Sampling information and sequence accessions for 18S-V4 sequences from surface water collected at the Santa Monica Pier (SMP) Santa Monica Bay, CA from 2018 to 2019

Website: https://www.bco-dmo.org/dataset/883924
Data Type: Other Field Results
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Project
» Protistan, prokaryotic, and viral processes at the San Pedro Ocean Time-series (SPOT)

Contributors	Affiliation	Role
Caron, David	University of Southern California (USC)	Principal Investigator
Hu, Sarah K.	University of Southern California (USC)	Scientist
Ollison, Gerid A.	University of Southern California (USC)	Scientist
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This dataset contains sampling information and sequence accessions for 18S-V4 sequences at the National Center for Biotechnology (NCBI) from surface water collected at the Santa Monica Pier (SMP) from 2018 to 2019. Sequences can be found under NCBI BioProject PRJNA480318. These data were published in Ollison et al (2022). These data were collected as part of a study of high frequency (daily) changes in relative abundance dynamics of the metabolically active protistan community were followed via expressed 18S V4 rRNA genes (RNA) throughout two algal blooms during the spring of 2018 and 2019 in Santa Monica Bay (central Southern California Bight) to examine the environmental factors that influence protistan community dynamics during algal blooms.

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Coverage

Spatial Extent: **Lat**:34.009 **Lon**:-118.497
Temporal Extent: 2018-04-16 - 2019-05-05

Methods & Sampling

Blooms were targeted in Santa Monica Bay during spring 2018 and 2019 using local meteorological information to anticipate coastal upwelling events (Figure S1, Ollison et al., 2022). Sampling from the Santa Monica Pier (SMP) was conducted daily at 0900 (PST/PDT) from the same location and orientation on the SMP from the 16th through the 30th in April 2018 (15 days), and in 2019 from the 13th April through 6th May (22 days; no sample was collected on the 14th April 2019). Sampling periods are henceforth referred to as 2018 and 2019, respectively.

An RBR Concerto (https://rbr-global.com) was deployed in surface water for 15 minutes at the time of each sample collection to obtain temperature, conductivity, chlorophyll a fluorescence, and dissolved oxygen concentrations (See "Related Datasets" section for CTD data). A 20 µm mesh plankton net was drift towed from the pier (15 min), and samples were examined via light microscopy to identify the dominant planktonic taxa and their relative abundances.

Surface water for 18S rRNA sequencing, nutrient analyses (see Related Datasets section), cell counts, extracted chlorophyll concentrations, and domoic acid concentrations was collected via bucket toss from the pier; an extended funnel was used to gently fill a single acid-washed-3x-rinsed 20 L carboy per established lab protocol (https://www.protocols.io/view/sample-collection-from-the-field-for-downstream-mo-hisb4ee). The carboy was protected from the light and immediately transported approximately 300 meters to the Heal the Bay Aquarium located at the SMP for sample processing.

Sample Processing:

Molecular community analyses were performed in triplicate on 2 L seawater samples pre-filtered with nitex mesh (80 µm) and collected on 45 mm GF/F filters (nominal pore size 0.7 µm, Whatman, International Ltd. Florham Park, NJ) to capture the unicellular eukaryote community while excluding most metazoa from the samples. The filters were placed in a 15 ml RNAse-free Falcon tube containing 1.5 ml of RLT buffer + betamercaptoethanol, immediately flash frozen in liquid nitrogen, and subsequently stored at -80 ˚C until RNA extraction. Chlorophyll *a* (hereafter referred to as chlorophyll) was used as a proxy for biomass and bloom magnitude. Major blooms were categorized using two standard deviations (SD = 4.6) added to the 15-year average (~2.8 µg/L; 2008 through 2020, minus year 2015) at the Santa Monica Pier (Kim *et al.*, 2009; Seubert *et al.*, 2013). Extracted chlorophyll and particulate domoic acid concentrations (pDA in 2018 only) were determined by filtering up to 300 ml of seawater onto 25 mm GF/F filters in duplicate. Less than 300 ml of seawater was collected for chlorophyll samples in 2019 when the filters clogged during periods of high biomass.

RNA extraction and sequencing:

Total RNA was extracted per a previously established protocol (Hu, S., 2017; doi:10.17504/protocols.io.hk3b4yn)(Ollison *et al.*, 2021). Briefly, each GF/F filter was shredded by vortexing after the addition of silica beads to each tube containing a GF/F filter and lysis buffer. The mixture was transferred to a syringe that was used to obtain the lysate from the filter/water slurry. RNA was extracted from the lysate via Qiagen All Prep DNA/RNA Mini Kit (Qiagen, #80204) per manufacturer instructions. Genomic DNA was removed prior to RNA extraction using an RNase-Free Qiagen DNase (Qiagen, #79254). RNA was reverse transcribed to cDNA using the Bio-Rad iScript Reverse Transcription Supermix with random hexamers (Bio-RAD, #170-8840).

The 18S rRNA V4 region in each sample was PCR amplified using 18S 565F (5'-CCAGCASCYGCGGTAATTCC-3') and 948R (5'-ACTTTCGTTCTTGATYRA-3') primers (Stoeck *et al.*, 2010) via Q5 High-Fidelity 2x Master Mix (NEB, #M0492S). PCR reactions were carried out in two steps due to the difference in annealing temperature between primer pairs. The PCR reaction consisted of an initial 98 ˚C denaturation for 2 min, and 10 cycles of 98 ˚C for 10s, 53 ˚C for 30s, and 72 ˚C for 30s. The final 15 cycles consisted of 98 ˚C for 10s, 48 ˚C for 30s and 72 ˚C for 30s. A final extension at 72 ˚C for 2 min was performed after both steps. PCR products were subsequently purified using Agencourt AMPure XP beads (Beckman Coulter #A63881), indexed using Illumina-specific P5 and P7 indices, quantified using a QuBit 2.0 fluorometer (ThermoFisher, #Q32866), and normalized to 10 µM prior to sequencing. Normalized samples were quality checked on an Agilent Bioanalyzer 2100 and paired-end sequenced (250X250) on an Illumina MiSeq (Laragen Inc. Culver City, CA).

Data Processing Description

BCO-DMO Data Manager Processing Notes:
* Data from source file SMP_sample_inventory.xlsx Sheet1 were imported into the BCO-DMO data system.
* lat_lon column split into separate lat, lon columns in decimal degrees. Longitude was made negative as is correct for decimal degrees.
* date format converted to ISO8601 format

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Related Publications

Hu, S. (2017). RNA (and optional DNA) extraction from environmental samples (filters) v2 (protocols.io.hk3b4yn). Protocols.io. doi:10.17504/protocols.io.hk3b4yn
Methods

Kim, H.-J., Miller, A. J., McGowan, J., & Carter, M. L. (2009). Coastal phytoplankton blooms in the Southern California Bight. Progress in Oceanography, 82(2), 137–147. https://doi.org/10.1016/j.pocean.2009.05.002
Methods

Ollison, G. A., Hu, S. K., Hopper, J. V., Stewart, B. P., Smith, J., Beatty, J. L., Rink, L. K., & Caron, D. A. (2022). Daily dynamics of contrasting spring algal blooms in Santa Monica Bay (central Southern California Bight). Environmental Microbiology. Portico. https://doi.org/10.1111/1462-2920.16137
Results

Ollison, G. A., Hu, S. K., Mesrop, L. Y., DeLong, E. F., & Caron, D. A. (2021). Come rain or shine: Depth not season shapes the active protistan community at station ALOHA in the North Pacific Subtropical Gyre. Deep Sea Research Part I: Oceanographic Research Papers, 170, 103494. https://doi.org/10.1016/j.dsr.2021.103494
Methods

Seubert, E. L., Gellene, A. G., Howard, M. D. A., Connell, P., Ragan, M., Jones, B. H., Runyan, J., & Caron, D. A. (2013). Seasonal and annual dynamics of harmful algae and algal toxins revealed through weekly monitoring at two coastal ocean sites off southern California, USA. Environmental Science and Pollution Research, 20(10), 6878–6895. https://doi.org/10.1007/s11356-012-1420-0 https://doi.org/10.1007/S11356-012-1420-0
Methods

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Related Datasets

IsRelatedTo

Caron, D., Ollison, G. A., Hu, S. K. (2022) **CTD data from daily sampling at the Santa Monica Pier (SMP), Santa Monica Bay, CA from 2018 to 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-17 http://lod.bco-dmo.org/id/dataset/883908 [view at BCO-DMO]
Relationship Description: Data collected as part of targeted sampling of blooms in the Santa Monica Bay during spring 2018 and 2019.

Caron, D., Ollison, G. A., Hu, S. K. (2022) **Nutrients (phosphate and nitrite+nitrate) from daily sampling at the Santa Monica Pier (SMP), Santa Monica Bay, CA from 2018 to 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-17 http://lod.bco-dmo.org/id/dataset/883916 [view at BCO-DMO]
Relationship Description: Data from the same samples collected as part of targeted sampling of blooms in the Santa Monica Bay during spring 2018 and 2019.

University of Southern California (2018). 18S Amplicon sequencing, SMPier, Amplicon sequencing Raw sequence reads. NCBI:BioProject: PRJNA480318.. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA480318.

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Parameters

Parameter	Description	Units
date	Sample date	unitless
lat	latitude of sample location	decimal degrees
lon	longitude of sample location	decimal degrees
depth	depth sample was taken (nominal sampling position)	unitless
BioSample	NCBI BioSample ID	unitless
SRA	NCBI SRA ID	unitless
Accession	NCBI Accession number (SRS# = SRA sample accession, SRR = SRA run accession)	unitless
name	Name of sample on NCBI	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Instrument used for deciphering the order of bases in a strand of DNA.
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.

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Project Information

Protistan, prokaryotic, and viral processes at the San Pedro Ocean Time-series (SPOT)

Coverage: San Pedro Channel off the coast of Los Angeles

Planktonic marine microbial communities consist of a diverse collection of bacteria, archaea, viruses, protists (phytoplankton and protozoa) and small animals (metazoan). Collectively, these species are responsible for virtually all marine pelagic primary production where they form the basis of food webs and carry out a large fraction of respiratory processes. Microbial interactions include the traditional role of predation, but recent research recognizes the importance of parasitism, symbiosis and viral infection. Characterizing the response of pelagic microbial communities and processes to environmental influences is fundamental to understanding and modeling carbon flow and energy utilization in the ocean, but very few studies have attempted to study all of these assemblages in the same study. This project is comprised of long-term (monthly) and short-term

(daily) sampling at the San Pedro Ocean Time-series (SPOT) site. Analysis of the resulting datasets investigates co-occurrence patterns of microbial taxa (e.g. protist-virus and protist-prokaryote interactions, both positive and negative) indicating which species consistently co-occur and potentially interact, followed by examination gene expression to help define the underlying mechanisms. This study augments 20 years of baseline studies of microbial abundance, diversity, rates at the site, and will enable detection of low-frequency changes in composition and potential ecological interactions among microbes, and their responses to changing environmental forcing factors. These responses have important consequences for higher trophic levels and ocean-atmosphere feedbacks. The broader impacts of this project include training graduate and undergraduate students, providing local high school student with summer lab experiences, and PI presentations at local K-12 schools, museums, aquaria and informal learning centers in the region. Additionally, the PIs advise at the local, county and state level regarding coastal marine water quality.

This research project is unique in that it is a holistic study (including all microbes from viruses to small metazoa) of microbial species diversity and ecological activities, carried out at the SPOT site off the coast of southern California. In studying all microbes simultaneously, this work aims to identify important ecological interactions among microbial species, and identify the basis(es) for those interactions. This research involves (1) extensive analyses of prokaryote (archaean and bacterial) and eukaryote (protistan and micro-metazoan) diversity via the sequencing of marker genes, (2) studies of whole-community gene expression by eukaryotes and prokaryotes in order to identify key functional characteristics of microorganismal groups and the detection of active viral infections, and (3) metagenomic analysis of viruses and bacteria to aid interpretation of transcriptomic analyses using genome-encoded information. The project includes exploratory metatranscriptomic analysis of poorly-understood aphotic and hypoxic-zone protists, to examine their stratification, functions and hypothesized prokaryotic symbioses.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1737409

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