RNA-Seq and NCBI accessions of microbial communities in the western basin of Lake Erie during summer-fall from 2017-2019

Website: https://www.bco-dmo.org/dataset/945401
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Project
» The role of heterotrophic bacteria in protecting cyanobacteria from hydrogen peroxide in coastal ecosystems (Lake Erie H2O2)

Contributors	Affiliation	Role
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Abstract
Hydrogen peroxide is an oxidative stressor that may influence aquatic microbial community composition and function. It has been hypothesized that hydrogen peroxide may influence the toxicity of cyanobacterial harmful algal blooms (CHABs) in Lake Erie and other eutrophic waters, yet the sources and sinks of hydrogen peroxide are not fully understood. We assessed the relationship between hydrogen peroxide concentrations and CHABs by measuring production and decay of hydrogen peroxide in filtered and unfiltered waters from western Lake Erie with and without UV-visible light. Absolute H2O2 production rates and H2O2 decay rate constants were quantified in the western basin of Lake Erie before, during, and after Microcystis blooms from June – September, 2017-2019 and 2021. Experiments were conducted in whole and filtered waters with natural sunlight or visible light and in the dark to assess relative contributions of major microbial and photochemical processes to production and decay of H2O2. Absolute rates of H2O2 production depended on visible light and were significantly, positively correlated with concentration of chlorophyll a, chromophoric dissolved organic matter (CDOM), and rates of whole-water respiration and primary production. Rate constants for H2O2 decay were highest in waters containing high bloom biomass, and were significantly, positively correlated with whole-water respiration rates and with a proxy for labile dissolved organic nitrogen. Microcystis abundance was not a significant predictor of absolute H2O2 production rates, and microbial production and decay of H2O2 were primarily controlled by microorganisms smaller than 105 µm. Light-dependent production of H2O2 by microorganisms smaller than 105 µm suggests that photosynthesizing organisms other than Microcystis are responsible for H2O2 production. High microbial production and decay of H2O2 are favored by Microcystis bloom conditions (e.g., high light, high biomass) but are not directly due to Microcystis.

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Coverage

Location: western basin of Lake Erie
Spatial Extent: **N**:41.94841 **E**:-82.63979 **S**:41.51771 **W**:-83.42189
Temporal Extent: 2017-05-30 - 2019-09-19

Methods & Sampling

Water samples were collected in the western basin of Lake Erie during the summer and fall of 2017, 2018, and 2019. In 2017, water was collected approximately weekly from NOAA station WE2 in conjunction with the NOAA Great Lakes Environmental Research Lab (GLERL) harmful algal bloom monitoring program. During August and October 2017, lake water was also collected by Environment and Climate Change Canada's monitoring program. In 2018 and 2019, lake water was collected at several stages of bloom development (pre-bloom, early bloom, late bloom, and post bloom). In 2018, lake water was collected at NOAA's monitoring stations WE2 and WE12 and at the drinking water intake for the City of Toledo (TWI). During summer 2019, the goal was to sample lake waters containing high bloom biomass as predicted by the NOAA HAB forecast model and HAB tracker bulletins (Wynne et al. 2013). Sampling sites were chosen based on the presence of surface scums comprised of dense cyanobacterial colonies (i.e., "bloom chase" sites).

For all sites, a depth-integrated water sample was collected in acid-washed carboys. Water samples were collected from the NOAA stations using a peristaltic pump. The pump hose was moved down the water column from the surface to 1 meter above the bottom. For the TWI, bloom chase, and Environment Canada cruise sites, a depth-integrated sample was collected by pooling water collected at 1 m intervals from surface to 1 meter above the lake bottom using a Niskin (Environment Canada) or Van Dorn (TWI and bloom chase sites) bottle. Integrated water samples were stored in carboys in an outdoor aquaculture tank until the start of the bottle experiments the following morning. The water temperature in the aquaculture tank was controlled using copper piping attached to a NESLAB RTE refrigerated water bath (Thermo Scientific, Newington, NH) and maintained at the lake temperature measured at the time of sample collection. During the Environment Canada cruises, bottles and carboys were stored in a plexiglass tank continuously circulated with fresh lake water.

Subsamples for supporting water quality analyses were taken from each carboy. Upon arrival in the laboratory at the University of Michigan, a subsample of whole (unfiltered) water was taken for analysis of total phosphorus. During 2017, pH of the water from each site was obtained from NOAA monitoring buoys. For samples collected in 2018-2019, pH of the whole water was measured upon arrival in the laboratory. Subsamples of the whole water were filtered through a 0.22 µm polyethersulfone (PES) filter for subsequent analysis of total dissolved phosphorus (TDP), soluble reactive phosphorous, nitrate and ammonium, dissolved organic carbon (DOC), and chromophoric and fluorescent dissolved organic matter (CDOM and FDOM, respectively). DOC samples were preserved by addition of 6N trace metal grade hydrochloric acid to pH 3. TDP, SRP, DOC, CDOM and FDOM were stored in the dark at 4 °C until analysis. Nitrate and ammonium samples were stored at -20 °C until analysis at GLERL.

To measure microbial community composition, DNA was extracted from the filters collected from each bottle at the beginning (T=0) of each experiment using a Qiagen Dneasy Blood and Tissue Kit with QIAshredder columns (QIAGEN, MD, USA). The extraction protocol is included as a supplemental file. For absolute quantification of sequence data, genomic DNA from *Thermus thermophilus* strain DSM 7039 was added to the samples after the cell lysis step of the extraction as an internal standard. *Thermus thermophilus* DNA was obtained from the American Type Culture Collection (ATCC; product number BAA-163D-5). The internal standard was added as ~ 1 % of DNA yield, which was estimated based on an empirically determined relationship between total mass of chlorophyll *a* on the filter and DNA yield. DNA yields were measured with Quant-iT Picogreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA). The true percentage of the internal standard was 0.72 ± 0.37 % of total DNA yield, on average.

The V4 region of the bacterial 16S rRNA gene was amplified using a dual-indexed primer set (Kozich et al. 2013), and amplicon sequencing was performed using Illumina MiSeq V2 500 cycle chemistry (Illumina cat# MS102-2003) at the University of Michigan Microbial Community Analysis Core following Schloss and Bishop (2019). Forward and reverse reads were quality screened to remove sequences below 250 bases and trimmed to Q20 using the BBDuk tool in BBTools (Bushnell 2018). Following trimming, overlapping forward and reverse reads were assembled into contigs, aligned, screened for chimeras, and clustered into operational taxonomic units (OTUs) using MOTHUR v. 1.43.0, following the protocol as of February 2020 (Kozich et al. 2013). OTU clustering was performed using a 97 % similarity cutoff with the OptiClust algorithm (Westcott and Schloss 2017). Contigs were aligned with the align.seqs function in MOTHUR, and taxonomy was assigned using the Wang method (Wang et al., 2007). The Silva v. 138 SSU database (Pruesse et al. 2007) was used as the reference to align and classify contigs. The absolute abundance of each OTU per volume of lake water was estimated from the recovery of the internal standard as described in (Lin et al. 2019). All OTUs classified as

Genus *Thermus* were removed from downstream analysis. The raw sequence data are available in NCBI under BioProject PRJNA646259.

BCO-DMO Curation Notes

Processed the submitted file "NCBI accession numbers.xlsx" with the BCO-DMO processing tool Laminar.

- Imported the submitted file into Laminar

- Renamed the parameters to conform with the BCO-DMO naming convention by replacing spaces with underscores.

- Added a column containing the BioProject accession.

- Converted dates from the format %d-%b-%y into the ISO 8601 format of %Y-%m-%d.

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Related Publications

Bushnell, B. (2014). BBTools software package. http://bbtools.jgi.doe.gov
Software

Cory, R. M., Davis, T. W., Dick, G. J., Johengen, T., Denef, V. J., Berry, M., Page, S. E., Watson, S. B., Yuhas, K., & Kling, G. W. (2017). Corrigendum: Seasonal Dynamics in Dissolved Organic Matter, Hydrogen Peroxide, and Cyanobacterial Blooms in Lake Erie. Frontiers in Marine Science, 4. https://doi.org/10.3389/fmars.2017.00377
Related Research

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Applied and Environmental Microbiology, 79(17), 5112–5120. doi:10.1128/aem.01043-13
Methods

Lin, Y., Gifford, S., Ducklow, H., Schofield, O., & Cassar, N. (2019). Towards Quantitative Microbiome Community Profiling Using Internal Standards. Applied and Environmental Microbiology, 85(5). https://doi.org/10.1128/aem.02634-18 https://doi.org/10.1128/AEM.02634-18
Methods

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glockner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Research, 35(21), 7188–7196. doi:10.1093/nar/gkm864
Methods

Schloss, P. D., Bishop, L. (2019). MiSeq Wet Lab SOP. Retrieved from https://github.com/SchlossLab/MiSeq_WetLab_SOP.
Methods

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology, 73(16), 5261–5267. https://doi.org/10.1128/aem.00062-07 https://doi.org/10.1128/AEM.00062-07
Methods

Wynne, T. T., Stumpf, R. P., Tomlinson, M. C., Fahnenstiel, G. L., Dyble, J., Schwab, D. J., & Joshi, S. J. (2013). Evolution of a cyanobacterial bloom forecast system in western Lake Erie: Development and initial evaluation. Journal of Great Lakes Research, 39, 90–99. https://doi.org/10.1016/j.jglr.2012.10.003
Methods

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Related Datasets

IsRelatedTo

Smith, D., Kling, G., Cory, R., Dick, G. J. (2025) **Hydrogen peroxide influence on toxicity of cyanobacterial harmful algal blooms (CHABs) in Lake Erie and other eutrophic waters from 2017 - 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-02-03 http://lod.bco-dmo.org/id/dataset/944935 [view at BCO-DMO]

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Parameters

Parameters for this dataset have not yet been identified

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Project Information

The role of heterotrophic bacteria in protecting cyanobacteria from hydrogen peroxide in coastal ecosystems (Lake Erie H2O2)

Coverage: Western Basin of Lake Erie (41N, 83W)

NSF Award Abstract:
Toxic cyanobacterial harmful algal blooms (CHABs) are now a worldwide problem that poses dangers for humans and aquatic organisms including life-threatening sickness, beach closures, health alerts, and drinking water treatment plant closures. This project focuses on the basic science needed to understand interactions between the microorganisms present in CHABs and the chemistry of the lakes they inhabit. In particular, it will study the sources, fate, and effects of hydrogen peroxide, which is a potentially important control on the toxicity and species present within these blooms. This research will be conducted in Lake Erie, a source of drinking water for 11 million people that is threatened by CHABs annually. Results will be directly integrated into two water quality models that are widely used by water managers and other stakeholders. This project will support the training of two PhD students, including a first-generation college attendee, and undergraduate students from backgrounds that are underrepresented in the earth sciences. Research will also be integrated into outreach aimed at increasing diversity in the earth sciences by involving women and underrepresented minorities in K-12 as well as college and adult educational settings.

The overall goal of this project is to determine the influence of hydrogen peroxide (H2O2) on cyanobacterial community composition and function in nearshore ecosystems. Preliminary results from Lake Erie show that dominant primary producers rely on heterotrophic bacteria to draw down H2O2 from transiently high environmental levels that are likely inhibitory to members of the cyanobacterial community. This suggests that H2O2 plays important and still poorly understood roles in aquatic microbial ecology. A combination of field sampling, experiments, and state-of-the art "-omics" will be used to test the overall hypothesis that H2O2 decomposition by heterotrophic "helpers" is an important determinant of microbial interactions and community structure and function. Lake Erie will be studied because (i) it is a model system for shallow coastal areas receiving high terrestrial nutrient runoff, (ii) it offers strong inshore-offshore gradients of light and nutrients for comparative studies, and (iii) existing sampling infrastructure, archived samples, and preliminary data can be leveraged. Field and laboratory experiments and measurements will be integrated to answer the following questions: Q1: What drives the temporal dynamics of H2O2 concentrations? Q2: Which enzymes and organisms are responsible for protecting the community via biological H2O2 decay? Q3: How does protection from H2O2 by helpers influence the composition and function of the community? The study will perform controlled lab experiments on cultures and on natural waters during different points of the bloom. Measures of H2O2 concentrations and rates of production and decay, along with supporting chemical and biological measurements, will be used to assess the major sources and sinks of H2O2. Molecular tools will be used to determine the pathways underpinning H2O2 decay and the effect of H2O2 on cyanobacterial community composition function. In parallel, impacts of varying H2O2 concentrations on growth rates of major cyanobacteria will be assessed experimentally. These experimental results will be placed into context through comparisons with the structure and function of microbial communities from field samples across spatial, temporal, and chemical gradients in this coastal ecosystem. The approach of integrating studies of H2O2 with

"-omics" in natural systems is novel, and will advance our fundamental knowledge and understanding of the relationship between microbial community composition and function.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736629

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