# Diatom cultures used to generate DNA reference library from samples collected from sites in Alpena, Michigan and Palm Coast, Florida between July 2021 & 2022.

**Website**: https://www.bco-dmo.org/dataset/911008
**Data Type**: experimental
**Version**: 1
**Version Date**: 2023-10-17

**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 information for each of the cultures generated from samples collected from three sites in Alpena, Michigan, one site in Monroe, Michigan, and one site in Palm Coast, Florida. Data are for cultures sequenced using Sanger sequencing and include taxonomic identification, location and water parameter information from samples used to develop the cultures, and growth medium. Each of these cultures was developed from 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. Sequences from these cultures contribute to tying molecular data to morphologically identified isolates, providing a bridge between these two data types that can be used to improve metabarcoding analyses.

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## Coverage

**Spatial Extent**: **N**:45.1984 **E**:-83.3245 **S**:41.7679 **W**:-83.456
**Temporal Extent**: 2021-07-20 - 2022-07-03

### 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.

Individual diatom cells were isolated from each culturing subsample via micropipette serial dilution to establish unialgal cultures. Monocultures were maintained in WC+Si liquid medium (Guillard & Lorenzen 1972) at 10 °C and a 12:12 light cycle. For morphological identification of cultures, live material was boiled in HNO3 for one hour, repeatedly washed and settled with ddH2O, dried on coverslips, and mounted on slides using Naphrax®. Each culture was identified to species under 1000× using a Nikon Eclipse Ni-U light microscope with DIC and Krammer and Lange-Bertalot (1986, 1988, 1991a,b). When monocultures had grown to a sufficient density for DNA extraction, cells were harvested by centrifugation and a Chelex extraction was performed following Richlen & Barber (2005). The rbcL region of each culture was amplified using primers rbcL66+ (Alverson et al. 2007) and DPrbcL7- (Jones et al. 2005) using Cytiva PuReTaq ™ Ready-To-Go ™ PCR beads (Cytiva, Marlborough, MA, USA) and a thermocycler protocol of 94°C for 3 mins 30 s, then 36 cycles of 94°C for 50 s, 52°C for 50 s, 72°C for 80 s, with a final extension at 72°C for 15 mins (Stepanek et al. 2015). The PCR products were frozen and sent to Eurofins Scientific (Louisville, Kentucky) for Sanger sequencing using the PCR primers as well as internal primers CfD+ (Hamsher et al. 2011) and rbcL1255- (Alverson et al. 2007).

### Data Processing Description

Sequences were assembled, edited, and aligned using Geneious Prime (Version 11.0.15+10). The final alignment of rbcL sequences included data from 43 cultures (~1370 bp with no indels).

Missing water parameters for Middle Island Sinkhole samples/cultures

### BCO-DMO Processing Description

* Changed NA values to blanks to comply with our data system.
* Adjusted parameter names (with underscores, rather than points) to comply with data system.

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## Data Files

| File | |
|---|---|
| **911008_v1_diatoms.csv** | (Comma Separated Values (.csv), 7.51 KB) MD5:effeb079fe18c7e1c94a42f70ac46b3c |
| Primary data file for dataset 911008. | |

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## Related Publications


Alverson, A. J. (2008). Molecular Systematics and the Diatom Species. Protist, 159(3), 339–353. https://doi.org/10.1016/j.protis.2008.04.001
*Methods*

Guillard, R. R. L., & Lorenzen, C. J. (1972). YELLOW-GREEN ALGAE WITH CHLOROPHYLLIDE C 1, 2. Journal of Phycology, 8(1), 10–14. Portico. https://doi.org/10.1111/j.1529-8817.1972.tb03995.x
*Methods*

Hamsher, S. E., Evans, K. M., Mann, D. G., Poulíčková, A., & Saunders, G. W. (2011). Barcoding Diatoms: Exploring Alternatives to COI-5P. Protist, 162(3), 405–422. https://doi.org/10.1016/j.protis.2010.09.005
*Methods*

In prep: Callahan McGovern, Davis Fray, Sarah Hamsher, Bopaiah Biddanda, and Dale Casamatta. Multi-marker metabarcoding reveals unexpected diversity of microbial mats in low-oxygen, high-sulfur springs in the Lake Huron basin.
*Results*

In prep: Davis Fray, Callahan McGovern, Dale Casamatta, Bopaiah Biddanda, and Sarah Hamsher. Life in the Extreme: Metabarcoding reveals increased diversity and evidence of biogeographic influence in microbial mats from low-oxygen, high-sulfur springs.
*Results*

Jones, H., Simpson, G. E., Stickle, A. J., & Mann, D. G. (2005). Life history and systematics ofPetroneis(Bacillariophyta), with special reference to British waters. European Journal of Phycology, 40(1), 61–87. https://doi.org/10.1080/09670260400024675 https://doi.org/:10.1080/09670260400024675
*Methods*

K. Krammer and H. Lange-Bertalot, “Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae,” In: H. Ettl, J. Gerloff, H. Heynig and D. Mollenhauer, Eds., Susswasserflora von Mitteleuropa, Gustav Fisher Verlag, Stuttgart, 1991, pp. 1-576.
*Methods*

Krammer, K. and Lange-Bertalot, H. (1986) Bacillariophyceae. Teil 1. Naviculaceae. Suβwassweflora von Mitteleuropa, Bd. 2, berg. Von A. Pascher. Gustav Fisher Verlag, Stuttgart.
*Methods*

Krammer, K. and Lange-Bertalot, H. (1988) Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In: Ettl, H., Gerloff, J., Heynig, H. and Mollenhauer, D., Eds., Süsswasserflora von Mitteleuropa, Vol. 2/2, VEB Gustav Fischer Verlag, Jena.
*Methods*

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*

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*


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## Parameters

| Parameter | Description | Units |
|---|---|---|
| NCBI_Accession | Accession number assigned by NCBI | unitless |
| Culture_ID | ID associated with culture flask | unitless |
| Genus | Genus name | unitless |
| Species | Species name | unitless |
| Variety | Variety name | unitless |
| Collection_ID | ID associated with prepared slide for sample | unitless |
| Media | Type of culture medium used to grow cells | unitless |
| Collection_Date | Date sample was collected | Month/Day/Year |
| Location | What spring sample was collected from | unitless |
| Sample_Type | Benthic biofilm, epiphytic biofilm, or plankton tow sample | unitless |
| Lat | Latitude of sampling site | Decimal degrees |
| Long | Longitude of sampling site | Decimal degrees |
| Temp | Temperature | Celsius (°C) |
| Cond | Conductivity | Microsiemens / centimeter (µS/cm) |
| TDS | Total dissolved solids | Grams/liter (g/L) |
| pH | Potential hydrogen | unitless |
| ORP | Oxidation-reduction potential | Millivolts (mV) |
| 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) |

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## Instruments

| | |
|---|---|
| **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** | 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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