

Water column methane, methane oxidation, and pmoA gene copies above methane seeps determined from samples collected off the Aleutian Islands, Gulf of Alaska on R/V Atlantis cruise AT50-24 in May to June 2024

Website: <https://www.bco-dmo.org/dataset/986875>

Data Type: Cruise Results

Version: 1

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Project

» [Collaborative Research: Redefining the footprint of deep ocean methane seepage for benthic ecosystems](#)
(Methanosphere)

Contributors	Affiliation	Role
Treude, Tina	University of California-Los Angeles (UCLA)	Co-Principal Investigator
Klonicki-Ference, Emily	University of California-Los Angeles (UCLA)	Student
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract

Cold seeps along the eastern Aleutian subduction zone in the Gulf of Alaska fuel benthic ecosystems through microbial methane (CH₄) consumption, yet the structure and controls of water column CH₄ oxidation in these deep, cold waters remain poorly resolved. During a May–June 2024 expedition with the R/V Atlantis and HOV Alvin, we studied CH₄ and its bacterial oxidation from surface to seafloor above three deep seep sites (2000 to 5000 meters): Edge, Shumagin, and Sanak, by combining radiotracer incubations with pmoA gene profiling. CH₄ oxidation occurred throughout the water column, with peak rates (1 to 242 nanomoles per liter per day) in near-seafloor Alvin samples and 0.1 to 0.25 nanomoles per liter per day in CTD rosette samples 10 to 30 meters above bottom. Rates varied by site and depth. CH₄ oxidation in surface waters, coinciding with an algal bloom, suggests cryptic cycling via in situ production and consumption. A ~325-meter near-bottom CTD transect at Sanak revealed lateral gradients in CH₄ and oxidation aligned with bottom currents, with oxidation highest near hydrate-bearing gas vents and at the off-seep distal end. These findings show that aerobic CH₄ oxidation peaks near the seafloor to ~30 meters above but extends laterally and vertically beyond active seepage. Oxidation was detected even where methanotroph gene abundance was low, potentially indicating the influence of lateral CH₄ transport and tidal currents. The methanosphere thus emerges as a dynamic and spatially diffuse microbial system shaped by CH₄ availability and physical transport processes.

Table of Contents

- [Coverage](#)
- [Dataset Description](#)
 - [Methods & Sampling](#)
 - [Data Processing Description](#)
 - [BCO-DMO Processing Description](#)
- [Related Publications](#)
- [Parameters](#)
- [Instruments](#)
- [Deployments](#)
- [Project Information](#)
- [Funding](#)

Coverage

Location: Gulf of Alaska off the Aleutian Islands (Edge, Shumagin, Sanak)

Spatial Extent: N:57.4803 E:-147.9998 S:53.747437 W:-162.590273

Temporal Extent: 2024-05-17 - 2024-06-05

Dataset Description

Funding note: OCE-2205998 and OCE-2126631 are Postdoctoral Research Fellowships awarded to Dr. Kira Homola and Dr. Daniel Utter. Dr. Homola supported geophysical maps and helped with the identification of methane plumes for the CTD sampling using echosounder. Dr. Utter supported the DNA extraction and qPCR (pmoA data).

Methods & Sampling

Water column sampling and profiling was conducted over two weeks during the AT50-24 expedition aboard the R/V Atlantis using the HOV Alvin and a CTD/Rosette system. Water samples were collected from surface to near-seafloor at all three methane (CH₄) seep sites using Niskin-bottle rosettes (Ocean Test Equipment, Inc., Fort Lauderdale, FL): a 12 × 10 liter (L) configuration for Edge and Shumagin to accommodate the cable-tension limits at these depths, and a 23 × 10 L configuration for Sanak. A Seabird Scientific pumped CTD system was mounted on the rosette frames outfitted with Niskin bottles and sensors.

Vertical CTD casts were deployed above active seep sites and inactive off-seep locations selected based on available information, such as real-time EK80 acoustic backscatter data, multibeam seafloor mapping, and/or prior observations of seep-associated organisms and features from Alvin dives. At all three seep sites, vertical CTD/rosette casts were conducted to profile the water column above areas of active seepage. At Sanak, we additionally conducted a horizontal near-bottom CTD transect spanning active vent zones and off-seep background areas, with water collected at multiple waypoints along the direction of bottom current flow. To achieve this, the CTD/rosette was maintained approximately 5 meters (m) above the seafloor and towed horizontally along the transect that extended from the background area, across the seep site, and back into the background area. During CTD/rosette casts, the ship followed a pre-defined set of coordinates using dynamic positioning, a computer-controlled thruster system that uses satellite and/or acoustic transponder inputs to maintain or adjust the vessel's location. A CTD-mounted acoustic beacon was used to track the position of the CTD package relative to the ship, allowing real-time monitoring of its trajectory during movement between waypoints for the horizontal transect at Sanak. Niskin bottles were triggered after the rosette stabilized, following the ship's arrival at a new waypoint. To ensure representative sampling and minimize current-related offsets, the CTD was gently raised and lowered within a 5-meter vertical range at the target depth to flush the bottles.

Samples collected by Alvin were obtained via Niskin bottles mounted on the submersible's basket. Sample locations were chosen based on either previously known coordinates from literature (e.g. Suess et al., 1998; Wallmann et al., 1997) or *in situ* observations of gas bubble releases and chemosynthetic habitats during the dive. To prevent sediment contamination, Niskin bottles were triggered while the vehicle hovered approximately 0.5 m above the seafloor, just prior to contact and/or following landing on the seafloor after the observed current removed resuspended sediment. For all sampling events, water from each Niskin bottle (CTD and Alvin) was subsampled in the following order: (1) CH₄ concentration, (2) CH₄ oxidation rate, and (3) DNA preservation. All depths are reported as meters below sea level.

Water column CH₄ samples were collected in 125 milliliter (mL) glass vials sealed with grey stoppers and crimp caps, filling each vial three times to eliminate bubbles before final sealing. Vials for CH₄ determination were injected with 7.5 mL of 50% NaOH and a 2.5 mL air headspace while a total volume of 10 mL water sample was removed. The amount of CH₄ in the headspace was subsequently analyzed via gas chromatography. Specifically, a Shimadzu Gas Chromatograph (GC-2014) was used, equipped with a Haysep-D packed column and a flame ionization detector. The column temperature was set to 80 degrees Celsius (°C), and helium served as the carrier gas at a flow rate of 12 mL per minute. CH₄ concentrations were calibrated using CH₄ standards (Scotty Analyzed Gases), with a precision of ±5%. Water column CH₄ concentrations were calculated using Henry's law and the Bunsen solubility coefficient (Yamamoto et al., 1976) to account for CH₄ in both the gas and liquid phase of the preserved samples.

Water column CH₄ oxidation samples were collected in 30 mL glass vials sealed with non-toxic chlorobutyl stoppers (blue Belco stoppers, 20 millimeters (mm); Niemann et al., 2015), filling each vial three times to eliminate bubbles before final sealing. CH₄ oxidation rates were determined onboard through *ex situ* incubations with tritium-labelled CH₄ (³H-CH₄) applying established methods (Bussmann et al., 2015; Steinle et al., 2015). Samples (triplicates) were incubated between 36 and 105 hours (the exact duration for each sample set was determined by workflow constraints) with 10 µL gaseous ³H-CH₄ (~2 kBq, specific activity 20 Curies per millimole (Ci/mmol), American Radiolabeled Chemicals, USA). Control samples were injected with 100 microliters (µL) of 25% H₂SO₄ prior to radiotracer injection to stop microbial activity. To determine the total

radioactivity of the sample, the crimped vials were opened, and a 2 mL subsample was pipetted into a 6 mL scintillation vial and filled with 3 mL of Ultima Gold LLT scintillation cocktail from Perkin Elmer. For the determination of $^3\text{H-CH}_4$ that was metabolized to $^3\text{H-H}_2\text{O}$, 2 mL from the incubation was subsampled into an additional 6 mL scintillation vial and bubbled with air for 5 minutes to remove $^3\text{H-CH}_4$ prior to the addition of the scintillation cocktail. Both subsamples were mixed by gentle inversion and counted onboard in a PerkinElmer Tri-Carb liquid scintillation counter. The *in-situ* temperatures of the samples ranged from 1.5 to 6 °C. Due to the availability of only a single incubator, all samples were incubated at 5 °C in the dark. To correct for abiotic tracer turnover, the reported rate values are at least the mean tracer turnover in the killed controls plus one standard deviation of the killed-control value. Assuming first-order kinetics, the rate constant (k) was determined using the following equation:

$$k = ^3\text{H-H}_2\text{O} / (^3\text{H-H}_2\text{O} + ^3\text{H-CH}_4) / t$$

In this equation, $^3\text{H-H}_2\text{O}$ refers to the amount of tritiated water produced (in counts per minute, cpm), $^3\text{H-CH}_4$ represents the remaining unoxidized tritiated CH_4 (cpm), and t is the incubation duration in days.

CH_4 oxidation rates (r_{ox}) were calculated again assuming first-order kinetics, using the fractional turnover rate multiplied by the CH_4 concentration (nanomolar (nM)). Rates were determined with the following equation:

$$r_{\text{ox}} = k \times [\text{CH}_4]$$

For DNA extraction, seawater was collected into pre-rinsed 1.5 L polycarbonate bottles and stored at 6 °C until filtration. Microbial biomass was collected by filtering the samples using a peristaltic pump through 0.22 micrometer (μm) Sterivex filters (Millipore, Cat. No. SVGP0150). Filters were immediately frozen after filtration for downstream molecular analyses. Genomic DNA was extracted from Sterivex filters using a modified phenol-chloroform procedure followed by purification on Zymo silica spin columns (part C1006). Briefly, each filter was sealed, and 1.8 mL of filter-sterilized lysis buffer (containing 50 millimolar (mM) EDTA, 50 mM Tris-Cl [pH 8], 0.75 M sucrose, and 0.01% Tween 20) was injected into the cartridge. Lysozyme (40 μL ; 50 milligrams per milliliter (mg mL^{-1})) was then added, and the filter was incubated at 37 °C for 45 minutes under rotation. 50 μL of Proteinase K (800 U mL^{-1}) and 150 μL of 20% SDS were added to achieve a final SDS concentration of ~1%, followed by incubation at 55 °C for 2 hours. The lysate was transferred to a polypropylene tube, and total nucleic acids were extracted via successive phenol-chloroform-isoamyl alcohol (25:24:1) extractions at 65 °C, with centrifugation steps (10 minutes at 16,000 $\times g$) to separate the aqueous and organic phases. Residual phenol was removed through an additional chloroform-isoamyl alcohol wash, and DNA was precipitated with 0.4 vol of 5 M NaCl and 0.8 vol of isopropanol. The precipitated DNA was bound to Zymo Spin-Away columns by repeated loading at 6,000 $\times g$ for 1 minute, washed with 70% ethanol, and eluted in nuclease-free water or 10 mM Tris. Final DNA extracts were stored at -80 °C until further analysis.

For the qPCR assays, each 10 μL reaction consisted of 5 μL Phusion SYBR® Green PCR Master Mix (BioRad), 0.5 μL (0.5 μM) each primer, 3.5 μL PCR-grade H_2O , and 0.5 μL of DNA (concentration pre-determined through Qubit™ dsDNA Quantification, High Sensitivity Assay Kit). Duplicate bacterial *pmoA* assays were run using water column specific primers *wcpmoA189f* and *wcpmoA661r* (Tavormina et al., 2010) and the following cycling parameters: 98 °C for 2 minutes followed by 40 cycles of 98 °C for 10 seconds (s), 52 °C for 20s, 72 °C for 30s, detection, then a melt curve from 65 °C to 95 °C in 0.5 °C increments, plate reading every 5s. Standard curves were constructed with 10-fold dilutions of PCR products from 0 (negative control) to 10^6 gene copies total DNA from extracted *Methylomonas* sp. LW13 cells.

Data Processing Description

All data were processed in Excel.

BCO-DMO Processing Description

- Imported original file "Klonicki et al., AT50-24_dataset_BCO-DMO_final.xlsx" into the BCO-DMO system.
- Marked "N/A" as a missing data value (missing data are empty/blank in the final CSV file).
- Renamed fields to comply with BCO-DMO naming conventions.
- Created date-time field in ISO 8601 format.
- Saved the final file as "986875_v1_at50-24_water_column_methane.csv".

Related Publications

Bussmann, I., Matousu, A., Osudar, R., & Mau, S. (2015). Assessment of the radio 3 H-CH₄ tracer technique to measure aerobic methane oxidation in the water column. *Limnology and Oceanography: Methods*, 13(6), 312–327. doi:[10.1002/lom3.10027](https://doi.org/10.1002/lom3.10027)
Methods

Niemann, H., Steinle, L., Blees, J., Bussmann, I., Treude, T., Krause, S., Elvert, M., & Lehmann, M. F. (2015). Toxic effects of lab-grade butyl rubber stoppers on aerobic methane oxidation. *Limnology and Oceanography: Methods*, 13(1), 40–52. Portico. <https://doi.org/10.1002/lom3.10005>
Methods

Steinle, L., Graves, C. A., Treude, T., Ferré, B., Biastoch, A., Bussmann, I., Berndt, C., Krastel, S., James, R. H., Behrens, E., Böning, C. W., Greinert, J., Sapart, C.-J., Scheinert, M., Sommer, S., Lehmann, M. F., & Niemann, H. (2015). Water column methanotrophy controlled by a rapid oceanographic switch. *Nature Geoscience*, 8(5), 378–382. <https://doi.org/10.1038/ngeo2420> <https://doi.org/10.1038/NGEO2420>
Methods

Suess, E., Bohrmann, G., von Huene, R., Linke, P., Wallmann, K., Lammers, S., Sahling, H., Winckler, G., Lutz, R. A., & Orange, D. (1998). Fluid venting in the eastern Aleutian Subduction Zone. *Journal of Geophysical Research: Solid Earth*, 103(B2), 2597–2614. Portico. <https://doi.org/10.1029/97jb02131>
<https://doi.org/10.1029/97JB02131>
Methods

Tavormina, P. L., Ussler, W., Joye, S. B., Harrison, B. K., & Orphan, V. J. (2010). Distributions of putative aerobic methanotrophs in diverse pelagic marine environments. *The ISME Journal*, 4(5), 700–710. <https://doi.org/10.1038/ismej.2009.155>
Methods

Wallmann, K., Linke, P., Suess, E., Bohrmann, G., Sahling, H., Schlüter, M., Dähmann, A., Lammers, S., Greinert, J., & von Mirbach, N. (1997). Quantifying fluid flow, solute mixing, and biogeochemical turnover at cold vents of the eastern Aleutian subduction zone. *Geochimica et Cosmochimica Acta*, 61(24), 5209–5219. [https://doi.org/10.1016/S0016-7037\(97\)00306-2](https://doi.org/10.1016/S0016-7037(97)00306-2)
[https://doi.org/10.1016/S0016-7037\(97\)00306-2](https://doi.org/10.1016/S0016-7037(97)00306-2)
Methods

Yamamoto, S., Alcauskas, J. B., & Crozier, T. E. (1976). Solubility of methane in distilled water and seawater. *Journal of Chemical & Engineering Data*, 21(1), 78–80. <https://doi.org/10.1021/jc60068a029>
Methods

Parameters

Parameter	Description	Units
Date.UTC	Date when sample was collected in Coordinated Universal Time	unitless
CTD_Start_Time_or_Alvin_Niskin_Closure.UTC	CTD start time or Alvin Niskin closure time in Coordinated Universal Time	unitless
ISO_DateTime.UTC	Date and time (UTC) in ISO 8601 format	unitless
Site_Name	Name of the site	unitless
CTD_or_Alvin_Sample_Number	CTD or Alvin sample number (AD)	unitless
Waypoint	Sampling locations (approximately 5 meters above ground) along horizontal CTD transects (CTD Tow-yo)	unitless
Latitude	Latitude of sample collection	decimal degrees
Longitude	Longitude of sample collection	decimal degrees
Water_Depth	Water depth at sample collection site	meters below sea level (mbsl)
Methane	Methane concentration	nanomoles per liter (nmol L ⁻¹)
Methane_oxidation_Replicate_1	Methane oxidation rate, replicate 1	nanomoles per liter per day (nmol L ⁻¹ d ⁻¹)
Methane_oxidation_Replicate_2	Methane oxidation rate, replicate 2	nanomoles per liter per day (nmol L ⁻¹ d ⁻¹)
Methane_oxidation_Replicate_3	Methane oxidation rate, replicate 3	nanomoles per liter per day (nmol L ⁻¹ d ⁻¹)
pmoA_gene_copy_number	log numbers of pmoA gene copies per liter	Log pmoA copies per liter

[[table of contents](#) | [back to top](#)]

Instruments

Dataset-specific Instrument Name	Seabird Scientific pumped CTD system
Generic Instrument Name	CTD Sea-Bird
Dataset-specific Description	A Seabird Scientific pumped CTD system was mounted on the rosette frames outfitted with Niskin bottles and sensors to continuously measure conductivity (SBE4), temperature (SBE3), pressure (SBE9), dissolved O ₂ (SBE43), and light transmission (C-Star) and chlorophyll-a fluorescence (WET Labs ECO-AFL). Fluorescence data were only collected at Sanak, as rosette size constraints prevented installation of the fluorometer at the other sites.
Generic Instrument Description	A Conductivity, Temperature, Depth (CTD) sensor package from SeaBird Electronics. This instrument designation is used when specific make and model are not known or when a more specific term is not available in the BCO-DMO vocabulary. Refer to the dataset-specific metadata for more information about the specific CTD used. More information from: http://www.seabird.com/

Dataset-specific Instrument Name	flame ionization detector
Generic Instrument Name	Flame Ionization Detector
Dataset-specific Description	A Shimadzu Gas Chromatograph (GC-2014) was used, equipped with a Haysep-D packed column and a flame ionization detector.
Generic Instrument Description	A flame ionization detector (FID) is a scientific instrument that measures the concentration of organic species in a gas stream. It is frequently used as a detector in gas chromatography. Standalone FIDs can also be used in applications such as landfill gas monitoring, fugitive emissions monitoring and internal combustion engine emissions measurement in stationary or portable instruments.

Dataset-specific Instrument Name	Shimadzu Gas Chromatograph (GC-2014)
Generic Instrument Name	Gas Chromatograph
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	HOV Alvin
Generic Instrument Name	HOV Alvin
Dataset-specific Description	Water column sampling and profiling was conducted over two weeks during the AT50-24 expedition aboard the R/V Atlantis using the HOV Alvin and a CTD/Rosette system.
Generic Instrument Description	Human Occupied Vehicle (HOV) Alvin is part of the National Deep Submergence Facility (NDSF). Alvin enables in-situ data collection and observation by two scientists to depths reaching 6,500 meters, during dives lasting up to ten hours. Commissioned in 1964 as one of the world's first deep-ocean submersibles, Alvin has remained state-of-the-art as a result of numerous overhauls and upgrades made over its lifetime. The most recent upgrades, begun in 2011 and completed in 2021, saw the installation of a new, larger personnel sphere with a more ergonomic interior; improved visibility and overlapping fields of view; longer bottoms times; new lighting and high-definition imaging systems; improved sensors, data acquisition and download speed. It also doubled the science basket payload, and improved the command-and-control system allowing greater speed, range and maneuverability. With seven reversible thrusters, it can hover in the water, maneuver over rugged topography, or rest on the sea floor. It can collect data throughout the water column, produce a variety of maps and perform photographic surveys. Alvin also has two robotic arms that can manipulate instruments, obtain samples, and its basket can be reconfigured daily based on the needs of the upcoming dive. Alvin's depth rating of 6,500m gives researchers in-person access to 99% of the ocean floor. Alvin is a proven and reliable platform capable of diving for up to 30 days in a row before requiring a single scheduled maintenance day. Recent collaborations with autonomous vehicles such as Sentry have proven extremely beneficial, allowing PIs to visit promising sites to collect samples and data in person within hours of their being discovered, and UNOLs driven technological advances have improved the ability for scientific outreach and collaboration via telepresence Alvin is named for Allyn Vine, a WHOI engineer and geophysicist who helped pioneer deep submergence research and technology. (from https://www.whoi.edu/what-we-do/explore/underwater-vehicles/hov-alvin/ , accessed 2022-09-09)

Dataset-specific Instrument Name	Niskin-bottle rosettes (Ocean Test Equipment, Inc., Fort Lauderdale, FL)
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Water samples were collected from surface to near-seafloor at all three CH ₄ seep sites using Niskin-bottle rosettes (Ocean Test Equipment, Inc., Fort Lauderdale, FL): a 12 × 10 L configuration for Edge and Shumagin to accommodate the cable-tension limits at these depths, and a 23 × 10 L configuration for Sanak.
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	qPCR assays
Generic Instrument Name	qPCR Thermal Cycler
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

Deployments

AT50-24

Website	https://www.bco-dmo.org/deployment/984569
Platform	R/V Atlantis
Start Date	2024-05-16
End Date	2024-06-07
Description	See more information from R2R: https://www.rvdata.us/search/cruise/AT50-24

Project Information

Collaborative Research: Redefining the footprint of deep ocean methane seepage for benthic ecosystems (Methanosphere)

Coverage: Gulf of Alaska and Southern California Bight

NSF Award Abstract:

This research examines the role of deep-sea organisms in determining the fate and footprint of methane, a potent greenhouse gas, on Pacific continental margins. The investigators are evaluating the deep ocean methanosphere defined by the microbial communities that consume methane and the animals that directly feed on or form symbioses with methane-consuming microbes. They are also investigating animal communities that gain energy indirectly from methane, as well as those that take advantage of carbonate rocks, the physical manifestation of methane consumption in seafloor sediments. The study of methane seeps in the deep waters of both Alaska (4400-5500 meters) and Southern California (450-1040 meters) is enabling comparisons of the methanosphere under different food-limitation and oxygen regimes. By applying diverse chemical, isotopic, microscopy, and genetic-based analyses to seep microbes and fauna, this study is advancing understanding of the contribution of methane to deep-sea biodiversity and ecosystem function, information that can inform management and conservation actions in US waters. In addition to training for graduate and undergraduate students at their home institutions, the investigators are collaborating with the Alaska Native Science and Engineering Program (ANSEP). They are recruiting Alaskan undergraduates to participate in the research, contributing to ANSEP's online resources that promote interaction between scientists and middle and high school students, and participating in ANSEP's annual residential Career Exploration in Marine Science programs to engage middle school students in learning about deep-sea ecosystems and the variety of career pathways available in marine related fields.

Microbial production and consumption of methane is dynamic and widespread along continental margins, and some animals within deep-sea methane seeps rely on the oxidation and sequestration of methane for nutrition. At the same time, understanding of methane-dependent processes and symbioses in the deep-sea environment is still rudimentary. The goals of this study are to 1) examine the diversity of animals involved in methane-based symbioses and heterotrophic consumption of methane-oxidizing microbes and how these symbioses extend the periphery of seeps, contributing to non-seep, continental slope food webs; and 2) determine whether carbonates on the seep periphery sustain active methanotrophic microbial assemblages, providing a localized food source or chemical fuel for thiotrophic symbioses, via anaerobic oxidation of methane, or free-living, sulfide-oxidizing bacteria consumed by animals. The investigators are addressing these goals by surveying, sampling, and characterizing microbes, water, sediments, carbonates and animals at a deep seep site on the Aleutian Margin and a shallow site off Southern California. Shipboard experiments and laboratory analyses are using molecular, isotopic, geochemical, and radiotracer tools to understand transfer of methane-sourced carbon from aerobic methanotrophs under multiple oxygen levels, pressures, and photosynthetic food inputs. This approach offers a wide lens by which to examine the methane seep footprint, allow reinterpretation of past observations, and identify new scientific areas for future study. Improved

characterization of the deep continental margin methanosphere informs climate science, biodiversity conservation, and resource management.

[[table of contents](#) | [back to top](#)]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2048597
NSF Division of Ocean Sciences (NSF OCE)	OCE-2048666
NSF Division of Ocean Sciences (NSF OCE)	OCE-2048720
NSF Division of Ocean Sciences (NSF OCE)	OCE-2126631
NSF Division of Ocean Sciences (NSF OCE)	OCE-2205998

[[table of contents](#) | [back to top](#)]