Flow cytometric counts from grazing saturation culture experiment using single prey (Isochrysis galbana) and predator (Ochromonas danica) from March to April 2020

Website: https://www.bco-dmo.org/dataset/905496
Data Type: experimental
Version: 1
Version Date: 2023-08-03

Project
» EAGER: A Saturation Approach to Microzooplankton Grazing Rate Determination (Grazing Saturation)

Contributors	Affiliation	Role
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Abstract
Flow cytometric counts of the nanoeukaryote prey Isochrysis galbana, the mixotrophic predator Ochromonas danica and 2 um green fluorescent bead abundance in laboratory culture based experiments to demonstrate the saturation approach.

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Coverage

Spatial Extent: **Lat**:43.8597 **Lon**:-69.58
Temporal Extent: 2020-03-24 - 2020-04-17

Methods & Sampling

The culture experiments used the mixotrophic chrysophyte *Ochromonas danica*, ~ 6 to 9 µm in diameter, as a predator, and the prymnesiophyte *Isochrysis galbana*, ~ 4 to 6 µm in diameter, as prey. The experiments followed the methods of 'Tests Using Single Prey and Predator Combinations' as described in Archer et al. (2022).

- prey: *Isochyris galbana;* AphiaID=573884
- predator: *Ochromonas danica,* CCMP 1391; AphiaID=1305802 (recommended name of *Chlorochromonas danica*).

Culture experiment
The cultures were maintained in sterile L1 media in a 21˚C incubator with a 14 h light/10 h dark cycle and light levels of ~ 90 µE m^{-2} sec^{-1}. Prior to the start of the experiments, *O. danica* was transitioned from K media and rice to sterile L1 media and then fed every other day on *I. galbana*. The prey, *I. galbana* was maintained in semi-

continuous growth through regular transfers (2 - 3 days). Similar ratios of predator-to-prey were generated in tubes containing a total volume of 40 ml. Fluorescent polystyrene microspheres (beads) of 2 µm in diameter (Fluoresbrite Plain YG microspheres, Polysciences, Inc., Warrington, PA), were used as surrogate prey. To minimize clumping, the beads were blocked in a solution of 1 % bovine serum albumin overnight then centrifuged for 5 minutes at 2000 rpm, after which the pellet was resuspended in 0.2 µm filtered seawater. A new solution of beads was prepared at the start of each experiment. During the incubations, the experimental tubes were rotated at 1.2 rpm on a plankton wheel to keep particles in suspension. Two subsamples of 1 ml were removed from each tube at the start (T_0) and the final time point (T_{24}) after ~24 hours, for flow cytometric analysis.

Note: It was not possible to accurately obtain *Ochromonas danica* abundance at the final time point (T_{24})

Flow Cytometric Measurements
Particles were excited with a 488 nm blue excitation laser (100 mW). Data acquisition was triggered on forward scatter (FSC). Signals were recorded from detectors with bandpass filters for right angle light scatter and fluorescence emission in red (692 nm/80 nm band pass) indicative of chlorophyll a, orange for phycoerythrin (593/52 nm), and green (525/35 nm). To ensure accurate calibration of the flow cytometer, ZE5 QC beads (Bio-Rad, Hercules, CA, USA) were run daily.

Data Processing Description

Flow cytometric data files were analyzed from logarithmic dot plots based on fluorescence and characteristic light scattering properties (DuRand and Olson, 1996) using FlowJo 10.6 Software (Becton Dickinson & Company, San Jose, CA, USA)

Model fitting of the experimental data was carried out using the nonlinear least squares regression function (nls) in R (R Core Team 2021).

BCO-DMO Processing Description

- Imported data from source file "Grazing_Saturation_Culture_experiments_2.xlsx"
- Modified field (parameter/column) names to conform to BCO-DMO naming conventions. The only allowed characters are A-Z,a-z,0-9, and underscores. (NO spaces, hyphens, commas, parentheses, or Greek letters.)
- Converted yyyy/mm/dd date format to ISO Date format yyyy-mm-dd
- Added columns for latitude and longitude of Bigelow lab
- Added column for Ochromonas at end of incubation (T24) to indicate that it was not possible to accurately obtain abundance at the final time point, so viewers know there is a reason for no data.
- Taxonomic names were checked using the World Register of Marine Species (WoRMS) taxa match tool at http://www.marinespecies.org/
. * Isochyris galbana (AphiaID=573884)
. * Ochromonas danica is NOT accepted taxon name. Accepted name is Chlorochromonas danica (AphiaID=1305802).

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Related Publications

Archer, S. D., Lubelczyk, L. C., Kunes, M., McPhee, K., Dawydiak, W., Staiger, M., Posman, K. M., & Poulton, N. J. (2022). Saturation Approach to Determine Grazing Mortality in Picoeukaryote and Synechococcus Populations. Frontiers in Marine Science, 9. https://doi.org/10.3389/fmars.2022.844620
Methods

Durand, M. D., & Olson, R. J. (1996). Contributions of phytoplankton light scattering and cell concentration changes to diel variations in beam attenuation in the equatorial Pacific from flow cytometric measurements of pico-, ultra- and nanoplankton. Deep Sea Research Part II: Topical Studies in Oceanography, 43(4–6), 891–906. https://doi.org/10.1016/0967-0645(96)00020-3

Methods

FlowJo™ Software Version 10.6 (2023) [software application] Becton, Dickinson and Company. https://docs.flowjo.com/flowjo/getting-acquainted/10-6-release-notes/10-6-exhaustive-release-notes/
Software

R Core Team (2021). R: A language and environment for statistical computing. R v4.0.5. (March 2021) R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Software

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Related Datasets

IsRelatedTo

Archer, S. D., Poulton, N. J. (2023) **Flow cytometric counts from grazing saturation culture experiment using single prey (Micromonas pusilla) and predator (Ochromonas danica) in October 2019.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-08-02 http://lod.bco-dmo.org/id/dataset/905469 [view at BCO-DMO]

Archer, S. D., Poulton, N. J. (2023) **Flow cytometric counts of picoeukaryotes, Synechococcus, and beads using natural waters from the Gulf of Maine during Jul-Aug 2019 and Jun-Jul 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-08-04 http://lod.bco-dmo.org/id/dataset/905568 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Latitude	Latitude of experiment location (Bigelow Lab)	decimal degrees
Longitude	Longitude of experiment location (Bigelow Lab)	decimal degrees
Date	Start date of experiment	unitless
Experiment	Code ID for each experiment (IG1, IG2, IG3)	unitless
Duration	Duration of the incubation	day (d)
Beads_T0	Bead abundance at time zero (T0)	beads per milliliter (beads/ml)
Isochrysis_T0	Abundance of *Isochrysis galbana* at time zero	cells per milliliter (cells/ml)
Beads_T24	Bead abundance at end of incubation	beads per milliliter (beads/ml)
Isochrysis_T24	Abundance of *Isochrysis galbana* at end of incubation	cells per milliliter (cells/ml)
Ochromonas_T0	Abundance of *Ochromonas danica* at time zero	cells per milliliter (cells/ml)
Ochromonas_T24	Abundance of *Ochromonas danica* at end of incubation	cells per milliliter (cells/ml)

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Instruments

Dataset-specific Instrument Name	Bio-Rad ZE5 Cell Analyzer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	A ZE5 Cell Analyzer (Bio-Rad, Hercules, CA, USA) was used to measure optical properties and abundance of single cells from each sample
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)

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Project Information

EAGER: A Saturation Approach to Microzooplankton Grazing Rate Determination (Grazing Saturation)

Coverage: Gulf of Maine

NSF Award Abstract:

Heterotrophic protists are the dominant consumers of the 50% of global primary production by phytoplankton in the oceans. Hence, they play a key role in influencing ocean biogeochemistry, the composition of microbial communities, and transfer of energy to higher trophic levels. The aim of the project is to develop a novel saturation approach to quantify the rates of grazing on phytoplankton by phagotrophic protists in the ocean. As a proof-of-concept, this study will focus on determining grazing rates on picophytoplankton. This smallest size-class of phytoplankton often dominates oceanic primary production and can contribute up to 50% of annual primary production in coastal waters. Understanding grazing is of critical importance to understanding how planktonic communities function and respond to environmental change has the important societal benefit of potentially more accurately predicting the future of global fisheries and interactions between ocean and atmosphere that influence our climate. The project incorporates experiential education of undergraduates in the research environment and biological oceanography and will be a feature of an Advanced Aquatic Flow Courses designed for graduate students, faculty members and commercial entities. Public engagement in the science will be through Cafe Scientifique presentations and the series of Open House events that occur at Bigelow Laboratory through the year.

The motivation behind this project is that challenges in performing and interpreting current experimental measurements of herbivory by protists in the ocean constrain our understanding of this key process. The basis of the present approach is saturation of the grazers with a surrogate prey, resulting in release of grazing pressure on the natural prey. Measurement of the resulting increased growth rate of the natural prey provides a value for the rate of grazing. The project involves laboratory experiments using cultures of model predator-prey combinations to select suitable surrogate prey and test the underlying theoretical assumptions of the approach. This information will then be used to inform the design of experiments on natural planktonic communities. The objectives of these experiments are to test the efficacy of the saturation approach and to compare results to traditional experimental approaches run in parallel. This research will introduce a new approach to biological oceanography that will have been thoroughly tested, with recommendations for optimum set-up procedures and an assessment of the factors that influence uncertainty in the results. The saturation approach has potential advantages over previous methods. It lends itself to analysis by flow cytometry allowing high throughput and accurate measurements, avoids manipulation of the natural seawater and microbial communities, and provides growth and grazing information on defined components of the phytoplankton community.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1738061

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