Cyanobacterial and diatom samples collecteced between April and September 2022, and sequenced for metabarcodig of 16S and rbcL.

Website: https://www.bco-dmo.org/dataset/911441
Data Type: experimental
Version: 1
Version Date: 2023-10-19

Project
» Collaborative Research: RUI: OCE-BO: Tango in the Mat World: Biogeochemistry of diurnal vertical migration in microbial mats of Lake Huron's sinkholes (Tango in the Mat World)

Contributors	Affiliation	Role
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Abstract
These data are the sample information for each of the samples collected for metabarcoding of 16S and rbcL to describe Cyanobacterial and diatom diversity, respectively, in three sites in Alpena, Michigan, one site in Monroe, Michigan, and one site in Palm Coast, Florida. Sample data are for sequenced samples and include their associated water parameter information that was collected simultaneously. Each of these sites are high-sulfur, low-oxygen environments formed by underwater sinkholes and springs that create extreme habitats populated by microbial mat communities. Our study investigated previously undescribed diatom diversity in these habitats, and further explored the bacterial communities as well. Our results provide novel information on microbial mat community composition, and present evidence that microbial biogeography influences these unique communities.

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Coverage

Spatial Extent: **N**:45.1984 **E**:-81.2084 **S**:29.6317 **W**:-83.456
Temporal Extent: 2022-04-25 - 2022-09-16

Methods & Sampling

Each site was visited in the spring (April-May), summer (June-July), and fall (September) periods. Exceptions include MIS and OAK, which were only sampled during the summer period. During each visit, a YSI multiprobe

(Yellow Springs Instruments, Inc., Yellow Springs, OH, USA) was used to measure temperature, specific conductance, and percent dissolved oxygen. Due to multiprobe malfunction, data from a summer 2021 YSI deployment was used to characterize MIS water parameters. In addition to YSI parameters, 250 mL acid-washed Nalgene bottles were used to collect water samples for nutrient analyses at each sampling point. Each water sample was subsampled into two vials, of which one was refrigerated, and one was frozen within 24 h of collection. The refrigerated subsample was used to determine orthophosphate (SRP) concentrations using USEPA method 365.1 (O'Dell 1996). The frozen subsample was used to determine dissolved silica concentrations using USEPA method 370.1 (USEPA) and chloride, sulfate, and nitrate using USEPA method 300.0 (Pfaff 1993).
Mats from wadable sites were collected using a suction device and placed in sterile Whirlpak® bags, then put on ice for transport to the Annis Water Resources Institute (AWRI, Muskegon, MI, USA). Three replicate mat samples were collected from each habitat type at each site during each sampling event. Mats from MIS were collected by NOAA divers using a coring device, and transported to AWRI as cores in plastic tubes on ice. Plankton tow samples were also collected at GSS and ECB to determine taxa that may be considered part of the surrounding planktonic community, rather than active members of the microbial mat community. Each mat sample collected was subsampled, with one subsample used for generating unialgal cultures and the other for metabarcoding.

Subsamples for metabarcoding were frozen at -80 °C within 36 h of collection, except for MIS samples which were stored at 10 °C for 72 hours prior to harvesting, then frozen at -80 °C, due to logistical limitations. DNA was extracted from the metabarcoding subsamples using the Qiagen PowerSoil DNA Extraction Kit (Qiagen, Crawley, UK) according to the manufacturer's protocol, with a negative control consisting of autoclaved nanopore water included for each subset of extractions and for each primer to assess potential processing contamination. To prepare samples for Illumina amplicon sequencing, a two-step PCR approach was employed. The initial PCR was completed to amplify the two barcode markers (rbcL and 16S) in individual reactions using specific primers with the attached Illumina adapter. The primary PCR amplification was completed in 25 µl reactions using 12.5 µl of Q5 High-Fidelity2X Master Mix (New England BioLabs Inc., Ipswich, MA, USA), 1.0 µl of each primer (1µM), 9.5 µl RNase-free H2O, and 1 µl DNA. For the 16S marker, the primer pair and thermocycler protocol from Walters et al. (2015) were employed. For the rbcL marker, we targeted a 312 bp region of the rbcL plastid gene using an equimolar mix of the three forward and two reverse degenerate primers from Vasselon et al. (2017), along with the thermocycler protocol.

Following PCR amplification, samples were sent to the University of Tennessee, Knoxville for processing and sequencing. PCR products were cleaned with Agencourt AmPure XP beads (Beckman Coulter Inc., Indianapolis, IN, USA) and quantified using a Qubit Fluorometer (v.2.0; ThermoFisher Scientific, Waltham, MA, USA). Samples were normalized, and a second PCR reaction (50 µl) enriched with Q5 High-Fidelity 2X Master Mix was performed to apply indexing primers, following cycling conditions: 95° C for 3 min followed by 10 cycles of 95° C for 30 s, 55° C for 30 s, 72° C for 30 s, with a final extension of 72° C for 5 min, modified from the 16S protocol (Illumina 2013). A second PCR clean-up was performed, and samples were quantified using a Qubit Fluorometer. Libraries were loaded with 25% PhiX clustering control on the Illumina MiSeq platform for 300 bp × 2 paired end reads using the V3 kit.

Data Processing Description

The resulting sequence datasets were analyzed separately for each marker region. Sequences were demultiplexed and adapters removed. Primers were trimmed using cutadapt version 4.2 (Martin 2011). Using the DADA2 pipeline (Callahan et al. 2016), reads were quality filtered based on Q30 scores and trimmed to remove low-quality reads. Filtered reads were denoised and dereplicated using DADA2 to produce amplicon sequence variants (ASVs). Singletons, doubletons, and chimeric sequences were removed from the dataset. ASVs identified as chloroplast or mitochondria in the 16S dataset were removed. The SILVA database (release 138.1, Quast et al. 2013) appended with CyanoSeq (Lefler et al. 2023) was employed to assign taxonomy to the 16S ASVs. For the rbcL dataset, taxonomy was assigned using the curated reference database Diat.barcode (Rimet et al. 2019). For both datasets, ASVs matching our culture-generated sequences were assigned to the taxa we identified them as, and reference taxonomy assignment (from SILVA/CyanoSeq or Diat.barcode) was replaced if taxonomy assignment differed. Only ASVs assigned to diatom taxa were kept for the rbcL marker.

Issues: Missing water parameters for Middle Island Sinkhole samples/cultures

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Data Files

File
911441_v1_sequences.csv (Comma Separated Values (.csv), 10.73 KB) MD5:b8fa213ff7f75dd2b090f73140cd1316 Primary data file for dataset ID 911441, version 1

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Related Publications

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869
Software

Fray, D., McGovern, C., Casamatta, D., & Hamsher, S. (2023). *Metabarcoding data processing using dada2 - rbcL*. Zenodo. https://doi.org/10.5281/ZENODO.10019983 https://doi.org/10.5281/zenodo.10019983
Software

Lefler, F. W., Berthold, D. E., & Laughinghouse, H. D. (2023). Cyanoseq: A database of cyanobacterial 16S rRNA gene sequences with curated taxonomy. Journal of Phycology, 59(3), 470–480. Portico. https://doi.org/10.1111/jpy.13335
Methods

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal, 17(1), 10. doi:10.14806/ej.17.1.200
Software

McGovern, C., Fray, D., Hamsher, S., & Casamatta, D. (2023). *Metabarcoding data processing using dada2 - 16S*. Zenodo. https://doi.org/10.5281/ZENODO.10019989 https://doi.org/10.5281/zenodo.10019989
Software

O'Dell, J. W. (1996). DETERMINATION OF PHOSPHORUS BY SEMI-AUTOMATED COLORIMETRY. Methods for the Determination of Metals in Environmental Samples, 479–495. https://doi.org/10.1016/b978-0-8155-1398-8.50027-6 https://doi.org/10.1016/B978-0-8155-1398-8.50027-6
Methods

Pfaff, J.D. 1993. USEPA Method 300.0: Determination of Inorganic Anions by Ion Chromatography, U.S. Environmental Protection Agency, Cincinatti, OH 45268, p. 30
Methods

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590–D596. doi:10.1093/nar/gks1219
Methods

RICHLEN, M. L., & BARBER, P. H. (2005). A technique for the rapid extraction of microalgal DNA from single live and preserved cells. Molecular Ecology Notes, 5(3), 688–691. https://doi.org/10.1111/j.1471-8286.2005.01032.x
Methods

Rimet, F., Gusev, E., Kahlert, M., Kelly, M. G., Kulikovskiy, M., Maltsev, Y., Mann, D. G., Pfannkuchen, M., Trobajo, R., Vasselon, V., Zimmermann, J., & Bouchez, A. (2019). Diat.barcode, an open-access curated barcode library for diatoms. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-51500-6
Methods

Stepanek, J. G., Mayama, S., & Kociolek, J. P. (2015). Description and phylogenetic position of Amphora aliformis (Bacillariophyta), a new species from Tokyo Bay. Phycologia, 54(1), 78–86. https://doi.org/10.2216/14-081.1
Methods

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Parameters

Parameter	Description	Units
SampleID	ID from file name used in data analysis	unitless
Collection_ID	ID associated with prepared slide for sample	unitless
Collection_Date	Date sample was collected	unitless
Location	What spring sample was collected from	unitless
Lat	Latitide of sampling site	Decimal degrees
Long	Longitude of sampling site	Decimal degrees
Sample_Type	Benthic biofilm, epiphytic biofilm, or plankton tow sample	unitless
Temp	Temperature	Celsius (°C)
Cond	Conductivity	Microsiemens / centimeter (µS/cm)
TDS	Total dissolved solids	Grams/liter (g/L)
pH	Potential hydrogen	unitless
NTU	Turbidity	Nephelometric Turbidity Units (NTU)
ODO	Percent dissolved oxygen	Percentage saturation
ODO_mg_L	Dissolved oxygen	Milligrams/liter (mg/L)
Cl_mg_L	Chloride (Cl) concentration	Milligrams/liter (mg/L)
SO4_mg_L	Sulfate (SO4) concentration	Milligrams/liter (mg/L)
NO3_N_mg_L	Nitrate (NO3) concentration	Milligrams/liter (mg/L)
Si_mg_L	Silica (Si) concentration	Milligrams/liter (mg/L)

SRP_P_mg_L	Soluble reactive phosphorus concentration	Milligrams/liter (mg/L)
NCBI_BioProject	NCBI Accession number for the metabarcoding data	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	250mL Nalgene bottles
Generic Instrument Name	Bottle
Generic Instrument Description	A container, typically made of glass or plastic and with a narrow neck, used for storing drinks or other liquids.

Dataset-specific Instrument Name	Qubit Fluorometer
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	YSI 650MDS multiprobe
Generic Instrument Name	YSI Professional Plus Multi-Parameter Probe
Generic Instrument Description	The YSI Professional Plus handheld multiparameter meter provides for the measurement of a variety of combinations for dissolved oxygen, conductivity, specific conductance, salinity, resistivity, total dissolved solids (TDS), pH, ORP, pH/ORP combination, ammonium (ammonia), nitrate, chloride and temperature. More information from the manufacturer.

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Project Information

Collaborative Research: RUI: OCE-BO: Tango in the Mat World: Biogeochemistry of diurnal vertical migration in microbial mats of Lake Huron's sinkholes (Tango in the Mat World)

Coverage: Middle Island, Lake Huron, Great Lakes N 45.19843°N, W083.32721°W

NSF Award Abstract:
Modern-day microbial mats living on the bottom of sinkholes underneath Lake Huron experience an oxygen-poor, sulfur-rich environment resembling life on early Earth. These mat worlds are dominated by motile filaments of microbes that variably use sunlight and chemicals in their daily routines and offer opportunities for discovering novel microorganisms and ecosystem processes. Recently, complex patterns of daily vertical migration has been observed in the field, suggesting different microbes migrate vertically to the surface of the mat during daylight and nighttime. This project is unraveling the who, why and how of daily microbial migration through integration of microscopy, cultures, molecular approaches, and process rate measurements in response to changing gradients of light, sulfide and oxygen over the day-night cycle. This project places the vertical migration of microbial mats into a broader geobiological context through comparisons with other globally distributed cyanobacterial mat systems such as terrestrial springs and ice-covered Antarctic lakes. Furthermore, the diverse and versatile sinkhole mats may serve as a useful working model for robotic exploration of similar life in extraterrestrial waters like that of Jupiter's Europa or Saturn's Enceladus. This project is generating compelling student projects, attracting public imagination, and fueling active collaboration between two predominantly undergraduate institutions and a National Marine Sanctuary.

The functioning of cyanobacteria under sulfidic, low O2-conditions is a major gap in our understanding of Earth's oxygenation in the past. Recently, time-lapse images of diel vertical migration (DVM) were collected revealing alternating waves of vertically migrating photosynthetic and chemosynthetic filaments that followed daily fluctuating light in microbial mats in Lake Huron's sinkholes; observations corroborated with intact mats under simulated day-night conditions in the laboratory. Such synchronized diel movement, might have played a critical role in optimizing photosynthesis, chemosynthesis, carbon burial, and oxygenation during the Precambrian. This project is evaluating the taxa involved in DVM and is probing geobiological controls on DVM under low-O2, sulfidic conditions using macro- and microscopic imaging, physico-chemical microprofiling, culturing, genetics, and allelopathic studies. Three central issues are being addressed: (1) what taxa are responsible for the DVM? (2) how and why do they perform DVM? and (3) what are the ecosystem consequences of DVM community and activity synergies? The project is revealing specific microbial populations, metabolic pathways, and geochemical processes that underpin mat biogeochemistry over the diel cycle. Studying microbial communities that have regular and measurable daily rhythms in processes that can also be tracked at micrometer scales yields an unprecedented view of the molecular underpinnings of microbial mat biogeochemistry and lays the foundation for future studies aimed at re-defining the role of autotrophic communities in ancient seas and modern ecosystems.

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.

Logo photo credit:
Diver image of microbial mats in Middle Island Sinkhole, Lake Huron. Photo credit: Phil Hartmeyer, NOAA-NMS

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2046958
NSF Division of Ocean Sciences (NSF OCE)	OCE-2045972

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