ATP and cell numbers in laboratory experiments with Thalassiosira weissflogii conducted in 2019

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Project
» Collaborative Research: Transforming Carbon in the Deep Sea (Carbon in the Deep Sea)

Contributors	Affiliation	Role
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Abstract
This dataset includes ATP and cell numbers from batch culture experiments with Thalassiosira weissflogii. ATP was measured with a new extraction and analytical protocol and cell numbers were determined using a Coulter Counter at three temperatures (15, 20, 25 degrees Celsius). Experiments were conducted in 2019. These data are published in Figure 3 of Bochdansky et al., 2021 (doi:10. 1002/lom3.10409)

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Coverage

Spatial Extent: **Lat**:36.88493 **Lon**:-76.30765
Temporal Extent: 2019-06-19 - 2019-07-10

Methods & Sampling

Complete methodology published in Bochdansky et al. (2021). Data are in Figure 3 of Bochdansky et al. (2021). The experiments were conducted from June 19 to July 10, 2019 at Old Dominion University.

Growth rate experiments with *Thalassiosira weissflogii*
Experiments were performed at room temperature (~ 22° Celsius). In all experiments, sampling occurred daily, beginning at the day of inoculation of the f/2^{+} medium. After agitating the flasks to resuspend and evenly mix settled cells, 25 ml were withdrawn from each flask using a sterile disposable pipette. Then, 5 ml were immediately filtered through a 25 mm diameter, 0.2 um polycarbonate filter (Isopore type GTTP) at a vacuum of 200 mbar in a filter station preloaded with six filters. The filtrate was captured in 15 ml polypropylene centrifuge tubes (Falcon) placed below the filter funnel inside the vacuum flasks. The filters were quickly transferred into 15 ml polypropylene centrifuge tubes, and ~ 4.5 ml boiling-hot ultrapure water (18.2 MΩ) was added. The centrifuge tubes were vortexed for a few seconds and then immersed in a beaker containing hot water (> 90 °C). The hot ultrapure water breaks up cells and inactivates ATPases. For unfiltered whole water, triplicate samples of 0.5 ml were added to 15 ml centrifuge tubes chased by ~ 4 ml boiling-hot water and

vortexed. Of the filtrate, 0.5 ml was transferred to the centrifuge tubes and treated in the same fashion as the whole water samples. All tubes were kept in hot-water baths for approximately 15 minutes. The samples were subsequently cooled to room temperature and analyzed the same day. Using a muffled Pasteur pipette, ultrapure water was added dropwise to exactly the 5 ml mark (markings on the 15 ml Falcon centrifuge tubes are sufficiently accurate). For analysis, 50 ul of sample, 3 ml of ultrapure water, and 50 ul of the ATP working stock (for the internal standard only, see below), and 50 ul of firefly extract (CellTiter-Glo 2.0, Promega Corp.) were combined in 6 ml plastic scintillation vials (Pico Prias, Perkin Elmer) and briefly vortexed.

The scintillation counter was programmed to run samples in sequence repeatedly up to 10 times. Data of the second cycle only were used in the analysis. Samples and corresponding internal standards were run alternately.

Standards
Adenosine-5'-triphosphate disodium salt hydrate (Millipore Sigma) was dissolved ultrapure water (Barnstaed Nanopure, 18.2 MΩ) and serially diluted to a stock concentration of ~10 uM using ultrapure water. The stock solution was then divided into individual 15-ml polypropylene centrifuge vials and stored frozen at -20 °C. The exact concentration of the ~10 uM standard was determined using a spectrophotometer (Shimadzu UV-2401PC) at a wavelength of 259 nm, a 1 cm cuvette and a molar absorptivity coefficient of 15.4 x 10^3 M^{-1} cm^{-1} (Karl 1993) in the equation:

$C = A/(l*e)$

where C is the molar concentration of ATP (M), A the absorption, l the pathlength of the cuvette (cm), and e the molar absorptivity coefficient (M^{-1} cm^{-1}). The concentration of the stock solution was 16.4 uM throughout the experiment. A working stock solution was prepared daily by diluting 250 ul of the stock solution to 250 ml with ultrapure water in a volumetric flask (1000x dilution to 10 nM). This working stock was added as the internal standard (see above).

Calculations
ATP concentrations in the samples were calculated as displayed in Equation 2:

ATP (nM) = ([ATP] * (CPM_sample - CPM_blank)) / (CPM_standards - CPM_samples) * (V_{std} * R) / V_{extr}

where [ATP] is the concentration of the internal standard (i.e., 16.4 nM), CPM_sample the photon counts per minute for the sample, CPM_blank the average value of 4 to 6 blanks (50 ul of firefly reagent added to 3 ml ultrapure water only), CPM_standards the average counts per minute for the standard vials, CPM_samples the average value of the samples, V_{std} the volume of the standard added to the scintillation vial in ul, R the ratio between volume of the extract (numerator) and volume of sample filtered (denominator), and V_{extr} the volume of the extract added to the scintillation vial (ul). Internal standard values were regressed against time. This regression was applied to every sample based on its time stamp in the counting protocol. This procedure, rather than using the values from the adjacent vials, reduces variance caused by standard-to-standard variability. To arrive at the final ATP values in the results, the values were further multiplied by two to correct for the extraction efficiency difference between material collected on filters and whole water samples.

Cell counts
Thalassiosira weissflogii was enumerated in a Z2 Coulter Counter Multisizer with a 100 um orifice tube. Seven measurements each were made for particle sizes larger than 10 um, the first values discarded, and the next six measurements recorded (the first measurement may still contain air bubbles).

Data Processing Description

BCO-DMO Processing:
- renamed fields to conform with BCO-DMO naming conventions.

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Data Files

File
2019_exp_data.csv (Comma Separated Values (.csv), 5.01 KB) MD5:e72dafcc7c1d99ea1bc29e9f7c7f2bef Primary data file for dataset ID 858440

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Related Publications

Bochdansky, A. B., Stouffer, A. N., & Washington, N. N. (2021). Adenosine triphosphate (ATP) as a metric of microbial biomass in aquatic systems: new simplified protocols, laboratory validation, and a reflection on data from the literature. Limnology and Oceanography: Methods, 19(2), 115–131. doi:10.1002/lom3.10409
Results

Karl, D. M. 1993. Total microbial biomass estimation derived from the measurement of particulate adenosine-5′-triphosphate, p. 359–368. In P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole [eds.], Handbook of methods in aquatic microbial ecology. Routledge. https://isbnsearch.org/isbn/0873715640
Methods

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Parameters

Parameter	Description	Units
Elapsed_days	Elapsed time since the beginning of the experiment	number of days
Elapsed_hours	Elapsed time since the beginning of the experiment	number of hours
Temperature	Incubation temperature	degrees Celsius
Temperature_group	One of three temperature groups (15, 20, 25 C)	unitless
Flask	Bottle number	unitless
Cell_concentration	Coulter Counter cell counts > 10 micrometer	cells per milliliter
Corrected_ATP	Particulate ATP corrected for filtration losses	nanomolar (nM)
ATP_filtrate	ATP in the 0.2 micrometer filtrate; bd = below detection	nanomolar (nM)

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Centrifuge
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	Z2 Coulter Counter Multisizer
Generic Instrument Name	Coulter 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-2401PC
Generic Instrument Name	Spectrophotometer
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

Collaborative Research: Transforming Carbon in the Deep Sea (Carbon in the Deep Sea)

Coverage: Mesopelagic North Atlantic

NSF Award Abstract:
Through understanding the biological pump (the ocean's biologically driven sequestration of carbon from the atmosphere to the ocean interior and seafloor sediments), scientists know that the world's oceans absorb more carbon dioxide than it returns to the atmosphere. While much is known about the biological processes largely responsible for the transfer of carbon into the deep sea, very little is known about the microbial decay and subsequent remineralization processes that occur when the carbon reaches the deep sea. Using newly-designed deep-sea incubators deployed off the east coast of the United States, researchers will explore the microbial communities and remineralization processes that transform carbon in the deep sea. The incubators will be filled with tracer-labeled algae or fecal material mimicking the diet and waste products of animal plankton. The tracers allow the researchers to follow the material through the microbial food web, and simultaneously determine the net release of carbon dioxide during the incubations. Using a combination of genetic analysis and novel analytical techniques, the researchers will be able to identify the organisms involved in the decay processes and rates at which changes occur at the single-cell level. Results will shed light on these understudied biological phenomena and contribute to an improved understanding of the global carbon cycle. In addition to novel advancements in oceanographic technology, the research supports graduate and

undergraduate student education, and public outreach through partnerships with the Virginia Aquarium and National Ocean Sciences Bowl to increase ocean science literacy.

In this project, researchers will study the organisms, mechanisms, and physical and ecological factors that modulate the remineralization of organic material in the deep sea. The methods include using in situ incubations of well-defined and stable isotope-labeled sources of organic carbon (live and dead phytoplankton and fecal pellets of zooplankton) with natural microbial communities. The incubations will take place northeast of Cape Hatteras, a region characterized by strong offshore transport of phytoplankton carbon. Net carbon dioxide release rates will be measured over time by conversion of Carbon-13 labeled organic carbon to 13CO2 . The dependence of degradation rates on the source material, seasonality, oxygen concentration, and the type of microbial colonizers will be assessed. Parallel laboratory experiments will elucidate the exact shape of the time course of carbon release by phytoplankton into dissolved organic and inorganic fractions as well as determine how representative laboratory and ship-board generated values are relative to those obtained in situ. Target eukaryotic and prokaryotic taxa are identified by fluorescence in-situ hybridization (FISH) after the incubations and individually interrogated using Raman microspectrometry to investigate the relative Carbon-13-enrichment rates in organisms assimilating labeled detrital carbon. This multi-faceted approach will provide better constrained parameters for ecosystem and biological pump models and shed light on carbon balances of the deep sea. The research contributes to the development of new oceanographic technology, including new deep-sea incubators and application of single-cell Raman microspectrometry to natural microbial communities.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1851368

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