Flow cytometry measurements from HHQ experiments conducted during the MesoHux mesocosm experiment, May 2017, Bergen, Norway

Website: https://www.bco-dmo.org/dataset/753431
Data Type: experimental
Version: 1
Version Date: 2019-01-23

Project
» Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics (HHQSignals)

Contributors	Affiliation	Role
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Abstract
This dataset includes flow cytometry measurements from HHQ experiments conducted during the MesoHux mesocosm experiment, May 2017, Bergen, Norway. Microbial mesocosms were spiked with 2-heptyl-4-quinolone (HHQ).

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Coverage

Spatial Extent: **Lat**:60.221 **Lon**:5.281
Temporal Extent: 2017-05-16 - 2017-05-31

Dataset Description

This dataset includes flow cytometry measurements from HHQ experiments conducted during the MesoHux mesocosm experiment, May 2017, Bergen, Norway. Microbial mesocosms were spiked with 2-heptyl-4-quinolone (HHQ).

Methods & Sampling

Triplicate 5 mL samples were preserved for flow cytometry with 0.5% glutaraldehyde (final concentration), incubated at 4°C for 10 min and frozen (-80°C) until analysis (within 2-3 weeks; Kemp et al. 1993). To calculate phytoplankton group abundances, 200 µl aliquots of fixed sample were added to a 96-well plate and run on a Guava flow cytometer (Millipore). Filtered seawater (0.45 µm) was run as a blank and instrument-specific beads

were used to calibrate the cytometer. Samples were analyzed at low flow rate (0.24 µl s-1) for 3 min. Three major phytoplankton groups were distinguishable based on plots of forward scatter vs. orange (phycoerythrin-containing, Synechococcus spp.) or red (pico- and nanoeukaryotes) fluorescence signals (Worden and Binder 2003).

Samples for enumerating bacteria were stained prior to running on the Guava in 0.5% v/v SybrGreen I DNA stain for 1 hour at room temperature in the dark.

Mesocosm treatment for all HHQ experiments was as follows:
Redfield: N:P added in a 16:1 ratio during the first 3 days of the experiment, no shading

HHQ treatments here are as follows:
High HHQ - 100 ng mL-1 (410 uM) added to triplicate 5L bottles.
DMSO control - equivalent (v:v) DMSO added to triplicate 5L bottles.

All bottles were incubated for 24h in a flow-through tank, that was shaded to mimic in situ conditions. Chlorophyll samples were taken at T0 and T24 for all experiments.

Data were processed in Excel with statistics run in Excel, R, or Matlab.

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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Data Files

File
flow_cytometry.csv (Comma Separated Values (.csv), 4.15 KB) MD5:97828df2a961d41e5cbeab4c75cd05ce Primary data file for dataset ID 753431

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Related Publications

Kemp, P., B. F. Sherr, E. B. Sherr, and J. J. Cole (Eds.). (1993). Handbook of methods in aquatic microbial ecology. Lewis Publishing, Boca Raton, FL 33431, 777 pp. https://isbnsearch.org/isbn/0-873-71564-0
Methods

Worden, A., & Binder, B. (2003). Application of dilution experiments for measuring growth and mortality rates among Prochlorococcus and Synechococcus populations in oligotrophic environments. Aquatic Microbial Ecology, 30, 159–174. doi:10.3354/ame030159
Methods

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Parameters

Parameter	Description	Units
Date	sampling date formatted as Mon dd yyyy	unitless
Sample	sample identifier	unitless
Experiment_num	experiment number	unitless
Time	time since start of experiment	hours
Replication	replicate number	unitless
Bacteria	number of bacterial cells	cells/milliliter
Synechococcus	number of Synechococcus cells	cells/milliliter
Picoeukaryotes	number of Picoeukaryotes cells	cells/milliliter
Nanoeukaryotes	number of Nanoeukaryotes cells	cells/milliliter
Total_Phytoplankton_lt_15um	total number of phytoplankton cells less than 15 microns in diameter	cells/milliliter

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Instruments

Dataset-specific Instrument Name	Millipore Guava inCyte BG HT flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Used for cell counts
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	5 L Niskin
Generic Instrument Name	Niskin bottle
Dataset-specific Description	Used to collect water samples.
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

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Project Information

Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics (HHQSignals)

Coverage: Bergen, Norway

NSF Award Abstract:
Bacteria and phytoplankton play a central role in the modification and flow of materials and nutrients through the marine environment. While it has been established that interactions between these two domains are complex, the mechanisms that underpin these interactions remain largely unknown. There is increasing recognition, however, that dissolved chemical cues govern these microbial interactions. This project focuses on establishing a mechanistic framework for how bacterially derived signaling molecules influence interactions between phytoplankton and bacteria. The quorum-sensing (QS) molecule, 2-heptyl-4-quinolone (HHQ) will be used as a model compound for these investigations. Previously published work suggests that exposure to very low levels of HHQ results in phytoplankton mortality. Gaining a mechanistic understanding of these ecologically important interactions will help to inform mathematical models for the accurate prediction of the cycling of material through the marine microbial loop. This work initiates a new, hybrid workshop-internship undergraduate research program in chemical ecology, with a focus

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Interactions between phytoplankton and bacteria play a central role in mediating biogeochemical cycling and microbial trophic structure in the ocean. The intricate relationships between these two domains of life are mediated via excreted molecules that facilitate communication and determine competitive outcomes. Despite

their predicted importance, identifying these released compounds has remained a challenge. The PIs recently identified a bacterial QS molecule, HHQ, produced by globally distributed marine gamma-proteobacteria, which induces phytoplankton mortality. The PIs therefore hypothesize that bacteria QS signals are critical drivers of phytoplankton population dynamics and, ultimately, biogeochemical fluxes. This project investigates the timing and magnitude of HHQ production, and the physiological and transcriptomic responses of susceptible phytoplankton species to HHQ exposure, and quantifies the influence of HHQ on natural algal and bacterial assemblages. The work connects laboratory and field-based experiments to understand the governance of chemical signaling on marine microbial interactions, and has the potential to yield broadly applicable insights into how microbial interactions influence biogeochemical fluxes in the marine environment.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657898

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