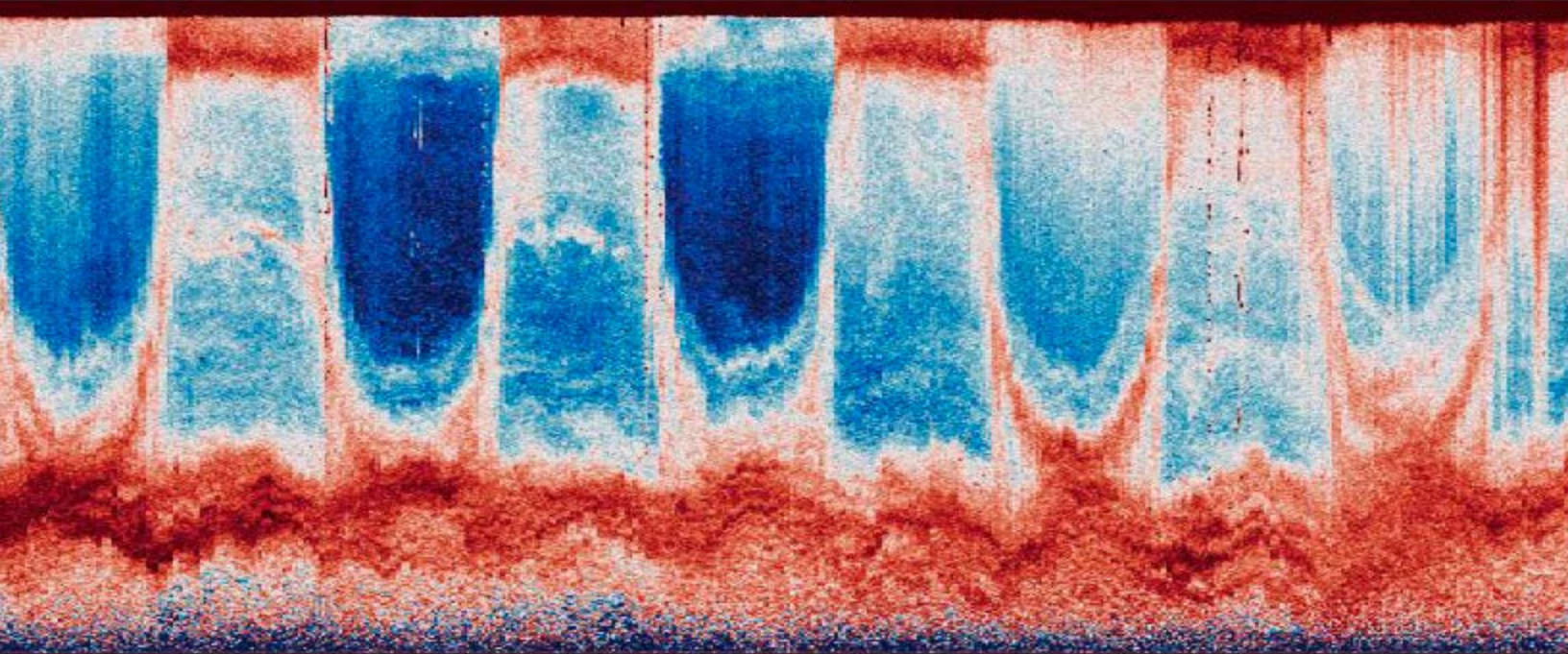
OTZ Observation Network funded by the Happel Foundation

\$1 MILLION

Technology innovation grant funded by the Simons Foundation

\$550,000

Grant funded by an anonymous donor to harden ROAM tag technology



This ecogram (above) depicts acoustic data of migration through the twilight zone taken over several days from the Ocean Twilight Zone Observation Network, a first of its kind persistent record of fish migrating from the twilight zone to the surface at night and back again. This is one of three different large philanthropic gifts over the past couple years. The OTZ project has had success in fundraising efforts due to the solid foundation this project has laid enabling nimble pivots when opportunities arise.



Raising public awareness

WE'VE SEEN A REAL SEA CHANGE IN PUBLIC AWARENESS OF THE OCEAN TWILIGHT ZONE OVER THE PAST FIVE YEARS. Our continued focus on high-quality imagery and well-crafted narratives has potentially reached billions over the past five years. The OTZ project has not only made an impact on people's awareness of this little known zone in the ocean—it has also had a significant influence on the way that the rest of WHOI communicates with the public about its research.

This project has given us the capacity to try new types of marketing campaigns, participate in large-scale events like TED and COP, and realize our very first children's book. "Where the Weird Things Are: An Ocean Twilight Zone Adventure" received great acclaim in March by Inside Editions, an imprint of Simon and Schuster. The book made several Amazon bestseller lists for weeks after publication, and has been very popular with our followers on social media. We've continued to maintain our strong presence with that audience, leveraging our investments in compelling visuals. We have also enjoyed great success with earned media, attracting coverage in dozens of local and national media outlets.



Public Engagement

RELEASED WHO'S FIRST EVER CHILDREN'S BOOK, *Where the Weird Things Are: An Ocean Twilight Zone Adventure*. The book is performing strongly for the publisher with an estimated sales of over 4,000 copies within the first couple months.

LAUNCHED "KEEP IT WEIRD" TWILIGHT ZONE AWARENESS CAMPAIGN. Garnered over 21 million impressions, 2.1 million media post engagements and over 8 million impressions via billboards and other out-of-home ads.

EARNED WIDESPREAD MEDIA COVERAGE OF MAJOR SCIENTIFIC PAPERS. A paper published in *Science Robotics* on Mesobot, WHOI's semi-autonomous underwater robot designed to study the twilight zone, was covered by 20+ media outlets including *Wired*, *Newsweek*, *Science Magazine* and PBS. Estimated combined reach was over 50 million.

COVERED RESEARCH LIVE IN THE FIELD. Each expedition had a dedicated engagement team that was able to capture valuable imagery used for earned media, social, and video.

GENERATED SEVERAL DOCUMENTARY FEATURES ON TZ RESEARCH. The Ocean Twilight Zone+ project has been featured in several different documentary length videos on platforms including Curiosity Stream, YouTube, and more.

INCREASED TZ VISIBILITY AT PUBLIC AND PRIVATE POLICY FORUMS. The OTZ video installation in New York, seen by millions of people during the 76th General Assembly and Climate Week NYC, led to an article in *The New York Times* and recognition of the twilight zone as central to global climate by U.N. delegates, including U.N. Secretary General António Guterres. The OTZ project team has developed two highly-acclaimed policy reports that have been distributed at the Biodiversity Beyond National Jurisdiction (BBNJ) negotiation sessions at the U.N., The Our Ocean Conference, U.N. Decade of the Ocean events, and others.

FEATURED THE TZ IN EXHIBITS AT PROMINENT EVENTS. In 2022 the TZ was featured as part of COP27 and the first-ever ocean pavilion in the exclusive blue zone. In 2019 the team put together an impressive exhibit at the TED conference that exposed a very high-profile audience to the TZ.

Early in the project we created a WHOI's first large scale temporary exhibit at the 2019 TED Conference (above). It was a huge success and has led to several other large events that WHOI might not have otherwise participated in. Photo courtesy of Marla Aufmuth/TED

Social Media and Earned Media

These numbers represent project to date efforts.

Social Media

3 million
TOTAL ENGAGEMENTS

36 million
TOTAL IMPRESSIONS

1000+
POSTS

Media

1,188
MEDIA MENTIONS

3.16 billion
POTENTIAL REACH

FEATURED POST

Woods Hole Oceanographic Institution (WHOI) • March 21

NEW BOOK RELEASE!

103K IMPRESSIONS 127K ENGAGEMENT

HIGHLIGHTS

Vox

212M POTENTIAL REACH
7 ocean mysteries scientists haven't solved yet
vox.com

yahoo!

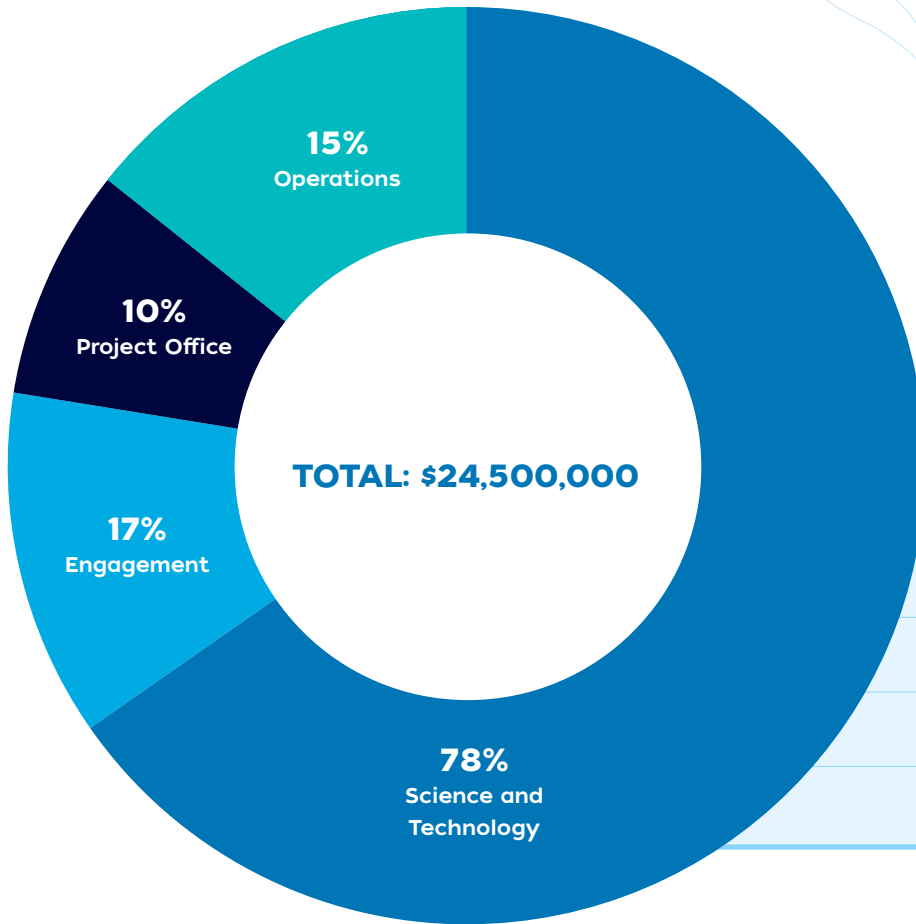
65M POTENTIAL REACH
The ocean could store vast amounts of carbon captured from the atmosphere in its twilight zone – but first we need a 4D internet of deep ocean sensors to track the effects
Associated Press

CAPE COD TIMES

200K POTENTIAL REACH
Entering 'the twilight zone.' Woods Hole scientists, author team up on new children's book
capecodtimes.com

Project Financial Report

Inception through June 2023



ACTUAL EXPENSES

Science and Technology	\$16,000,000
Operations	\$3,000,000
Project Office	\$2,000,000
Engagement	\$3,500,000
Total	\$24,500,000

Thanks to our generous Audacious Project donors, the pledged amount for the six-year, three-phase OTZ Project is: **\$32,100,000**.

RECEIVED
\$28M

Amount the project has received since inception (April 2018)

EXPENSED
\$24.5M

Project expenditures from inception (April 2018) through April 2022

ANTICIPATED
\$4.1M

Funds anticipated to be received during Phase II (July 2020–June 2022)



OCEAN TWILIGHT ZONE

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

Questions or feedback? Contact:

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