

# Microbial cell counts in sediment cores collected during R/V Knorr cruise KN195-03 in the Pacific Ocean in 2009 (Subseafloor Microbial Life project)

**Website:** <https://www.bco-dmo.org/dataset/567215>

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## Project

» [Oceanographic control and global distributions of subseafloor microbial life and activity](#) (Subseafloor Microbial Life)

## Program

» [Center for Dark Energy Biosphere Investigations](#) (C-DEBI)

Contributors	Affiliation	Role
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## Dataset Description

Microbial cell counts in sediment cores collected during KN195-03 (Equatorial Pacific 86°W to 148°W and North Pacific Gyre 15°N to 30°N and 149°W to 158°W); cell counts from 12 coring stations.

## Methods & Sampling

*Coring systems used:*

EQP-01: Multi Corer, Piston Corer (long core)

EQP-02: Multi Corer, Gravity Corer

EQP-03: Gravity Corer

EQP-03a: Multi Corer, Gravity Corer (used long core system as a gravity corer)

EQP-04: Multi Corer, Gravity Corer (used long core system as a gravity corer), Piston Corer (long core)

EQP-05: Multi Corer, Gravity Corer, Piston Corer (long core)

EQP-06: Multi Corer, Gravity Corer, Piston Corer (long core)

EQP-06a: Gravity Corer

EQP-07: Multi Corer, Gravity Corer, Piston Corer (long core)

EQP-08: Multi Corer, Gravity Corer, Piston Corer (long core)  
EQP-09: Gravity Corer  
EQP-10: Multi Corer, Gravity Corer, Piston Corer (long core)  
EQP-11: Multi Corer, Gravity Corer, Piston Corer (long core)

*Excerpt from Kallmeyer et al. (2012) Supplemental Information:*

For each cell enumeration, we took 2-cm<sup>3</sup> samples from the center of a freshly cut core end using a sterile cutoff 3-cm<sup>3</sup> syringe. We carried out cell counts according to the method of Kallmeyer et al. (2008). We extruded the 2-cm<sup>3</sup> sediment plug into a sterile 15-mL centrifuge tube containing 8 mL of 2.5% (wt/vol) NaCl solution with 2% (vol/vol) formalin as a fixative and then thoroughly shook the tube to form a homogenous suspension. In cases where cell densities were high enough (> 10<sup>5</sup> cells/cm<sup>-3</sup>), we made direct cell counts by staining this slurry with SYBR Green I, placing a small aliquot of the slurry directly on a 0.2-μm pore size filter and enumerating manually under a fluorescence microscope (Noble and Fuhrman, 1998). Counts obtained with SYBR Green I have been found to be indistinguishable from acridine orange direct counts (AODCs) (Morono et al., 2009).

*References:*

Kallmeyer, J., Smith, D.C., D'Hondt, S.L., & Spivack, A.J. 2008. New cell extraction procedure applied to deep subsurface sediments. *Limnol Oceanogr Methods* 6:236–245. doi:[10.4319/lom.2008.6.236](https://doi.org/10.4319/lom.2008.6.236)

Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., & D'Hondt, S. 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. *Proceedings of the National Academy of Sciences*, 109(40), 16213-16216. doi:[10.1073/pnas.1203849109](https://doi.org/10.1073/pnas.1203849109)

Noble, R.T., Fuhrman, J.A. 1998. Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. *Aquat Microb Ecol* 14(2):113–118. doi:[10.3354/ame014113](https://doi.org/10.3354/ame014113)

Morono, Y., Terada, T., Masui, N., & Inagak,i F. 2009. Discriminative detection and enumeration of microbial life in marine subsurface sediments. *ISME J* 3:503–511. doi:[10.1038/ismej.2009.1](https://doi.org/10.1038/ismej.2009.1)

## Data Processing Description

*Excerpt from Kallmeyer et al. (2012) Supplemental Information:*

Independent of the stain used, direct counting has a minimum detection limit (MDL) around 10<sup>5</sup> cells/cm<sup>-3</sup> (Kallmeyer, 2011). For samples with lower cell abundances, we found it necessary to detach and separate the cells from the mineral matrix using a cell extraction protocol (Kallmeyer et al. 2008). Most counts at North Pacific Gyre sites were of extracted cells because gyre cell abundances drop below the direct count MDL within decimeters to meters below the seafloor; for the same reason, a few counts of the deepest equatorial Pacific sediment were also of cell extracts.

*References:*

Kallmeyer J. 2011. Detection and quantification of microbial cells in subsurface sediments. *Advances in Applied Microbiology*, eds Laskin AI, Sariaslani S, Gadd GM (Elsevier, San Diego), Vol 76. doi:[10.1016/B978-0-12-387048-3.00003-9](https://doi.org/10.1016/B978-0-12-387048-3.00003-9)

Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., & D'Hondt, S. 2012. Global distribution of microbial abundance and biomass in subseafloor sediment. *Proceedings of the National Academy of Sciences*, 109(40), 16213-16216. doi:[10.1073/pnas.1203849109](https://doi.org/10.1073/pnas.1203849109)

Kallmeyer, J., Smith, D.C., D'Hondt, S.L., & Spivack, A.J. 2008. New cell extraction procedure applied to deep subsurface sediments. *Limnol Oceanogr Methods* 6:236–245. doi:[10.4319/lom.2008.6.236](https://doi.org/10.4319/lom.2008.6.236)

*BCO-DMO Processing:*

- Modified parameter names to conform with BCO-DMO naming conventions;
- Converted lat and lon from degrees and decimal minutes to decimal degrees.

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## Data Files

File
<b>KN195-03_cell_counts.csv</b> (Comma Separated Values (.csv), 25.97 KB) MD5:10b8c7bc2891f7f3cf0815fb01856a4c
Primary data file for dataset ID 567215

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## Parameters

Parameter	Description	Units
cruise_id	Cruise identification number.	dimensionless
station_id	Station identification number.	dimensionless
lat	Latitude of station.	decimal degrees
lon	Longitude of station.	decimal degrees
MBSF	Meters Below Seafloor (MBSF).	meters
cell_density	Cell density.	cells per cubic centimeter (cells cm-3)
st_dev	Standard deviation of cell density.	cells per cubic centimeter (cells cm-3)

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## Instruments

<b>Dataset-specific Instrument Name</b>	fluorescence microscope
<b>Generic Instrument Name</b>	Fluorescence Microscope
<b>Dataset-specific Description</b>	Direct cell counts were obtained by placing a small aliquot of the slurry directly on a 0.2-um pore size filter and enumerating manually under a fluorescence microscope.
<b>Generic Instrument Description</b>	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

<b>Dataset-specific Instrument Name</b>	Gravity Corer
<b>Generic Instrument Name</b>	Gravity Corer
<b>Generic Instrument Description</b>	The gravity corer allows researchers to sample sediment layers at the bottom of lakes or oceans. The coring device is deployed from the ship and gravity carries it to the seafloor. ( <a href="http://www.whoi.edu/instruments/viewInstrument.do?id=1079">http://www.whoi.edu/instruments/viewInstrument.do?id=1079</a> ).

<b>Dataset-specific Instrument Name</b>	Multi Corer
<b>Generic Instrument Name</b>	Multi Corer
<b>Generic Instrument Description</b>	The Multi Corer is a benthic coring device used to collect multiple, simultaneous, undisturbed sediment/water samples from the seafloor. Multiple coring tubes with varying sampling capacity depending on tube dimensions are mounted in a frame designed to sample the deep ocean seafloor. For more information, see Barnett et al. (1984) in Oceanologica Acta, 7, pp. 399-408.

<b>Dataset-specific Instrument Name</b>	Piston Corer (Long Core)
<b>Generic Instrument Name</b>	Piston Corer
<b>Generic Instrument Description</b>	The piston corer is a type of bottom sediment sampling device. A long, heavy tube is plunged into the seafloor to extract samples of mud sediment. A piston corer uses a "free fall" of the coring rig to achieve a greater initial force on impact than gravity coring. A sliding piston inside the core barrel reduces inside wall friction with the sediment and helps to evacuate displaced water from the top of the corer. A piston corer is capable of extracting core samples up to 90 feet in length.

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## Deployments

### KN195-03

<b>Website</b>	<a href="https://www.bco-dmo.org/deployment/58736">https://www.bco-dmo.org/deployment/58736</a>
<b>Platform</b>	R/V Knorr
<b>Start Date</b>	2009-01-12
<b>End Date</b>	2009-02-23
<b>Description</b>	The cruise went from Puntarenas, Costa Rica to Honolulu, Hawaii and was the first cruise during which scientists used the long coring system (developed at Woods Hole Oceanographic Institution) to recover sediments from the seafloor. Cruise information and original data are available from the NSF R2R data catalog.

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## Project Information

### Oceanographic control and global distributions of subseafloor microbial life and activity (Subseafloor Microbial Life)

**Coverage:** Pacific Ocean

Recent studies of subseafloor life, that is microbes living deep below the ocean's seafloor, have produced astonishing results that challenge fundamental ideas about the limits and distributions of life. These include: (1) that the microbial biomass of subseafloor sediments is spatially much more variable and possibly much smaller than previously believed; (2) that rates of subseafloor sedimentary microbial activity are far below the rate required for cell maintenance, implying that either most subseafloor cells are inactive or that the energy required for their cellular maintenance is lower than anticipated; and (3) the global distributions of subseafloor sedimentary microbes and their activities are significantly affected by the oceanographic properties of the overlying water column. This proposal will conduct fieldwork to test these ideas at a range of sites in the equatorial Pacific. To do this the principal investigators will conduct a transect study where the following samples and measurements will be taken: (1) coring the sediment to ~18 meter or more below seafloor (mbsf) at 12 sites in the Pacific Ocean; (2) conducting extensive microbiological and biogeochemical analyses of these cores; (3) surveying the oceanographic and geologic characteristics of each site; and (4) using the results to test and refine models for the global distribution of subseafloor microbial abundances and their metabolic activities. Using these data the investigators will then address four important questions: (1) What are the principal controls on the magnitude and geographic distribution of subseafloor sedimentary cell abundance and steady-state rates of microbial activities? (2) Can we accurately estimate the magnitude and global distribution of subseafloor sedimentary cell abundance? (3) Can we accurately estimate the global distribution of organic carbon-fueled microbial activity in subseafloor sediment? and (4) Do different subseafloor sediments with very different cell abundances and rates of metabolic activity characterized by different groups of organisms? This study will significantly advance our understanding of life in the subseafloor ocean and will provide samples for diverse independent studies, including the International Census of Marine Microbes. This project will also have a strong research and training impact at both the graduate and undergraduate levels as the inherently multidisciplinary nature of subsurface life provides an ideal entry into collaborative modern science.

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## Program Information

### Center for Dark Energy Biosphere Investigations (C-DEBI)

**Website:** <http://www.darkenergybiosphere.org>

**Coverage:** Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:

- (1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
- (2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep seafloor ecosystems;
- (3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
- (4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

### **Data Management:**

C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their [Data Management Plan \(PDF\)](#) and in compliance with the [NSF Ocean Sciences Sample and Data Policy](#). The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

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## **Funding**

Funding Source	Award
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-0752336</a>

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