TEP concentrations of co-cultures and axenic cultures of Minutocellus polymorphus and particle-associated marine bacteria

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Data Type: experimental
Version: 1
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Project
» Aggregation of Marine Picoplankton (Marine Plankton Aggregation)

Contributors	Affiliation	Role
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Abstract
The aggregation of phytoplankton leads to the settling of particulate organic carbon in the form of marine snow, making it an important process in marine biogeochemical cycles. To better understand the particle behavior of diatoms <20 microns in size, laboratory growth experiments to study diatom aggregation and production of transparent exopolymeric particles were performed. Triplicate cultures of Minutocellus polymorphus (CCMP497) with and without the addition of known particle-associated marine bacteria (M. adhaerens, P. carrageenovora, and V. thalassae) were sampled and measured for TEP concentration.

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Coverage

Spatial Extent: **Lat**:33.41941012097 **Lon**:-111.92828937936
Temporal Extent: 2021 - 2022

Methods & Sampling

In this study, one diatom and three bacteria species were grown and measured. *Minutocellus polymorphus* were incubated with or without the addition of bacteria in flasks and sampled throughout their growth (representative of bloom conditions), to determine the production of transparent exopolymeric particles (TEP) and the formation of micro-aggregates, and in roller tanks to investigate the formation of sinking aggregates (representative of end of bloom conditions). Additional details are in Cruz & Neuer, 2022.

Two growth experiments were carried out independently:
1. Growth experiment 1 with the addition of *Marinobacter adhaerens*
2. Growth experiment 2 with the addition of *Pseudoalteromonas carrageenovora* and *Vibrio thalassae*

Stock cultures of marine Minutocellus polymorphus (CCMP497, National Center for Marine Algae and Microbiota, NCMA) were maintained in L1 medium prepared in artificial seawater and incubated in an environmental growth chamber (Conviron) at 23 ± 1 °C. Stock cultures of *Vibrio thalassae* (DSM102810, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH), *Pseudoalteromonas carrageenovora* (DSM6820, DSMZ), and *Marinobacter adhaerens* HP15 were maintained on Marine Agar (BD Difco 2216, Becton Dickinson, NJ; ZoBell, 1941) plates at 23 ± 1 °C.

Triplicate cultures of *Minutocellus polymorphus* (CCMP497) with and without the addition of known particle-associated marine bacteria (*M. adhaerens*, *P. carrageenovora,* and *V. thalassae*) were sampled every other day for 19-23 days for the quantification of:

- single cells [Cell abundance dataset]
- suspended microaggregates (aggregates ca. 5-60 µm) [Suspended Microaggregate dataset]
- TEP (Transparent Exopolymeric Particles) [this dataset]

This dataset presents Transparent Exopolymeric Particle (TEP) concentrations. The other measurements can be found in the Related Datasets listed below.

TEP concentrations in the co-cultures and axenic cultures were determined as described by Passow and Alldredge (1995). 10 mL of glutaraldehyde-fixed culture samples were filtered through duplicate 0.4 µm pore-size polycarbonate membranes (GVS Life Technologies, ME) at a low and constant vacuum pressure (100 mm Hg). The retained TEP was subsequently stained with 0.5 mL of the acidic polysaccharide-specific Alcian Blue (AB) dye (8GX, Sigma-Aldrich), followed by a 0.5 mL rinse with MilliQ water for the removal of excess stain and stored at −40 °C until analysis. Prior to staining, the pre-calibrated 0.02% (w/v) AB working solution that was pH-adjusted with 0.06% (v/v) acetic acid (final pH 2.5) was passed through a 0.2 µm Acrodisc syringe filter (Pall Corporation, NY) to remove the undissolved dye. Membranes were soaked in 6 mL of 80% (v/v) sulfuric acid for 3 h to extract the AB-stained TEP and absorption was then measured using a spectrophotometer (Shimadzu UV-1900i, Shimadzu, Kyoto, JP) at 787 nm. Duplicate stained filters with sterile media functioned as blanks.

TEP concentrations were calculated using a calibration factor of the Alcian-Blue dye determined with xanthan gum (f-factors: 81.70 for experiments with M. adhaerens and 83.83 for experiments with V. thalassae and P. carrageenovora) and expressed in µg of xanthan gum equivalent units (µg XG eq.) as described by Bittar et al. (2018). To compare TEP concentrations between treatments and with other phytoplankton groups, concentrations were normalized to diatom cell abundances and biovolumes, respectively.

For additional Methods details, see Cruz & Neuer, 2022.

Data Processing Description

BCO-DMO Processing Notes:
- Excel sheet "M.polymorphus_Microaggregates_and_TEP-TEP Concentration.xlsx " was processed and saved as a CSV file
- added a conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- blank values in this dataset are displayed as "nd" for "no data." nd is the default missing data identifier in the BCO-DMO system.

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Related Publications

Bittar, T. B., Passow, U., Hamaraty, L., Bidle, K. D., & Harvey, E. L. (2018). An updated method for the calibration of transparent exopolymer particle measurements. Limnology and Oceanography: Methods, 16(10), 621–628. doi:10.1002/lom3.10268
Methods

Cruz, B. N., & Neuer, S. (2022). Particle-associated bacteria differentially influence the aggregation of the marine diatom Minutocellus polymorphus. ISME Communications, 2(1). https://doi.org/10.1038/s43705-022-00146-z
Results

,
Methods

Iuculano, F., Mazuecos, I. P., Reche, I., & Agustí, S. (2017). Prochlorococcus as a Possible Source for Transparent Exopolymer Particles (TEP). Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.00709
Methods

Passow, U., & Alldredge, A. L. (1995). A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP). Limnology and Oceanography, 40(7), 1326–1335. doi:10.4319/lo.1995.40.7.1326
Methods

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Related Datasets

IsRelatedTo

Neuer, S., Cruz, B. N., Cadillo-Quiroz, H. (2022) **Abundance of aggregates of the marine diatom Minutocellus polymorphus and particle-associated marine bacteria from culture and roller tank experiments in 2021-2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-06-27 http://lod.bco-dmo.org/id/dataset/876461 [view at BCO-DMO]

Neuer, S., Cruz, B. N., Cadillo-Quiroz, H. (2022) **Laboratory data on cell abundance of Minutocellus polymorphus in experiments measuring TEP and production of microaggregates.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-11-04 http://lod.bco-dmo.org/id/dataset/882570 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Experiment_ID	Experiment ID describing the experiment in which the organism grew	unitless
Culture	Experimental culture, listing the organisms grown	unitless
Culture_type	Culture type, either co-culture or axenic (diatom-only control)	unitless
Days	Days since start of experiment	days
TEP_Conc_rep1	Replicate 1 concentration of transparent exopolymeric particles	micrograms of xanthan gum equivalents per milliliter (ug XG per mL)
TEP_Conc_rep2	Replicate 2 concentration of transparent exopolymeric particles	micrograms of xanthan gum equivalents per milliliter (ug XG per mL)
TEP_Conc_rep3	Replicate 3 concentration of transparent exopolymeric particles	micrograms of xanthan gum equivalents per milliliter (ug XG per mL)

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Instruments

Dataset-specific Instrument Name	Multisizer 3 Particle Counter
Generic Instrument Name	Coulter Counter
Dataset-specific Description	Suspended microaggregates were quantified using a Multisizer 3 Particle Counter
Generic Instrument Description	An apparatus for counting and sizing particles suspended in electrolytes. It is used for cells, bacteria, prokaryotic cells and virus particles. A typical Coulter counter has one or more microchannels that separate two chambers containing electrolyte solutions. from https://en.wikipedia.org/wiki/Coulter_counter

Dataset-specific Instrument Name	Shimadzu UV-1900i
Generic Instrument Name	UV Spectrophotometer-Shimadzu
Dataset-specific Description	TEP absorption was measured using a spectrophotometer (Shimadzu UV-1900i, Shimadzu, Kyoto, JP) at 787 nm.
Generic Instrument Description	The Shimadzu UV Spectrophotometer is manufactured by Shimadzu Scientific Instruments (ssi.shimadzu.com). Shimadzu manufacturers several models of spectrophotometer; refer to dataset for make/model information.

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Project Information

Aggregation of Marine Picoplankton (Marine Plankton Aggregation)

Coverage: Bermuda Atlantic Time-Series station

NSF abstract:

Marine phytoplankton are microscopic algae that live in the sunlit zone of the ocean. They play an important role in the uptake of carbon dioxide from the atmosphere through photosynthesis, similar to what plants do on land, and are the basis of the marine food web. However, instead of storing this organic carbon in leaf tissue and roots, marine phytoplankton are grazed by planktonic animals, or die and subsequently sink out of the sunlit zone in the form of aggregates, also called "Marine Snow". These particles not only export the organic carbon contained in their cells to the deep ocean, but also serve as food for animals and bacteria that live in the deep. A considerable portion of these phytoplankton are extremely small, among the tiniest of all organisms known. These extremely small cells have not been thought to play an important role in the formation and sinking of marine snow; however, recent findings challenge this view. This project will investigate how the smallest of these phytoplankton contribute to the rain of sinking particles from the sunlit surface to the deep ocean. This research is important because, in some of the largest expanses of the open oceans, these minute cells dominate the phytoplankton community, and larger plankton organisms are very sparse. The project, through a combination of work in the laboratory and at a field station, will shed light on how these tiny phytoplankton cells make aggregates, which ultimately enable them to sink as "Marine Snow". The project also provides unique opportunities for undergraduate students at Arizona State University, a land-locked public university, to gain experience in working with marine research. The project will serve to educate one PhD student, one MS student in an accelerated BS-MS program, and 8-10 undergraduate students/semester in a unique, inquiry based learning effort termed Microbial EducatioN Training and OutReach (MENTOR). The undergraduate students will also participate in Arizona State University (ASU)'s School of Life Sciences, Undergraduate Research Program (SOLUR), which seeks to increase the participation of minorities in science. They will also contribute towards developing web and classroom materials, based on this project, which will then be distributed through a partnership with the award-winning ASU-sponsored Ask A Biologist K-12 web site.

The oceanic "biological carbon pump", the photosynthetically mediated transformation of dissolved inorganic carbon into particulate and dissolved organic carbon and its subsequent export to deep water, functions as a significant driver of atmospheric carbon uptake by the oceans. The traditional view of the biological carbon pump in the ocean is that of sinking of large aggregates (marine snow) or fecal pellets, which are made up of large, mineral ballasted cells of phytoplankton. However, recent evidence, stemming from in situ investigations of particulate matter, trap studies and modelling studies, have shown that micron-sized phytoplankton such as picocyanobacteria as well as picoeukaryotes can contribute significantly to the sinking of particulate matter. The specific mechanisms behind the sinking of these micrometer sized cells remain elusive as the cells are too small to sink on their own, and mesozooplankton is likely unable to ingest single cells. Intriguingly, recent research by the investigators has shown that the ubiquitous picocyanobacteria Synechococcus are able to

form aggregates and sink at velocities comparable to those of marine snow. They found that the matrix of the Synechococcus aggregates was made of Transparent Exopolymeric Particles (TEP), and that TEP production was enhanced under nutrient limited culture conditions. Interaction with clays and presence of heterotrophic bacteria also enhanced aggregation and sinking velocity. This study aims to further investigate aggregation of other common picoplankton in the laboratory and aggregation occurring in natural settings at an oligotrophic open ocean site, the Bermuda Atlantic Time-series Site (BATS). Ultimately, this project will increase and refine our understanding of the role of the smallest phytoplankton in aggregation and sinking - information vital to understanding carbon cycling processes in the oceans.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1658527

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