Picocyanobacteria (Prochlorococcus or Synechococcus) and picoeukaryote (Micromonas and Ostreococcus) cell concentrations and hydrogen peroxide (HOOH) concentrations during batch culture at the University of Tennessee, Knoxville

Website: https://www.bco-dmo.org/dataset/913181
Data Type: experimental
Version: 1
Version Date: 2023-10-16

Project
» Characterizing the effects of exogenous reactive oxygen species on marine microbial ecosystem dynamics (ROS and Microbial Dynamics)

Contributors	Affiliation	Role
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Abstract
These data include picocyanobacteria (Prochlorococcus or Synechococcus) and picoeukaryote (Micromonas and Ostreococcus) cell concentrations and hydrogen peroxide (HOOH) concentrations during batch culture. Cultures were either amended with ~400 nanomolar (nM) HOOH on day 0 or left unamended, and cell counts and HOOH concentrations were quantified over several days of incubation. The ability of Synechococcus and picoeukaryotes to protect Prochlorococcus from HOOH was addressed and compared to the protection conferred by the 'helper' heterotroph Alteromonas macleodii EZ55.

Table of Contents

Coverage

Temporal Extent: 2020-01-31 - 2020-02-10

Methods & Sampling

Cultures:
The strains used in this study included axenic cultures of *Prochlorococcus* MIT9215, *Synechococcus* WH7803, *Synechococcus* CC9605, *Micromonas commoda* RCC299, *Micromonas pusilla* CCMP1545, *Ostreococcus lucimarinus* CCMP2972, and *Alteromonas macleodii* EZ55. All phytoplankton strains were routinely assayed for heterotrophic contaminants by staining cells with purity test broth as previously described (Morris, et al. 2008).

Cultivation methods for mono- and co-cultures:
Experiments were conducted at the University of Tennessee, Knoxville (Tennessee, USA). All cyanobacterial

stock cultures were maintained in an artificial seawater medium, AMP-A (Morris and Zinser, 2013). Picoeukaryote stock cultures were maintained and all experiments were performed using an AMP-A derivative, AMP-PE (for& Pico-Eukaryotes), which allowed for efficient and consistent growth of all photosynthetic microbes in mono- and coculture. This medium has an identical recipe and preparation as AMP-A except for the following alterations: 10x addition of trace metal working stock, $1.06e^{-4}$ molar (M) silica, $2.96e^{-7}$ M thiamine, $2.05e^{-9}$ M biotin, and $3.69e^{-10}$ M cyanocobalamin. Stocks of these nutrients were filter sterilized and added after sterilization of the base saltwater medium. Axenic heterotrophic bacteria *Alteromonas macleodii* strain EZ55 was grown in 5 milliliters (mL) YTSS (Sobecky et al., 1996) and incubated shaking at 140 RPM at 24° Celsius (C) overnight. Before inoculation into experimental cultures, the heterotroph was washed three times in 1.5 mL microcentrifuge tubes by centrifugation at 8,000 RPM for two minutes in a tabletop microcentrifuge and resuspension in 1 mL AMP-A. All experiments were carried out in unshaken test tubes containing 20 mL cultures incubated at 24°C in Percival I36VLX incubators (Percival, Boone, IA) that allowed for gradual increase and decrease of cool white light to simulate sunrise and sunset with peak midday light intensity of 150 millimoles quanta per square meter per second (mmol quanta $m^{-2}s^{-1}$) on a 14 hour:10 hour light:dark cycle (Zinser et al. 2009).

Sampling:
Cultures and uninoculated controls were monitored periodically during the incubations by removing small volumes (<1 mL) of culture to determine total cell concentrations with flow cytometry and hydrogen peroxide (HOOH) concentrations with the acridinium luminescence method.

HOOH concentration assay:
The concentration of HOOH in the medium and cultures was measured on an Orion L Microplate Luminometer (Titertek Instruments Inc, Berthold Detection Systems, Pforzheim, Germany) using an acridinium ester (Cayman Chemical Company, Ann Arbor, MI) chemiluminescence method (Morris et al. 2011). Concentrations in cultures were adjusted via both instantaneous and incremental (over 14-hour period) addition to achieve specific exposure conditions.

Cell concentration assay:
Abundances of cyanobacteria were quantified by flow cytometry using a CytoFLEX S flow cytometer (Beckman Coulter, Brea, CA) with populations of *Prochlorococcus* and *Synechococcus* differentiated in co-cultures by their red (675 nanometers (nm)) and red / yellow (675 nm / 578 nm) fluorescence, respectively (Cavender-Bares et al. 1998; Morris et al. 2008). Picoeukaryotes were quantified by red (675 nm) and far red (770 nm) fluorescence. Detection of red and yellow fluorescence was achieved after excitation with a blue (488 nm) laser, while detection of far-red fluorescence required excitation by a yellow (565 nm) laser. Quantification of *Prochlorococcus* in coculture was achieved by observing events determined by red fluorescence after events that corresponded to the fluorescence properties of either *Synechococcus* (red / yellow) or picoeukaryotes (red / far red) were removed from abundance calculation.

BCO-DMO Processing Description

- Imported original file named "data.xlsx" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved the final file as "913181_v1_cell_growth_and_hooh.csv".

[table of contents | back to top]

Data Files

File	
913181_v1_cell_growth_and_hooh.csv	(Comma Separated Values (.csv), 6.80 KB) MD5:28aafd66343a9278bacebc21aea7e09a
Primary data file for dataset ID 913181, version 1.	

[table of contents | back to top]

Related Publications

Cavender-Bares, K. K., Frankel, S. L., & Chisholm, S. W. (1998). A dual sheath flow cytometer for shipboard analyses of phytoplankton communities from the oligotrophic oceans. Limnology and Oceanography, 43(6), 1383–1388. Portico. https://doi.org/10.4319/lo.1998.43.6.1383
Methods

Morris, J. J., & Zinser, E. R. (2013). Continuous hydrogen peroxide production by organic buffers in phytoplankton culture media. Journal of Phycology, 49(6), 1223–1228. Portico. https://doi.org/10.1111/jpy.12123
Methods

Morris, J. J., Johnson, Z. I., Szul, M. J., Keller, M., & Zinser, E. R. (2011). Dependence of the Cyanobacterium Prochlorococcus on Hydrogen Peroxide Scavenging Microbes for Growth at the Ocean's Surface. PLoS ONE, 6(2), e16805. https://doi.org/10.1371/journal.pone.0016805
Methods

Morris, J. J., Kirkegaard, R., Szul, M. J., Johnson, Z. I., & Zinser, E. R. (2008). Facilitation of Robust Growth of Prochlorococcus Colonies and Dilute Liquid Cultures by "Helper" Heterotrophic Bacteria. Applied and Environmental Microbiology, 74(14), 4530–4534. https://doi.org/10.1128/aem.02479-07 https://doi.org/10.1128/AEM.02479-07
Methods

Sobecky, P. A., Schell, M. A., Moran, M. A., & Hodson, R. E. (1996). Impact of a genetically engineered bacterium with enhanced alkaline phosphatase activity on marine phytoplankton communities. Applied and Environmental Microbiology, 62(1), 6–12. https://doi.org/10.1128/aem.62.1.6-12.1996
Methods

Zinser, E. R., Lindell, D., Johnson, Z. I., Futschik, M. E., Steglich, C., Coleman, M. L., Wright, M. A., Rector, T., Steen, R., McNulty, N., Thompson, L. R., & Chisholm, S. W. (2009). Choreography of the Transcriptome, Photophysiology, and Cell Cycle of a Minimal Photoautotroph, Prochlorococcus. PLoS ONE, 4(4), e5135. https://doi.org/10.1371/journal.pone.0005135
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Experiment	Experimental set defined by days examined for monocultures, cocultures, and relevant controls. A= days 0,1,2,5 for Prochlorococcus and/or picoeukaryotes. B = days 0,1,4,6,8 for Prochlorococcus and/or Synechococcus / Alteromonas.	unitless
Strain_1	Strain name for monoculture type and first strain for coculture type. 1 = Prochlorococcus MIT9215. 2= Micromonas commoda RCC299. 3= Micromonas pusilla CCMP1545. 4= Ostreococcus lucimarinus CCMP2972. 5= Synechococcus WH7803. 6= Synechococcus CC9605. 7= Alteromonas macleodii EZ55.	unitless
Strain_2	Strain name for second strain for coculture type. 2= Micromonas commoda RCC299. 3= Micromonas pusilla CCMP1545. 4= Ostreococcus lucimarinus CCMP2972. 5= Synechococcus WH7803. 6= Synechococcus CC9605. 7= Alteromonas macleodii EZ55.	unitless
HOOH_amend	Concentration of hydrogen peroxide (HOOH) added to medium on day 0	nanomolar (nM)
time	Time (in days) of data collection measured from time of inoculation	days
strain_1_rep_1	Cell concentration for Strain 1 Replicate 1	cells per milliliter (cells/mL)
strain_1_rep_2	Cell concentration for Strain 1 Replicate 2	cells per milliliter (cells/mL)
strain_1_rep_3	Cell concentration for Strain 1 Replicate 3	cells per milliliter (cells/mL)
strain_1_rep_4	Cell concentration for Strain 1 Replicate 4	cells per milliliter (cells/mL)
strain_2_rep_1	Cell concentration for Strain 2 Replicate 1	cells per milliliter (cells/mL)
strain_2_rep_2	Cell concentration for Strain 2 Replicate 2	cells per milliliter (cells/mL)
strain_2_rep_3	Cell concentration for Strain 2 Replicate 3	cells per milliliter (cells/mL)
strain_2_rep_4	Cell concentration for Strain 2 Replicate 4	cells per milliliter (cells/mL)
ave_HOOH	Average concentration of HOOH for all 4 replicate cultures	nanomolar (nM)
SD_HOOH	Standard deviation of the average concentration of HOOH for all 4 replicate cultures	nanomolar (nM)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	CytoFLEX S flow cytometer (Beckman Coulter, Brea, CA)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	The flow cytometer passes liquid in a narrow stream through the path of light emitted by a laser. Detectors can measure light scatter and fluorescence generated when the photons interact with particles (e.g. cells) moving in the stream. Phytoplankon can be distinguished by the fluorescence profiles established by their suite of fluorescent photopigments.
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	Orion L Microplate Dual injector luminometer (Titertek Instruments Inc, Berthold Detection Systems, Pforzheim, Germany)
Generic Instrument Name	Luminometer
Dataset-specific Description	The dual injector luminometer uses acridinium ester to quantify hydrogen peroxide (HOOH) in liquid. Injectors simultaneously add acridinium ester (originally, colorless) and a buffer, and the ester reacts at a 1:1 ratio to HOOH and emits light that can be converted to [HOOH] through use of a standard curve.
Generic Instrument Description	A luminometer is an instrument that measures light and other optical properties of specimens in chemiluminescent and bioluminescent applications.

[table of contents | back to top]

Project Information

Characterizing the effects of exogenous reactive oxygen species on marine microbial ecosystem dynamics (ROS and Microbial Dynamics)

Coverage: Station ALOHA and UTK laboratory growth chambers

NSF Award Abstract:
The microbes that constitute the phytoplankton community of the ocean account for about half of all photosynthesis on the earth and as a consequence are critically important to the climate and major nutrient cycles of the planet. The vast majority of the ocean surface is relatively depleted in nutrients essential for organisms to grow. This is especially true for the ocean gyres that are hundreds or thousands of miles from the coast and thousands of meters above the ocean floor. In these regions, competition among different phytoplankton for nutrients is thought to be especially strong. The most abundant member of the photosynthetic community, the single-celled bacterium *Prochlorococcus*, is also its smallest, measuring a half a micrometer in length. Interestingly, *Prochlorococcus* is very sensitive to reactive oxygen species (ROS) such as hydrogen peroxide and cannot grow in the absence of “helper” microbes which detoxify the ROS generated when sunlight reacts with pigmented organic material in the seawater. The true extent to which *Prochlorococcus* depends on helpers is currently unknown: thus far, experiments have only assessed *Prochlorococcus* survival of ROS stress under otherwise optimal growth conditions which are rare in the natural environment. Recent evidence that *Prochlorococcus* and helpers can compete for nutrients adds

another layer of complexity. This project combines experimental culture work, field measurements, and ecosystem modeling to characterize the roles of ROS in surface ocean community dynamics. Laboratory cultures of *Prochlorococcus* and other microbes are being examined for growth and survival changes when exposed to ROS under a range of nutrient concentrations, temperatures, and light intensities, when grown separately and in co-culture with each other. Outcomes from the laboratory experiments are then being used in mathematical ecosystem models to simulate the natural marine environment. Finally, laboratory results and mathematical models are being compared to natural communities in the North Pacific Ocean exposed to a range of ROS concentrations. In this way, this research is developing a deeper and more predictive understanding of how microbial community composition and mortality depend upon ROS production and decay. Broader impacts of the project include the training of undergraduate and graduate students in oceanographic research and public outreach about microbiology and oceanography to the local Knoxville community, as well as dependents of active duty Marines.

The overarching goal of this project is to empirically parameterize ecosystem models using a combination of lab experiments and field manipulations to explore the coupled dynamics of HOOH and oligotrophic microbial communities. *Prochlorococcus* is the most abundant phytoplankter in the oligotrophic ocean and contributes significantly to global carbon cycling. Key to its abundance is its ability to outcompete other microbes for nutrients. This ecological advantage is thought to involve an evolutionary process of genomic streamlining, including a loss of hydrogen peroxide (HOOH) resistance mechanisms and reliance of *Prochlorococcus* on the microbial community to degrade photochemically-generated HOOH in the sun-exposed surface mixed layer. When temperature deviates from optimal, however, sensitivity of *Prochlorococcus* to HOOH – and thus reliance upon helpers - is heightened. The same may hold true for light and nutrient conditions. Similarly, little is known about the environmental sensitivity of HOOH-detoxifying “helper” microbes, including fellow members of the phytoplankton community. Therefore, the extent to which *Prochlorococcus* requires help, and to which different HOOH-consuming microbes provide this function, is not currently understood. This project is providing the quantitative measurements in HOOH and microbial dynamics to understand the rules of microbial community assembly in the surface mixed layer. Several strains of *Prochlorococcus*, potential helper, and competitor microbes are being grown under a range of nutrient-limiting and HOOH conditions in chemostats to assess growth, mortality, and - for the phytoplankton – photosynthesis. Co-cultures with the other microbes, including *Synechococcus* and several photosynthetic picoeukaryotes, are being used to test hypotheses about HOOH detoxification and the impacts of competition on *Prochlorococcus*–ROS dynamics under optimal and suboptimal conditions These ecosystem model predictions are being interpreted alongside field manipulations which directly assess ROS mediated impacts in the context of other loss processes, primarily grazing and viral lysis.

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2023680

[table of contents | back to top]