# Bacterial transcriptional response to picoeukaryote Micromonas commoda

**Website**: https://www.bco-dmo.org/dataset/928039
**Data Type**: experimental
**Version**: 1
**Version Date**: 2024-07-15

**Project**
» Effects of Climate Change Variables on Microbial Autotroph-Heterotroph Carbon Flux (CC_Auto_Hetero_Fluxes)

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|---|---|---|
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**Abstract**
Marine biogeochemical cycles are built on interactions between surface ocean microbes, particularly those connecting phytoplankton primary producers to heterotrophic bacteria. However, direct influences of bacteria on phytoplankton physiology are poorly known. In this study, three marine bacteria (Ruegeria pomeroyi DSS-3, Stenotrophomonas sp. SKA14, and Polaribacter dokdonensis MED152) were co-cultured with green alga Micromonas commoda, and the phytoplankter's transcriptome was studied by RNASeq. The presence of each bacterium invoked transcriptomic remodeling by M. commoda after 8 h in co-culture. Some aspects of the algal transcriptomic response were conserved across all three bacteria, while others were restricted to a single bacterium. M. commoda had both rapid and extensive responses to heterotrophic bacteria.

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## Coverage

**Spatial Extent**: **Lat**:33.9519 **Lon**:-83.3576
**Temporal Extent**: 2021 - 2021

## Dataset Description

The data are linked to Hamilton et al., 2024 (see related publications).

### Methods & Sampling

Axenic cultures of *Micromonas commoda* RCC299 (National Center for Marine Algae, NMCA) were grown in 1 L of organic-carbon free defined medium L1-Si [31] as modified by NCMA (https://ncma.bigelow.org/) at a salinity of 35 in 1900 mL vented polystyrene tissue culture flasks. Flasks were maintained at 18 oC under 16 h light at 160 µmol photons m−2s−1 and 8 h dark. Pre-cultures of *Micromonas* were sequentially upscaled (50 ml, 200 ml, 1 L) with transfers occurring during the exponential growth phase. After growing for 7 d (early stationary growth phase; ~2.7 × 106 cells ml−1), three marine bacteria pre-grown in YTSS medium (*Ruegeria pomeroyi* DSS-3, *Stenotrophomonas* sp. SKA14, and *Polaribacter dokdonensis* MED152 were washed 5 times in sterile L1 medium at 6000 RCF and inoculated into the axenic cultures at ~106 cells ml−1. Three or four replicate co-cultures were established for each bacterial strain and also for an axenic phytoplankton control. Three additional treatments were established with bacterial strains introduced individually into L1 medium with 400 µM C glucose as the sole carbon source (which supports all 3) at the same initial cell concentration as the co-cultures. As this treatment contained a single, known metabolite, it served as a control for co-culture transcriptome analysis. Bacterial contamination of the axenic phytoplankton cultures was ruled out based on lack of colony formation from culture aliquots spread onto YTSS plates and absence of bacterial-size particles in flow cytometry scattergrams.

### Data Processing Description

Bacterial cell counts: Cell counts were periodically obtained by flow cytometry. Samples were fixed at a final concentration of 1% glutaraldehyde, incubated at 4°C for 20 min, and stored at −80°C. Just prior to analysis, an internal standard of 5 µm fluorescent particles (ACFP-50-5; Spherotech, Lake Forest, IL, USA) was added, followed by staining for 15 min with SYBR Green I (final concentration 0.75X; Life Technologies, Waltham, MA, USA). Samples were analyzed on an Agilent Quanteon flow cytometer (Acea, Biosciences Inc, San Diego CA) with a 405 nm laser using a 530/30 bandpass filter for SYBR Green.

Micromonas cell counts: Cell counts were periodically obtained by flow cytometry. Samples were fixed at a final concentration of 1% glutaraldehyde, incubated at 4°C for 20 min, and stored at −80°C. Just prior to analysis, an internal standard of 5 µm fluorescent particles (ACFP-50-5; Spherotech, Lake Forest, IL, USA) was added, followed by staining for 15 min with SYBR Green I (final concentration 0.75X; Life Technologies, Waltham, MA, USA). Samples were analyzed on an Agilent Quanteon flow cytometer (Acea, Biosciences Inc, San Diego CA) with a 405 nm laser using a 695/40 bandpass filter for chlorophyll a (phytoplankton).

Nutrient analysis: At the 8 h time point, 50 ml of each sample was used for nutrient analysis. Samples were filtered through 0.2 µm pore-size 47 mm Supor filters to remove cells. The filtrate was frozen and stored at -20°C. Nutrient analyses were performed by the University of Georgia Laboratory of Environmental Analysis. Concentrations of nitrate (NO3-), nitrite (NO2-), and phosphate (PO43-) were measured using ion chromatography on a DX500 Ion Chromatograph (Dionex Co.) with an initial cartridge treatment (OnGuard-Ag cartridge from Dionex) performed to remove chloride ions. Measurements for ammonium (NH4+) were done separately via the phenate method with spectrophotometric analysis on a Model Spectronic 21D (Spectronic Instrumentation).

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## Data Files

| File | |
|---|---|
| **928039_v1_micromonas.csv** | (Comma Separated Values (.csv), 3.63 KB)<br>MD5:8c540761991125d327926458cc9e6aa5 |
| Primary data file for dataset ID 928039, version 1 | |

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## Related Publications

Ferrer-González, F. X., Hamilton, M., Smith, C. B., Schreier, J. E., Olofsson, M., & Moran, M. A. (2023). Bacterial

transcriptional response to labile exometabolites from photosynthetic picoeukaryote Micromonas commoda. ISME Communications, 3(1). https://doi.org/10.1038/s43705-023-00212-0
*Methods*

Guo, J., Wilken, S., Jimenez, V., Choi, C. J., Ansong, C., Dannebaum, R., Sudek, L., Milner, D. S., Bachy, C., Reistetter, E. N., Elrod, V. A., Klimov, D., Purvine, S. O., Wei, C.-L., Kunde-Ramamoorthy, G., Richards, T. A., Goodenough, U., Smith, R. D., Callister, S. J., & Worden, A. Z. (2018). Specialized proteomic responses and an ancient photoprotection mechanism sustain marine green algal growth during phosphate limitation. Nature Microbiology, 3(7), 781–790. https://doi.org/10.1038/s41564-018-0178-7
*Methods*

Hamilton, M., Ferrer-González, F. X., & Moran, M. A. (2024). Heterotrophic bacteria trigger transcriptome remodelling in the photosynthetic picoeukaryote Micromonas commoda. Environmental Microbiology Reports, 16(3). Portico. https://doi.org/10.1111/1758-2229.13285
*Results*

Price, M. N., Wetmore, K. M., Waters, R. J., Callaghan, M., Ray, J., Liu, H., Kuehl, J. V., Melnyk, R. A., Lamson, J. S., Suh, Y., Carlson, H. K., Esquivel, Z., Sadeeshkumar, H., Chakraborty, R., Zane, G. M., Rubin, B. E., Wall, J. D., Visel, A., Bristow, J., … Deutschbauer, A. M. (2018). Mutant phenotypes for thousands of bacterial genes of unknown function. Nature, 557(7706), 503–509. https://doi.org/10.1038/s41586-018-0124-0
*Methods*

Worden, A. Z., Lee, J.-H., Mock, T., Rouzé, P., Simmons, M. P., Aerts, A. L., Allen, A. E., Cuvelier, M. L., Derelle, E., Everett, M. V., Foulon, E., Grimwood, J., Gundlach, H., Henrissat, B., Napoli, C., McDonald, S. M., Parker, M. S., Rombauts, S., Salamov, A., … Grigoriev, I. V. (2009). Green Evolution and Dynamic Adaptations Revealed by Genomes of the Marine Picoeukaryotes Micromonas. Science, 324(5924), 268–272. https://doi.org/10.1126/science.1167222
*Methods*

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Bottle_ID | Bottle ID | unitless |
| Treatment | Lab treatment description: DSS-3, MED152, SKA14, axenic | unitless |
| Time_h | Time after start of experiment | hour (h) |
| Micromonas_cells_ml | Micormonas cell count | cells per milliliter (cell/ml) |
| Bacteria_cells_ml | Bacteria cell count | cells per milliliter (cell/ml) |
| NH4_uM | Ammonium (NH4+) concentration | micromolar (uM) |
| NO3_uM | Nitrate (NO3-) concentration | micromolar (uM) |
| PO4_uM | Phosphate (PO43-) concentration | micromolar (uM) |
| NCBI_Sample_ID | Identifier of lab sample | unitless |
| Accession | NCBI Biosample Accession | unitless |
| BioProject | NCBI Bioproject Accession | unitless |
| Organism | Organism name | unitless |
| Taxonomy_ID | Taxonomy ID | unitless |
| Description | Sample description | unitless |

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## Instruments

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|---|---|
| **Dataset-specific Instrument Name** | Agilent Quanteon flow cytometer (Acea, Biosciences Inc, San Diego CA) |
| **Generic Instrument Name** | Flow Cytometer |
| **Dataset-specific Description** | Agilent Quanteon flow cytometer (Acea, Biosciences Inc, San Diego CA) with a 405 nm laser using a 530/30 bandpass filter for SYBR Green for bacterial cell counts. 405 nm laser using a 695/40 bandpass filter for chlorophyll a (phytoplankton) was used for micromonas 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) |

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|---|---|
| **Dataset-specific Instrument Name** | DX500 Ion Chromatograph (Dionex Co.) |
| **Generic Instrument Name** | Ion Chromatograph |
| **Dataset-specific Description** | DX500 Ion Chromatograph (Dionex Co.) with an initial cartridge treatment (OnGuard-Ag cartridge from Dionex) performed to remove chloride ions. |
| **Generic Instrument Description** | Ion chromatography is a form of liquid chromatography that measures concentrations of ionic species by separating them based on their interaction with a resin. Ionic species separate differently depending on species type and size. Ion chromatographs are able to measure concentrations of major anions, such as fluoride, chloride, nitrate, nitrite, and sulfate, as well as major cations such as lithium, sodium, ammonium, potassium, calcium, and magnesium in the parts-per-billion (ppb) range. (from http://serc.carleton.edu/microbelife/research_methods/biogeochemical/ic....) |

| | |
|---|---|
| **Dataset-specific Instrument Name** | Model Spectronic 21D (Spectronic Instrumentation) |
| **Generic Instrument Name** | Spectrophotometer |
| **Dataset-specific Description** | Measurements for ammonium (NH4+) were done separately via the phenate method with spectrophotometric analysis on a Model Spectronic 21D (Spectronic Instrumentation). |
| **Generic Instrument Description** | An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples. |

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## Project Information

**Effects of Climate Change Variables on Microbial Autotroph-Heterotroph Carbon Flux (CC_Auto_Hetero_Fluxes)**

**Coverage**: Laboratory in Athens, GA, USA, and waters around Sapelo Island, GA, USA

*NSF Award Abstract:*
Phytoplankton in the surface ocean are responsible for roughly half of all photosynthesis on the planet. Much of the organic material created by these photosynthetic organisms is ultimately consumed by diverse marine bacteria with differing preferences for specific types of chemical compounds. This project investigates how climate change (temperature and CO2) might alter the types and amounts of organic compounds produced by different species of marine phytoplankton and the types and amounts of compounds transferred from phytoplankton to marine bacteria. Shifts in organic compounds transferred to bacteria could alter the distribution of bacterial species in the ocean, their growth rates and efficiencies, and flows of energy through the global ocean. This project helps scientists better understand the effects of climate change on marine ecosystems. Two graduate students and a postdoctoral researcher are supported by the project, receiving interdisciplinary training in biology, chemistry, and ocean sciences. Summer research internships in the PIs' laboratories are offered to AP Biology students enrolled at Cedar Shoals High School in Athens, GA, a school that serves a diverse social and economic community.

Much of the bacterial secondary production in the surface ocean is supported by rapid uptake of labile metabolites released from phytoplankton, either directly through excretion and diffusion or indirectly through lysis and predation. This project investigates the effects of two climate change variables (temperature and CO2) on the metabolite pools produced and released by three model phytoplankton species (a diatom, a coccolithophore, and a cyanobacterium) and assesses changes in the composition and fate of metabolites transferred to bacteria. Phytoplankton species are being grown axenically at two different temperatures and CO2 concentrations in a factorial design and endo- and exometabolite composition is determined using NMR. A suite of phytoplankton physiological characteristics is measured and evaluated in the context of metabolite composition. Experiments with heterotrophic bacteria (either model bacteria or natural bacterial communities) are being conducted to assess the effects of climate change variables on metabolite transfer from phytoplankton to marine bacteria. In the first experiment type, bacteria are co-cultured with the phytoplankton at different temperatures and CO2 concentrations, and changes in bacterial gene expression and metabolite concentrations are used to assess shifts in the composition of metabolites transferred. In the second type, bacteria are grown on phytoplankton metabolite pools produced at different temperatures and CO2 concentrations in high-throughput bioassays, and changes in bacterial traits (growth rate, carrying capacity, growth efficiency) resulting from the different climate scenarios are used to indicate changes in metabolite quality. Knowledge of how the heterotrophic processing of phytoplankton metabolites might shift in response to climate change allows better prediction of Earth's future carbon cycle.

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.

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## Funding


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


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