ETNP OMZ 2023 Total Prokaryoplankton Cell Count Metadata R/V Atlantis AT50-08 in the Eastern Tropical Pacific Minimum Oxygen Zone from February to March 2023 (MicroPro project)

Website: https://www.bco-dmo.org/dataset/1005974
Data Type: Cruise Results
Version: 1
Version Date: 2026-08-25

Project
» Collaborative Research: Key Microbial Processes in Oxygen Minimum Zones: From In Situ Community Rate Measurements to Single Cells (MicroPro)

Contributors	Affiliation	Role
Pachiadaki, Maria G.	Woods Hole Oceanographic Institution (WHOI)	Co-Principal Investigator
Taylor, Gordon T.	Stony Brook University (SUNY Stony Brook)	Co-Principal Investigator
Yakubovskaya, Elena	Stony Brook University (SUNY Stony Brook)	Scientist
Butkevich, Natalie	Stony Brook University (SUNY Stony Brook)	Student
Mejia, Leslie	Stony Brook University (SUNY Stony Brook)	Student
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Data are depth profiles of total prokaryoplankton cell counts from two stations in Eastern Tropical North Pacific Oxygen Minimum Zone collected February and March of 2023. Whole water samples were preserved at sea. In the laboratory, samples were filtered, stained with 4', 6-diamidino-2-phenylindole (DAPI) and manually enumerated under a Zeiss Axioscope epifluorescence microscope. Samples were collected by Natalie Butkevich, Dr. Gordon Taylor and Dr. Elena Yakubovskaya. Samples were processed by Natalie Butkevich and Leslie Mejia.

Table of Contents

Coverage

Location: Eastern Tropical North Pacific
Spatial Extent: **N**:18.90883 **E**:-104.94333 **S**:18.17717 **W**:-106.33917
Temporal Extent: 2023-02-22 - 2023-03-07

Methods & Sampling

Methods + Sampling:

Shipboard Protocol:

Whole water samples of either 13mL or 45mL were collected in 15mL or 50mL Falcon tubes, respectively, on deck at the rosette. Samples were fixed by adding borate-buffered formaldehyde (2% final concen.) and stored at 4 °C.

Laboratory Protocol:

Storage: Samples were stored at 4°C before processing.

Sample Processing: Black-stained (Millipore Sigma) polycarbonate membrane filters (0.2 µm pore size, 25 mm diameter) were washed with dd-H2O then briefly dipped into very dilute Triton X-100 detergent for better cell dispersion (range between 0.001-0.01%). Washed membranes were placed on top of moistened glass fiber backing filters (Whatman GF/C) on the filtration base and funnel was secured. 14-16mL of sample was filtered, vacuum was applied until approx. 2 mL of the sample remained in funnel. Samples were then stained with DAPI (1µg/ml final concen.) and incubated for 5 min. The remaining 2mL of stained sample was passed through the filter and rinsed with ~5mL distilled water. Filters were dried for >15 minutes at room temperature. Filters were mounted on slides with 4:1 Citifluor:Vectashield. Before enumeration, slides were stored at -20 °C. Separate reagent blanks were prepared during each filtration session.

Slide Enumeration: At least 10 grids and >300 cells were enumerated per filter to minimize analytical error (Kirchman et al., 1982). Individual fields/grids were randomly selected along north-south and east-west transects before viewing to minimize operator bias. Manual counting and statistical calculations were facilitated by our NewCount macro within Excel which, once initialized, transforms a computer keyboard into blind data entry portal that tallies a single count from any keystroke (except two control keys), recognizes end of a single field count and tabulates cell concentrations, S.D., S.E., C.O.V. and 95% confidence limits once designated number of fields is counted (developed by G. Taylor and D. Zheng; available upon request). Cells/L were calculated by using the following formula, where dilution factor compensates for the volume of formaldehyde fixative, and multiplication factor is the constant required to convert average grid/field count to the total count for the entire filter area exposed to sample: (((adjusted mean cells per grid)/(dilution factor*mL filtered))*multiplication factor)*1000 = cells/L. Coefficient of variation was calculated using the following formula: (standard error(cells/L)*100)/average(cells/L)=CoV. Total prokaryotic counts were corrected for storage decay between sample collection and filter preparation via the following exponential decay curve: Ncorr=Nobs/e-0.000605(ts-tc), where Ncor is the corrected cell abundance at the time of sample collection, Nobs is the observed cell abundance after sample preparation, ts is date of sample preparation, and tc is date of sample collection. Raw cell count data available from N. Butkevich or G. Taylor by request. The correction factor is based on empirical observations of stored samples from the CARIACO time series. Details are available within Scranton and Taylor (2019) BCO-DMO repository metadata.

Data Processing Description

Total prokaryotic counts were corrected for storage decay between sample collection and filter preparation via the following exponential decay curve: Ncorr=Nobs/e-0.000605(ts-tc), where Ncor is the corrected cell abundance at the time of sample collection, Nobs is the observed cell abundance after sample preparation, ts is date of sample preparation, and tc is date of sample collection. Raw cell count data available from N. Butkevich or G. Taylor by request. The correction factor is based on empirical observations of stored samples from the CARIACO time series. Details are available within Scranton and Taylor (2019) BCO-DMO repository metadata.

BCO-DMO Processing Description

Curation Actions Performed on Data

- Loaded ENTP_Prokaryotic_Census_FINAL_nb.xlsx (sheet 1) as table 1005974_v1_ETNP_OMZ_AT50_Prokaryoplankton_Cell_Counts, using xlsx preserve_formatting enabled, floating point error adjustment enabled, header row 1, empty strings and "nd" set as missing values
- Combined columns "Cast Date" (format %m/%d/%y) and "Cast Time (UTC)" (format %H:%M:%S), interpreted as UTC, into new datetime column ISO_Cast_Start_DateTime_UTC formatted as %Y-%m-%dT%H:%M:%SZ
- Reformatted column "Cast Date" (format %m/%d/%y) into date column output as %Y-%m-%d, keeping the same column name
- Renamed columns: "CTD Cast filename" to CTD_Cast_filename, "Cast Date" to Cast_Date, "Cast Time (UTC)" to Cast_Time_UTC, "Average cells/L" to Average_cells_per_L, "Standard deviation" to Standard_deviation, "Standard error" to Standard_error, "Coefficient of Variation (percent)" to Coefficient_of_Variation_percent, "Grids counted" to Grids_counted
- Reordered columns to: Depth, Station, Lat, Long, CTD_Cast_filename, ISO_Cast_Start_DateTime_UTC, Cast_Date, Cast_Time_UTC, Average_cells_per_L, Standard_deviation, Standard_error, Coefficient_of_Variation_percent, Grids_counted
- Set column types and formats: Average_cells_per_L as number (scientific notation output), CTD_Cast_filename as string, Cast_Date as date (%Y-%m-%d), Cast_Time_UTC as time (%H:%M:%S), Coefficient_of_Variation_percent as number, Depth as number, Grids_counted as integer, ISO_Cast_Start_DateTime_UTC as datetime (%Y-%m-%dT%H:%M:%SZ), Lat as number, Long as number, Standard_deviation as number (scientific notation output), Standard_error as number (scientific notation output), Station as string
- Updated column metadata with descriptions, standard name IDs, supplied units, and primary parameter flags for all columns, including calculation formulas for Average_cells_per_L, Coefficient_of_Variation_percent, Standard_deviation, and Standard_error
- Dumped final table to CSV output, with unique lat/lon extraction enabled and pipeline specification saved

Curation Actions Performed on Metadata
- Standard metadata entry and text formatting steps were performed.

Problem Description

No problems or issues have been reported by the authors for this dataset.

[table of contents | back to top]

Related Publications

Hobbie, J. E., Daley, R. J., & Jasper, S. (1977). Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology, 33(5), 1225–1228. https://doi.org/10.1128/aem.33.5.1225-1228.1977
Methods

Kirchman, D., Sigda, J., Kapuscinski, R., & Mitchell, R. (1982). Statistical analysis of the direct count method for enumerating bacteria. Applied and Environmental Microbiology, 44(2), 376–382. https://doi.org/10.1128/aem.44.2.376-382.1982
Methods

Scranton, M., & Taylor, G. (2019). Biogeochemistry and microbiology from the B/O Hermano Gines HG93_CARIACO cruise in the CARIACO basin from 1995 to 2015 (CARIACO project) [Dataset]. In (Editor), Biological and Chemical Oceanography Data Management Office. Biological and Chemical Oceanography Data Management Office. https://doi.org/10.1575/1912/bco-dmo.3120.1
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units

Depth	Recorded depth at which Niskin bottle was closed and water sample was taken. Manually recorded from CTD cast at time of sample acquisition.	meters (m)
Station	Broadly categorizes cast location into 'Mex1' or 'Mex2', which corresponds to a nearshore or an offshore station respectively.	unitless
Lat	Latitude at which sample was taken, recorded in CTD cast header. A positive value indicates a northern latitude coordinate.	decimal degrees
Long	Longitude at which sample was taken, recorded in CTD cast header. A negative value indicates a western longitude coordinate.	decimal degrees
CTD_Cast_filename	Filename associated with CTD cast corresponding to these samples.	unitless
ISO_Cast_Start_DateTime_UTC	Start datetime of the assocated cast in UTC. The values of this column were created by combining the original Cast Date and Cast Time column.	unitless
Cast_Date	Date the cast was made as recorded by CTD cast.	unitless
Cast_Time_UTC	Time the cast began recorded by CTD cast in UTC.	unitless
Average_cells_per_L	Cells/L were calculated by using the following formula, where dilution factor compensates for the volume of formaldehyde fixative, and multiplication factor is the constant required to convert average grid/field count to the total count for the entire filter area exposed to sample: (((blank adjusted mean cells per grid)/(dilution factor*mL filtered))*multiplication factor)*1000 = cells/L.	cells per liter (cells/L)
Standard_deviation	Standard deviation is calculated by taking the sum of all cells, dividing it by L, squaring the result, dividing by the number of grids counted, and then taking the square root. (Standard deviation = sqrt(((sum of all cells/L)^2)/# grids counted)).	cells per liter (cells/L)
Standard_error	Standard error is calculated by dividing the standard deviation by the square root of the number of grids counted. (Standard error = standard deviation/sqrt(# grids counted)).	cells per liter (cells/L)
Coefficient_of_Variation_percent	The coefficient of variation (CoV) was calculated by dividing the standard error of the cell concentration (cells per liter) by the average cell concentration (cells per liter), then multiplying by 100. (Coefficient of variation = (standard error(cells/L)*100)/average(cells/L)=CoV).	cells per liter (cells/L)

Grids_counted	Number of grids counted to acquire the average cells per liter value.	grids (count)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Zeiss Axioscope Epifluorescence Microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	A Zeiss Axioskop epifluorescence microscope equipped with a DAPI filter set and a 60× or 100× objective was used to visualize and manually enumerate DAPI-stained prokaryotic cells collected on black polycarbonate membrane filters; a calibration factor accounted for the objective used. At least 10 randomly selected fields or grids and more than 300 cells were counted per filter. The resulting counts were used to calculate total prokaryotic cell abundance in cells per liter.
Generic Instrument Description	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.

[table of contents | back to top]

Deployments

AT50-08B

Website	https://www.bco-dmo.org/deployment/995472
Platform	R/V Atlantis
Start Date	2023-02-10
End Date	2023-03-16
Description	Project: Collaborative Research: Key Microbial Processes in Oxygen Minimum Zones: From In Situ Community Rate Measurements to Single Cells Chief: Pachiadaki, Maria G Start port: Putarenas, Costa Rica End port: Puntarenas, Costa Rica See additional information at R2R: https://www.rvdata.us/search/cruise/AT50-08B

[table of contents | back to top]

Project Information

Collaborative Research: Key Microbial Processes in Oxygen Minimum Zones: From In Situ Community Rate Measurements to Single Cells (MicroPro)

Coverage: East Tropical North Pacific Ocean

NSF Award Abstract:

Oxygen availability shapes the distributions and activities of marine organisms. Ongoing human activities and climate change are expected to lead to expansion and intensification of already large oxygen-stressed areas of the coastal and open ocean. Decreases in ocean oxygen have significant ecological consequences, including habitat loss for migratory and bottom-dwelling organisms, modification of the marine food web, and production of trace gases with pronounced feedbacks on climate, such as methane and nitrous oxide. Intense chemical cycling by microorganisms occurs in oxygen-depleted marine habitats. However, a full understanding of the consequences for marine ecosystems is hampered by limited knowledge of actual rates of key microbiological processes and dynamics of the microorganisms mediating them. This study combines novel methods and sampling techniques to understand how these processes are influenced by changes in oxygen concentration to inform predictions of important chemical exchanges within a changing ocean and its production of climate-active gases. This deeply collaborative project trains undergraduates (four of whom participate on the cruise), a graduate student and a postdoctoral fellow. Outreach takes place in middle and high schools and through social media. Data and samples from the cruise are integrated in coursework.

Oxygen depletion alters cycling of major elements (especially carbon, nitrogen, and sulfur) as well as food web functionality. This project addresses major gaps in our knowledge of oxygen minimum zone (OMZ) processes by applying in situ approaches to more accurately measure rates of several key microbial processes (chemoautotrophy, denitrification, anammox, sulfate reduction and sulfide oxidation) central to marine biogeochemical cycling. This work studies the Eastern Tropical North Pacific OMZ, the largest open ocean oxygen-depleted system, to 1) determine the in situ rates of microbial processes involved in carbon, nitrogen, and sulfur cycling, 2) reveal the genomic blueprint of active single cells involved in these processes, and 3) obtain estimates of the relative contributions of the dominant chemoautotrophic and heterotrophic groups to the measured rates. This work include applies cutting-edge equipment for in situ sampling and incubations that minimize artifacts associated with traditional water sampling approaches, allowing more accurate estimates of rates of important biogeochemical processes. Additionally, rate measurements of relatively undisturbed bulk and fractionated water samples make it easier to distinguish the potential role of particle-associated microorganisms in these OMZ processes. Single cell sorting of microorganisms using a fluorescent dye indicative of cell activity together with metatranscriptomics informs on metabolic pathways used for key processes by active microbial community members, as well as the potential coupling of chemoautotrophy and nitrogen or/and sulfur cycling. By combining stable isotope probing, fluorescence in situ hybridization and single cell Raman microspectrometry the relative activity levels of different microbial phylotypes involved in chemoautotrophic and heterotrophic elemental cycling are assessed.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1924492
NSF Division of Ocean Sciences (NSF OCE)	OCE-1924424

[table of contents | back to top]