KByT 2017-2019, 16S rRNA gene amplicons, flow cytometry, chlorophyll a, and surface seawater measures taken at the Kāneʻohe Bay, Oʻahu (Hawaiʻi) between 2017 and 2019

Website: https://www.bco-dmo.org/dataset/930084
Data Type: Other Field Results
Version: 1
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Project
» Population genomics and ecotypic divergence in the most dominant lineage of marine bacteria (Pelagibacteromics)

Contributors	Affiliation	Role
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Abstract
These data include temperature, pH, salinity, chlorophyll a concentrations, cellular abundances of Prochlorococcus, Synechococcus, photosynthetic picoeukaryotes, and heterotrophic bacteria, and 16S ribosomal RNA gene amplicon libraries from 200 surface seawater samples collected as part of the Kāneʻohe Bay Time-series (KByT). Near-monthly sampling of surface seawater was conducted between August 2017 to June 2019 at 10 sites within coastal waters of Kāneʻohe Bay, Oʻahu, Hawaiʻi and in the adjacent offshore. Instruments used were a YSI 6,600 sonde, a Turner 10AU fluorometer, a EPICS ALTRA flow cytometer, and Illumina MiSeq v2 platform. These data characterize the partitioning of microbial communities across sharp physiochemical gradients in surface seawaters connecting nearshore and offshore waters in the tropical Pacific. This study provides evidence for ecological differentiation of SAR11 marine bacteria across nearshore to offshore waters in the tropical Pacific and further increases our understanding of how SAR11 genetic diversity partitions into distinct ecological units. Data were collected by Sarah J. Tucker, Kelle C. Freel, Elizabeth A. Monaghan, Clarisse E. S. Sullivan, Oscar Ramfelt, Yoshimi M. Rii, and Michael S. Rappé.

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
  - BCO-DMO Processing Description
- Related Publications
- Related Datasets
- Parameters
- Instruments
- Project Information
- Funding

Coverage

Location: Kāneʻohe Bay, Oʻahu, Hawaiʻi and adjacent offshore waters, 21° 28′ N, 157° 48′ W, depth 2m

Spatial Extent: **N**:21.526 **E**:-157.7663 **S**:21.43635 **W**:-157.83585
Temporal Extent: 2017-08-23 - 2019-06-14

Dataset Description

Other Grants:

* "National Science Foundation Graduate Research Fellowship Program" (Grant ID 1842402, National Science Foundation)
* "NOAA Margaret A. Davidson Fellowship" (Grant ID NA20NOS4200123, National Oceanic and Atmospheric Administration)

Methods & Sampling

Sample collection and environmental parameters

Between August 2017 and June 2019, seawater was collected from a depth of 2 m at 10 sites in and around Kāneʻohe Bay, Oʻahu, Hawaiʻi, on a near-monthly basis (20 sampling events over 23 months). At each station, seawater samples for biogeochemical analyses and nucleic acids were collected, and *in situ* measurements of seawater temperature, pH, and salinity were made with a YSI 6,600 sonde (YSI Incorporated, Yellow Springs, OH, USA). Approximately one L of seawater was prefiltered using an 85 µm Nitex mesh and subsequently collected on a 25-mm diameter 0.1-µm pore-sized polyethersulfone (PES) membrane for nucleic acids (Supor-100, Pall Gelman Inc., Ann Arbor, MI, USA). The filters were submerged in DNA lysis buffer (Suzuki et al., 2001; Yeo et al., 2013) and stored at −80 °C until extraction.

Subsamples for chlorophyll *a* were collected by filtering 125 mL of seawater onto 25-mm diameter GF/F glass microfiber filters (Whatman, GE Healthcare Life Sciences, Chicago, IL, USA), and stored in aluminum foil at −80 °C until extraction in 100% acetone and subsequent measurement of fluorescence with a Turner 10AU fluorometer (Turner Designs, Sunnyvale, CA, USA) followed standard techniques (Welschmeyer, 1994). Seawater for cellular enumeration was preserved in two-mL aliquots in a final concentration of 0.95% (v:v) paraformaldehyde (Electron Microscopy Services, Hatfield, PA, USA) at −80 °C until analyzed *via* flow cytometry. Cellular enumeration of cyanobacterial picophytoplankton (marine *Synechococcus* and *Prochlorococcus*), eukaryotic picophytoplankton, and non-cyanobacterial (presumably heterotrophic) bacteria and archaea (hereafter referred to as heterotrophic bacteria) was performed on an EPICS ALTRA flow cytometer (Beckman Coulter Inc., Brea, CA, USA), following the method of Monger and Landry (Monger & Landry, 1993).

Data Processing Description

DNA extraction and sequencing

Genomic DNA was extracted using a Qiagen Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA) with modifications (Becker, Brandon & Rappé, 2007). For each sample, 16S rRNA gene fragments were amplified by polymerase chain reaction using a dual-index sequencing strategy where barcoded universal primers 515-Y-F and 926R (Parada, Needham & Fuhrman, 2016) are complete with Illumina sequencing adapters, barcode, and index. The 25 µL reactions included 13 µL H2O, 0.5 µL each of forward and reverse primer at 0.2 µm final concentration, one µL gDNA (0.5 ng), and 10 µL 1 × 5PRIME Hot Master Mix (0.5 U *Taq* DNA polymerase, 45 mm KCl, 2.5 mm Mg2+, 200 µm dNTPs) (Quantabio, Beverly, MA, USA). PCR conditions included an initial denaturation at 95 °C for 2 min followed by 30 cycles of 95 °C for 45 s, 50 °C for 45 s, and 68 °C for 90 s, and a final 5 min extension at 68 °C. PCR products were inspected on a 1.5% agarose gel and quantified using the Qubit dsDNA HS Kit (Qubit 2.0, Life Technologies, Foster City, CA, USA). PCR products were normalized to 240 ng each, pooled, and purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Pooled libraries were then sequenced on an Illumina MiSeq v2 250 bp paired-end run at the Oregon State University Center for Genome Research & Biocomputing.

BCO-DMO Processing Description

* No data value NA set to blank
* Split latitude and longitude value into own column.

[table of contents | back to top]

Related Publications

Becker, J. W., Brandon, M. L., & Rapp&#65533;, M. S. (2007). Cultivating Microorganisms from Dilute Aquatic Environments: Melding Traditional Methodology with New Cultivation Techniques and Molecular Methods. Manual of Environmental Microbiology, 399–406. Portico. https://doi.org/10.1128/9781555815882.ch32
Methods

Monger, B. C., & Landry, M. R. (1993). Flow Cytometric Analysis of Marine Bacteria with Hoechst 33342 †. Applied and Environmental Microbiology, 59(3), 905–911. doi:10.1128/aem.59.3.905-911.1993
Methods

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2015). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18(5), 1403–1414. doi:10.1111/1462-2920.13023
Methods

Suzuki, M. T., Béjà, O., Taylor, L. T., & DeLong, E. F. (2001). Phylogenetic analysis of ribosomal RNA operons from uncultivated coastal marine bacterioplankton. Environmental Microbiology, 3(5), 323–331. Portico. https://doi.org/10.1046/j.1462-2920.2001.00198.x
Methods

Tucker, S. J., Rii, Y. M., Freel, K. C., Kotubetey, K., Kawelo, A. H., Winter, K. B., & Rappe, M. S. (2024). Sharp transitions in phytoplankton communities across estuarine to open ocean waters of the tropical Pacific. https://doi.org/10.1101/2024.05.23.595464
Results

Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnology and Oceanography, 39(8), 1985–1992. doi:10.4319/lo.1994.39.8.1985
Methods

Yeo, S. K., Huggett, M. J., Eiler, A., & Rappé, M. S. (2013). Coastal Bacterioplankton Community Dynamics in Response to a Natural Disturbance. PLoS ONE, 8(2), e56207. https://doi.org/10.1371/journal.pone.0056207
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

University of Hawaii at Manoa. Spatial and temporal dynamics of SAR11 marine bacteria sampled across a nearshore to offshore transect in the tropical Pacific Ocean. 2021/03. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA706753. NCBI:BioProject: PRJNA706753.

[table of contents | back to top]

Parameters

Parameter	Description	Units
Sample_ID	Sample ID. Sample ID is composed of the station ID abbreviation and a uniqe sample ID #. In most cases a month abbreviation is also included.	unitless

collection_date	Collection date.	unitless
depth	Depth of sample collection.	m
env_broad_scale	Environment description, broad.	unitless
env_local_scale	Environment description, local. Defined by analyses included in associated publication.	unitless
env_medium	Source of sample.	unitless
geo_loc_name	Geographic location of sampling.	unitless
latitude	Station latitude, south is negative	decimal degrees
longitude	Station longitude, west is negative	decimal degrees
Site_name	Site ID for collection.	unitless
chlorophyll_a_ug_per_L	Extracted chlorophyll a concentrations from surface seawater.	micrograms per Liter
ph	pH of surface seawater in situ.	no unit
salinity	Salinity of surface seawater in situ.	ppt
Seawater_temperature	Temperature of surface seawater in situ.	degrees Celsius (°C)
Prochlorococcus_cells_per_mL	Surface seawater cellular abundances of Prochlorococcus cells counted on EPICS ALTRA flow cytometer.	cells per mL
Synechococcus_cells_per_mL	Surface seawater cellular abundances of Synechococcus cells counted on EPICS ALTRA flow cytometer.	cells per mL
Heterotrophic_bacteria_cells_per_mL	Surface seawater cellular abundances of non-cyanobacterial (presumably heterotrophic) bacteria and archaea (referred to as heterotrophic bacteria) counted on EPICS ALTRA flow cytometer.	cells per mL
Eukaryotic_picophytoplankton_cells_per_mL	Surface seawater cellular abundances of photosynthetic picoeukaryotes counted on EPICS ALTRA flow cytometer.	cells per mL
Universal_Sample_ID	Universal Sample ID. Universal Sample ID is used to connect multiple sample types within the Kāneʻohe Bay Time-series. It is composed of the Project ID "KBT", the date (mm.dd.yyyy), and Station ID.	unitless
SRA_accession	NCBI SRA Accession for amplicon data.	unitless
study	NCBI Study ID.	unitless
bioproject_accession	NCBI Bioproject Accession for amplicon data.	unitless
biosample_accession	NCBI Biosample Accession.	unitless
library_ID	Unique barcode sample identifier for amplicon sequencing.	unitless
library_strategy	Sequencing library type.	unitless
library_source	Source of sequencing library.	unitless
library_selection	Mode of library creation.	unitless
library_layout	Single or paired end sequencing reads.	unitless
platform	Platform used for library creation.	unitless
instrument_model	Sequencer model.	unitless

design_description	Description of ampicon library.	unitless
filetype	File type.	unitless
filename	Forward reads file name.	unitless
filename2	Reverse reads file name.	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Illumina MiSeq v2
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	EPICS ALTRA flow cytometer (Beckman Coulter Inc., Brea, CA, USA
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Turner 10AU fluorometer (Turner Designs, Sunnyvale, CA, USA)
Generic Instrument Name	Turner Designs Fluorometer 10-AU
Generic Instrument Description	The Turner Designs 10-AU Field Fluorometer is used to measure Chlorophyll fluorescence. The 10AU Fluorometer can be set up for continuous-flow monitoring or discrete sample analyses. A variety of compounds can be measured using application-specific optical filters available from the manufacturer. (read more from Turner Designs, turnerdesigns.com, Sunnyvale, CA, USA)

Dataset-specific Instrument Name	YSI 6,600 sonde (YSI Incorporated, Yellow Springs, OH, USA)
Generic Instrument Name	YSI Sonde 6-Series
Generic Instrument Description	YSI 6-Series water quality sondes and sensors are instruments for environmental monitoring and long-term deployments. YSI datasondes accept multiple water quality sensors (i.e., they are multiparameter sondes). Sondes can measure temperature, conductivity, dissolved oxygen, depth, turbidity, and other water quality parameters. The 6-Series includes several models. More from YSI.

[table of contents | back to top]

Project Information

Population genomics and ecotypic divergence in the most dominant lineage of marine bacteria (Pelagibacteromics)

Website: https://rappelab.wordpress.com/marine-bacterioplankton/population-genomics-of-sar11-marine-bacteria/

Coverage: Moorea, Oahu, and Gulf of Alaska

In the upper water column of Earth's coastal and open oceans, roughly one million microscopic, single-celled bacteria inhabit each milliliter of seawater, where they play important roles in driving nutrient cycles and other processes that are vital to the habitability of these systems to other marine life. While some marine bacteria are similar to plants in that they use energy from the sun to transform the greenhouse gas carbon dioxide into living material and produce oxygen as a byproduct, other marine bacteria known as chemoheterotrophs are similar to humans and other animals in that they consume organic matter and oxygen, producing carbon dioxide as a consequence of their growth. Although they are limited in size and shape when observed under a microscope, genetic techniques such as DNA sequencing have revealed tremendous functional (i.e. what they are doing) and phylogenetic (i.e. how they are related) biodiversity in natural communities of marine bacteria. Despite this high genetic diversity, a single group of phylogenetically related chemoheterotrophic bacteria known as SAR11 can sometimes make up over 50% of the microscopic cells inhabiting seawater systems around the globe; it is considered one of the most abundant organisms on Earth and thus an important aspect of ocean ecology. While it is known that the SAR11 group consists of many distinct "types" that differ in abundance with location, depth and time, we know little about what genetically encoded features distinguish the different types, or how genetic characteristics are gained and lost within the group. The goal of this study is to use a genomics approach to understand the evolutionary processes that shape one of the most abundant groups of organisms on our planet, and to improve our theoretical understanding of the evolutionary processes that shape natural microbial biodiversity in general. This project will provide advanced, cross-disciplinary professional training for a postdoctoral scientist and a graduate student, and will increase the participation of underrepresented groups in scientific research by mentoring undergraduate students of native Hawaiian or Pacific Island ancestry in hands-on research and training. Results will be incorporated into a new university course offering on comparative genomics and microbial evolution. A culture collection of marine microorganisms will also be expanded and maintained, providing a valuable resource for other marine scientists.

This project will take advantage of recent advances in DNA sequencing technology and a high throughput extinction culturing approach in order to investigate the evolutionary characteristics of genomes from sympatric populations of the globally important SAR11 marine bacterial lineage. The major objectives of this project are to understand the forces that shape genomic diversity in large bacterial populations such as SAR11, and to determine the nature by which this diversity is reflected in functional differences between populations, as inferred from genomics. SAR11 cells will be isolated from similar ecosystems in the tropical North and South Pacific, as well as the coastal ocean of the subpolar North Pacific, in order to investigate the effect of geographic distance versus habitat similarity on the population genetics of free-living, planktonic

marine bacteria. By opening a unique genomic window that encompasses SAR11 lineages of varying degrees of genetic divergence simultaneously, this study will facilitate the investigation of evolutionary dynamics that spans a continuum between macro- and microevolutionary processes. Quantitative information regarding the mechanisms by which genetic diversity is generated, propagated, and removed from native SAR11 populations will also help efforts to model the fate of SAR11 and other large marine bacterioplankton populations in the face of predicted climate-induced changes to the global ocean.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1538628

[table of contents | back to top]