Microbial community structure influences seasonal variability in phosphorus fluxes at a coastal time series site from November 2021 to March 2023

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Project
» OCE-PRF Predicting OMZ and seasonal microbial activity from community structure using machine learning and novel measurements of ATP turnover (Predicting OMZ and seasonal microbial activity)

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Abstract
These data include metabolic rates, stocks of nutrients and biomass, and cell counts collected from November 2021-March 2023 at the end of the Scripps Pier in La Jolla, CA. Samples were collected roughly twice weekly across the dataset, with varying degrees of coverage based on the parameter: stocks were measured most consistently, while metabolic rates were collected as feasible. Metabolic rates were measured using radioisotope incubations and both bul microbial energy production rates (ATP phosphorylation rates) and phosphate uptake rates are provided. Stocks are provided for all phosphorus pools, as well as certain biochemicals (chlorophyll, pheophytin, ATP). Cell counts are provided for both cells measured via flow cytometry as well as select eukaryotic cells enumerated via microscopy. Understanding how microbial community and season can vary with metabolic rates is important for understanding what drives nutrient and energy transformation, as well as the efficiency of energy and biomass assimilation at the base of the food web in this highly productive coastal zone. Data was coordinated and compiled from a few time series including the Southern California Coastal Ocean Observing System (microscopy counts of eukaryotic cells, macronutrients aside from phosphorus, chlorophyll, pheophytin, temperature), Scripps Ecological Observatory (genomics data), as well as this time series (metabolic rates, phosphorus stocks, ATP stock). This highly collaborative time series was conducted within the Bowman lab at Scripps, and run by Dr. Kaycie Lanpher.

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Parameters
- Instruments
- Project Information

- Funding

Coverage

Location: Ellen Browning Scripps Memorial Pier ("Scripps Pier") surface waters (32°52′1.1″N, 117°15′25.7″W)
Spatial Extent: **Lat**:32.866972 **Lon**:-117.257139
Temporal Extent: 2021-11-22 - 2023-03-30

Dataset Description

Citation note: Sabrina Glynn and Kaycie Lanpher are co-first authors for this dataset

Methods & Sampling

This was a highly collaborative project, and methods regarding community composition are provided by the Scripps Ecological Observatory while environmental and chlorophyll methods are provided by the Southern California Coastal Ocean Observing System.

Metabolic rates were measured using two assays: phosphate uptake and ATP phosphorylation. Seawater was collected from the Ellen Browning Scripps Memorial Pier at the Scripps Institution of Oceanography (SIO) in La Jolla, California (32°52′1.1″N, 117°15′25.7″W). The seawater was collected just below the surface in acid clean, sample rinsed HDPE bottles using a crane located in the Scripps Pier Laboratory. The seawater was immediately pre-filtered with 250 µm nylon mesh into acid clean, sample-rinsed polycarbonate bottles.

For both assays, seawater was collected, prefiltered, and aliquoted into triplicate incubation bottles. Radioactive 32P-PO4 was added to each bottle, with this tracer addition marking the beginning of the time course (time zero), with samples collected at specified intervals afterward. For the phosphate uptake assay, incubation water was filtered after a designated incubation period of less than 30 minutes, and particulate material was captured on a GF-75 filter with a nominal pore size of 0.3um). For the ATP-phosphorylation assay, samples were filtered as quickly as possible post-spike (after addition of radioactive 32P-PO4). The filters were then processed to preserve ATP by boiling them in Tris buffer and transferring the supernatant to HPLC. Radioactive ATP was purified and collection in fractions using preparative HPLC. Radioactivity in all samples was quantified using a liquid scintillation counter. Additonal details are provided in Lanpher & Popendorf, 2022.

Flow cytometry cell counts were collected on a BD Accuri in two methods-- one for autofluorescence (chlorophyll containing small cells) and one for total cells (DNA stain was used to fluoresce all cells).

Phosphorus stocks were quantified using the molybdenum blue method and spectrophotometry (phosphate) or combustion and acid hydrolysis to convert organic phosphorus to phosphate then subsequent molybdenum blue processing (dissolved organic phosphorus, and particulate phosphorus but from a filter). ATP concentration was measured using the firefly luciferin-luciferase method.

Data Processing Description

RStudio (v. 2024.04.2)

[table of contents | back to top]

Parameters

Parameters for this dataset have not yet been identified

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	BD Accuri flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Flow cytometry cell counts were collected on a BD Accuri in two methods-- one for autofluorescence (chlorophyll containing small cells) and one for total cells (DNA stain was used to fluoresce all cells).
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. Description from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm

Dataset-specific Instrument Name	Beckman LS60000A liquid scintillation counter
Generic Instrument Name	Liquid Scintillation Counter
Dataset-specific Description	All radioactive samples were quantified using a liquid scintillation counter.
Generic Instrument Description	Liquid scintillation counting is an analytical technique which is defined by the incorporation of the radiolabeled analyte into uniform distribution with a liquid chemical medium capable of converting the kinetic energy of nuclear emissions into light energy. Although the liquid scintillation counter is a sophisticated laboratory counting system used to quantify the activity of particulate emitting (ß and a) radioactive samples, it can also detect the auger electrons emitted from 51Cr and 125I samples. Liquid scintillation counters are instruments assaying alpha and beta radiation by quantitative detection of visible light produced by the passage of rays or particles through a suitable scintillant incorporated into the sample.

Dataset-specific Instrument Name	Tecan Spark luminometer
Generic Instrument Name	Luminometer
Dataset-specific Description	A Tecan Spark luminometer was used to measure particulate ATP concentration
Generic Instrument Description	A luminometer is an instrument that measures light and other optical properties of specimens in chemiluminescent and bioluminescent applications.

Dataset-specific Instrument Name	Spectrophotometer (unknown)
Generic Instrument Name	Spectrometer
Dataset-specific Description	A spectrophotometer was used for quantifying phosphorus stocks (phosphate, dissolved organic phosphorus, particulate phosphorus) via the molybdenum blue colorimetric method.
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

[table of contents | back to top]

Project Information

OCE-PRF Predicting OMZ and seasonal microbial activity from community structure using machine learning and novel measurements of ATP turnover (Predicting OMZ and seasonal microbial activity)

Coverage: Subtropical waters, California Current Ecosystem

NSF Award Abstract:

This award is funded in whole or in part under the American Rescue Plan Act of 2021 (Public Law 117-2).

Marine microbes, including single-celled bacteria, archaea, and eukarya, are the main drivers of carbon and energy transformations in the ocean. The efficiency of the microbial loop at transforming dissolved nutrients into biomass and up the food web is defined by the individual activities of the microbial community. Unfortunately, current methods for characterizing microbial activities in the marine environment are biased, with most direct measurements focusing on heterotrophic microbes that respire carbon dioxide in oxygenated waters. These measurements exclude a large diversity of microbes that play major roles in ocean nutrient cycles, including photosynthesizes and chemotrophs as well as microbes in low oxygen, anoxic waters. This project will measure the activity of microbial organisms through novel applications of the adenylate energy system, which is used by all organisms and in almost all metabolic pathways. This work will focus on sites in the California Current Ecosystem with a wide range of oceanographic conditions and variable microbial communities. These locations include low oxygen canyons representative of oxygen minimum zones (OMZs), upwelling locations reflecting eastern boundary currents, and temporally dynamic coastal sites. Within the planned sampling, support will be allocated for undergraduate students to join shipboard efforts to help with seawater sampling and gain experience conducting oceanographic research. The datasets produced from these sampling efforts will be used to develop predictive models of microbial metabolism (activity) that can be applied to other oceanographic regions, based on community structure, abundance, and geochemical parameters across time and environmental concentrations.

The highly productive California Current Ecosystem is subjected to seasonal successions of phytoplankton communities, with diatom and dinoflagellate dominance coinciding with upwelling. The ability of these phytoplankton groups to convert nutrients into biomass have impacts on primary productivity, biogeochemical cycles, and the microbial loop. Hypoxic environments shift the microbial community towards anaerobes and chemotrophs, which have unique metabolisms and differential efficiencies converting acquired carbon sources into biomass and energy. Therefore, the microbial loop efficiency in OMZs is likely to be directly impacted by microbial community structure, but precisely defining these differences in microbial activity has been hampered by biased sampling techniques. This project will address the hypotheses that microbial activity, in terms of energy storage, metabolic rates, and growth rates will be directly liked to and predicted by phytoplankton community structure and abundances in seasonal upwelling systems. Additionally, microbial activities in OMZs are linked directly to the implementation of anaerobic metabolic strategies and can thus be predicted by changes in community structure. Microbial activity will be characterized using high throughput applications of novel measures of the energy charge and adenosine triphosphate (ATP) turnover rates while the microbial

community structure will be defined with 16S and 18S rRNA gene amplicon sequencing. Two dedicated, single day cruises to the La Jolla and Scripps Canyons located within the California Bight, and containing known OMZs will be conducted. Additionally, microbial activity measurements will be incorporated into an on-going time-series that is sampled for microbial community structure twice-a-week at the Scripps Institute of Oceanography pier in La Jolla, CA. Measurements from the cruises and time-series will be used to develop predictive models of microbial metabolism based on community structure, abundance, and geochemical parameters across time and within known OMZs using self-organizing maps (SOMs) and random forest regression. Therefore, this project will produce predictive models that can be generalized to broader ocean regions in addition to datasets with unprecedented resolution of natural microbial activity across time and environmental conditions.

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-2126668

[table of contents | back to top]