Cell concentrations, percent of host infected, and bulk dissolved organic carbon (DOC) from laboratory experiments examining parasite-host metabolites in 2023

Website: https://www.bco-dmo.org/dataset/962736
Data Type: experimental
Version: 1
Version Date: 2025-05-29

Project
» Characterizing plankton parasite-host metabolites and the response of heterotrophic bacteria (Plankton parasite-host metabolites)

Program
» Center for Chemical Currencies of a Microbial Planet (C-CoMP)

Contributors	Affiliation	Role
Anderson, Sean	University of New Hampshire (UNH)	Principal Investigator
Harvey, Elizabeth	University of New Hampshire (UNH)	Co-Principal Investigator
Poulson-Ellestad, Kelsey	Roosevelt University (RU)	Co-Principal Investigator
Place, Philip	University of New Hampshire (UNH)	Scientist
Gray, Laura	Woods Hole Oceanographic Institution (WHOI)	Data Manager
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Laboratory experiments were carried out to characterize plankton infection dynamics and extracellular metabolites over an infection cycle in two parasite-host pairings. Data reported here are cell concentrations, percent of host infected, and bulk DOC concentrations measured daily over an infection cycle (four days) that coincides with collection of extracellular metabolites for analysis via liquid chromatography-mass spectrometry. Separate experiments were performed for two strains of the parasite Amoebophrya sp. (4390 and 4401) that infected the same host dinoflagellate species, Scrippsiella acuminata. Metabolomics data collected from these experiments will be available on MetaboLights (MTBLS11219).

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
  - BCO-DMO Processing Description
  - Problem Description
- Data Files
- Supplemental Files
- Related Publications
- Related Datasets
- Parameters
- Instruments
- Project Information
- Program Information
- Funding

Coverage

Location: Laboratory culture studies
Temporal Extent: 2023-10 - 2023-12

Methods & Sampling

Culturing and experimental set-up:

Phytoplankton cultures used in this study were obtained from the Roscoff Culture Collection (RCC; Vaulot et al. 2004). Host cultures of *Scrippsiella acuminata* (RCC 1627) were maintained in 0.2 µm sterile-filtered autoclaved seawater that was enriched with f/2 minus silica (Guillard 1975). Hosts were transferred into fresh media every 7-10 d to maintain exponential growth. Two strains of *Amoebophrya* sp. parasite spores (RCC 4390 and 4401) were inoculated every 2-3 d with fresh and exponentially growing host culture at a ratio of 1:1 spore to host by volume to maintain infection. Parasite and host cultures were kept at 18 °C on a 12:12 hr light: dark cycle at 80-100 µmol photon m^{-2} s^{-1}. Spore cultures were spot-checked each week on a Nikon eclipse TE300 microscope at 20x magnification.

To prepare for the infection experiment, fresh parasite spores were filtered through 10 µm mesh to separate the smaller spores (2-5 µm) from any remaining host cells (Chen et al. 2021). Spores were added separately (4401 and 4390) into healthy host cultures in triplicate 1.2-L bottles at a ratio of 1:1 spore to host based on their cell densities (Long et al. 2021). Several control treatments, including spore-only, host-only, f/2 media, and Milli-Q water, were also included in triplicate. Separate infection experiments were conducted for each parasite strain (Infection_experiment column in data file), with their own set of treatments and controls (Treatment column). Bottles were sampled daily for 4 d to capture a single infection cycle.

Flow cytometry:

Samples for flow cytometry were run daily to estimate changes in host and parasite spore abundances in the infected and control treatments. Two separate runs were performed for host and spores. For the hosts, 200 µl aliquots were sampled from triplicate bottles, added to a 96-well plate, and run live on a Guava easyCyte HT (Millipore) flow cytometer at low flow rate (0.24 µl s^{-1}) for 3 min per well. Host populations were distinguished using predefined gates that were based on plots of forward scatter and red fluorescence (692 nm). For the spores, 198 µl aliquots were added to a 96-well plate, stained with 2 µl of 100x SYBR Green (Thermo Fisher), and allowed to incubate in the dark at room temperature for 20-30 min (Kayal et al. 2021). Parasite spore populations were detected based on forward scatter (proxy for cell size) and green fluorescence (512 nm) originating from SYBR-Green stained DNA (Kayal et al. 2021).

In addition, samples (1.8 mL) from each bottle were fixed with 1% glutaraldehyde, stored at 4 °C, and run on a Sony SH800Z sorting flow cytometer (Sony) within 4-6 months to provide an estimate on the prevalence of infection (% of hosts infected). *Amoebophrya* sp. emit a natural green autofluorescence (Long et al. 2021), and so, we used predefined gates of the hosts using red and green fluorescence vs. forward scatter to bin infected from healthy hosts. This resulted in distinct populations of likely infected hosts that were larger and had stronger fluorescence, which may signify active intracellular infection and growth via spores. The difference in cell abundance from predefined gates were used as a conservative estimate of host infection (% infected).

DOC analysis:

Bulk DOC samples (40 mL) were filtered per bottle at each time point through pre-combusted GF/F filters and stored in HDPE plastic bottles at -20 °C until analysis. DOC samples were analyzed using high temperature combustion on a TOC-V analyzer (Shimadzu) at the University of New Hampshire (Carlson et al. 2010). Bulk DOC samples were not run for the 4390 strain infection experiment.

Organism information:

TaxonomicName, Life Science Identifier (LSID), strain_identifier, organism_type
Scrippsiella acuminata, urn:lsid:marinespecies.org:taxname:1321853, RCC 1627, host
Amoebophrya sp., urn:lsid:marinespecies.org:taxname:109448, RCC 4390 and 4401, parasite

Data Processing Description

Cell concentrations (cells mL^{-1}) were calculated within the Guava InCyte software based on absolute cell counts measured in each gate and the volume of sample analyzed. Percent of hosts infected were estimated by

subtracting host cell counts from pre-defined gates on the cell sorter. DOC concentrations were calculated based on the instrument absorbance responses to known concentration standards.

Any samples that were not collected are represented by ‘nd’ (no data) [Note: at BCO-DMO, the missing data format will match the filetype you download (e.g. NaN for Matlab, and the default is blank for .csv files]. For example, percent of hosts infected was not considered for spore-only treatments. Some samples collected for metabolites were re-run on the mass spectrometer as a second aliquot of the same sample (e.g., 4401_infected_T4_A_v2). In these cases, the corresponding cell count data is marked by ‘nd’, as only one aliquot was run per sample on the flow cytometer.

BCO-DMO Processing Description

* Data table within the submitted file "parasite_counts.csv" was imported into the BCO-DMO data system for this dataset. Values "nd" imported as missing data values. Table will appear as Data File: 962736_v1_parasite_fcm_data.csv (along with other download format options).

Missing Data Identifiers:
* In the BCO-DMO data system missing data identifiers are displayed according to the format of data you access. For example, in csv files it will be blank (null) values. In Matlab .mat files it will be NaN values. When viewing data online at BCO-DMO, the missing value will be shown as blank (null) values.

* Taxonomic identifiers added from name matches at the World Register of Marine Species (WoRMS), they exactly matched known names there as of 2025-05-29.
* References added for the RCC strains cited in metadata.

Problem Description

The same gating was used for the host and spore samples across time points. There is not a corresponding .xml for every .fcs file; however, the .fcs files convey the same information as the xml files (like gating, settings, etc.), since the same gating information was used for all the .fcs files.

[table of contents | back to top]

Data Files

File	
962736_v1_parasite_fcm_data.csv	(Comma Separated Values (.csv), 5.34 KB) MD5:02988b0b5563d1e40f833a358fff1558
Primary data file for dataset ID 962736, version 1	

[table of contents | back to top]

Supplemental Files

File

Flow Cytometry Standard (FCS) and raw .xml files

filename: parasite_FCM_fcs_and_xml.zip

(ZIP Archive (ZIP), 2.91 MB)
MD5:7f73699155fb5256d3a2802fdc5d8267

Flow Cytometry Standard (FCS) and raw .xml files for flow cytometry samples that were measured as part of the parasite infection experiments to quantify cell concentrations of parasite spores (strains 4390 and 4401) and hosts daily for four days. Hosts samples were run live and parasite samples were run separately by staining cells with 100x SYBR Green (Thermo Fisher). All samples were run on a 96-well plate on a Guava easyCyte HT (Millipore) flow cytometer.

File naming convention for the 4390 strain infection experiment:

Filenames include the strain number, "host" or "spore," and the timepoint. Example filename "4390_host_T3.fcs"
The filenames are consistent with the prefix of the sample ID (excluding replicate number) included in the data table "962736_v1_parasite_fcm_data.csv"

The same gating was used for the host and spore samples across time points. There is not a corresponding .xml for every .fcs file; however, the .fcs files convey the same information as the xml files (like gating, settings, etc.), since the same gating information was used for all the .fcs files.

[table of contents | back to top]

Related Publications

Carlson, C. A., Hansell, D. A., Nelson, N. B., Siegel, D. A., Smethie, W. M., Khatiwala, S., Meyers, M. M., Halewood, E. (2010). Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. Deep Sea Research Part II: Topical Studies in Oceanography, 57(16), 1433–1445. doi:10.1016/j.dsr2.2010.02.013
Methods

Chen, T., Liu, Y., Hu, Z., Song, S., & Li, C. (2021). Chloroplast Ultrastructure and Photosynthetic Response of the Dinoflagellate Akashiwo sanguinea Throughout Infection by Amoebophrya sp. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.742498
Methods

Guillard, R. R. L. (1975). Culture of Phytoplankton for Feeding Marine Invertebrates. Culture of Marine Invertebrate Animals, 29–60. doi:10.1007/978-1-4615-8714-9_3
Methods

Kayal, E., Alves-de-Souza, C., Farhat, S., Velo-Suarez, L., Monjol, J., Szymczak, J., Bigeard, E., Marie, D., Noel, B., Porcel, B. M., Corre, E., Six, C., & Guillou, L. (2020). Dinoflagellate Host Chloroplasts and Mitochondria Remain Functional During Amoebophrya Infection. Frontiers in Microbiology, 11. https://doi.org/10.3389/fmicb.2020.600823
Methods

Long, M., Marie, D., Szymczak, J., Toullec, J., Bigeard, E., Sourisseau, M., Le Gac, M., Guillou, L., & Jauzein, C. (2021). Dinophyceae can use exudates as weapons against the parasite Amoebophrya sp. (Syndiniales). ISME Communications, 1(1). https://doi.org/10.1038/s43705-021-00035-x
Methods

Station Biologique de Roscoff (n.d.). RCC1627: Scrippsiella_acuminata. Roscoff Culture Collection. https://www.roscoff-culture-collection.org/rcc-strain-details/1627
Methods

Station Biologique de Roscoff (n.d.). RCC4390: Amoebophrya_sp. Roscoff Culture Collection. https://www.roscoff-culture-collection.org/rcc-strain-details/4390
Methods

Station Biologique de Roscoff (n.d.). RCC4401: Amoebophrya_sp. Roscoff Culture Collection. https://www.roscoff-culture-collection.org/rcc-strain-details/4401
Methods

Vaulot, D., Gall, F., Le Marie, D., Guillou, L., & Partensky, F. (2004). The Roscoff Culture Collection (RCC): a collection dedicated to marine picoplankton. Nova Hedwigia, 79(1–2), 49–70. https://doi.org/10.1127/0029-5035/2004/0079-0049
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

Anderson, S.R., Place, P.F., Poulson-Ellestad, K. & Harvey, E.L. (2025). Strain level differences in marine microbial parasite-host metabolites and host mortality dynamics. MetaboLights. https://www.ebi.ac.uk/metabolights/MTBLS11219

[table of contents | back to top]

Parameters

Parameter	Description	Units
Sample_name	Unique name specifying treatment, day of infection, and replicate	unitless
Infection_experiment	Experiment with 4401 or 4390 spores	unitless
Treatment	Infected cells, spore-only, or host-only	unitless
Time	Day of the infection cycle	days
Replicate	Treatment bottle replicate	unitless
Date	Date of sample collection	unitless
Cell_concentration_host	Concentration of hosts	Cells per ml (cells mL-1)
Cell_concentration_spore	Concentration of spores	Cells per ml (cells mL-1)
Percent_infected	Prevalence of infection	Percent infected hosts (%)
DOC_concentration	Bulk DOC concentration	Milligrams per liter (mg L-1)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Guava easyCyte HT flow cytometer (Millipore)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Guava easyCyte HT flow cytometer (Millipore) to measure host and spore concentrations.
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	Sony SH800Z sorting flow cytometer (Sony)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Sony SH800Z sorting flow cytometer (Sony) to estimate the percent of infected hosts.
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	Nikon eclipse TE300
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Nikon eclipse TE300 inverted microscope (Nikon) for visual inspection of the spores.
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

Dataset-specific Instrument Name	Shimadzu TOC-V CSH with TNM-1 and ASI-V Autosampler (Shimadzu)
Generic Instrument Name	Shimadzu TOC-V Analyzer
Dataset-specific Description	Shimadzu TOC-V CSH with TNM-1 and ASI-V Autosampler (Shimadzu) to analyze DOC samples for 4401 infection.
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

[table of contents | back to top]

Project Information

Characterizing plankton parasite-host metabolites and the response of heterotrophic bacteria (Plankton parasite-host metabolites)

Nearly half of annual primary production in the ocean is released by phytoplankton as labile metabolites, making up a large portion of dissolved organic carbon (DOC) that can fuel microbial food webs and mediate cell signaling and species interactions. Phytoplankton mortality is a major source of labile DOC, and yet, the contribution of different sources of mortality to carbon cycling remains unclear. This is especially true for parasitism. In recent genomics surveys, parasitic protists have been found to be widespread in the ocean and well-connected to potential phytoplankton hosts via network analysis. The family Amoebophryaceae (Syndiniales) are one of the ubiquitous and phylogenetically diverse groups of protist parasites, known to infect a range of hosts and drive shifts in microbial diversity and bloom phenology. Though not well quantified, these parasites are thought to have a similar impact on carbon cycling as viruses, with rapid host infection (2-3 d) and a rerouting of particulate carbon to labile DOC. In this project, we performed culture-based experiments and used untargeted metabolomics to profile labile DOC released over an infection cycle. Phytoplankton cultures included two strains of *Amoebophrya* sp. that infect the same dinoflagellate host (*Scrippsiella acuminata*), allowing us to explore new insights into strain-level infection dynamics. Separate incubation

experiments were also conducted to expose parasite-derived filtrate (collected fresh from cultures after host lysis) to natural bacterial communities. Samples were collected for metabarcoding and metatranscriptomics at discrete time points to characterize changes in bacterial community assembly and gene expression in the presence of labile DOC. This work aims to provide new perspectives on plankton parasitism and its role in biogeochemical cycling, which can inform future research directions in microbial parasite-host systems and lead to more accurate food web and ecosystem models in a changing ocean environment.

[table of contents | back to top]

Program Information

Center for Chemical Currencies of a Microbial Planet (C-CoMP)

Website: https://ccomp-stc.org/

Coverage: North Atlantic, BATS, global/other

Functions carried out by microscopic inhabitants of the surface ocean affect every aspect of life on our planet, regardless of distance from the coast. Ocean phytoplankton are responsible for half of the photosynthesis on Earth, the first step in a complex system that annually withdraws 50 billion metric tons of carbon from the atmosphere to sustain their growth. Of this, 25 billion metric tons participate in a rapid cycle in which biologically reactive material is released into seawater and converted back into carbon dioxide by marine bacteria within hours to days. The chemical-microbe network at the heart of this fast cycle remains poorly constrained; consequently, its primary currencies and controls remain elusive; its sensitivities to changing ocean conditions are unknown; and its responses to future climate scenarios are not predictable. The Center for Chemical Currencies of a Microbial Planet (C-CoMP) integrates research, education and knowledge transfer activities to develop a mechanistic understanding of surface ocean carbon flux within the context of a changing ocean and through increased participation in ocean sciences. C-CoMP supports science teams that merge biology, chemistry, modeling, and informatics to close long-standing knowledge gaps in the identities and dynamics of organic molecules that serve as the currencies of elemental transfer between the ocean and atmosphere. C-CoMP fosters education, outreach, and knowledge transfer activities that engage students of all ages, broaden participation in the next generation of ocean scientists, and extend novel open-science approaches into complementary academic and industrial communities. The Center framework is critical to this mission, uniquely facilitating an open exchange of experimental and computational science, methodological and conceptual challenges, and collaborations that establish integrated science and education partnerships. With expanded participation in ocean science research and ocean literacy across the US society, the next generation of ocean scientists will better reflect the diverse US population.

Climate-carbon feedbacks on the marine carbon reservoir are major uncertainties for future climate projections, and the trajectory and rate of ocean changes depend directly on microbial responses to temperature increases, ocean acidification, and other perturbations driven by climate change. C-CoMP research closes an urgent knowledge gap in the mechanisms driving carbon flow between ocean and atmosphere, with global implications for predictive climate models. The Center supports interdisciplinary science teams following open and reproducible science practices to address: (1) the chemical currencies of surface ocean carbon flux; (2) the structure and regulation of the chemical-microbe network that mediates this flux; and (3) sensitivity of the network and its feedbacks on climate. C-CoMP leverages emerging tools and technologies to tackle critical challenges in these themes, in synergy with existing ocean programs and consistent with NSF’s Big Ideas. C-CoMP education and outreach activities seek to overcome barriers to ocean literacy and diversify participation in ocean research. The Center is developing (1) initiatives to expand ocean literacy in K-12 and the broader public, (2) ocean sciences undergraduate curricula and research opportunities that provide multiple entry points into research experiences, (3) post-baccalaureate programs to transition undergraduates into graduate education and careers in ocean science, and (4) interdisciplinary graduate student and postdoctoral programs that prepare the next generation of ocean scientists. The C-CoMP team includes education faculty who evaluate the impacts of education and outreach activities and export successful STEM initiatives to the education community. C-CoMP is revolutionizing the technologies for studying chemical transformations in microbial systems to build understanding of the outsized impact of microbes on elemental cycles. Open science, cross-disciplinary collaborations, community engagement, and inclusive practices foster strategic advances in critical science problems and STEM initiatives. C-CoMP science, education, and

knowledge-transfer themes are efficiently addressed through a sustained network of scientists addressing critical research challenges while broadening the workforce that will tackle multi-disciplinary problems with academic, industrial and policy partners.

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.

The Program's Data Management Plan (DMP) is available as a PDF document.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2019589

[table of contents | back to top]