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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.

ABOVE and COVER: Jellyfish, strawberry squid, swordfish, and *Phronima* illustrations by Natalie Renier as part of the Keep It Weird campaign to raise awareness of the ocean twilight zone. © WHOI Creative





ABOVE: Glass squid. Photo by Paul Caiger © Woods Hole Oceanographic Institution

The Ocean Twilight Zone Project team has met the "new normal" of COVID-era research with remarkable creativity, pressing forward with innovative scientific work and a major public engagement campaign while prioritizing the safety and health of staff, students, and colleagues.

Throughout the summer and fall of 2020, when ship-based expeditions were canceled or postponed due to COVID restrictions, the team took advantage of the time ashore to analyze existing data and generate a number of new publications and manuscripts. They continued to develop and refine machine-learning techniques, enabling data to be processed more quickly, and they pioneered laboratory experiments and computer models to improve the accuracy of sample analyses.

With the new year and new safety measures in place, planning began in earnest for two exciting expeditions—the OTZ Project's first since the start of the pandemic. In February, a subset of the OTZ team carried out a unique, monthlong field operation at a new rapid-development center for ocean technology in Bermuda. Then, in May, other team members departed for Vigo, Spain, for a rescheduled research cruise on the *Sarmiento de Gamboa*, joining our NASA EXPORTS partners as part of a three-ship research expedition.

On the engagement front, planning continued for the largest public engagement campaign that Woods Hole Oceanographic Institution (WHOI) has ever undertaken—reconceived to be more inclusive than ever and to celebrate the wonderful diversity of life in the twilight zone (visit keepitweird.org). In addition, we redesigned the OTZ Project website and launched visually striking social media campaigns, which resulted in significant growth of our web and social media audiences. We also continued to build strong relationships at the United Nations, create robust international policy recommendations for the ocean twilight zone, and present (virtually) at a number of conferences—including one held by the World Economic Forum.

The following pages highlight some of the OTZ Project's major achievements over the past year in scientific discovery, technology innovation, and public impact.

Key Performance Indicators

KNOWLEDGE GAINED

At the core of this work is a goal to better understand the ocean twilight zone in order to inform decision making around it. Here we highlight some of the most impactful knowledge gains our project has made.

HOW DOES IT AFFECT CLIMATE?

KNOWLEDGE GAINED

By using the depth of light penetration to delineate the top of the twilight zone, scientists found that the biological carbon pump is twice as efficient as previously estimated.

IMPACT

Using this method could lead to more accurate climate models, such as those used by the Intergovernmental Panel on Climate Change, to set global climate policy.

WHO IS EATING WHAT?

KNOWLEDGE GAINED

Twilight zone animals fall into at least two categories, animals that feed within a food web supported by plankton in the upper ocean, and animals that are part of a deeper web that is fueled by sinking detritus.

IMPACT

This information helps us understand the complicated interactions among different fish and is critical for informed decision making.

WHAT LIVES THERE?

KNOWLEDGE GAINED

Used genetic analysis, or DNA barcoding, to identify 100+ unique mesopelagic fish and jellyfish species.

IMPACT

Most of the animals in the twilight zone have never been genetically analyzed and are not yet in genetic libraries. Adding them enables new avenues of research, including eDNA analysis and species-specific ecological insights.

HOW MUCH LIFE IS THERE?

KNOWLEDGE GAINED

Improved accuracy of acoustic biomass measurements by comparing data from sonar systems deployed in the twilight zone with those from ship-mounted sonar.

IMPACT

Accurate estimates of biomass help us in two ways, they inform sustainable fishing management and help us quantify the role the twilight zone plays in our climate.

HOW MANY EGGS?

KNOWLEDGE GAINED

Improved estimates of the age at which different fish species first reproduce and how many eggs they produce.

IMPACT

Age at maturity and egg numbers are critical factors in determining the resilience of a fishery, or how quickly a fish population will be able to recover.



WHAT IS THE VALUE OF THIS RESEARCH

KNOWLEDGE GAINED

The global economic benefit of studying the ocean's biological pump is \$500 billion.

IMPACT

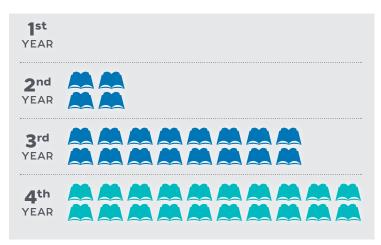
Quantifying the economic benefits of ocean twilight zone research enables funding on a larger scale and showcases the importance of WHOI's work.



PUBLICATIONS

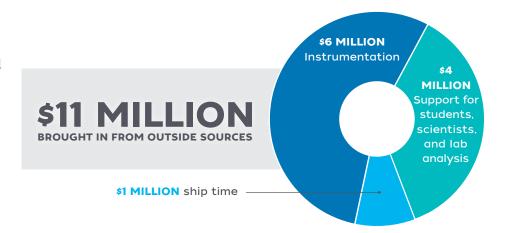
Academic publications are the standard metric used for validating impact within the scientific community. Publications allow scientists to share vetted research to their peers. Our robust publication track record is a testament to the scientific advances we've accomplished.





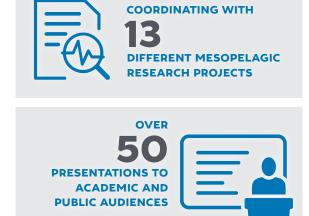
LEVERAGED FUNDING

The most impactful research is collaborative. 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.



NETWORK REACH

Network reach is a measure of how and to whom our team has shared their findings and advances. Collaborations, partnerships, and external outreach and engagement have all served to expand the project's network reach.





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. To better illustrate progress we categories technologies by "readiness level."

READINESS LEVELS

- prototype
- lab test
- ocean test
- science mission



DEEP-SEE

Start lab test

Current science mission

At project start Deep-See had been tested in the lab. It has since gone through engineering tests and has been used to collect robust science data for the OTZ Project.



MESOBOT

Start prototype

Current science mission

At project start Mesobot was designed and a prototype was built. Since then it has undergone ocean and engineering tests and completed several scientific missions. Mesobot is a collaboration between WHOI, Stanford, and MBARI.



MINIONS

Start concept

Current ocean test

At project start MINIONS were in the concept phase. The prototypes have been tested. MINIONS were first deployed in a scientific mission in May, 2021. MINIONS development is led by URI.



TZEx

Start concept

Current ocean test

In late 2019 the concept for Twilight Zone Explorer was designed. The instrument has completed successful engineering tests and will be used in its first scientific mission in May of 2021.



eDNA SAMPLER

Start concept

Current ocean test

The adjustable volume eDNA sampler was designed, prototypes were developed and used to obtain scientific data in 2019 and 2020. A new operational version is in development and will be used for science in 2022.



ROAM TAGS

Start concept

Current prototype

The ROAM tags are still in the concept and prototyping phase but are expected to go through a full ocean test in 2022.



DEEP-SEE MINI

Start concept

Current ocean test

The Deep-See mini was designed and built in the span of several months earlier this year. It will complete its first science mission in May of 2021.

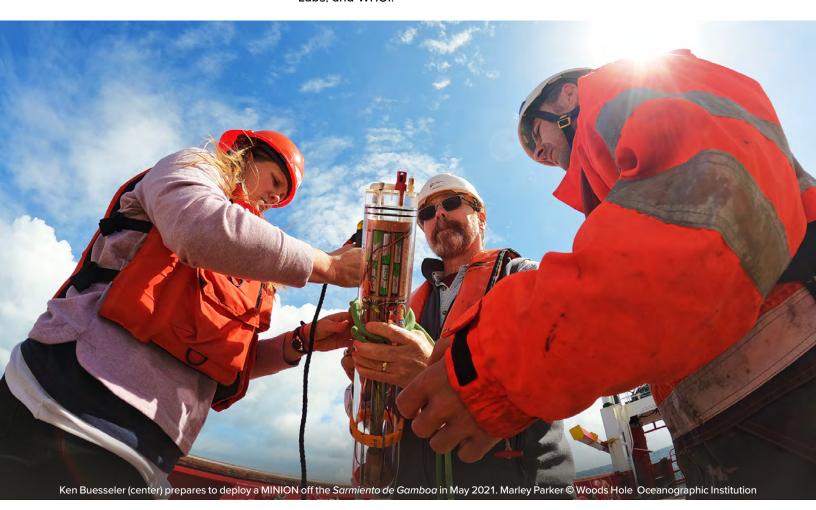


RADIOMETER

Start concept

Current science mission

At project start the low-cost highly sensitive radiometer was in concept phase. It has since completed successful science missions to collect data in March 2020 and Feb 2021. This effort is collaborative between MIT, Oceanic Labs, and WHOI.



Engagement by the numbers

JANUARY 2020-JANUARY 2021

EARNED MEDIA

282

MEDIA STORIES

400,000,000

IMPRESSIONS

WEB*

16,736

AUDIENCE 53% increase from last year

46,987

PAGEVIEWS

61% increase from last year

SOCIAL MEDIA

380,000

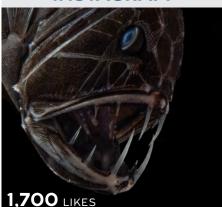
TOTAL ENGAGEMENTS
651% increase from last year

4,000,000

TOTAL IMPRESSIONS

100% increase from last year

INSTAGRAM



▲ A campaign posting twilight zone facts using #twilightzonetuesday and #weirdwednesday has been hugely popular.

FACEBOOK



During the Bermuda field operation, we posted 13 blog posts and over 25 unique social posts driving significant traffic to our website.

4

1,526,662 IMPRESSIONS **268,752** ENGAGEMENTS



1,454,686 IMPRESSIONS **36.943** ENGAGEMENTS



964,710 IMPRESSIONS **69,567** ENGAGEMENTS

in

94,772 IMPRESSIONS **5,882** ENGAGEMENTS

NEWSLETTER



They're wonderfully weird and downright creepy.

Here are 5 of the most chilling creatures of the deep, dark, and mysterious

▲ We saw record engagement numbers for some timely campaigns such as this one we did for Halloween.

*Web numbers are from April 2020 to April 2021

TWITTER



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 toward our goal of achieving a baseline of scientific understanding about the mesopelagic and informing policymakers and the public collectively to make sound decisions that ensure the long-term sustainable use of our ocean's twilight zone.





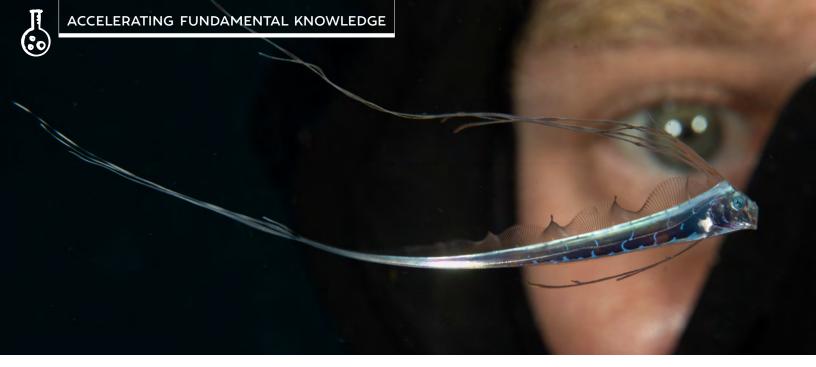
Understanding life in the twilight zone

To manage and sustain life in the twilight zone—and predict how commercial fishing might impact it—we need to understand how many and what kinds of animals live in this little-known part of the ocean, as well as their ages, growth rates, and reproductive behaviors. Surveying life in a region as vast and complex as the twilight zone is no easy task: It requires a multifaceted approach, including acoustic, imaging, and genetic technologies and the collection of thousands of physical samples.

KEY IMPACTS AND ACCOMPLISHMENTS

- Developed new tools to analyze acoustic measurements to more accurately estimate global twilight zone biomass.
- Visualized mesopelagic food webs to better understand the twilight zone ecosystem.
- Completed a review of existing data on how large marine predators use mesopelagic habitats.
- Conclusively proved the existence of two distinct food webs: one that uniquely
 exists in the twilight zone and one that is interconnected with the surface layer.
- Identified patterns in how twilight zone fish mature and lay eggs to inform fisheries management.
- Used genetic analysis to identify 70+ fish species and DNA bar coding to identify approximately 100 unique mesopelagic fish and jellyfish species.
- Quantified eDNA shedding and decay rates for different animal forms and created a model of eDNA transport and dispersal in the twilight zone.







ABOVE: Heidi Sosik puts the finishing touches on a new acoustic system mounted on the StingRay tow sled. Sosik and Lavery's labs collaborated to design and build this new, more versatile, acoustic system that, at the printing of this report, will have already been deployed on the May 2021 research cruise. Kayla Gardiner © Woods Hole Oceanographic Institution TOP: Juvenile oarfish with diver. © Paul Caiger

HOW MUCH LIFE IS IN THE TWILIGHT ZONE?

The twilight zone's total living biomass—the amount by weight of life contained in this vast region of the ocean—remains poorly understood, yet it is of paramount interest to scientists, commercial fisheries, and ocean policy experts. An accurate biomass estimate is essential to interpret ecosystem dynamics in the twilight zone, its potential to sustain exploitation, and its role in global climate.

During this past year, the OTZ Project team has developed new acoustic survey platforms and data processing techniques that will enable more accurate measurements of the quantity of life in the twilight zone than ever before possible. Lavery and Sosik's labs collaborated to create a miniaturized version of the multiband sonar system aboard the *Deep-See*, a large, ship-towed vehicle developed by Lavery's lab to measure biomass in the twilight zone, primarily using acoustics. The system, created in a matter of months, is mounted on the smaller and more easily deployed StingRay tow sled. The new miniaturized acoustic system is proving to be an effective and versatile tool for quantifying life in the twilight zone.

Lavery's team has also developed powerful new machine-learning systems that can analyze acoustic data patterns from both ship-based and submersible sonar systems to identify specific twilight zone animals. This new processing technique uses the more accurate acoustics collected at depth to identify individual fish to develop a more detailed model that will inform shipboard acoustic measurements. Shipboard acoustic systems are widespread and with this new model we'll be able to give a more accurate picture of how fish are distributed throughout the zone globally.

Looking to the near future, Lavery's team has begun designing an acoustic mooring for a newly funded Twilight Zone Observation Network in the Northwest Atlantic. The mooring, with its array of sensors and instruments, is scheduled to be deployed in the summer of 2021.



ABOVE: Cryptic species: In addition to contributing to bar code reference libraries, genetic identification of specimens, like the lantern fish pictured above, allows us to identify possible "cryptic species," which are organisms that are classified as a single species based on traditional morphological taxonomy but are revealed by genetic analysis to, in fact, be distinct. Paul Caiger © Woods Hole Oceanographic Institution

WHAT KIND OF ANIMALS LIVE IN THE TWILIGHT ZONE?

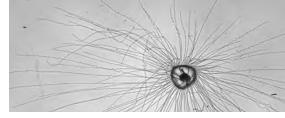
Identifying the full spectrum of species living in the ocean twilight zone has presented a challenge to researchers. Traditional sampling methods—such as net tows—cannot tell the whole story: Many fish can sense and avoid nets, and the twilight zone's ubiquitous gelatinous animals simply fall apart in them. Yet accurate species identification is necessary to understand life in the twilight zone, its ability to sustain commercial fishing or the environmental impacts of seafloor mining, and the role of twilight zone animals in removing carbon from the atmosphere.

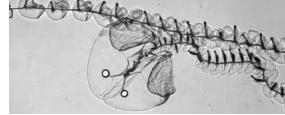
To supplement information derived from traditional sampling methods, the OTZ team is developing new methodologies to apply powerful environmental DNA (eDNA) techniques to the twilight zone, allowing the team to identify animals without needing to view or capture them. Biologist Annette Govindarajan and her lab have continued analyzing eDNA samples collected from the twilight zone, determining specific genetic markers that can help identify twilight zone animals. From these samples, the Govindarajan lab has also further developed a DNA bar code reference library—a database of DNA sequences that identify biological material as coming from specific fish or jellyfish species, for example, or higher-level taxonomic groupings. So far, the OTZ subgroup led by Govindarajan has generated DNA bar codes for more than 70 unique species of fishes.

One very exciting area of research that was recently published was an assessment of eDNA shedding and decay rates from different animal forms: fish, a shrimp, and two jellyfish. The rates of these processes varied between the animal forms-suggesting that these processes may impact eDNA signals detected in the field. A second published paper showcased the efficiency of eDNA by comparing eDNA biodiversity results with DNA results from the MOCNESS tows. While there was overlap, each method detected animal types that the other didn't. Govindarajan's team is currently working on drafts for two additional eDNA papers-one, that presents a model of eDNA dispersal and transport in the twilight zone, and another that assesses how well eDNA signals represent sampled communities using laboratory-constructed "mock" communities composed of mesopelagic fish DNA.

WHOI'S OTZ Project scientists are enhancing a valuable new method for detecting species, thus providing a more comprehensive understanding of biological communities in the twilight zone.







ABOVE: StingRay is particularly good for identifying and understanding gelatinous creatures like salps (TOP LEFT), siphonophores (BOTTOM RIGHT), and medusa (TOP RIGHT). These creatures are probably underestimated because traditional net sampling techniques don't preserve their fragile bodies. BELOW: Lyndsey Lefebvre, a research associate in the Llopiz lab is dissecting a bristlemouth to determine its age and fertility. Kathryn Baltes © Woods Hole Oceanographic Institution

IMAGE ANALYSIS

OTZ lead scientist Heidi Sosik and her lab worked to understand the tiny organisms at the base of ocean food webs: phytoplankton and zooplankton. Using a StingRay platform outfitted with an imaging system—the in situ Ichthyoplankton Imaging System, or ISIIS—her team has begun to create a photographic database of plankton that spans from the surface ocean into the twilight zone. The towed platform is especially good at imaging fragile gelatinous species, which abound in the twilight zone but are difficult to collect in nets.

ISIIS can collect millions of high-resolution "shadowgraph" images of plankton in a single deployment. To identify the imaged species, Sosik's team has developed new neural network algorithms capable of classifying each organism with an accuracy rate of more than 92%. They are currently adapting this processing technology so that it can be used at sea, enabling the team to identify and catalog species of plankton as soon as they are imaged, in near real time.

THE LLOPIZ LAB TAKES A HANDS-ON APPROACH

Although technologies such as acoustics, imaging, and eDNA analysis are vastly accelerating the pace of understanding of the amount and kinds of life in the twilight zone, these techniques depend on traditional, "hands-on" methods to inform their algorithms and validate their accuracy.

Over the past year, while ship-based expeditions were grounded by COVID, fish biologist Joel Llopiz and his team spent their days in the lab, painstakingly analyzing the thousands of physical specimens collected in net tows on previous twilight zone research cruises. This involves identifying each specimen, taking its measurements, and dissecting its tissues and structures for later analysis. To understand the feeding habits of twilight zone fish, the Llopiz lab analyzed the gut contents of more than 1,500 fish samples.

Using a device called a ZooScan—which is simply a modified office scanner—the team scanned hundreds of plankton samples to measure each individual's length, a critical component of interpreting biomass from acoustic measurements.

They also examined fish gonads under the microscope to pinpoint not only the sex of each animal but also its maturity, ability to spawn, and frequency of spawning. This information will be critical to future fisheries management of the twilight zone. They are able to determine the age of many of the fish by extracting otoliths (fish ear stones), which have rings on them like a tree. Knowing the age is critical to know when the fish become sexually mature, whether their diet changes as they grow, and how fast they grow. Otoliths aren't only used to determine age; the shape of a fish's otolith is species

specific and the Llopiz lab has compiled a photographic library of the otoliths of more than 40 twilight zone species. Being able to identify fish based on ear stones is just one more tool that will enable both OTZ Project scientists and the larger scientific community to use limited samples that are available to provide a more accurate picture of the ecosystem.





Connecting life in the twilight zone

Life in the ocean's twilight zone is connected to life at the surface through a complex network of food webs. These linkages mean that impacts to twilight zone species—from climate change, commercial-scale fishing, or seafloor mining—could have repercussions on species in other parts of the ocean, or vice versa.

> Food webs and their linkages across ocean zones also drive the transfer via biological processes of atmospheric carbon dioxide to the deep ocean, a phenomenon known as the ocean's "biological pump."

> Biologist Simon Thorrold and his lab used compound-specific isotopic analysis to continue to refine their understanding of food web structure in the twilight zone. Chemical analysis of stable isotopes gives a clear understanding of what eats what in the twilight zone. It's proof that "you are what you eat." Preliminary results suggest at least two distinct food webs are present, one consisting of species that migrate to the surface and back, the other of species that remain in the twilight zone. More work is needed to develop a detailed picture of these animals' feeding habits and prey, but the technique is already offering some exciting insights into these complex food webs that have historically been difficult to study.

> The Thorrold lab continued the development of a next-generation fish tag to track large pelagic predators such as swordfish and whale sharks as they move through the twilight zone. The tags will be tested in 2021 and deployed in 2022 as part of the Twilight Zone Observation Network.





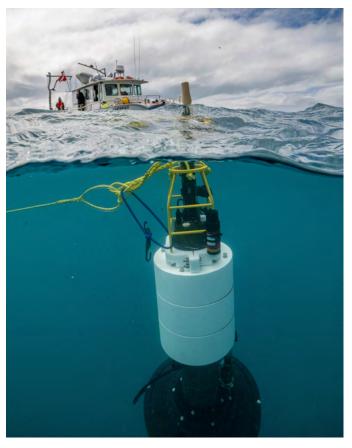
Revealing the twilight zone's impact on climate

The OTZ Project team continues to explore the important connections between the twilight zone and global climate. The daily migration of some twilight zone animals to and from the surface to feed—along with the sinking, carbon-rich detritus known as marine snow that forms the basis of many twilight zone food webs—together help drive the ocean's "biological pump," a process responsible for removing up to a third of all carbon dioxide from the atmosphere each year.

Yet the exact mechanisms controlling the biological pump are still poorly understood. This past year, marine chemist Ken Buesseler and his lab published four new research papers on the topic, with another five currently in review. Much of this work focused on quantifying and measuring elements of the biological carbon pump, creating valuable metrics on which future research can be based. By analyzing particles of marine snow, the Buesseler lab has been able to assess how the composition, size, and volume of the particles change as they sink through the twilight zone, and to use that information to better estimate the efficiency of the carbon pump at sequestering atmospheric carbon in the deep ocean.

Interestingly, the team found that the pump's efficiency seems to vary depending on how deep and where marine snow is measured. If carbon transport was measured at a fixed depth near the surface, for instance, it resulted in an underestimate of the pump's ability to remove carbon; conversely, recording measurements at a fixed point in deeper waters led to overestimating the pump's effectiveness.

Based on these findings—and the fact that animals in the twilight zone distribute themselves in response to light levels—Buesseler's team has proposed that light levels, not depth, should be used to guide the sampling of marine snow. The OTZ team's insights will help standardize future sampling efforts and should result in more accurate estimates of carbon flux through the twilight zone, a critical missing component of global climate change models.



ABOVE: Twilight Zone Explorer. Detailed measurements of carbon moving through the twilight zone allow scientists to learn more about exactly how the twilight zone helps regulate Earth's climate. Evan Kovacs © Woods Hole Oceanographic Institution.

Emerging technology to access the twilight zone

With the global pandemic entering its second year, access to ship time—the lifeblood of oceanographic research—remains difficult. In response, engineer Dana Yoerger and his team took an innovative approach to fieldwork, using shore-based operations in Bermuda to rapidly test new twilight zone technologies—chief among them the new Mesobot vehicle Yoerger invented. Using its powerful camera tracking system, Mesobot is designed to lock on to individual twilight zone animals and follow them for hours while recording their behavior.

KEY IMPACTS AND ACCOMPLISHMENTS

- Integrated a highly sensitive radiometer into Mesobot, programming it to follow a specific light level throughout the day, just like the migrating twilight zone organisms it observes.
- Completed the first full-ocean tests of the Twilight Zone Explorer, a device that will collect marine snow to help scientists understand how carbon flows through the zone.
- Developed a smaller version of the Deep-See vehicle's acoustic survey system, creating a flexible way to gather new acoustic readings.
- Continued development of a new satellite-enabled (ROAM) tag for tracking movements of large pelagic predators in the ocean twilight zone.
- Developed and deployed a new low-cost sensor for measuring carbon transport.

BELOW: Mesobot lead engineer Dana Yoerger and his team get ready to deploy at Station B in Bermuda. Evan Kovaks © Woods Hole Oceanographic Institution



WHILE IN BERMUDA, YOERGER'S TEAM RESOLVED SOME REMAINING ISSUES WITH MESOBOT'S CONTROL SYSTEMS AND FINE-TUNED ITS AUTONOMOUS TRACKING CAPABILITIES, ENSURING THAT FUTURE SHIP-BASED FIELDWORK WILL BE EVEN MORE SUCCESSFUL.

They also conducted the first successful test of a highly-sensitive radiometer, an instrument that enables *Mesobot* to detect light levels that govern the rhythm of life in the twilight zone. Team members also completed the first full-ocean tests of the Twilight Zone Explorer, a device that will gather marine snow to help scientists understand how carbon flows through the zone.

Back in New England, the OTZ Project team continued developing new technology for use in the field. Over the past year, the MINION floats developed by University of Rhode Island oceanographer Melissa Omand have moved beyond the initial prototype stage and are now ready to image marine snow as it sinks through the twilight zone.

Meanwhile, acoustic engineer Andone Lavery and Heidi Sosik created a smaller and more nimble acoustic package aboard the StingRay tow sled based on what we've learned from the larger *Deep-See* vehicle. This new instrument will provide only a subset of *Deep-See*'s sonar capabilities but on a smaller scale that can be deployed on a wider variety of ships with nonspecialized cables. *Deep-See* will remain a powerful tool for gathering high-level data to inform the scientific questions the team is asking, while the new smaller acoustic package will become a resourceful way to answer those questions with an easily deployable tool for gathering focused data.



Mesobot in the waters off Bermuda. Evan Kovacs © Woods Hole Oceanographic Institution



MINIONS lined up and ready to be deployed. Photo courtesy of Melissa Omand, University of Rhode Island.



WHOI engineers Molly Curran and Dana Yoerger plan out *Mesobot's* dives for the day. Evan Kovacs © Woods Hole Oceanographic Institution

Partnering for Success

WHOI'S PARTICIPATION IN NOAA'S NEW OCEAN EXPLORATION COOPERATIVE INSTITUTE (OECI) WILL ALLOW THE OTZ TEAM'S NEWLY DEVELOPED TECHNOLOGY TO BE USED BY A BROADER RANGE OF SCIENTISTS.

In late 2021, WHOI researchers will bring Mesobot, an automated eDNA sampler, and the high-sensitivity radiometer aboard the R/V Nautilus, with the goal of training a new cohort

of scientists on their deployment. During the cruise, these instruments will be used to continue studying the twilight zone's biodiversity, physical environment, and light levels, which determine how species are distributed throughout the zone's waters. Following the cruise, the team plans to build duplicate versions of an eDNA sampler and radiometer, and will make them freely available for use by OECI partners.

Making an impact on policy for the global ocean

The OTZ Project team has continued to work to inform and influence marine policy, aligning efforts with broader engagement objectives and with the planning process for the United Nations Decade of Ocean Science for Sustainable Development (UN Ocean Decade). The team has identified the UN Ocean Decade as offering the strongest near-term opportunity for science-based decision making, along with ongoing negotiations for the new treaty governing Marine Biodiversity of Areas Beyond National Jurisdiction (BBNJ).

A primary goal has been to raise the profile of the ocean twilight zone at key meetings and negotiations. To achieve this, WHOI OTZ team members have established and deepened targeted relationships with high-influence individuals and organizations in the ocean outreach and policy communities, including representatives of governmental agencies, intergovernmental organizations, civil society, and academia.

These include: The Intergovernmental Oceanic Commission leadership (IOC, part of UNESCO, the United Nations Educational, Scientific, and Cultural Organization), National Oceanic and Atmospheric Administration (NOAA) leadership, the International Union for the Conservation of Nature (IUCN), and the Deep-Ocean Stewardship Initiative (DOSI).

KEY IMPACTS AND ACCOMPLISHMENTS

- Advocated for inclusion of the twilight zone as a priority within the UN Ocean Decade in collaboration with the research consortium Joint Exploration of the Twilight Zone Ocean Network (JETZON).
- Estimated the value of the global economic benefit of studying the ocean's biological pump to be on the order of \$500 billion.
- The twilight zone was specifically mentioned in the White House National Strategy for Ocean Mapping, Exploration, and Characterization.
- Accepted to the National Academy of Sciences' National Committee for the Ocean Decade "Ocean Shot" initiative, resulting in a presentation at the UN Ocean Decade launch.

THE PAST YEAR HAS SEEN A REAL SHIFT IN THE OCEAN POLICY CONVERSATION WITH REGARDS TO THE OCEAN TWILIGHT ZONE.

In the national policy arena, our efforts and those of our colleagues led to the specific mention of the twilight zone in the White House National Strategy for Ocean Mapping, Exploration, and Characterization.

Internationally, progress has been even more significant. At the first BBNJ negotiating session in 2019, discussions focused almost exclusively on the surface ocean and the seabed; OTZ Project student Aria Finkelstein was the only speaker to mention the twilight zone during her presentation to the plenary. As of this year, the twilight zone has become an integral part of the policy programming

agenda, due in no small part to the dedicated efforts of WHOI's OTZ Project team and those of our partners.

The ocean twilight zone was highlighted on several occasions in the BBNJ negotiations through recent IUCN/DOSI webinars, including a panel presentation by OTZ Project science lead Heidi Sosik in which she shared recent data and developments. We have also deepened our relationship with the IOC to advocate for inclusion of the twilight zone as a priority within the UN Ocean Decade and members of the OTZ team worked with the international mesopelagic research consortium JETZON to apply for consideration as an official UN Ocean Decade "Programme."

In addition, The National Academy of Sciences' National Committee for

the Ocean Decade accepted the OTZ Project into its "Ocean Shot" initiative. Similar to NASA's "moon shot," this program recognizes ambitious, transformational research aligned with the goals of the United Nations Decade of Ocean Science for Sustainable Development.

All of our work to date has positioned the OTZ Project for continued progress in 2021 and beyond. The team has formed an internal Policy Advisory Committee to track opportunities for intervention and impact in the coming year, including those related to the United Nations General Assembly meeting in September, the 26th UN Climate Change Conference of the Parties (COP26), the first year of the UN Ocean Decade, and the Fourth Negotiation Session of the BBNJ.





Growing engagement in the twilight zone

The ocean's twilight zone presents a unique communications challenge: When it comes to the general public, few people have ever heard of it, much less will ever see it with their own eyes, so how can we make them care about it? From day one, it was clear this project was different from other projects at WHOI. Public communications, engagement, and awareness building have been woven into every level of this effort. We have communication professionals on every field effort helping us tell our story in new and exciting ways, and this past year was no exception. After such a focused and coordinated effort, we're starting to see measurable success. In the past year, we've seen a shift in general awareness of the twilight zone. More people are talking about it on social media, in print and online media, and, perhaps most important, in international policy discussions.



HIGHLIGHTS

- Web: Launched a redesigned website with more than 70 engaging pages featuring unusual creatures, new technology, missions at sea, and much more. This new, nimble web design has enabled us to tell the OTZ's highly visual story of exploration and discovery through a uniquely engaging medium.
- Social: Resulted in 2.8 million impressions and more than 1 million engagements across WHOI's social media channels.
- Media: Generated more than 282 media mentions with a potential reach of more than 400 million people since the start of the project.
- Partnerships: Increased our reach beyond existing WHOI audiences by partnering with OceanX and TED, as well as other organizations like the World Surf League.

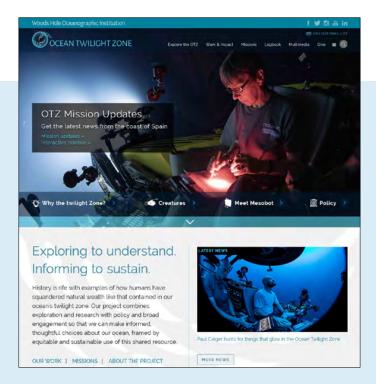


COUNTDOWN TO LAUNCH: NATIONAL OCEAN TWILIGHT ZONE AWARENESS CAMPAIGN

When we began to think in terms of how to accomplish our goal of driving breakthrough awareness, we realized that what we needed was a focused publicity campaign—a swing-for-the-fences marketing effort. Our goal is to make people aware, and it is also to make them care. Working with Edelman Inc., an international marketing and public relations firm, we designed a campaign called Keep It Weird. The campaign is fun, earnest, and clever. It's centered on our most engaging content: the weird and wonderful creatures of the ocean twilight zone.

The campaign is based on the idea that science has always been weird—and that is a good thing. Weird is the spark of creativity, the fresh perspective that turns established thoughts upside down that leads to new insights, innovation, and discovery. We want to celebrate and protect the weird world of the twilight zone by bringing families along on a journey predicated on the idea that, just as the child who feels they don't belong may one day grow up to change the world, the weird in everyone needs protecting and nurturing. The content is designed for a broad demographic, with millennial parents of young children at the core but really targeting the entire family.

We've been preparing for this campaign for over a year now, with several delays due to COVID, but we're ready to launch any day now and we couldn't be more excited to share it with the world.





MAXIMIZING ONLINE REACH AND IMPACT

We have increased the number and frequency of blog posts on the OTZ website and have launched a new quarterly email newsletter, which had 7,800 opens and referred nearly 1,100 people to the OTZ website. During both of our recent field expeditions—shore-based operations in Bermuda and the research cruise on Sarmiento de Gamboa—we embedded a science writer to post daily updates to give viewers a unique behind-the-scenes view of cutting-edge science and engineering. The recent cruise leveraged WHOI's existing and highly popular Dive and Discover platform, bringing the OTZ team's work to thousands of classrooms around the world with an expected reach of 5,000 views.

ABOVE LEFT: The completely redesigned OTZ website. ABOVE RIGHT: Media highlight: The \$500 billion question: What is the value of the ocean carbon pump? This article reached an audience of 41 million.

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 2020, WHOI and our work on the ocean twilight zone and/or the biological carbon pump were featured in 282 media stories, garnering 400 million impressions (pageviews).

- Daily Mail online: Oceans are capturing twice as much carbon as previously thought
- Yahoo.com: The \$500 Billion Question: What's The Value Of Studying The Ocean's Biological Carbon Pump?
- Popular Science (popsci.com): Scientists are tracking down deep sea creatures with free-floating DNA
- Scitechdaily.com: \$500 Billion: The Staggering Potential Value of Studying the Ocean's Biological Carbon Pump
- Snopes.com: Is This Deep-Sea Strawberry Squid Real?



BUILDING ONLINE AUDIENCES

Engagement with the OTZ Project on all social media channels grew by 651% from the previous year, due to a strategic increase in "snackable," socialfriendly content and targeted marketing tactics. Among these highly successful strategies was the social media launch of "#OceanTwilightZone Tuesdays" and "#WeirdWednesdays," with content released during strategic time slots to maximize reach. We also created themed, strategically timed campaigns, such as Halloween's "Creep from the Deep," which featured a visual slideshow of the creepiest ocean twilight zone animals.

The biggest growth channel for OTZ Project engagement was Instagram, where the primary audience is millennials. Among the year's Instagram highlights was an ocean twilight zone strawberry squid, whose captivating image went viral, leading to increased engagement across all WHOI channels. The photo by former WHOI postdoctoral researcher Paul Caiger gained even more attention when it was picked up by Snopes.com, a popular fact-checking site that concluded, yes, it really does exist.

In another engagement success, OTZ Project lead scientist Heidi Sosik joined actor Pierce Brosnan on TED'S Instagram channel for a live discussion of ocean conservation and the OTZ Project. That conversation, which took place in early December, has been viewed more than 100,000 times.

Exploring and protecting our oceans

WITH PIERCE BROSNAN AND OCEAN SCIENTIST HEIDI SOSIK

Thursday, 12/10 3pm ET / 12pm PT



TED

The OTZ engagement team's efforts not only raised awareness of the ocean twilight zone but also drew new followers to WHOI. Overall, WHOI increased its followership by 21% this past year across all social media channels, and the OTZ Project was one of the key drivers of this success.

The videos in WHOI's OTZ YouTube playlist collectively have over 28,000 views. The YouTube premiere of our first

foray into long-form storytelling, the OTZ Project documentary *A Window into the Twilight Zone*, proved to be highly successful with almost 2,000 people tuning in live for its premiere showing. In addition, "The Ocean Twilight Zone: Earth's Final Frontier," a short video funded by WHOI Trustee George Moss showcasing some of the captivating research being conducted by the OTZ Project team, has received more than 4,000 views.



Collaborating and engaging academia

The OTZ Project team continues to make it a priority to network and collaborate with other mesopelagic research groups worldwide and to share findings through academic channels such as publications and conferences.

Featured publications:

Comparison of mesopelagic organism abundance estimates using in situ target strength measurements and echo-counting techniques published in *Journal of the Acoustical Society of America Express Letters* and selected as the editor's pick.

The value of scientific research on the ocean's biological carbon pump published in Science of the Total Environment

See all publications: twilightzone.whoi.edu/reports

ACADEMIC PUBLICATIONS AND CONFERENCES

The OTZ Project team took advantage of the time ashore imposed by COVID restrictions to accelerate their analysis of previously collected data and to make their findings available to the ocean twilight zone research community through publications in academic journals. In all, OTZ team scientists and collaborators published 20 academic articles in the past year, with more than 13 additional publications currently underway. Their work appeared in the pages of the prestigious *Proceedings of the National Academy of Sciences*, *Science Robotics*, and *Journal of the American Statistical Association*.

Team members also completed or planned more than 35 online presentations at national and international scientific conferences.

GROWING INTERNATIONAL COLLABORATIONS

WHOI's twilight zone research is primarily focused on the Northwest Atlantic and Caribbean; by coordinating efforts with the more than a dozen national research programs around the world focused on different geographic regions and research angles, the WHOI team is maximizing the efficient use of its own resources while enabling rapid overall progress toward understanding this critical part of the ocean.

The WHOI OTZ team continues to work closely with the Joint Exploration of the Twilight Zone Ocean Network (JETZON), a network of more than 12 different mesopelagic research efforts. JETZON has provided a framework to coordinate global research and share data, ensuring that the international scientific community is able to gain the greatest insight possible.

Members of the WHOI OTZ team also have strong connections with several specific projects within JETZON. For example, lead scientist Heidi Sosik serves on advisory committees for both the Sustainable Management of Mesopelagic Resources (SUMMER) project and the Ecologically and Economically Sustainable Mesopelagic Fisheries (MEESO) project. In addition, policy experts from the WHOI team are working on a joint research project with MEESO. By fostering partnerships and connections like these, the OTZ team is helping to lead a global effort to inform the sustainable management of twilight zone resources.

Funding and fundraising

The OTZ Project team continues to make it a priority to network and collaborate with other mesopelagic research groups worldwide and to share findings through academic channels such as publications and conferences.

PHILANTHROPIC SUPPORT

At the start of 2021, the OTZ team secured a new \$1 million grant from German philanthropist Otto Happel. The Happel Foundation grant is being used to develop and build the first long-term observation network focused on life in the twilight zone. Located in the Atlantic Ocean off the U.S. East Coast—out beyond the continental shelf, where water depths are measured in thousands of meters—the network will provide an around-the-clock view of life in the twilight zone over an area spanning more than 155,000 square miles (250,000 square kilometers). Sound sources anchored at the network's four corners will send out acoustic signals that can be "heard" by tiny receivers in tags attached to big-eye tuna and swordfish, two commercially important species known to dive into the twilight zone for prey. Another major component of the network, a central mooring equipped with active sonars, will complement the predator tracking data with information about the location and abundance of their prey: the small fish found in huge numbers throughout the twilight zone. A fleet of 20 MINIONS will complete the network, tracking carbon-rich particles as they sink to deeper waters.

LEVERAGING EXPERTISE

In its first three years, the WHOI OTZ Project successfully extended its impact by leveraging over \$11 million from external sources beyond the \$35.1 million raised through the TED Audacious Project.

OTZ Project efforts to understand the twilight zone are complemented by those of our partners and colleagues at other organizations. These existing partnerships—and the new ones we are forming—are enabling the WHOI team to do more with its resources. For example, the OTZ Project was able to expand its research on carbon flux through the twilight zone by adding a third research vessel to a two-ship mission led by EXPORTS, a \$40 million project funded by NASA and the National Science Foundation to study the transport of carbon from the atmosphere into the deep ocean. Having three research vessels studying different aspects of the biological carbon pump in the same location in the Northeast Atlantic allowed for more comprehensive and robust research and greatly increased the capacity of both teams.

The OTZ Project has also maintained a strong partnership with the Northeast U.S. Shelf Long-Term Ecological Research project, also led by Heidi Sosik, and the Ocean Observatories Initiative Pioneer Array, a multi-institution project led by WHOI, both located off the Northeast U.S. coast. These collaborations have provided the OTZ Project with access to additional ship time and have expanded the reach of the team's research in space and time.

TOTAL RAISED TO DATE: \$33,220,000

Audacious Project

\$32,050,000

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

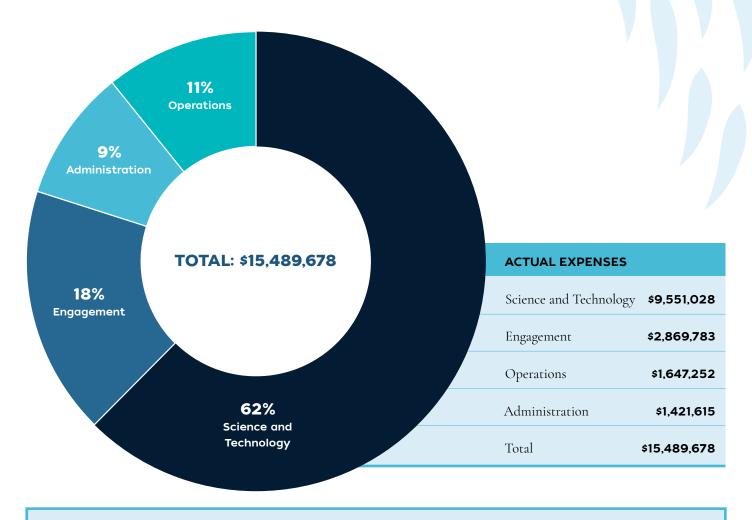
\$15,000

LEVERAGED FUNDING: \$11,000,000

Leveraged funding is a metric that shows us how interconnected our project is in the field.

Project financial report

Inception through April 2021



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

\$19.7M

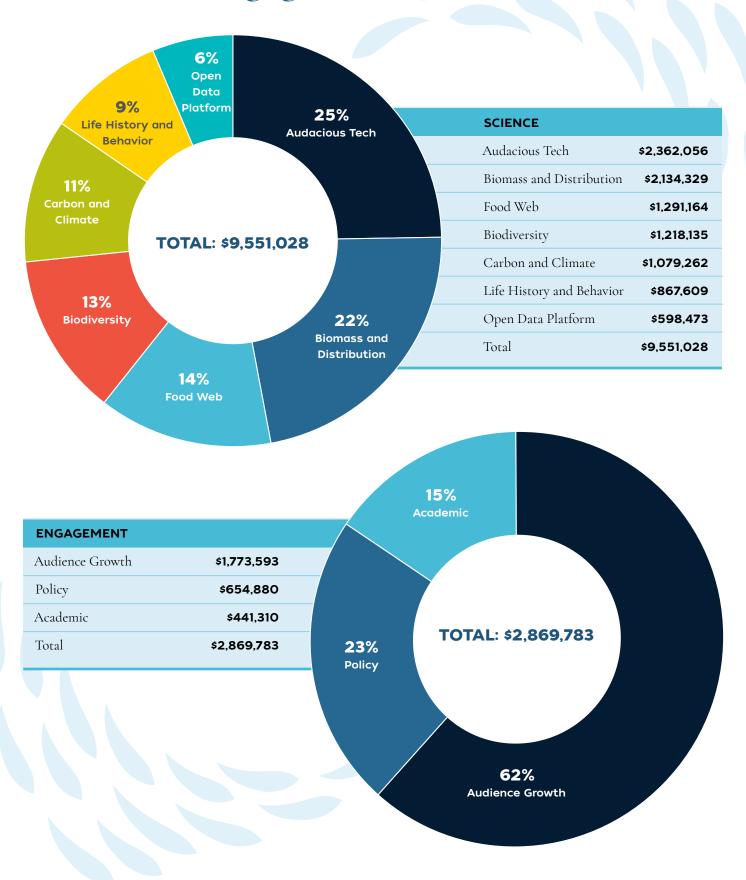
Amount the project has received since inception (April 2018)

\$15.3M

Project expenditures from inception (April 2018) through April 2021 ANTICIPATED \$12.4M

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

Science and engagement





LEADERSHIP COMMITTEE

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Scientist Emeritus

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Andy Bowen, Lead

Ken Buesseler

Annette Govindarajan

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Jonathan Howland

Di Jin

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Joel Llopiz

Larry Madin

Simon Thorrold

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Dana Yoerger

PROGRAM OFFICE

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

STUDENTS AND POSTDOCS

Elizabeth Allan

Postdoctoral Investigator (Annette

Govindarajan)

Paul Caiger

Postdoctoral Investigator (Joel Llopiz)

Emma Cotter

Postdoctoral Scholar (Andone Lavery)

Montserrat Roca Marti

Postdoctoral Investigator (Ken Buesseler)

Kevin Archibald

MIT-WHOI Joint Program PhD student

(Heidi Sosik)

Samantha Clevenger

MIT-WHOI Joint Program PhD student

(Ken Buesseler)

Kayla Gardner

MIT-WHOI Joint Program PhD student

(Simon Thorrold)

Rachel Kahn

MIT-WHOI Joint Program PhD student

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Levi Kai

MIT-WHOI Joint Program PhD student

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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)

John San Souce

MIT-WHOI Joint Program PhD student

(Heidi Sosik)

Jackson Sugar

University of Rhode Island MS student

(Ken Buesseler and Melissa Omand)

Riley Sennott

Undergraduate student, Santa Clara

University (Di Jin)

Helena McMonagle

Postgraduate research assistant, now at the University of Washington (Joel Llopiz)

Shadowgraph image from StingRay © Woods Hole Oceanoagraphic Inisitiution

OCEAN TWILIGHT ZONE

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

Questions or feedback? Contact: Philip Renaud, OTZ Program Manager prenaud@whoi.edu or (508) 289-2216

twilightzone.whoi.edu



