

# Cyanobacteria cultures used to generate DNA reference library from samples collected from sites in Alpena and Monroe, Michigan and Palm Coast, Florida between May and June 2022.

**Website:** <https://www.bco-dmo.org/dataset/911338>

**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 sample type for samples used to develop the cultures. 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:45.0854 W:-83.3251

**Temporal Extent:** 2022-05-02 - 2022-06-20

## Methods & Sampling

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.

To isolate cyanobacterial taxa, mat samples were spread onto solid Z-8 medium (Rippka et al. 1988) and nitrogen-free Z-8 medium to isolate a wider range of cyanobacteria, and grown under ambient conditions (23 °C, ~16:8 h light:dark photoperiod). Colonies were individually picked and plated until unialgal cultures were achieved. Morphology of the strains was analyzed via light microscopy (Nikon Eclipse Ni with DIC), and taxonomic identification was assessed using Wehr et al. (2015) and Komárek and Anagnostidis (2005). Images were taken with a high-resolution camera (Nikon digital sight DS-U3). Direct PCR was performed as follows: cells were placed into -20 °C for 30 mins, centrifuged, and the supernatant containing DNA collected. The partial 16S rRNA and the whole 16S-23S ITS region (Gaylarde et al. 2004) was amplified using primers CYA8F and CYAB23R (Neilan et al. 1997). The 50 µL PCR reaction contained: 27 µL DNA containing supernatant, 0.5 µL of each primer (0.01 mM concentration), and 22 µL PCR Master Mix (Promega, Madison, WI, USA). PCR amplification proceeded as detailed in Casamatta et al. (2005), and products were frozen and sent to Eurofins Scientific (Louisville, Kentucky) for Sanger sequencing.

## Data Processing Description

Sequences were assembled, edited, and aligned using Geneious Prime (Version 11.0.15+10).

## BCO-DMO Processing Description

\* Adjusted parameter names to comply with database requirements

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## Data Files

File
<b>911338_v1_cyanobact.csv</b> (Comma Separated Values (.csv), 1.81 KB) MD5:8dd96359df999de834da18134c955294
Primary data file for dataset ID 911338, version 1

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## Related Publications

Casamatta, D. A., Johansen, J. R., Vis, M. L., & Broadwater, S. T. (2005). MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF TEN POLAR AND NEAR-POLAR STRAINS WITHIN THE OSCILLATORIALES (CYANOBACTERIA)1. Journal of Phycology, 41(2), 421–438. Portico. <https://doi.org/10.1111/j.1529-8817.2005.04062.x>

### Methods

Gaylarde, C. C., Gaylarde, P. M., Copp, J., & Neilan, B. (2004). Polyphasic Detection of Cyanobacteria in Terrestrial Biofilms. Biofouling, 20(2), 71–79. <https://doi.org/10.1080/08927010410001681237>

### 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

Komárek J, Anagnostidis K (2005) "Cyanoprokaryota; Oscillatoriales" in in Süßwasserflora von Mitteleuropa, Book 19/2. Elsevier/Spektrum, Heidelberg, Germany.

#### Methods

Neilan, B. A., Jacobs, D., Therese, D. D., Blackall, L. L., Hawkins, P. R., Cox, P. T., & Goodman, A. E. (1997). rRNA Sequences and Evolutionary Relationships among Toxic and Nontoxic Cyanobacteria of the Genus *Microcystis*. *International Journal of Systematic Bacteriology*, 47(3), 693–697.

<https://doi.org/10.1099/00207713-47-3-693>

#### Methods

Rippka, R. (1988). [1] Isolation and purification of cyanobacteria. *Cyanobacteria*, 3–27.

[https://doi.org/10.1016/0076-6879\(88\)67004-2](https://doi.org/10.1016/0076-6879(88)67004-2)

#### Methods

Wehr, J. D., Sheath, R. G., Kocielek, R. P., & Kocielek, J. P. (2015). *Freshwater algae of North America: Ecology and Classification*. Academic Press. <https://isbnsearch.org/isbn/9780123858764>

#### 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
Media	Type of culture medium used to grow cells	unitless
Collection_Date	Date sample was collected	unitless
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

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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 O<sub>2</sub>-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-O<sub>2</sub>, 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
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-2046958</a>
<a href="#">NSF Division of Ocean Sciences (NSF OCE)</a>	<a href="#">OCE-2045972</a>

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