Bacterial 16S rRNA barcodes associated with methane seep invertebrates collected off of 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/1007825
Version: 1
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Project
» Collaborative Research: Redefining the footprint of deep ocean methane seepage for benthic ecosystems (Methanosphere)

Abstract
Seep invertebrates were collected from the Sanak seep off of Alaska in June 2024 (53.74865 N/ −162.58970 W; 2,020 m) aboard the R/V Atlantis (expedition AT50-24) with HOV Alvin (operated by the Woods Hole Oceanographic Institution). Specimens for molecular analysis were preserved within 2 h of collection in ~90% ethanol and stored at 4 °C. Total genomic DNA was extracted from whole specimens preserved in ethanol using the Qiagen DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total DNA extracted was quantified via a Qubit 3.0 fluorometer using the dsDNA BR Assay Kit (Thermo Fisher Scientific). The V4-V5 hypervariable region of the 16S rRNA gene was PCR amplified using bacterial primers [515F: GTGYCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT] with Illumina adapters on the 5′ end (San Diego, CA). Each PCR product was secondarily barcoded with Illumina NexteraXT index v2 Primers that included unique 8-bp barcodes, with NEB Q5 Hot Start High-Fidelity Mix at an annealing temperature of 66 °C for 11 cycles. Barcoded products were purified using Millipore-Sigma (St. Louis, MO) MultiScreen Plate MSNU03010 with a vacuum manifold and quantified using the QuantIT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific) on a BioRad CFX96 Touch Real-Time PCR Detection System. Barcoded samples were combined in approximately equimolar amounts, purified again with Promega's Wizard SV Gel and PCR Clean-up System (#A9281), and quantified again using the QuBit system. This sample was submitted to Laragen, Inc. (Culver City, CA) for 2× 250 bp paired-end analysis on the Illumina MiSeq platform with 20% PhiX addition.

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Coverage

Location: Sanak seep off of Alaska in June 202
Spatial Extent: **Lat**:0 **Lon**:0
Temporal Extent: 2024-05-17 - 2024-06-05

Methods & Sampling

Sampling

Seep invertebrates were collected from the Sanak seep off of Alaska in June 2024 (53.74865 N/ −162.58970 W; 2,020 m) aboard the R/V Atlantis (expedition AT50-24) with HOV Alvin (operated by the Woods Hole Oceanographic Institution). Specimens for molecular analysis were preserved within 2 h of collection in ~90% ethanol and stored at 4 °C. Total genomic DNA was extracted from whole specimens preserved in ethanol using the Qiagen DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Data Collection Methods

Total DNA extracted was quantified via a Qubit 3.0 fluorometer using the dsDNA BR Assay Kit (Thermo Fisher

Scientific). The V4-V5 hypervariable region of the 16S rRNA gene was PCR amplified using bacterial primers [515F: GTGYCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT] with Illumina adapters on the 5′ end (San Diego, CA). Each PCR product was secondarily barcoded with Illumina NexteraXT index v2 Primers that included unique 8-bp barcodes, with NEB Q5 Hot Start High-Fidelity Mix at an annealing temperature of 66 °C for 11 cycles. Barcoded products were purified using Millipore-Sigma (St. Louis, MO) MultiScreen Plate MSNU03010 with a vacuum manifold and quantified using the QuantIT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific) on a BioRad CFX96 Touch Real-Time PCR Detection System. Barcoded samples were combined in approximately equimolar amounts, purified again with Promega's Wizard SV Gel and PCR Clean-up System (#A9281), and quantified again using the QuBit system. This sample was submitted to Laragen, Inc. (Culver City, CA) for 2× 250 bp paired-end analysis on the Illumina MiSeq platform with 20% PhiX addition.

Data Processing Description

Raw reads were processed as follows: CutAdapt v4.1 was used to remove the primer sequences, which allowed one error for every 10 bp in the primer sequence. FastQC v1.13 was used to quality control the raw sequence data and identify trim cutoffs for both the forward and reverse reads, ahead of pairing. Raw sequences were then processed with DADA2 [for initial quality trimming, error rate estimation, merging of read pairs, chimeric sequence removal and community data matrix construction] and taxonomy was assigned to the processed ASVs (ASVs at 100% identity) using the SILVA database v138.1.

Problem Description

No problems or issues have been reported by the dataset author.

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Parameters

Parameters for this dataset have not yet been identified

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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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