Phytoplankton exometabolite concentrations from laboratory cultures of Crocosphaera watsonii, Micromonas commoda, Prochlorococcus marinus, Synechococcus, Gephyrocapsa huxleyi, and Thalassiosira pseudonana collected from September to December 2022

Website: https://www.bco-dmo.org/dataset/991360
Data Type: experimental
Version: 1
Version Date: 2026-01-14

Project
» Phytoplankton Exometabolites (C-CoMP Phytoplankton Exometabolites)

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

Contributors	Affiliation	Role
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Abstract
Blank-corrected concentration data of 56 exometabolites were characterized in laboratory cultures of Gephyrocapsa huxleyi CCMP371, Crocosphaera watsonii WH8501, Micromonas commoda RCC299, Prochlorococcus marinus MIT9301, Synechococcus WH8102, and Thalassiosira pseudonana CCMP1335. Cultures were harvested during the exponential growth phase. Cell count data (available at BCO-DMO project 984095) were used to calculate cell-specific concentrations. Blank-corrected dissolved organic carbon (DOC) concentrations (available at BCO-DMO project 984095) were used to calculate each exometabolite's fraction contribution to phytoplankton-excreted DOC. Raw mass spectrometry files have been deposited to the MetaboLights repository (dataset MTBLS12895).

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Coverage

Temporal Extent: 2022-09-17 - 2025-02-09

Methods & Sampling

Standards, filtrate samples, and media blanks were derivatized with benzoyl chloride (BC) (Widner et al., 2021). The derivatization and sample preparation protocols together with pictures and video clips are available online (https://www.protocols.io/workspaces/kujawinski-lab). Briefly, each 5-milliliter (mL) sample was basified with 150 microliters (µL) of 8 M NaOH, derivatized with 1 mL BC working solution for 5 minutes, and then acidified with 75 µL phosphoric acid. The BC working solution was prepared by mixing 95 mL acetone (Optima, ACROS Organics) with 5 mL BC (99%, ACROS Organics) and was used within 36 hours after preparation. Two sets of stable isotopically labeled internal standards (SIL-IS) were prepared by derivatizing standard mixes of metabolites in seawater (aged, 0.1-µm filtered seawater collected from offshore Caribbean Sea) using BC working reagent prepared with $^{13}C_6$-BC (99% ^{13}C, Sigma-Aldrich) or D_5-BC (99% D, Cambridge Isotope Laboratories, Inc.), respectively. SIL-IS were derivatized following the same protocol as the samples.

Each derivatized standard, filtrate, or media blank was spiked with 498 pg addition of $^{13}C_6$-labeled SIL-IS and 9.96 nanograms (ng) addition of D_5-labeled SIL-IS and was dried using a vacufuge (Eppendorf) until ≥ 95% (by weight) of acetone was removed. Upon acetone removal, the liquid was transferred onto a preconditioned (6 mL of methanol followed by 24 mL of 0.01 M HCl) Bond Elut PPL cartridge (1g/6 mL, Agilent) and loaded by gravity. The samples were eluted with 6 mL of methanol by vacuum followed by evaporation to near dryness in a vacufuge. Samples were reconstituted with 100 µL 5% acetonitrile in Milli-Q water, vortexed thoroughly, and then centrifuged at 12000 g, 22 degrees Celsius (°C) for 5 minutes. The supernatant was transferred to LC vials with glass inserts, which were previously spiked with 5 µL of acetonitrile. The samples were stored at 4°C until LC-MS analyses.

Targeted metabolomics analyses were performed using an ultrahigh performance liquid chromatography system (Vanquish UHPLC, Thermo Scientific) coupled to a heated electrospray ionization source (H-ESI) and an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific). A Waters Acquity HSS T3 column (2.1 mm × 100 mm, 1.8 µm), equipped with an Acquity HSS T3 VanGuard Pre-column, was used for chromatographic separation at 40°C. The column was eluted at 0.5 mL per minute with a combination of solvents: A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile. The chromatographic gradient was as follows: 1% B (0-0.5 min), 10% B (0.5-2.0 min), 10% B (2.0-5.0 min), 25% B (5.0-7.0 min), 25% B (7.0-9.0 min), 50% B (9.0-12.5 min), 95% B (12.5-13.0 min), 95% B (13.0-14.5 min), 1% B (14.5-14.6 min), and 1% B (14.6-16.0 min). The autosampler was set at 4°C. Individual autosampler injections (5 µL each) were used for negative and positive ion mode analyses. The first 0.8 minutes of flow was diverted to waste after passing through the column. Other instrument parameters were: ESI voltages = 3600 V (positive) and 2600 V (negative); source gases = 55 (sheath), 20 (auxillary), and 1 (sweep); capillary temperature = 350°C; vaporizer temperature = 400°C. MS data from 170-1000 m/z were collected at resolution 60,000 FWHM (at m/z 200), automatic gain control (AGC) at 4e5, and max injection time 50 msec. MS/MS data were triggered using a targeted mass list with m/z and retention time window for each derivatized analyte, and collected at resolution 7,500 FWHM, AGC 5e4, and max injection time 22 milliseconds using higher energy collisional dissociation (HCD) with 35% collision energy and intensity threshold 2e4. Parent ions were isolated within the quadrupole at a width of 1 m/z.

Data Processing Description

Metabolite identification and peak area integration were performed using Skyline. MS data and retention times were used for peak identification and integration. MS/MS data were used to confirm the compound identification. Metabolite concentrations were quantified using calibration curves with peak area ratios of the non-labeled (light) metabolite added (ng) to the heavy-D_5 or heavy-$^{13}C_6$ internal standard. The MATLAB script (considerSkyline.m) used for processing Skyline output is available at the Kujawinski Lab SkyMat repository (https://github.com/KujawinskiLaboratory/SkyMat). For several metabolites, calculated concentrations exceeded the 11-point calibration curve's upper limit (e.g., 100 ng). To determine the limit of linearity (LOL), the standard curve was extended to 1500 ng. Glycerol 3-phosphate was the only metabolite with its concentrations in *T. pseudonana* filtrates (961-1108 ng) slightly exceeding the calculated LOL (1000 ng), while the concentrations of the rest of the 55 metabolites were all below the LOL. Therefore, no further correction was applied to the concentrations quantified using the 11-point calibration curve. Metabolite concentrations were filtered based on the calculated limit of detection (LOD), which was calculated using a subset standard curve taken from the

lower end of the 11-point calibration curve. The subset standard curve contains a minimum of 4 data points (a blank and three non-zero points) and encompasses the limit of quantification (LOQ). LOD and LOQ were calculated as 3.3 and 10 times the standard deviation of the y-intercept divided by the slope, respectively. Metabolite concentrations lower than the calculated LODs were replaced with zeros. For most of the metabolites, these steps yielded two data tables for each ionization mode, one based on $^{13}C_6$ normalization and the other based on D_5 normalization.

A matrix correction was conducted by comparing the 11-point calibration curve prepared in seawater and the 6-point matrix-matched standard curves prepared in media blanks and normalized with the corresponding SIL-IS. Statistical differences between the slopes of the two standard curves were assessed for each matrix type. If the slopes were statistically different, a matrix correction factor was calculated by dividing the slope of the matrix-matched calibration curve by the slope of the calibration curve prepared in seawater. The adjusted concentration was then determined by dividing the metabolite concentration by this matrix correction factor.

To choose which SIL-IS and ion mode to use, the 95% prediction interval for the metabolite concentration at each sample point was calculated using each calibration curve. For each metabolite, the SIL-IS that yielded a standard curve with the lowest average 95% prediction interval across the range of sample concentrations was selected. For metabolites analyzed in both positive and negative ion modes, we selected the ion mode with the lowest 95% prediction interval for the selected calibration curve.

In the final data table, presence of exometabolites was determined based on whether their concentrations in filtrates are statistically higher (Student's t-test, $P < 0.05$) than the media blanks.

BCO-DMO Processing Description

- Imported original file "Phytoplankton_exometabolite_abundance_table.xlsx" into the BCO-DMO system.
- Replaced the non-standard character "γ" with "gamma" in the "metabolites" column.
- Saved the final file as "991360_v1_pxm1_exometabolite_concentration.csv"

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Related Publications

Widner, B., Kido Soule, M. C., Ferrer-González, F. X., Moran, M. A., & Kujawinski, E. B. (2021). Quantification of Amine- and Alcohol-Containing Metabolites in Saline Samples Using Pre-extraction Benzoyl Chloride Derivatization and Ultrahigh Performance Liquid Chromatography Tandem Mass Spectrometry (UHPLC MS/MS). Analytical Chemistry, 93(11), 4809–4817. https://doi.org/10.1021/acs.analchem.0c03769
Methods

Zhu, Y., Anderson, H. S., Salcedo, E., Miller, S. E., Longnecker, K., Soule, M. C. K., Haley, S. T., Swarr, G. J., Braakman, R., Dyhrman, S. T., & Kujawinski, E. B. (2025). Characterization of Phytoplankton-Excreted Metabolites Mediating Carbon Flux through the Surface Ocean. https://doi.org/10.1101/2025.11.04.686593
Results

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Related Datasets

IsRelatedTo

Zhu, Y., Anderson, H., Gray, L., Kujawinski, E., Dyhrman, S. T., Braakman, R. (2026) **Phytoplankton cell count data from laboratory cultures of Crocosphaera watsonii, Micromonas commoda, Prochlorococcus marinus, Synechococcus, Gephyrocapsa huxleyi, and Thalassiosira pseudonana collected from September to December 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-01-14 http://lod.bco-dmo.org/id/dataset/991400 [view at BCO-DMO]

Zhu, Y., Anderson, H., Gray, L., Longnecker, K., Kujawinski, E., Dyhrman, S. T., Braakman, R. (2025)

Dissolved organic carbon data from laboratory cultures of Crocosphaera watsonii, Micromonas commoda, Prochlorococcus marinus , Synechococcus, Gephyrocapsa huxleyi, and Thalassiosira pseudonana collected from September to December 2022. Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-01-16 http://lod.bco-dmo.org/id/dataset/991509 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
species	phytoplankton strain grown in the experiment	unitless
strain	CCMP strain identifier of the phytoplankton species	unitless
metabolites	name of the identified metabolite	unitless
presence	whether an exometabolite was identified as present (Y/N)	unitless
metabolite_concentration	blank-corrected, nanomolar metabolite concentration	nanomoles per liter (nmol/L)
carbon_concentration	blank-corrected, metabolite carbon concentration	nanomoles carbon per liter (nmol C/L)
cell_specific_concentration	blank-corrected, cell-specific concentration of metabolites	attomoles per cell (amol/cell)
carbon_fraction	blank-corrected, metabolite's contribution to phytoplankton-excreted DOC	unitless

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Instruments

Dataset-specific Instrument Name	Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific)
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Ultrahigh performance liquid chromatography system (Vanquish UHPLC, Thermo Scientific) coupled to a heated electrospray ionization source (H-ESI) and an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific)
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

Dataset-specific Instrument Name	Ultrahigh performance liquid chromatography system (Vanquish UHPLC, Thermo Scientific)
Generic Instrument Name	Ultra-high-performance liquid chromatography
Dataset-specific Description	Ultrahigh performance liquid chromatography system (Vanquish UHPLC, Thermo Scientific) coupled to a heated electrospray ionization source (H-ESI) and an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific)
Generic Instrument Description	Ultra high-performance liquid chromatography: Column chromatography where the mobile phase is a liquid, the stationary phase consists of very small (< 2 microm) particles and the inlet pressure is relatively high.

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Project Information

Phytoplankton Exometabolites (C-CoMP Phytoplankton Exometabolites)

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

Coverage: Lab study

The Center for Chemical Currencies of a Microbial Planet (C-CoMP) is focused on understanding marine chemical currencies. This project examines exometabolites released from representative taxa of marine phytoplankton to better characterize the composition of labile marine dissolved organic matter and understand the biological sources of these metabolites to the marine environment. Specifically, this project integrates novel metabolomics, genomics, transcriptomics, and proteomics methods to identify extracellular metabolites and link them with their production pathways under environmentally relevant conditions.

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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.

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Funding

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

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