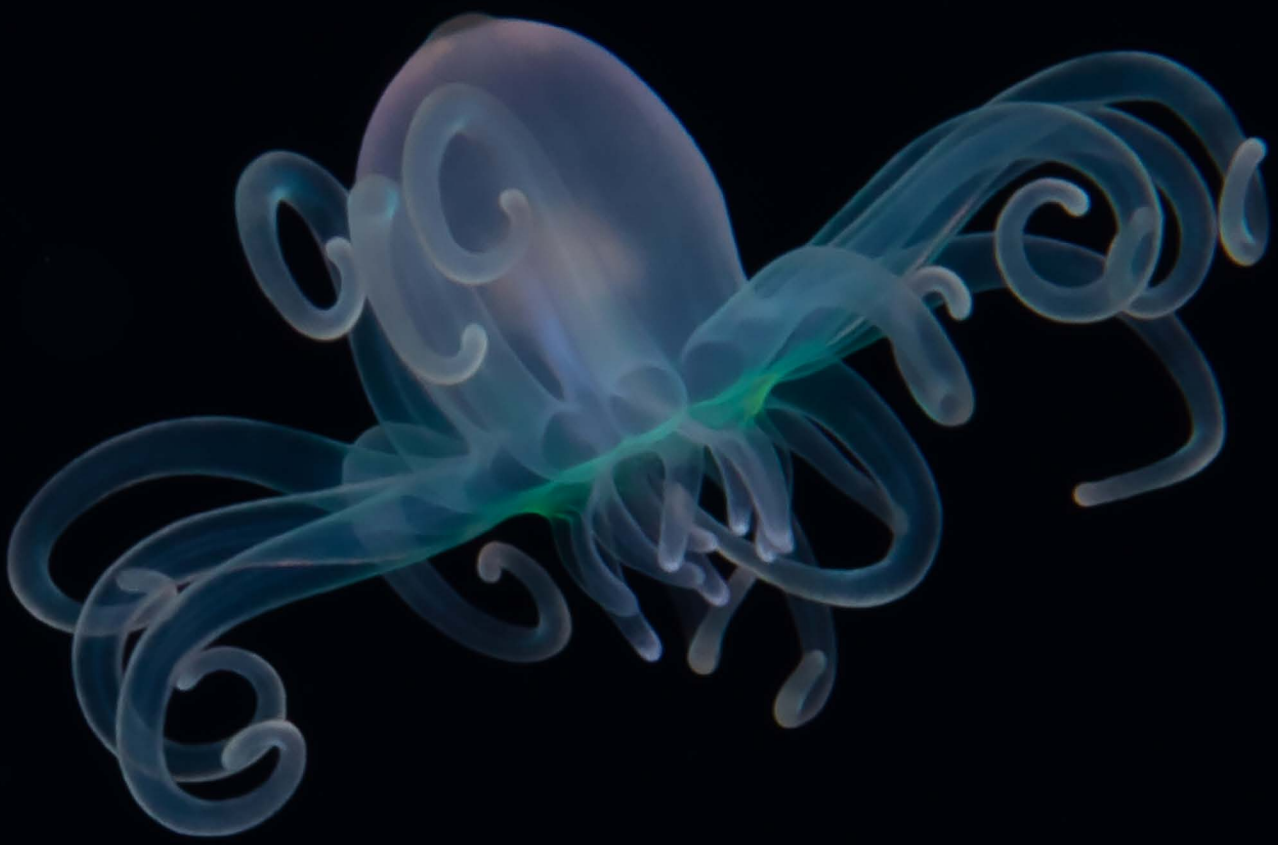
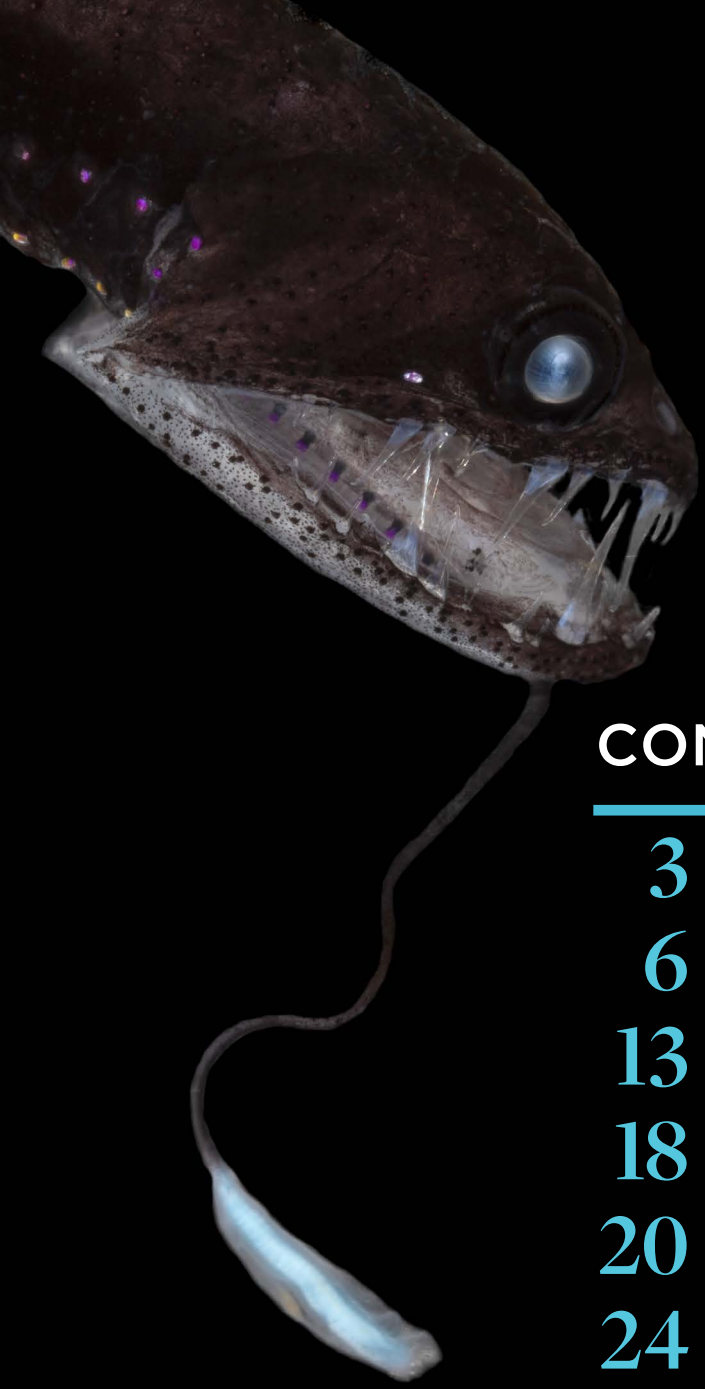


OCEAN TWILIGHT ZONE

WOODS HOLE OCEANOGRAPHIC INSTITUTION

AUDACIOUS PROJECT ANNUAL REPORT

Fiscal Year 2020



CONTENTS

We have embarked on a bold new journey to explore one of our planet's final frontiers—the ocean twilight zone (OTZ), 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.

Cover: Larval tube anemone (*Ceriantharia*). Above: Black dragonfish (*Idiacanthus*). (Photos by Paul Caiger, © Woods Hole Oceanographic Institution)

A shadowgraph image of a siphonophore taken by ISIIS. (© Woods Hole Oceanographic Institution)

Introduction

In the first two years of the Ocean Twilight Zone project, we formed a core, multi-disciplinary team of 12 scientists and developed an integrated work plan aligned with the project's phase one theme: "initiate, accelerate, and engage." It has created a firm foundation and infrastructure for future research; developed a growing arsenal of tools and techniques to understand the twilight zone; and dramatically improved awareness of this vast ecosystem's importance within international policy making groups.

The team's continued success is due in part to its powerful suite of sampling and analysis methods. It employs a wide range of different but complementary techniques, empowering the team to fill in gaps and shortcomings with higher confidence than any single method could produce. This approach leads to a bigger, more detailed view of the twilight zone than ever before possible, and enables researchers to unravel the mysteries of the zone at a faster pace.

KEY IMPACTS & ACCOMPLISHMENTS

Ocean Twilight Zone (OTZ) project researchers are employing cutting-edge tools like environmental DNA (eDNA) to explore the twilight zone, gathering crucial data on the types of species that exist in its waters—including cryptic species that net tows have missed. Using the *Deep-See* vehicle and the new autonomous *Mesobot*, the team has also been able to test new sampling devices, gather detailed acoustic information, and collect images using new holographic and high-speed cameras.

Combined data from the OTZ team as a whole is already generating surprising results. This year, researchers discovered that the twilight zone relies on not just one food web, but two: one involving migrating organisms; the other involving organisms at depth. The data also shows that undiscovered biomass in the twilight zone could be far lower than previous estimates, a finding that could have important implications for fisheries management.

In addition to these significant findings, team members produced a new, more nuanced definition of where the twilight zone begins and ends, using it to re-analyze estimates of carbon transport from the surface to the deep ocean. The early results of this work suggest that the ocean sequesters twice as much heat-trapping carbon from the atmosphere as previously thought.

DRIVERS OF SUCCESS

ACCELERATING FUNDAMENTAL KNOWLEDGE

- Calculated a more accurate estimate of twilight zone biomass.
- Discovered two different but connected food webs at surface and at depth.
- Created a new, more nuanced estimate of carbon transport in the twilight zone.

ADVANCING TECHNOLOGY

- Completed the first sea trial of *Mesobot* last summer with a successful scientific mission later that year.
- Gained critical insight on abundance and distribution of OTZ creatures from the first scientific mission of *Deep-See*.
- Developed and deployed a new high-volume eDNA sampler.
- Developed and deployed a new low-cost sensor for measuring carbon transport.

INFORMING POLICY

- Created the first quantitative assessment of twilight zone ecosystem services.
- Worked to inform major intergovernmental initiatives on the importance of the twilight zone, including the negotiations for a new treaty on Biodiversity Beyond National Jurisdiction (BBNJ) and UN Decade of Ocean Science for Sustainable Development.

RAISING PUBLIC AWARENESS

- Earned 2.8 million impressions through new social marketing campaigns.
- Placed op-eds in prominent media outlets with a reach of over 70 million to highlight the importance of understanding and protecting the twilight zone.
- Increased our engagement beyond existing WHOI audiences through partnerships with 4Oceans, OceanX, the Ocean Alliance, and other organizations.

COLLABORATING AND ENGAGING ACADEMIA

- Published or drafted more than a dozen papers in high-profile academic journals.
- Joined forces with leaders of large international research efforts to create JETZON to coordinate global research and share data.
- Developed collaborations with international twilight zone research groups SUMMER and MEESO.

ESTABLISHING BEST PRACTICES

- Designed new machine learning and processing techniques to analyze data autonomously.
- Developed new eDNA methods to identify species that net tows may miss.

Defining success

SCIENCE IS ITERATIVE—IT ADVANCES IN COUNTLESS SMALL STEPS AS EACH NEW DISCOVERY OR INSIGHT BUILDS ON ITS PREDECESSORS AND RAISES NEW QUESTIONS.

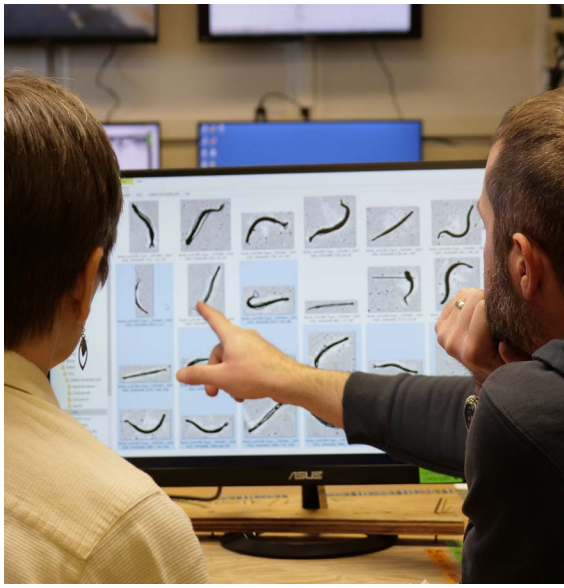
The Ocean Twilight Zone project has established a foundation to track progress towards our goal of achieving a baseline of scientific understanding about the mesopelagic and informing policy makers and the public collectively to make sound decisions that ensure the long-term sustainable use of our ocean's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

Understanding life in the twilight zone

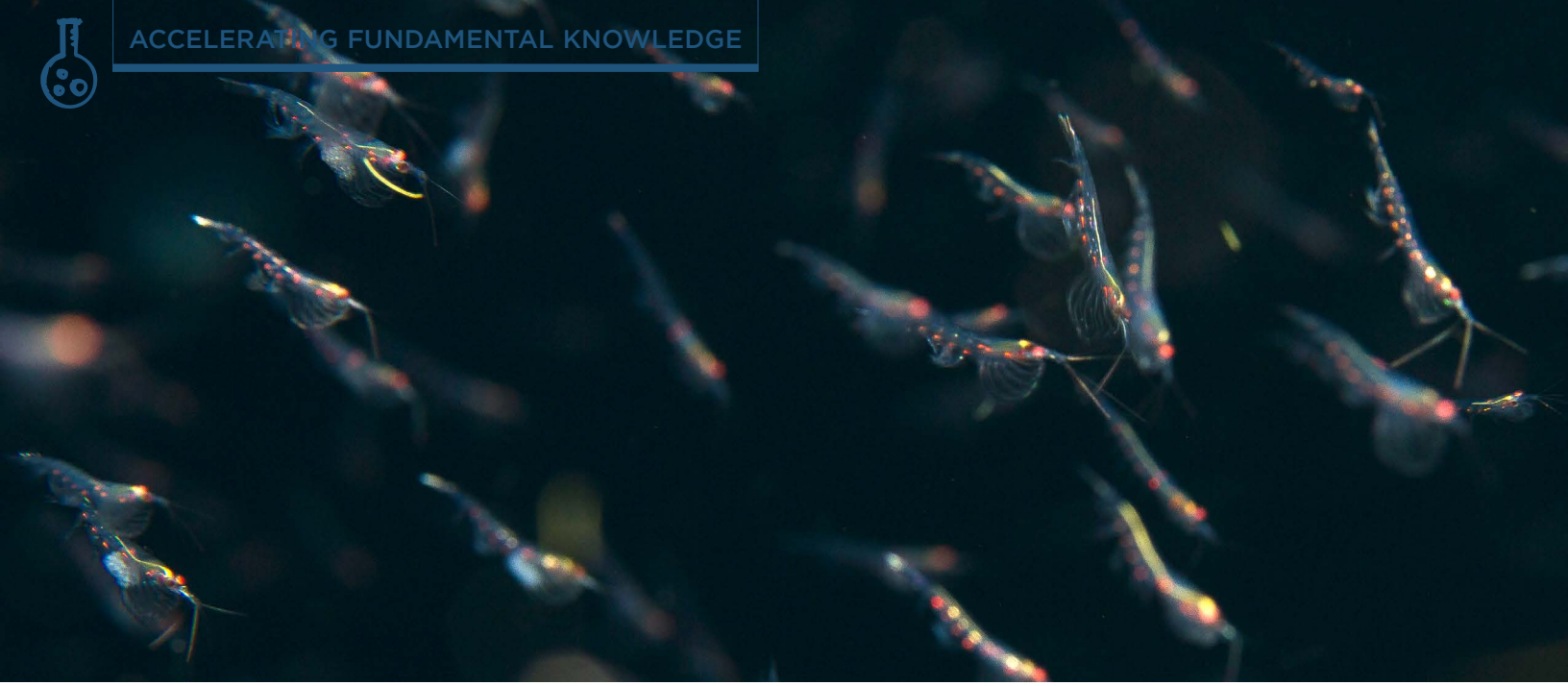
To sustainably manage an ocean twilight zone fishery, it is critical to understand how much overall life (or "biomass") the zone holds, and what types of animals exist there ("biodiversity"). That's no easy task. To date, researchers have been challenged by the fact that the twilight zone is vast, dark, and difficult to access. As new technology comes online and traditional technology is adapted in new ways, however, we have gained tantalizing insights that bring us closer to an answer.

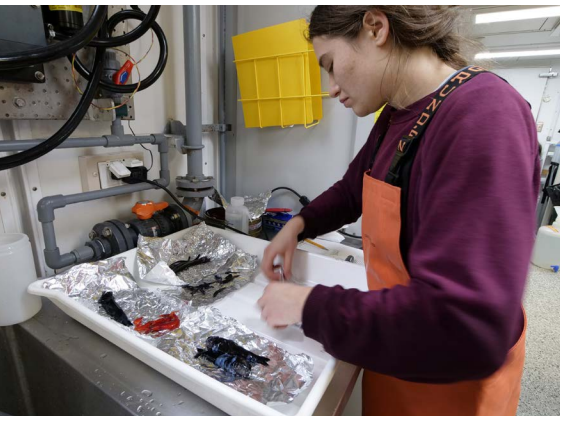
KEY IMPACTS & ACCOMPLISHMENTS

- Discovered that recent estimates of twilight zone fish biomass likely overstate the amount of life present. This finding arose from preliminary acoustic data taken from *Deep-See*, and was made possible by new automated analysis techniques.
- Collected detailed acoustic data from *Deep-See*, allowing the OTZ team to identify individual animals. These data revealed that life in the twilight zone is highly variable.
- Developed new methods and workflows for collecting and analyzing environmental DNA (eDNA) from mesopelagic depths.
- Identified numerous fish and invertebrates through DNA barcoding. These same animals are also being analyzed for their food web interactions, and the barcoding identifications allow us to draw species-specific conclusions.
- Identified a rare deep-sea jellyfish and revealed a potential new cryptic species of lanternfish using genetic analysis.
- Carried out experiments that showed high variability both in the rate that different organisms shed eDNA and the persistence of that eDNA in the twilight zone environment. These results will affect how scientists interpret eDNA signals from the twilight zone.



Heidi Sosik and Joel Llopiz identify shadowgraph images from ISIIS on the March 2020 *R/V Armstrong* Cruise. (Photo by Ken Kostel © Woods Hole Oceanographic Institution)





WHOI research assistant Julia Cox carefully sorts twilight zone animals collected during a net tow. (Ken Kostel © Wood Hole Oceanographic Institution) Above: Krill. (Photo by Paul Caiger © Woods Hole Oceanographic Institution)

HOW MUCH LIFE IS IN THE TWILIGHT ZONE?

Current estimates of fish biomass are highly uncertain due to assumptions about what the acoustic signatures of fish look like from the surface. The OTZ team's new vehicle, *Deep-See,* is uniquely capable of reducing this uncertainty. It places sophisticated acoustic systems directly into the twilight zone, creating precise measurements that let researchers develop more accurate estimates of biomass. This is particularly important today, since other recent estimates have suggested that fish biomass in the twilight zone may be more than 10 times greater than previously thought and have spurred the interest of the commercial fishing industry. Data gathered by *Deep-See,* however, indicates that this number is likely too high. The real number may be closer to two or three times more fish than historical estimates. The analysis also shows that the distribution of fish in the twilight zone is more variable than previously believed.

In addition to broadband acoustic instruments, *Deep-See* is equipped with a holographic camera designed to capture extremely high-resolution images of zooplankton less than one inch in size. Once analyzed, these images will add detail to the acoustic data and give the OTZ team the ability to characterize zooplankton in deep scattering layers. We need to know the relative abundance of common types of mesopelagic zooplankton so we can estimate their total biomass and better understand the role they play in marine ecosystem dynamics and global carbon cycling.

The holographic camera aboard *Deep See* can also image soft-bodied creatures like jellyfish, salps, and siphonophores—creatures that have been historically undercounted in both acoustic surveys and net tows. In addition, a camera aboard the towed system ISIIS is specially equipped to study these soft-bodied creatures, and the two platforms have each generated hundreds of hours of new footage.

All told, the team has completed over 200 hours of *Deep-See* tows, 25 trawl nets for fish, and 20 net tows for plankton throughout the twilight zone. These efforts have collected animal samples and data from the surface down to 1,000 meters at all times of day, capturing animals at multiple points during their migration through the water. In the process, the team is generating a more complete understanding of life across the entirety of the twilight zone and revealing how it changes over time.

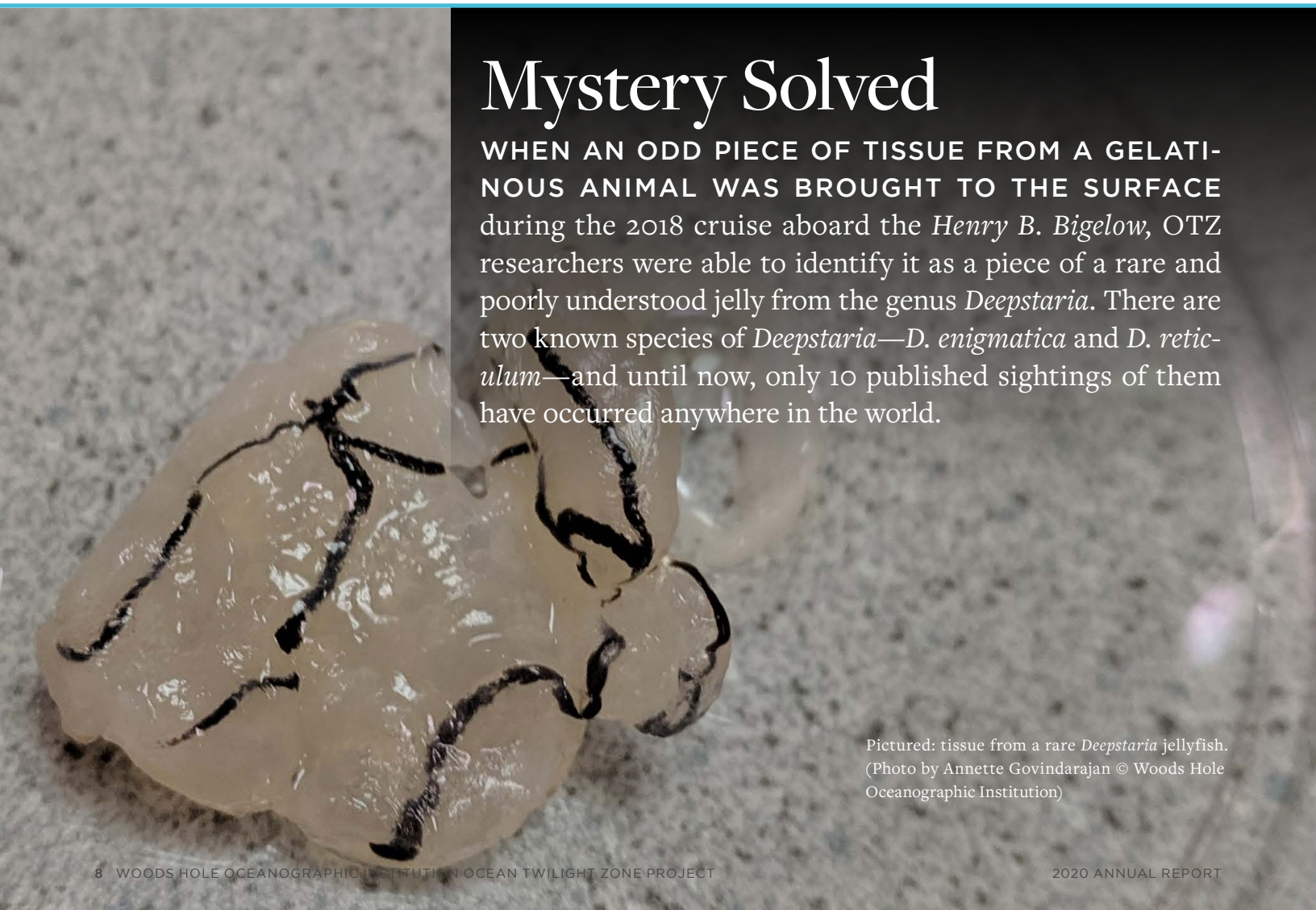
WHAT KIND OF ANIMALS LIVE IN THE TWILIGHT ZONE?

Species that live in the ocean twilight zone are remarkably understudied. As a result, genetic reference libraries don't exist for many of these creatures. The OTZ team is currently working to fill the gaps in genetic libraries, helping to both facilitate its own research and accelerate twilight zone research across the globe. To that end, team members have genetically analyzed over 200 individuals, adding DNA from 67 unique species to the library to date.

This effort has also revealed a probable new cryptic species—one that appears very similar or identical to existing species, but differs at the genetic level—in specimens of the lanternfish *Centrobranchus nigroocellatus*. The OTZ team found consistent genetic differences in samples originating in the Sargasso and Caribbean Seas and those from the Gulf of Mexico. Based on these differences, it is likely that there are actually at least two distinct and geographically separated species. Identifying cryptic species is important in order to obtain accurate estimates of biodiversity. We expect that as our database grows, we will find more examples of cryptic species.

Because net tows aren't capable of capturing everything, the team is pioneering a new genetic approach that uses environmental DNA (eDNA) to identify animals in the twilight zone that nets may have missed. The technique uses filtered seawater samples to reveal traces of DNA left by animals in the water, letting researchers identify animals without ever laying eyes on them. The OTZ team has collected more than 500 eDNA samples to date. While there is significant overlap between species identified through eDNA and net tows, each approach is recovering unique species that the other method missed.

The team has also conducted laboratory experiments to learn how different types of organisms might shed eDNA, and how long that eDNA may persist in the water. The results from these experiments have shown that gelatinous organisms shed more eDNA than fish, and organisms that have an exoskeleton, like shrimp, shed less. These findings will help create models that will let researchers better understand eDNA results from the twilight zone.



Mystery Solved

WHEN AN ODD PIECE OF TISSUE FROM A GELATINOUS ANIMAL WAS BROUGHT TO THE SURFACE during the 2018 cruise aboard the *Henry B. Bigelow,* OTZ researchers were able to identify it as a piece of a rare and poorly understood jelly from the genus *Deepstaria*. There are two known species of *Deepstaria*—*D. enigmatica* and *D. reticulum*—and until now, only 10 published sightings of them have occurred anywhere in the world.

Pictured: tissue from a rare *Deepstaria* jellyfish. (Photo by Annette Govindarajan © Woods Hole Oceanographic Institution)

Connecting life in the twilight zone

Measuring and understanding linkages in the twilight zone food web—and between the twilight zone and other parts of the ocean—are key objectives for the OTZ team. By describing what species are present and how they interact with each other, we can begin to piece together the function and importance of the twilight zone ecosystem as a whole.

KEY IMPACTS AND ACCOMPLISHMENTS

FOOD WEBS AND LIFE HISTORIES

- Discovered two different but connected food webs at surface and at depth.
- Published study describing blue sharks descending in warm-core ocean eddies to feed in the twilight zone.
- Dissected more than 600 individual animals to better understand each organism's lifespan, growth rate, and age at maturity. All of these are critical components for understanding a species' resilience to fishing pressures.

Blue sharks bask in the surface waters. (Photo by Nuna Sá)

DO ANIMALS NEAR THE SURFACE INTERACT WITH ANIMALS FROM THE TWILIGHT ZONE?

Over the past year, OTZ researchers have discovered that animals in the twilight zone don't just fit into a single food web. Instead, they make up at least two distinct, yet related systems: one that interacts with surface food webs and another that appears to remain at depth, largely separate from the surface.

OTZ researchers used stable isotope analysis to reveal the link between twilight zone and surface food webs. By measuring the ratio of carbon 13 and carbon 12 isotopes from amino acids in a fish's muscle tissue, the researchers were able to determine whether the animal's primary food source lived at the surface or at depth. Eventually, the stable isotope method may help determine which species migrate to the surface each night, and which ones do not, providing new information on the behavior and life cycles of poorly understood species.

WHO IS EATING WHOM IN THE TWILIGHT ZONE?

The OTZ team continues to use a variety of methods to determine predator-prey relationships in the twilight zone. These include placing tracking tags on top predators, stable isotope analysis of muscle tissue, eDNA analysis, and visual and genetic analysis of gut contents.

Data from satellite tags showed for the first time that blue sharks target the twilight zone on foraging dives in the center of warm-core ocean eddies—large, swirling water masses once thought to be ocean "deserts." This insight is critical to shaping flexible management strategies for individual species and entire ecosystems. Along with data collected through environmental DNA and the *Deep-See* vehicle, the OTZ team's research suggests that gelatinous organisms are likely underestimated, and could be one of the most common life forms in the twilight zone. Together, these complementary techniques provide a new picture of the twilight zone food web.

HOW COULD COMMERCIAL FISHING AFFECT THE TWILIGHT ZONE?

To determine how fishing might affect the twilight zone ecosystem, we need to first understand the life histories and behaviors of the many different species living there. Knowing their growth rate, maturation, and reproductive behavior will help the OTZ team lay the groundwork to predict such future impacts. To that end, OTZ researchers have completed over 600 dissections of animals spanning the depth range of the twilight zone and have conducted analyses to better understand their lifespan, growth rate, and age at maturity—all of which are critical components to understanding a species' resilience to fishing pressures.

FEATURED RESEARCHER

Andone Lavery

Andone Lavery is an acoustical oceanographer—a physicist who uses sound to learn more about life in the ocean. In the twilight zone, she applies sophisticated acoustic instruments to the problem of detecting and identifying animals who live in darkness. Lavery led the development of the new platform *Deep-See*, and she and her team are working to develop automated and machine learning processes to analyze its acoustic data. These new tools will enable the OTZ team to map the size and distribution of animals in the twilight zone more efficiently and precisely than ever before.

Revealing the twilight zone's impact on climate

Scientists have long understood that the world's oceans can remove heat-trapping carbon from the atmosphere and safely store it in deep waters or beneath the seafloor for long periods of time. However, estimates of how much carbon is captured and leaves the surface ocean are based on an arbitrary definition of the boundary between the sunlit upper ocean—where photosynthesis occurs—and the dark twilight zone, where photosynthesis ceases. Using actual measurements of light levels below the surface, OTZ researchers have revised where that boundary is, and have shown that the ocean may be sequestering twice as much carbon as previously thought. This finding, which was accelerated by OTZ support of a postdoctoral investigator, WHOI graduate students, and guest students, will help climate modelers refine their forecasts of climate change worldwide.

KEY IMPACTS AND ACCOMPLISHMENTS

CARBON AND CLIMATE

- Reanalyzed existing estimates of carbon transport from the surface to the deep ocean using a new, more nuanced definition of where the twilight zone begins. This reanalysis is changing our scientific understanding of the role the ocean plays in the global carbon cycle and Earth's climate.
- In collaboration with the University of Rhode Island, advanced from design to prototype stage of MINIONs, a new low-cost sensor for measuring carbon transport from the surface through the twilight zone.
- Analyzed the economic value of reducing the uncertainty in estimates of carbon sequestration by the world's oceans. OTZ policy experts and scientists concluded that making the estimates more accurate could have an economic value on the order of hundreds of billions of dollars, leading to improved decision making and policy formation.

Zooplankton under a microscope. (Photo by Erik Olsen © Woods Hole Oceanographic Institution)

HOW DOES CARBON MOVE FROM THE SURFACE TO THE DEEP OCEAN?

Excess carbon in the atmosphere forms a heat-trapping blanket that drives up temperatures and changes climate and weather patterns around the globe. It also dissolves into the surface ocean, where it is incorporated into organic forms of carbon via photosynthesis in marine algae. Some animals in the ocean, mainly microscopic zooplankton and small fish, feed on the algae, transferring carbon in the algae to their own bodies. As these animals die or defecate, that carbon slowly sinks down through the water, preventing it from returning to the atmosphere. Methods pioneered by OTZ team members allow researchers to measure how much carbon is sinking through the water to the deep ocean—where it remains for hundreds of thousands of years—and how much gets consumed or returns to the surface in forms that are no longer sequestered away from the ocean surface and the atmosphere.

HOW MUCH CARBON DOES THE TWILIGHT ZONE TRANSPORT?

Members of the OTZ science team recently helped reanalyze past efforts to calculate the efficiency of the biological carbon pump, or ability of the ocean to take up carbon. Those earlier estimates used a fixed depth to define the end of the sunlit upper ocean and the beginning of the twilight zone. The team found that this method underestimates the efficiency of the carbon pump because it does not take into account the depth of light penetration, which ultimately controls production of sinking organic carbon and varies by location and season. It also makes it difficult to make comparisons between studies if different groups use different depths to define the flow of carbon. Also of importance to this revised understanding of the biological carbon pump is the impact on the carbon cycle of diel vertical migration, the daily movement of plankton and fish from the twilight zone to feed at night on the surface. Understanding this movement, which is believed to be the largest animal migration on Earth—not just in terms of biomass, but in terms of its impact on carbon flow—is critical to a more nuanced and accurate view of the biological carbon pump and its role in global climate.

HOW COULD HUMAN ACTIVITY IN THE TWILIGHT ZONE AFFECT EARTH'S CLIMATE?

This is a critical question that is central to the OTZ project. If intensive fishing of the twilight zone follows the path of other fisheries around the globe, humans could quickly deplete species anywhere we fish. But if we overfish the twilight zone, would more or less carbon make it to the deep ocean? On one hand, removing grazers—fish that consume organic carbon particles in the water—would allow more of those particles to sink, sequestering the carbon for long periods of time. On the other hand, depleting the twilight zone of predators might cause populations of grazing species to expand, choking the flow of carbon into the deep ocean.

FEATURED RESEARCHER

Ken Buesseler

Ken Buesseler is a marine radiochemist who studies the fate and distribution of radioactive elements—some natural, some not—in the ocean. The properties of individual isotopes can tell us something about where and how quickly many of the physical, chemical and biological processes occur. Naturally occurring isotopes of thorium, for example reveal how quickly and how much carbon is carried on sinking particles from the sunlit surface ocean through the twilight zone to the deep ocean. Buesseler's work has been instrumental in refining scientific understanding of the biological carbon pump, its role in the global climate system, and how it links the twilight zone with the surface ocean and society. His lab has also applied this knowledge in responding to concerns over the spread and impacts of radioactivity from the Fukushima Daiichi nuclear power plant, and from earlier sources such as Chernobyl and atomic weapons testing at the Marshall Islands.

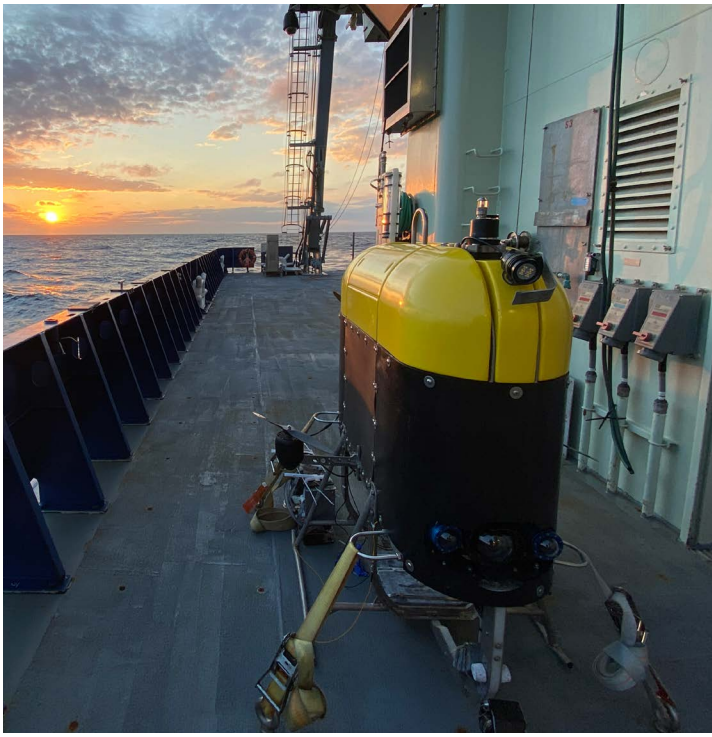
Emerging technology to access the twilight zone

The OTZ team continues to leverage new and emerging technologies to open access to the twilight zone. Advances in robotics, sensors, machine learning, molecular techniques, and low-cost manufacturing have allowed the team to develop new tools for research, to obtain measurements and samples across more of the twilight zone more rapidly than anticipated, and to expand access for other researchers around the globe.

KEY IMPACTS AND ACCOMPLISHMENTS

- Completed the first sea trial of *Mesobot* last summer with a successful scientific mission later that year
- Gained critical insight on abundance and distribution of twilight zone creatures from the first scientific mission of *Deep-See*
- Developed and deployed a new high-volume eDNA sampler
- Developed and deployed a new low-cost sensor for measuring carbon transport

An OTZ engineer deploys *Deep-See* during a 2019 research cruise. (Photo by Paul Caiger © Woods Hole Oceanographic Institution)



Mesobot on the deck of *R/V Armstrong*. (Photo by Ken Kostel © Woods Hole Oceanographic Institution)

DEEP-SEE

Data collected with the acoustic, optical, and biological sensors on *Deep-See* produced critical insights in 2019. First, it revealed that the distribution of organisms in the twilight zone is extremely uneven from one location to another. Second, it showed the value of placing submerged, *in situ* acoustic sensors directly into the twilight zone, where they were able to count and measure individual animals—a feat that would have been impossible using traditional acoustic techniques. The instruments on *Deep-See* have also shown that gelatinous organisms are more abundant than previously expected, and could be one of the most common life forms in the twilight zone.

Deep-See in mid-deployment aboard the NOAA ship *Henry B. Bigelow.* (Photo by Paul Caiger © Woods Hole Oceanographic Institution)

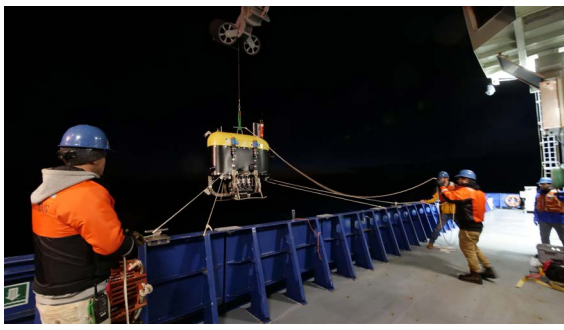
MESOBOT

The OTZ team finished building and testing the new autonomous underwater vehicle, *Mesobot*, which can carry a variety of camera and sampling instruments into the twilight zone while having minimal impact on the behavior of animals living there. Since its first scientific mission in summer 2019, OTZ researchers have used the platform to image aggregations of twilight zone animals, actively track moving animals, measure downwelling light, and take eDNA samples in layers identified by shipboard sonar. *Mesobot* has also been fitted with a newly designed, highly sensitive, high dynamic range radiometer capable of measuring the extremely low light levels that likely trigger the global daily vertical migration.

An OTZ research assistant prepares an eDNA sampler for deployment. (Photo by Dana Yoerger © Woods Hole Oceanographic Institution)

HIGH-VOLUME eDNA SAMPLER

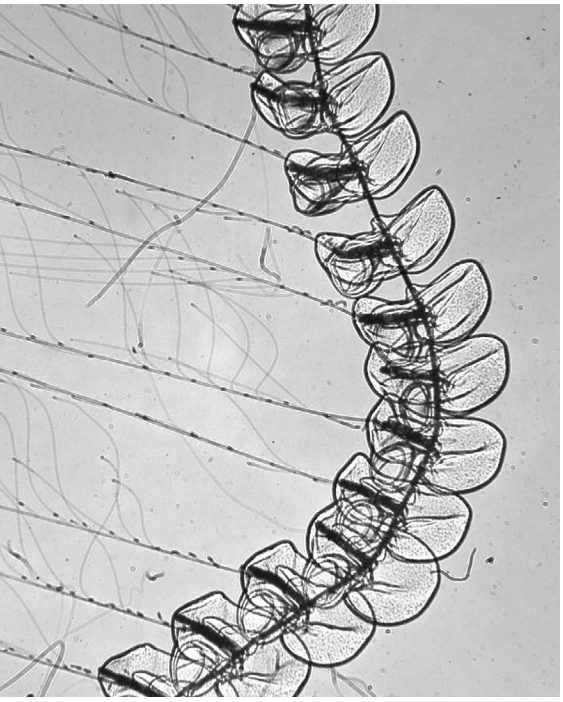
The OTZ team has continued to pioneer the use of environmental DNA (eDNA) analysis in the twilight zone. Throughout 2019, researchers and engineers worked closely to develop an eDNA sampler capable of filtering hundreds of liters of water in a single deployment, and used it for the first time in fall 2019 on *Mesobot*. With the samples it collected, the team is creating a more detailed view of biodiversity in the twilight zone and is extending the science community's use of this powerful analytical method in the open ocean.



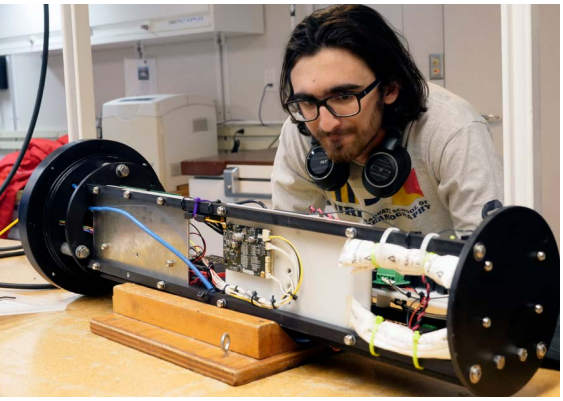
A nighttime deployment of *Mesobot* from the deck of the R/V *Armstrong.* (Photo by Ken Kostel © Woods Hole Oceanographic Institution)

RADIOMETER

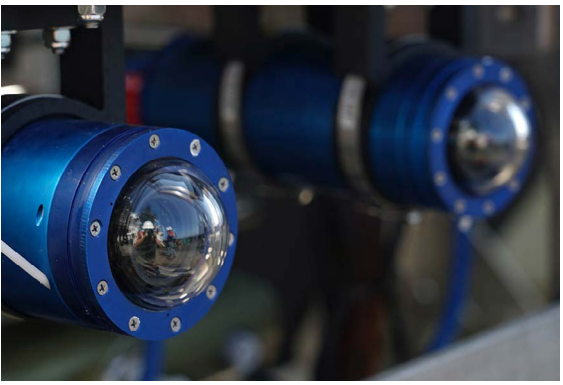
The low levels of light that reach the twilight zone play an important role in governing the rhythms and pulses of life. Light cues animals when to migrate, helps them hide, and much more—yet direct measurements of light levels in the deep ocean are normally extremely difficult to obtain. Our partners at MIT's Future Ocean Lab have created prototypes of a low-cost radiometer that will enable virtually any underwater vehicle or instrument to become an ultra-sensitive light sensor. Researchers deployed the first versions on *Mesobot* aboard R/V *Neil Armstrong* in March 2020 with early testing already returning useful data.



Shadowgraph image taken by ISIIS. (© Woods Hole Oceanographic Institution)



The computer module that controls ISIIS. (Photo by Ken Kostel © Woods Hole Oceanographic Institution)



High-resolution cameras aboard *Mesobot*. (Photo by Ken Kostel © Woods Hole Oceanographic Institution)

MINIONS

During a March 2020 cruise on R/V *Neil Armstrong*, the OTZ team deployed prototype MINIONs for the first time in the open ocean. These small, inexpensive devices—each costing approximately $1000 to build—are used to sample pre-selected depths in the OTZ where they photograph particles of marine snow as it falls from the surface. In the future, these instruments will become part of an armada of easily deployable devices to measure carbon flux in the twilight zone and enable more ubiquitous access to the twilight zone by researchers worldwide. MINIONs have been designed and developed In collaboration with the University of Rhode Island.

ROAM TAGS

Smaller, more capable ROAM satellite tags will soon permit the OTZ team to track sharks and other large predators in three dimensions as they move through the twilight zone over months or years. In 2019, the team continued development of prototypes that will be tested in fall 2020 using an autonomous glider to mimic the vertical movement of large mesopelagic predators. The team also tested deployment methods on sharks and swordfish using existing tags, providing data that will be useful for planning the first ROAM tag deployments in the field.

TWILIGHT ZONE EXPLORER (TZEx)

The movement of carbon in and through the twilight zone is a critical, but poorly understood part of Earth's climate system. In order to measure how much gets through and is sequestered in the deep ocean, the OTZ team is developing the Twilight Zone Explorer (TZEx), a combined sampling and imaging system that will collect marine snow as it sinks through the twilight zone. TZEx is being built entirely from off-the-shelf parts mounted to an existing French profiling float. In 2019, the team tested early prototypes of the explorer for buoyancy and other controls with plans to continue development in 2020.

ISIIS

The *in situ* Ichthyoplankton Imaging System (ISIIS) made it possible for the OTZ team to collect images of millions of plankton at all depths from the surface to the bottom of the twilight zone at different times of the day and night. The team is now applying automated image analysis and artificial intelligence to analyze this huge trove of biological and environmental data. The findings may change our understanding of plankton community dynamics, with implications for ocean food webs, fisheries, and global climate.

Bridge of NOAA Ship *Henry B. Bigelow.* (Photo by Erik Olsen © Woods Hole Oceanographic Institution)

In the Field: 2019-2020 Missions

The past 12 months have been remarkably active on almost every front, but perhaps none more so than expeditionary field work. In the summer, *Deep-See* made its first fully operational deployments, carrying its suite of acoustic and other sensors directly into the twilight zone. *Mesobot* also came fully online with a trip in the fall to Flower Garden Banks National Marine Sanctuary. The OTZ team capped its field work for the year in March 2020, with a cruise aboard the WHOI-operated research vessel *Neil Armstrong*, where it deployed *Mesobot*, a large MOCNESS towed net sampler, and a host of other research tools to examine a complex and dynamic part of the twilight zone just off the Northeast U.S. In all of these expeditions, environmental DNA (eDNA) sampling also took center stage, with WHOI researchers pushing the technology to help shape a new and powerful tool that will almost certainly rewrite what science can tell us about life in the twilight zone and the myriad ways it is affected by human activity.

R/V SARMIENTO DE GAMBOA, MAY 3-23, 2020*

Location: Northeastern Atlantic

Chief Scientists: Ken Buesseler and Heidi Sosik, WHOI

Science Objectives:

- Study processes that control carbon cycling during spring bloom
- Identify the key observations needed to characterize the biological pump
- Deploy new technologies within the EXPORTS field campaign

Technology: MOCNESS, MINION floats, *Mesobot*, *in situ* environmental DNA sampler, ISIIS

Engagement: Onboard journalist, videographer, children's book author / Dive & Discover Correspondent

Partner: NASA EXPORTS

**Postponed due to pandemic*

R/V NEIL ARMSTRONG, MARCH 11-16, 2020

Location: Northeastern US continental slope

Chief Scientists: Joel Llopiz and Heidi Sosik, WHOI

Science Objectives:

- Obtain concentrations of nutrients, chlorophyll, picoplankton and nanoplankton at depth
- Collect samples of animal life and eDNA
- Test and refine MINION floats, *Mesobot*, Stingray/ISIIS

Technology: MOCNESS, MINION floats, *Mesobot*, ISIIS

Engagement: Onboard journalist videographer, photographer

Partners: University of Rhode Island; MIT

R/V MANTA, SEPTEMBER 20-28, 2019

Location: Northwestern Gulf of Mexico

Chief Scientist: Santiago Herrera, Lehigh University

Science Objectives:

- Collect water samples for eDNA analysis
- Test functionality of AUV *Mesobot* for midwater navigation and sampling
- Filter water *in situ* with *Mesobot*

Technology: *Mesobot*

Engagement: Onboard videographer

Partners: Lehigh University; University of North Carolina, Wilmington; Flower Garden Banks National Marine Sanctuary

NOAA HENRY B. BIGELOW, JULY 24-AUGUST 8, 2019

Location: Northwest Atlantic
Chief Scientists: Michael Jech, NOAA-NMFS and Andone Lavery, WHOI
Science Objectives:

- Test, calibrate, and evaluate *Deep-See* acoustical and optical system
- Collect acoustical and optical data on deep-scattering layers
- Sample plankton and nekton using MOCNESS nets and midwater trawls
- Collect water samples for eDNA analysis

Technology: *Deep-See*, MOCNESS, *in situ* environmental DNA sampler
Engagement: Onboard videographer
Partner: NOAA Northeast Fisheries Science Center (NEFSC)

R/V RACHEL CARSON, JUNE 19-21, 2019

Location: Monterey Bay, CA
Chief Scientist: Dana Yoerger, WHOI
Science Objectives:

- Test and evaluation cruise for *Mesobot*

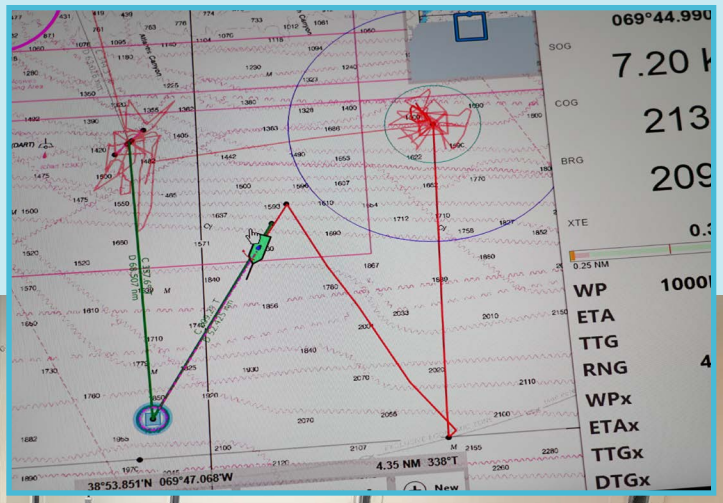
Technology: *Mesobot*
Engagement: Science team photographer
Partners: Monterey Bay Aquarium Research Institute; Stanford University; and the University of Texas Rio Grande Valley

SSV CORWITH CRAMER, APRIL 15-MAY 3, 2019

Location: Bermuda to Woods Hole
Chief Scientist: Kerry Whittaker, Sea Education Association
Science Objectives:

- Collect water samples for eDNA analysis

Engagement: Science team photographer
Partner: Sea Education Association



Researchers crowd into the *Deep-See* control room aboard the NOAA ship *Henry B. Bigelow*. (Photo by Jennifer Berglund © Woods Hole Oceanographic Institution). Inset: GPS tracks show the movement of the *Bigelow* throughout its mission.

Porter Hoagland and his student Aria Finkelstein at the United Nations (Photo by Michael Holland)

Informing Policy

Policy activities by members of the OTZ team have fallen into two categories: research and reporting, and leadership and influence. Both have established the policy group as a cross-theme within the project that draws on research findings and, at the same time, helps feed the science and technology themes with key unanswered questions at the heart of new or pending policy discussions.

New research and reports by the policy team have focused primarily on educating and informing policy-makers on the economic value of the twilight zone, both as a functional part of the broader ocean ecosystem and as a critical part of Earth's climate system. The OTZ report on twilight zone ecosystem services, an upcoming book chapter on natural capital in the mesopelagic, and a review of changes in marine capture fisheries for the Second World Ocean Assessment are prime examples of this work and its ability to reach targeted audiences.

On the leadership and influence front, team members from the policy and science themes have established and deepened relationships with representatives of governmental agencies, intergovernmental organizations, civil society, and academia to raise the profile of the mesopelagic region at key meetings and negotiations. The two efforts that the team has identified as offering the richest opportunity for far-reaching science-based decision making are the ongoing negotiations for the new treaty governing Biodiversity Beyond National Jurisdiction (BBNJ) and the upcoming UN Decade of Ocean Science for Sustainable Development.

Download the report here

KEY IMPACTS AND ACCOMPLISHMENTS

- **Value of Knowledge:** Showed that the efforts of ocean scientists to reduce uncertainty about the amount of carbon sequestered in the ocean could have an economic value on the order of hundreds of billions of dollars, and could lead to improved decision making and policy formation.
- **Bringing the Twilight Zone to Policy Makers:** Established and deepened relationships to facilitate inclusion of the twilight zone into major intergovernmental initiatives, including the BBNJ treaty and UN Decade of Ocean Science for Sustainable Development.
- **Reports:** Completed a report detailing the economic value of the twilight zone and ecosystem services it provides and distributed to U.S., European, and Canadian government officials as well as leaders from UN agencies, academia, and NGOs. Further, contributed chapters to high-profile publications detailing the economic importance of natural capital in the mesopelagic and reviewing changes in wild marine fisheries. The ecosystem services report in particular has been cited explicitly in a recent UN document summarizing extant proposals put forward to revise the BBNJ treaty language.

WHAT ARE THE POTENTIAL ECONOMIC EFFECTS IF THE COMMERCIAL HARVEST OF TWILIGHT ZONE FISH AFFECTS OTHER SPECIES?

At the moment, the economic value of twilight zone fisheries does not appear to support industrial-scale fishing, but this may change as technological developments and the demand for fishmeal and fish oil to supply aquaculture operations tip the scale towards profitability. Many large-scale fisheries have already forced alterations in the structure and function of their associated ecosystems as a result of overfishing and other human-caused environmental changes. Exactly how these ecosystem changes translate into economic impacts remain unknown, but developing nations that rely on fisheries for food security and export are likely to feel the effects of any changes more severely than those with more diversified economies. At the same time, recent research has shown that science-based management can lead to faster recovery of fisheries and more sustainable use of resources.

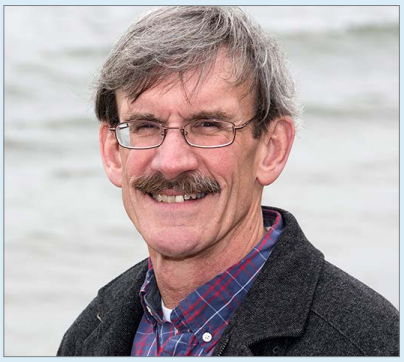
WHAT ARE THE POTENTIAL ECONOMIC EFFECTS IF THE COMMERCIAL HARVEST OF TWILIGHT ZONE FISH AFFECTS THE OCEAN'S ABILITY TO NATURALLY SEQUESTER CARBON?

The size and scope of the impending impacts of global climate change is one of the greatest uncertainties facing society. Disrupting the ocean's ability to remove excess heat-trapping carbon from the atmosphere by overfishing key species could mean warmer temperatures, higher sea levels, and greater climate disruptions in the future, all could cost society trillions of dollars by the end of the century. On the other hand, reducing uncertainty in estimates of the amount of carbon sequestered in the ocean could also have an economic benefit on the order of hundreds of billions of dollars.

WHAT ARE THE KEY OPPORTUNITIES TO HAVE THE OCEAN TWILIGHT ZONE RECOGNIZED AS AN INTEGRAL PART OF THE GLOBAL MARINE ECOSYSTEM?

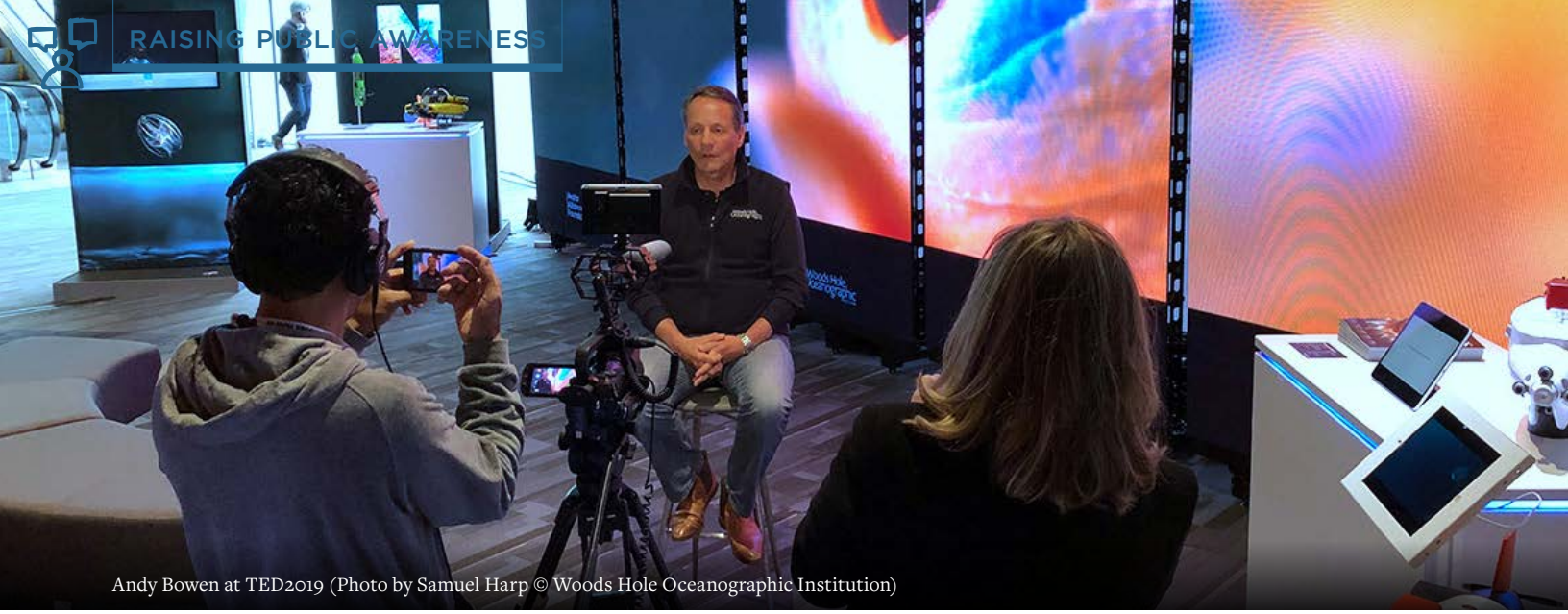
There are several critical international activities occurring now that present significant opportunities for the OTZ team to raise the visibility of the twilight zone on a global stage and to conserve its resources as a critical part of the marine ecosystem and global climate system, while also ensuring its long-term viability as a source of food for humans. Negotiations for a treaty to govern the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction have been occurring for more than a year, with OTZ team members taking part as observers with partners from the Deep Ocean Stewardship Initiative (DOSI). In addition, OTZ team members have been actively involved in the planning meetings for the upcoming UN Decade of Ocean Science for Sustainable Development as well as working with the Intergovernmental Oceanographic Commission of UNESCO to have the OTZ project recognized as an official Decade Activity and to ensure participation of WHOI in other ways.

FEATURED RESEARCHER



Porter Hoagland

Porter Hoagland has played a leadership role in advancing our policy efforts, including leading our partnership with Deep Ocean Stewardship Initiative, participating in multiple BBNJ negotiating sessions, and participating in a panel during the Informal Consultative Process meeting on the UN Decade of Ocean Science for Sustainable Development to educate officials from Member States on the objectives of the Decade. After nearly four decades at WHOI, Hoagland stepped down as a full-time member of the staff at the beginning of 2020, but has remained engaged in efforts to ensure the long-term sustainable use and conservation of the ocean twilight zone. In addition to attending a planning meeting for the Decade of Ocean Science for Sustainable Development in January, where he handed out copies of the team's ecosystem services report to participants, he also worked with WHOI colleagues on a book chapter focused on the importance of the twilight zone's natural capital and its relationship to ecosystem services.



Andy Bowen at TED2019 (Photo by Samuel Harp © Woods Hole Oceanographic Institution)

Raising Public Awareness

Increasing public awareness of the ocean twilight zone is key to achieving project success. The OTZ team aims to inspire a movement of informed citizens committed to its sustainable use—but in order to do so, it will be critical for the team to help lay audiences understand why the zone affects their daily lives. To reach that goal, the team has already implemented several digital campaigns, expanded our earned media efforts, and established new partnerships. It has also invested in filmmakers to document its activities, provided thought leadership through editorial commentary, and continued ongoing content marketing efforts across channels that prioritize visual storytelling.

THE HILL: The UN should protect the ocean's twilight zone

HIGHLIGHTS

- **Media**: Generated more than 500 media mentions with a potential reach of more than 800 million people since the start of the project.
- **Live Film Premier:** Attracted more than 1,500 viewers from around the globe with a watch time of 25,000 minutes during the live online premiere of a new documentary on the ocean twilight zone. Footage for the film was shot by an embedded journalist Jennie Berglund aboard the first OTZ expedition in 2018.
- **Thought Leadership:** Placed op-eds in prominent media outlets with a reach of over 70 million highlighting the importance of understanding and protecting the twilight zone for humans and the ocean.
- **Partnerships:** Increased our reach beyond existing WHOI audiences by developing partnerships with organizations such as OceanX, 4Ocean, and the Ocean Alliance.
- **Social**: Grew social audiences resulting in 2.8 million impressions and over one million engagements across social channels using content marketing campaigns.


Boston Sunday Globe

AUGUST 5, 2018

A6 Nation/World

BOSTON SUNDAY

Scientists head for the ocean's 'twilight zone'

OCEAN Continued from Page A1

Kaitlyn Tradd, engineer at Woods Hole Oc

EARNED MEDIA

Media coverage is the most cost-effective way to quickly build awareness and grow an audience. The OTZ team has aggressively sought content and media opportunities for the project, creating new coverage independently, through freelancers, and with media partners. In Phase I, the twilight zone has had over **500 media mentions, with a potential reach of more than 800 million people**.

Top Story: Carbon *Nature* article (14 million reach).
Appeared in 12 different outlets including *Phys.org*, *The New York Times*, and *New Atlas* with a potential reach of over 175,000,000

Other featured media highlights

Why we must protect the ocean's 'twilight zone'

- 2.7 million reach

Robot to study the marine twilight region completes sea trials

- 3.4 million reach

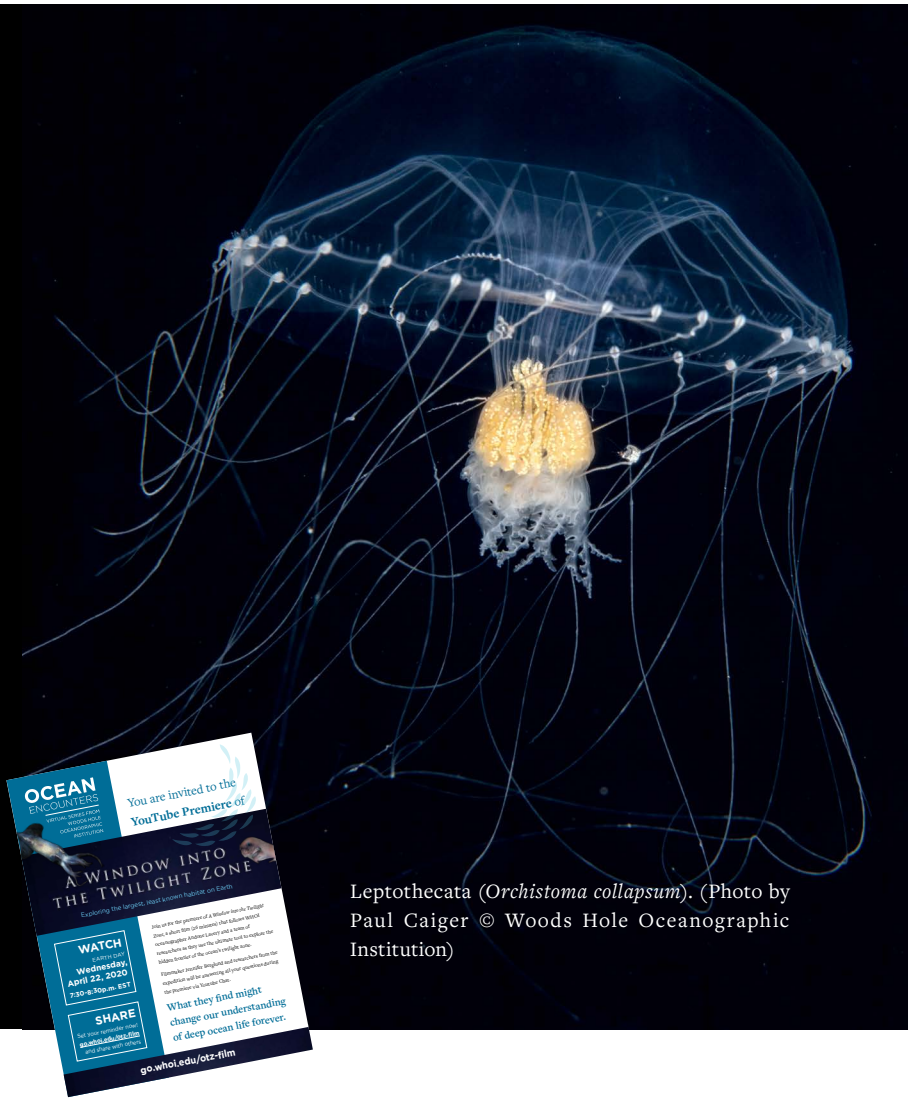
Blue sharks use eddies for fast track to food

- 7 million reach

VISUAL STORYTELLING

The OTZ team has emphasized visual storytelling to capture the public's interest and imagination. By using these techniques, the team saw a distinct increase in awareness of the twilight zone, its role in global climate and food security, and the threats it currently faces. Images of the remarkable animals that inhabit the twilight zone have appeared in major news outlets, and have recorded some of the highest engagement numbers WHOI has ever seen on social media. A regular drumbeat of short video pieces have taken viewers behind the scenes on expeditions and in labs to share the excitement of oceanographic research with new audiences. The videos in WHOI's OTZ YouTube playlist collectively have nearly 29,000 views, and the YouTube premiere of our first foray into long-form storytelling proved to be a welcome and fascinating event that reached almost 2,000 people in its first showing.

To make all of this possible, we've embedded filmmakers and videographers on nearly every research cruise to document activities. These media creators also provide real-time updates on social media and other important outreach channels.



Leptothecata (*Orchistoma collapsum*). (Photo by Paul Caiger © Woods Hole Oceanographic Institution)

THOUGHT LEADERSHIP

The OTZ team has continued to advance the importance of the ocean twilight zone by timing outreach to correspond with important events, like the anniversary of the first moon landing and the start of the BBNJ meeting. By using these events as a backdrop, our team was able to place several op-eds in high-profile national media outlets.

USA Today: We can protect the ocean 'twilight zone' with a new moonshot - authored by James Cameron

- 48 million reach

The Economist: The danger of creating a designer planet - authored by Ken Buesseler

- 4 million reach

The Hill: The UN should protect the ocean's twilight zone - authored by Mark Abbott and Christopher Scholin

- 18 million reach

Filmmaker James Cameron in the cockpit of the *Deepsea Challenger* scale model. (Photo by Peter Rae © EPA-EFE)

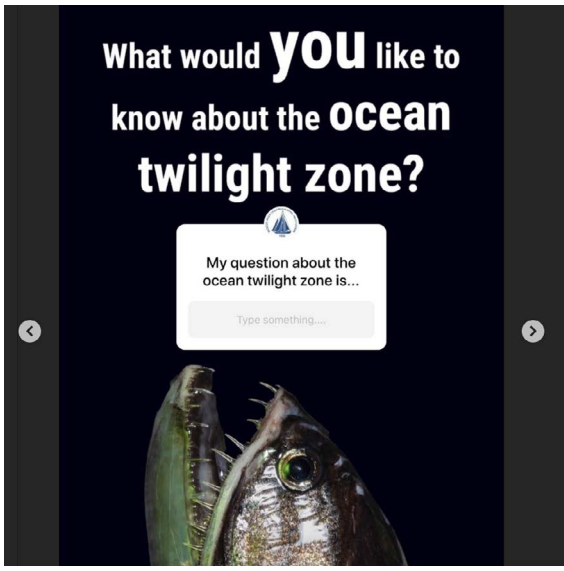
PARTNERSHIPS

The team continues to leverage partnerships that help increase awareness of the twilight zone among public audiences. A featured partner this past year was 4Ocean, an organization that produces bracelets out of plastic waste it recovers from the ocean. In November 2019, 4Ocean featured the Ocean Twilight Zone project with the release of its jellyfish bracelet, jewelry made entirely of recycled plastic. To support this effort, 4Ocean produced a video with OTZ researchers that was amplified across its channels, reaching more than 1.5 million viewers. Along with each purchase of the bracelet, 4Ocean was able to provide consumers with additional information about jellyfish and the twilight zone.

4Ocean bracelet (© 4Ocean)

DIGITAL CONTENT

Over the lifetime of this project, the OTZ team has made 2.8 million impressions across our social media channels, and generated more than 1 million engagements. To grow its social and online audiences, the team has implemented several new content campaigns, and has posted OTZ-related content weekly across WHOI's social media channels. It has also been a regular presence on WHOI's main website (whoi.edu)and its weekly e-newsletter, *Ocean Insights*, which reaches nearly 30,000 subscribers. Content drives include themed campaigns for Halloween and the December holidays, campaigns to launch publications like a report on the economic value of the twilight zone, and a publication about carbon storage in the journal *Nature*. The team also launched campaigns highlighting its real-time efforts in the field.



A screenshot of an OTZ Instagram story. (Photo by Paul Caiger © Woods Hole Oceanographic Institution)

MERCHANDISE

In 2019, the team created a new line of OTZ merchandise, including shirts, ornaments, and socks. It is currently planning to launch another new line of OTZ merchandise in 2020.

Engagement by the numbers

PROJECT INCEPTION THROUGH APRIL 2020

EARNED MEDIA

500
STORIES WORLDWIDE

800,000,000
POTENTIAL REACH

Top Story: Carbon *Nature* article (14 million reach). Appeared in 12 different outlets including *Phys.org*, *The New York Times*, and *New Atlas* with a potential reach of over 175,000,000.

WEB

56
STORIES

62,624
OTZ WEB PAGE VIEWS

65,332
TOTAL WHOI.EDU OTZ RELATED PAGE VIEWS

SOCIAL MEDIA

861
TOTAL POSTS

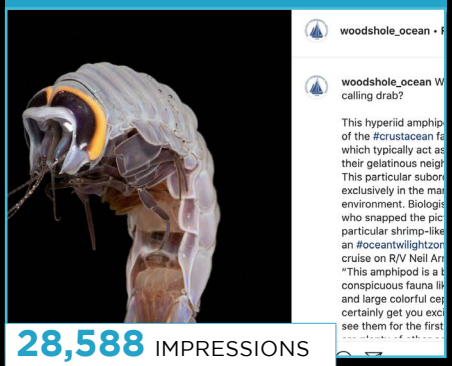
77,560
TOTAL ENGAGEMENTS

2,802,185
TOTAL IMPRESSIONS

TOP FACEBOOK POST

189,738 IMPRESSIONS

TOP INSTAGRAM POST



28,588 IMPRESSIONS

TOP YOUTUBE VIDEO

6,041 VIEWS

1,169,459
IMPRESSIONS

34,828
ENGAGEMENTS

402,266
IMPRESSIONS

32,768
ENGAGEMENTS

37,840
IMPRESSIONS

10,667
TOTAL VIEWS

27
OF VIDEOS ON CHANNEL

999.78
TOTAL WATCH HOURS

1,131,070
IMPRESSIONS

7,277
ENGAGEMENTS

61,550
IMPRESSIONS

2,687
ENGAGEMENTS

Heidi Sosik with Xabier Irigoien (left), lead of SUMMER, and Webjørn Melle (right), lead for MEESO, at the joint kick-off meeting for the two projects in Bilbao, Spain.

Collaborating and Engaging Academia

Heidi Sosik presenting at The Explorers Club in New York (Photo by Samuel Harp © Woods Hole Oceanographic Institution)

The OTZ project has made it a priority to reach out to other mesopelagic research groups worldwide and to develop collaborations with colleagues in order to accelerate research. There are more than a dozen national research programs around the world pursuing different angles of twilight zone research or focusing on different geographic regions of the ocean. In a time of ever-tightening research budgets, coordination will be critical to ensuring that resources are used efficiently and that the greatest strides in knowledge are made as quickly as possible.

FORGING INTERNATIONAL COLLABORATIONS

In 2019, members of the OTZ team joined forces with leaders of other large international efforts to create JETZON, the Joint Exploration of the Twilight Zone Ocean Network, and in February 2020 held its first meeting at the biennial Ocean Sciences Meeting in San Diego. JETZON is envisioned as a force multiplier that provides a framework to coordinate global research and share data, which will ensure that the international scientific community, from students to seasoned researchers, is able to gain the greatest insight possible into the twilight zone while also identifying new priority areas of research.

At the same time, OTZ lead scientist, Heidi Sosik, is serving on advisory committees for two new European Commission-funded projects: the Sustainable Management of Mesopelagic Resources (SUMMER) project and the Ecologically and Economically Sustainable Mesopelagic Fisheries (MEESO) project. By fostering partnerships and connections like these, the OTZ team is helping to lead a global effort to sustainably manage the resources of the mesopelagic zone.

CONFERENCES AND ACADEMIC PUBLICATIONS

OTZ researchers have continued to make significant academic contributions in the last year. Our scientists and collaborators published and submitted eight new papers in 2019 and have more than ten additional papers currently underway. Published work has been featured on the cover of the prestigious journal *Proceedings of the National Academies of Sciences* (*PNAS*), in the pages of the journal *Nature*, and in other major journals. The OTZ team has also completed or planned more than 30 presentations at a variety of international conferences.

RAISING AWARENESS THROUGH HIGH-PROFILE RELATIONSHIPS

Our team has steadily built a network of targeted relationships with high-influence individuals and organizations in the ocean outreach and policy communities. We have already begun working with partners such as the Deep Ocean Stewardship Initiative (DOSI), Ocean Alliance, and OceanX to expand awareness of the twilight zone, its role in climate and food security, and the threats it faces so that we can make inroads into key global policy discussions. Our team has also presented at the United Nations Decade of Ocean Science for Sustainable Development planning meetings, the New York Explorers Club, and other national and international events to expand our reach to new audiences and grow our network even larger.

Featured Publications:

TARGETING THE TWILIGHT ZONE

Mesoscale eddies release pelagic sharks from thermal constraints to foraging in the ocean twilight zone.

Cover story, PNAS, August 27, 2019

By tagging sharks, OTZ scientists showed for the first time that blue sharks can reach the twilight zone to feed by coasting down in the center of mesoscale eddies. Insight like this is critical to shape flexible management strategies for individual species and entire ecosystems.

OCEANS CAPTURE MORE CARBON THAN EXPECTED

Metrics that matter for assessing the ocean biological carbon pump

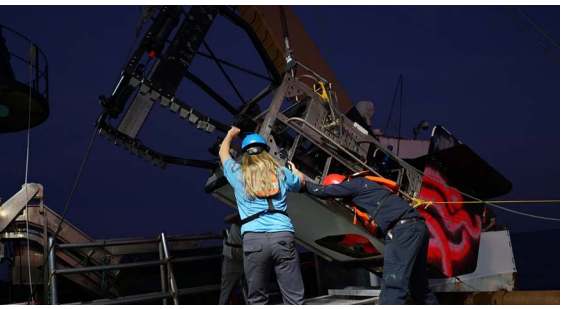
Cover story, PNAS, April 6, 2020

After defining the twilight zone by light levels rather than by depth, OTZ researchers discovered that the biological pump within it is actually twice as efficient as previously estimated. Climate modelers can use this to improve the accuracy of their predictions.

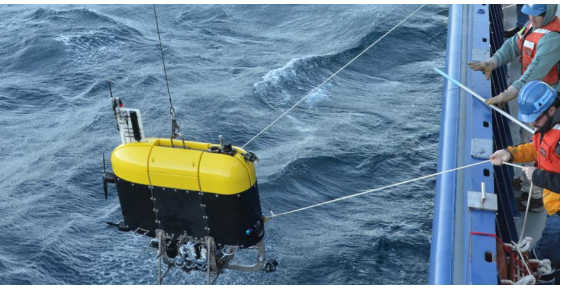
Vials of frozen eDNA samples collected aboard the *Henry B. Bigelow*. (Photo by Erik Olsen © Woods Hole Oceanographic Institution)

Establishing Best Practices

The twilight zone is one of the least explored habitats on Earth. Because of this, there is little precedent in many areas of the OTZ team's research. The team is constantly pioneering new methods and adapting others as it ramps up its study of the zone. Identifying best practices in the course of this work will be essential for accelerating new research in the future, since it will allow researchers to reliably compare findings and identify trends over time before large-scale fishing ramps up.



OTZ researchers and engineers recover *Deep-See* after a nighttime deployment. (Photo by Erik Olsen © Woods Hole Oceanographic Institution)



Mesobot hangs off the side of the R/V *Armstrong* after a 2019 test dive. (Photo by Ken Kostel © Woods Hole Oceanographic Institution)

A silver hatchetfish in mid-dissection. (Photo by Paul Caiger © Woods Hole Oceanographic Institution)

Kayle Hope, a journalist from the magazine *Quartz*, descends with OTZ researchers in submersible launched from the R/V *Alucia*. (Photo by Paul Caiger © Woods Hole Oceanographic Institution)

USING MACHINE LEARNING AND AUTOMATION TO ANALYZE DATA

In 2019 alone, *Deep-See* collected upwards of 50 terabytes of acoustic data and nearly 500,000 holograms, creating a trove of data that required new tools to analyze. We have focused early work on developing new machine learning techniques and automated processes that allow us to more efficiently examine the large volume of acoustic and image data. These tools significantly reduce the time needed to process the information. What used to take an individual researcher several minutes for each holographic image now takes less than one minute with a machine. The tools used to process acoustic data allow for near real-time analysis while at sea, giving researchers the ability to adapt sampling approaches based on what they are seeing. These tools will ultimately provide our team and other research groups a better and more detailed view of the mesopelagic's structure and estimated biomass.

DEVELOP NEW METHODS FOR HARNESSING DATA FROM eDNA

Environmental DNA, or eDNA, is a new tool used to identify animals without collecting physical specimens. Researchers extract genetic material that animals leave behind by filtering water samples, then analyze this material to identify the species of fish or jellyfish that shed it. This new method is particularly useful because nets don't capture animals that are especially mobile, and many parts of the twilight zone are sparsely populated with life. As a result, concentration of eDNA is significantly lower in the twilight zone than in coastal regions. The OTZ team is developing new protocols to analyze eDNA from the large volumes of water that must be filtered in order to obtain workable samples. Using eDNA alongside net tows and acoustic analysis will give the OTZ team unprecedented insight into these difficult-to-study regions.

CONDUCT NEW ANALYSIS OF FISH GUT CONTENTS

The team is also visually analyzing gut contents, metabarcoding gut contents, and conducting compound-specific stable isotope analysis to better understand the twilight zone food web. The individual animals being analyzed are also DNA-barcoded to obtain their species identification. Together, these methods create a powerful combination of tools capable of highlighting critical aspects of twilight zone food webs, allowing significant and species-specific conclusions to be drawn about food web structure and fisheries resilience that wouldn't be possible with any individual method.

BRINGING THE RESEARCH TO THE PUBLIC

Increasing public awareness of the ocean twilight zone is critical to inspiring a movement of informed citizens committed to its sustainable use. We've embedded writers, filmmakers, and videographers on nearly every research cruise to document behind-the-scenes activities and to create timely content that uses visual storytelling to capture the public's interest and imagination. The still images of the remarkable animals that inhabit the twilight zone and the documentary films produced have resulted in some of the highest engagement numbers WHOI has ever seen on social media. We've observed a distinct increase in awareness of the twilight zone, its role in global climate and food security, and the threats it faces.

Threadfin dragonfish (*Echiostoma barbatum*). (Photo by Paul Caiger, © Woods Hole Oceanographic Institution)

Funding and Fundraising

LEVERAGING EXPERTISE

In the first two-year phase of the OTZ project we leveraged over $10 million from external sources beyond the $35.1 million raised through the TED Audacious Project to expand the reach of the team's work. Our scientists are at the forefront of their fields, and they have behind them the reputation and legacy of the Woods Hole Oceanographic Institution. As a result, they are well positioned to use existing partnerships—and to develop new ones—in order to expand the reach and impact of the project. This audacious project is further bolstered by WHOI's long track record of partnerships between scientists and engineers, a tradition that lies at the core of the institution's many decades of high-impact ocean research.

OTZ project efforts to understand the twilight zone are complemented by those of other agencies as well. EXPORTS, a $40 million project funded by NASA, with some additional support from the National Science Foundation, focuses on how carbon moves through from the surface to the mesopelagic. These efforts are further enhanced by the Northeast U.S. Shelf Long-Term Ecological Research (NES-LTER) project and the Ocean Observatories Initiative (OOI) Pioneer Array, which are both located off the coast of Woods Hole. Partnerships with these efforts have provided access to ship time and have expanded the temporal and spatial coverage of the team's work.

In addition, OTZ funds have helped advance other research projects in notable and surprising ways. The initial construction of both *Deep-See* and *Mesobot* was funded by the National Science Foundation, the former through the Major Research Instrumentation Program and the latter through the Oceanographic Technology and Interdisciplinary Coordination Program, with OTZ program funds accelerating both projects. As a result, these innovative platforms are gathering crucial data faster than expected, giving the global research community a head start over commercial fishing efforts.

OTZ researchers prepare to collect black water diving footage off the coast of the northeastern U.S. (Photo by Paul Caiger, © Woods Hole Oceanographic Institution)

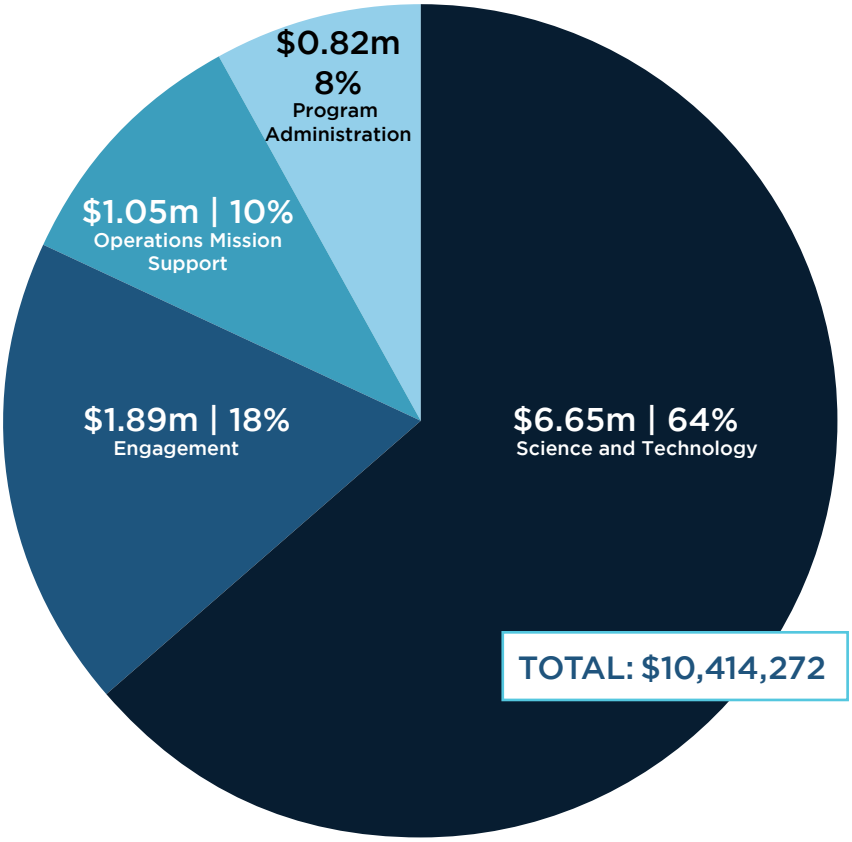
FUNDRAISING

Fundraising efforts have been focused on developing partnerships and cultivating prospective donors through events and other opportunities. This includes WHOI-organized donor events in New York City, Connecticut, and Martha's Vineyard, as well as outside opportunities like our presentation at the Explorers Club for Oceans Week. We have engaged WHOI's Board of Trustees through presentations, publications, and conversations designed to spark interest and equip them to serve as ambassadors. At the same time, our extensive engagement efforts serve to build a narrative and tool chest of assets—including film and video—that can be used to draw new donors into the fold.

TOTAL RAISED TO DATE:	$35,270,000
Audacious Project	$35,100,000
Private foundation grant to study target audiences in raising awareness of the twilight zone	$100,000
Short film production	$55,000
Charter boat off Florida for blackwater diving footage	$15,000
PENDING PROPOSALS	
National Science Foundation Grant to model the risks of resource overexploitation in the ocean's twilight zone	$1,250,000
Private foundation proposal for a demonstration project 'Ocean Internet of Things'	$1,000,000
DECLINED PROPOSALS	
Proposal for twilight zone research in the Galapagos	$1,000,000
Proposal for twilight zone research in the Indian Ocean	$1,130,000

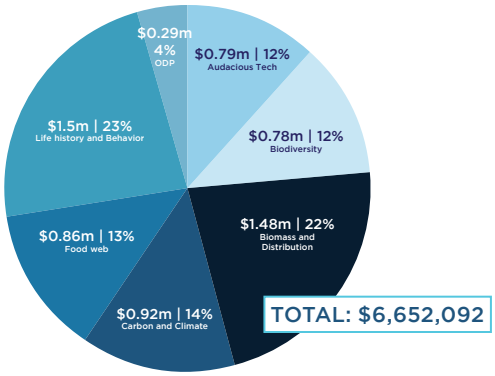
Project Financial Report

Inception though April 2020



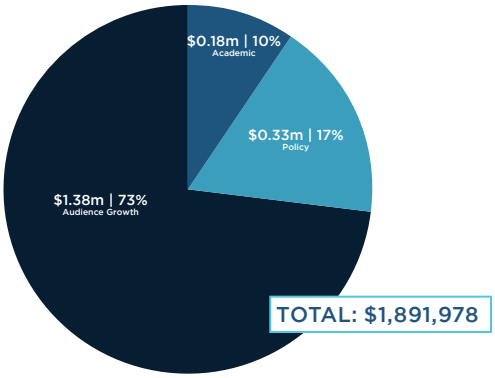
OTZ PROJECT ACTUAL EXPENSES

Science and Technology	$6,652,092
Engagement	$1,891,978
Operations Mission Support	$1,049,261
Program Administration	$820,941
Total	**$10,414,272**



OTZ PHASE I: SCIENCE AND TECH

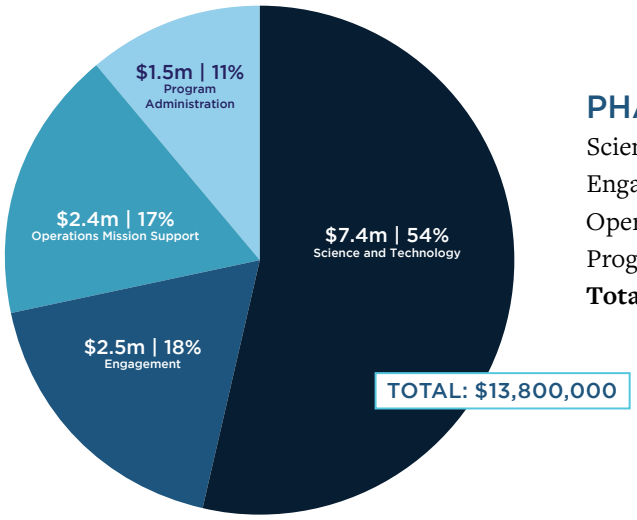
Audacious Tech	$790,249
Biodiversity	$784,518
Biomass and Distribution	$1,476,856
Carbon and Climate	$920,085
Food web	$859,942
Life History and Behavior	$1,528,092
Open Data Platform	$292,350
Total	**$6,652,092**



OTZ PHASE I: ENGAGEMENT

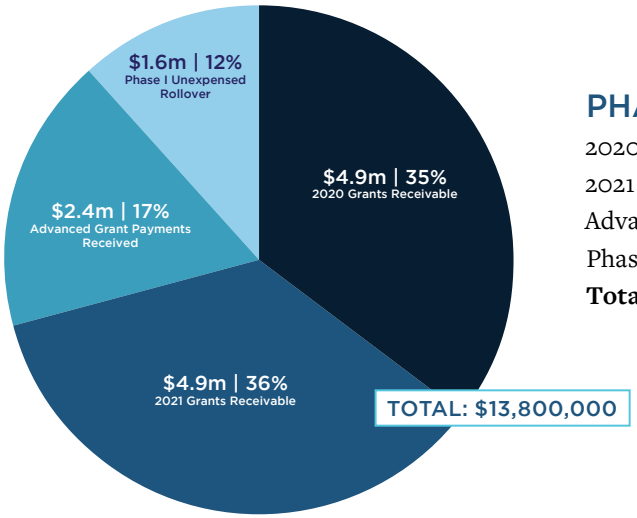
Academic	$181,661
Policy	$330,807
Audience Growth	$1,379,510
Total	**$1,891,978**

OTZ Phase II Budget Allocation and Revenue



PHASE II BUDGET (JULY 2020 - JUNE 2022)

Science and Technology	$7,400,000
Engagement	$2,500,000
Operations Mission Support	$2,400,000
Program Administration	$1,500,000
Total	**$13,800,000**



PHASE II REVENUES (JULY 2020 - JUNE 2022)

2020 Grants Receivable	$4,900,000
2021 Grants Receivable	$4,900,000
Advanced Grant Payments	$2,400,000
Phase I Unexpensed Rollover	$1,600,000
Total	**$13,800,000**

Thanks to our generous Audacious Project donors, the pledged amount for the six-year, three-phased OTZ project is: **$35,100,000**

RECEIVED
$15,496,850
Amount the project has received since inception (April 2018)

ANTICIPATED
$9,800,000
Funds anticipated to be received during Phase II (July 2020 – June 2022)

EXPENSED
$10,414,272
Project expenditures from inception (April 2018) through April 2020

Dragonfish (*idiacanthidae*) (Photo by Paul Caiger, © Woods Hole Oceanographic Institution)

LEADERSHIP COMMITTEE

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Annette Govindarajan
Porter Hoagland
Jonathan Howland
Di Jin
Andone Lavery
Joel Llopiz
Larry Madin
Simon Thorrold
Peter Wiebe
Dana Yoerger

PROGRAM OFFICE

Philip Renaud, *Program Manager*
Kathryn Baltes, *Project Manager*

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Paul Caiger
Postdoctoral Investigator (Joel Llopiz)
Emma Cotter
Postdoctoral Scholar (Andone Lavery)
Montserrat Roca Marti
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Zhaozhong Zhang
MIT-WHOI Joint Program PhD student (Andone Lavery)
Jessica Todd
MIT PhD student (Dana Yoerger)
Rune Oyerhamm
NORCE Norwegian Research Centre PhD student (Andone Lavery)
Jackson Sugar
University of Rhode Island MS student (Ken Buesseler and Melissa Omand)
Riley Sennott
Undergraduate student, Santa Clara University (Di Jin)
Helena McMonagle
Post-graduate research assistant, now at the University of Washington (Joel Llopiz)

5 Summer Student Fellows
22 Sea Education Association students

Confronting COVID

Like almost every other aspect of daily life, research into the twilight zone was affected by restrictions imposed by the spread of the COVID-19 pandemic worldwide.

One of the largest and most visible impacts was the need to postpone the long-planned EXPORTS cruise in May. This multi-ship expedition was slated to be a high-priority, high-visibility research effort to understand how carbon moves from the atmosphere into the surface ocean and then into deeper waters. The OTZ team chartered a third ship, adding to the two supported by the program's lead funder, NASA, to expand this focus. The additional ship would have permitted studying how physical and biological processes in the twilight zone help move carbon further down into the ocean, reaching depths that keep it safely away from the atmosphere for long periods of time. The team will resume planning for the expedition once travel restrictions are eased. All lab activities have stopped during stay-at-home orders while the Institution is closed. Fortunately, lab samples are safely stored and waiting for the return of scientists, students, and technicians. Data analysis has continued where possible and the restrictions have given researchers the opportunity to work on several new peer-reviewed publications.

Bobtail squid (*Heteroteuthis dispar*) (Photo by Paul Caiger, © Woods Hole Oceanographic Institution)

OCEAN TWILIGHT ZONE

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

Questions or feedback? Contact:
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Woods Hole Oceanographic Institution

THE AUDACIOUS PROJECT