# Flow cytometric counts of picoeukaryotes, Synechococcus, and beads using natural waters from the Gulf of Maine during Jul-Aug 2019 and Jun-Jul 2021

**Website**: https://www.bco-dmo.org/dataset/905568
**Data Type**: experimental
**Version**: 1
**Version Date**: 2023-08-04

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

| Contributors | Affiliation | Role |
|---|---|---|
| Archer, Stephen D. | Bigelow Laboratory for Ocean Sciences | Principal Investigator |
| Poulton, Nicole J. | Bigelow Laboratory for Ocean Sciences | Co-Principal Investigator |
| Gerlach, Dana Stuart | Woods Hole Oceanographic Institution (WHOI BCO-DMO) | BCO-DMO Data Manager |

**Abstract**
Flow cytometric counts of picoeukaryotes, Synechococcus, and 2 um green fluorescent bead abundance in experiments using natural waters to test the saturation approach.

## Table of Contents



## Coverage

**Spatial Extent**: **Lat**:43.8597 **Lon**:-69.5781
**Temporal Extent**: 2019-07-06 - 2021-07-26

### Methods & Sampling

The natural waters experiment used the experimental format established in culture experiments 1 and 2 (See Related Datasets section below), and was then adapted for use in natural waters. For detailed methods, refer to Archer et al. (2022) in the section 'Picophytoplankton Rates in Natural Waters'.

From 16th July to 15th August, 2019 (days of the year 197 to 227) and 8th June to 26th July 2021 (days 165 to 207), a series of saturation experiments were performed that focused on determining the growth rates and grazing mortality of populations of picoeukaryote and Syncechococcus species. Gulf of Maine coastal seawater was collected from the Damariscotta River Estuary at Bigelow Laboratory's dock off East Boothbay, Maine, USA (43.8604° N, 69.5781° W). Water for all experiments was collected within one hour of high tide at 1 meter depth using a 5 liter Niskin bottle and was gravity filtered through 200 µm mesh to remove zooplankton. Several Niskin casts of seawater were combined in an acid washed carboy before being siphoned into 600 ml polycarbonate bottles. For each saturation experiment, 12 to 14 bottles were used to generate a range of levels of surrogate prey addition. Fluorescent polystyrene microspheres of 2 µm in diameter, treated as for the laboratory experiments, were added to the bottles either as duplicate treatments or in a continuous series of

abundance that spanned the range from 0 to 1.7 x 106 beads ml-1 on day 197 to 0 to 3.8 x 106 beads ml-1 on day 226.

Bottles were incubated for 24 hours in a flow-through incubator on the dock under a nylon mesh that removed ~40 % of the surface photosynthetic active radiation (PAR). In an initial test of the flow-through incubator, five 600 ml bottles were filled with seawater from the same carboy and with no further treatment, were incubated for 24 hours. The counts of the t0 initial (time zero) abundance of picoeukaryotes from the five bottles showed a coefficient of variation of 1.3%, that increased in the T24 counts to only 4.1%, indicating consistent growth among the 5 replicate bottles. Following all experiments, the seawater to which beads had been added was filtered through a 0.45 µm capsule filter to recover the beads before discarding the water.

**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).

These data were published in Figure 2, Table 2, and Table S2 (Supplementary) of Archer et al. (2022)

**BCO-DMO Processing Description**

- Imported data from source file "Grazing_Saturation_Natural_experiments_1.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

[ table of contents | back to top ]

## 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, LLC. (2023) FlowJo™ Software Version 10.6 [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*

[ table of contents | back to top ]

## Related Datasets

### IsRelatedTo

Archer, S. D., Poulton, N. J. (2023) **Flow cytometric counts from grazing saturation culture experiment using single prey (Isochrysis galbana) and predator (Ochromonas danica) from March to April 2020.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-08-03 http://lod.bco-dmo.org/id/dataset/905496 [view at BCO-DMO]

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]

[ table of contents | back to top ]

## 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 |
| Day_of_year | Day of the year | day |
| Experiment | Code ID for each experiment (1 to 24) | unitless |
| Incubation_duration | Duration of the incubation | day |
| Picoeukaryote_T0 | Picoeukaryote abundance at start of incubation (time zero) | cells per milliliter (cells/ml) |
| Picoeukaryote_Tf | Picoeukaryote abundance at end of incubation (time final) | cells per milliliter (cells/ml) |
| Synechococcus_T0 | Synechococcus abundance at start of incubation (time zero) | cells per milliliter (cells/ml) |
| Synechococcus_Tf | Synechococcus abundance at end of incubation (time final) | cells per milliliter (cells/ml) |
| Beads_T0 | Bead abundance at time zero | beads per milliliter (beads/ml) |
| Beads_Tf | Bead abundance at end of incubation | beads per milliliter (beads/ml) |

[ table of contents | back to top ]

## 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) |

[ table of contents | back to top ]

## 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.

[ table of contents | back to top ]

## Funding


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


[ table of contents | back to top ]