Water column methane, methane oxidation, and pmoA gene copies above southern California methane seeps determined from samples collected on R/V Atlantis cruise AT50-12 in July 2023

Website: https://www.bco-dmo.org/dataset/986866
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
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Abstract
Marine methane (CH_4) seeps are dynamic biogeochemical systems that modulate carbon cycling and support high-biomass communities through microbial methane (CH_4) oxidation. While most CH_4 is consumed anaerobically in sediments, a fraction enters the water column, where aerobic methanotrophs form a biological filter limiting CH_4 flux to the atmosphere. However, the extent to which this microbial activity and CH_4 influence extend beyond visibly active seep zones remains poorly constrained, with implications for deep-sea food webs, biogeochemical gradients, and carbon cycling. We investigated CH_4 dynamics and methanotroph distribution across three seep sites on the Southern California margin (Del Mar, Santa Monica (800 meters) Mound, Lasuen Knoll; ~400–1200 meters depth). Using radiotracer (^3H-CH_4) incubations, CH_4 concentration profiling, and particulate methane monooxygenase (pmoA) gene quantification, we sampled vertical and horizontal transects and near-bottom waters via the HOV Alvin. CH_4 oxidation was active not only within seep plumes but also in off-seep regions, with the highest rate (454 nanomoles per liter per day) observed within a CH_4-rich bubble plume. pmoA gene abundances remained relatively stable across both seep and off-seep waters, suggesting a persistent CH_4-oxidizing potential. These findings support an expanded "methanosphere," a CH_4-influenced microbial zone shaped by physical transport and environmental gradients. This dataset is part of a PhD thesis: Klonicki-Ference, Emily F. "Microbial Regulation of Methane and Redox Dynamics in the Water Column: From a Proterozoic Ocean Analog to Modern Marine Seeps." PhD diss., University of California, Los Angeles, 2025.

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Coverage

Location: Southern California Borderland
Spatial Extent: **N**:33.8003 **E**:-117.7765 **S**:32.8339 **W**:-118.6488
Temporal Extent: 2023-07-17 - 2023-07-26

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

Sampling was conducted over two weeks during the AT50-12 expedition (July 16 to 29) aboard the R/V Atlantis using the HOV Alvin and a CTD/Rosette system for water column profiling and collection. Water samples were collected from various depths, spanning the surface to near-seafloor, across three methane (CH_4) seep sites (Del Mar, Santa Monica, and Lasuen Knoll) using a rosette equipped with 23 × 10 liter (L) Niskin bottles (Ocean Test Equipment, Inc., Fort Lauderdale, Florida). A Seabird Scientific pumped CTD system was mounted on a rosette frame outfitted with Niskin bottles and sensors to continuously measure conductivity (SBE4), temperature (SBE3), pressure (SBE9), dissolved O_2 (SBE43), fluorescence (FLNTURTD), and light transmission (C-Star). Salinity and density were calculated from conductivity and temperature, respectively, and depth was inferred from pressure. All sensors were calibrated prior to deployment according to manufacturer instructions.

Vertical CTD casts were deployed over active seep sites selected based on available information, which varied by location and included real-time EK80 acoustic backscatter data, multibeam seafloor mapping, and/or prior observations from Alvin dives. Horizontal CTD transects targeted both active features and surrounding background areas (no visible seep associated fauna, microorganisms, or structures), with the CTD rosette maintained approximately 5 meters (m) above the seafloor. For each cast, the ship maintained dynamic positioning using acoustic beacons to remain on station despite prevailing currents. Niskin bottles were only fired after the rosette stabilized at the target location to ensure representative sampling and to minimize current-related offsets. At the Del Mar site, a noticeable gap in horizontal sampling reflects the transit between two spatially distinct, acoustically active seep features (the northwest and southeast regions identified through bathymetry).

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 or observations of gas bubble releases (EK80 or Alvin observation) 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 after the observed current removed resuspended sediment. For all sampling events, water from each Niskin bottle was subsampled in the following order: (1) CH_4 concentration, (2) CH_4 oxidation rate, and (3) DNA preservation. All depths are reported as meters below sea level.

Water column CH_4 samples were collected in 100 milliliter (mL) glass vials sealed with grey stoppers and crimp caps, filling each vial three times to eliminate bubbles before final sealing. Vials for CH_4 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 partial pressure of CH_4 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. CH_4 concentrations were calibrated using CH_4 standards (Scotty Analyzed Gases), with a precision of ±5%. Sulfate samples were analyzed via ion chromatography (Metrohm 761). Water column CH_4 concentrations were calculated using Henry's law and the Bunsen solubility coefficient (Yamamoto et al., 1976) to account for CH_4 in both the gas and liquid phase of the preserved samples.

Water column CH_4 oxidation samples were collected in 30 mL glass vials sealed with non-toxic chlorobutyl stoppers (blue Bellco stoppers, 20 millimeters (mm), (Niemann et al., 2015)) filling each vial three times to eliminate bubbles before final sealing. CH_4 oxidation rates were determined onboard through *ex situ* incubations with tritium-labelled CH_4 (^3H-CH_4) applying established methods (Steinle et al., 2015; Bussmann et al., 2015). Samples (triplicates) were incubated between 35 and 88 hours (depending on sample set) with 10 microliter (µL) gaseous ^3H-CH_4 (~2 kiloBecquerel (kBq), specific activity 20 Curies per millimole (Ci/mmol), American Radiolabeled Chemicals, USA). Control samples were injected with 100 µL of 25% H_2SO_4 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 ^3H-CH_4 that was metabolized to

^3H-H_2O, 2 mL from the incubation was subsampled into an additional 6 mL scintillation vial and bubbled with air for 5 minutes to remove ^3H-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 ~4 to 20°C. Due to the availability of only a single incubator, all samples were incubated at 6 °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:

$k = {}^3H\text{-}H_2O / ({}^3H\text{-}H_2O + {}^3H\text{-}CH_4) / t$

In this equation, ^3H-H_2O refers to the amount of tritiated water produced (in counts per minute, cpm), ^3H-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:

$r_{ox} = k \times [CH_4]$

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. 50µL 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 × 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 × g for 1 min, 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., AT50-12_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 "986866_v1_at50-12_water_column_methane.csv".

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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
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

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

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/je60068a029
Methods

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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

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Instruments

Dataset-specific Instrument Name	Seabird Scientific pumped CTD system
Generic Instrument Name	CTD Sea-Bird
Dataset-specific Description	CTD/Rosette - A Seabird Scientific pumped CTD system was mounted on a rosette frame outfitted with Niskin bottles and sensors to continuously measure conductivity (SBE4), temperature (SBE3), pressure (SBE9), dissolved O_2 (SBE43), fluorescence (FLNTURTD), and light transmission (C-Star).
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	Sampling was conducted over two weeks during the AT50-12 expedition (July 16 to 29) aboard the R/V Atlantis using the HOV Alvin and a CTD/Rosette system for water column profiling and collection.
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	Metrohm 761
Generic Instrument Name	Ion Chromatograph
Generic Instrument Description	Ion chromatography is a form of liquid chromatography that measures concentrations of ionic species by separating them based on their interaction with a resin. Ionic species separate differently depending on species type and size. Ion chromatographs are able to measure concentrations of major anions, such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major cations such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. (from http://serc.carleton.edu/microbelife/research_methods/biogeochemical/ic....)

Dataset-specific Instrument Name	23 × 10 L Niskin bottles (Ocean Test Equipment, Inc., Fort Lauderdale, Florida)
Generic Instrument Name	Niskin bottle
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
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).

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Deployments

AT50-12

Website	https://www.bco-dmo.org/deployment/946261
Platform	R/V Atlantis
Start Date	2023-07-16
End Date	2023-07-29
Description	See more information in R2R: https://www.rvdata.us/search/cruise/AT50-12

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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.

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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

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