Bulk percentage of 14C, ATP, prokaryote and protist abundances from mesopelagic decay experiments with Thalassiosira weissflogii, Emiliania huxleyi, and Tetraselmis sp.

Website: https://www.bco-dmo.org/dataset/995456
Data Type: experimental
Version: 1
Version Date: 2026-03-24

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

Contributors	Affiliation	Role
Bochdansky, Alexander Boris	Old Dominion University (ODU)	Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
The mesopelagic zone is a site of strong microbially driven particle attenuation with depth and thus plays a crucial role in controlling the transfer efficiency of the ocean's biological pump. However, little quantitative information exists on the dependency of decay processes on the source material. Here, we followed the decay of 14C-labeled dead particulate carbon (POC) and dissolved organic carbon (DOC) from three different phytoplankton species, and two incubations of live diatoms, in mesopelagic water over three months. Commonly used first-order kinetics failed to adequately describe the decay of organic material as rate constants varied from day to day. Over extended periods, decay rates for organic material exhibited two distinct phases, with rates in the second phase being inversely related to rates in the first phase. Microbial biomass (measured via ATP and cell counts) increased substantially during phase 1 and ebbed during phase 2. Decay rates were significantly different among the three algal sources; however, differences were even more pronounced among carbon pools and followed a distinct pattern (combined average per-day decay rates at 12 degrees Celsius): fresh DOC (0.6) > fresh POC (0.1) > live cells (0.06) > aged DOC/POC (0.01). Separation of POC into four broad biochemical fractions showed that components in the operationally defined lipid fraction contained the most degradable compounds for fresh material. Our research highlights the need to include the dynamics of the most easily digestible fractions of freshly released organic material, and live plankton resilient to digestion, in calculations of vertical carbon flux budgets. This dataset includes bulk percent remaining 14C, ATP concentrations, and prokaryote and protist abundances, and instantaneous decay rate calculations from mesopelagic decay experiments using three different 14C-labeled algae (Thalassiosira weissflogii, Tetraselmis sp., and Emiliania huxleyi). See the "Related Datasets" section for complementary datasets from the same experiments.

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Coverage

Location: North Atlantic continental shelf break east of Virginia Beach

Spatial Extent: **Lat**:36.788 **Lon**:-74.629
Temporal Extent: 2020-10-06 - 2021-05-27

Dataset Description

This dataset is part of a group of related datasets from the same mesopleagic decay experiments. See the "Related Datasets" section on this page for access to other related datasets in this group.

Datasets from these experiments:
- Bulk percentage of 14C, ATP, and prokaryote and protist abundances
- Biochemical percentages (protein, polysaccharides, and lipids)
- Biochemical decay rates

Methods & Sampling

Experimental overview and design of treatments:
In the first experiment, live cells of the diatom *Thalassiosira weissflogii* were added to the mesopelagic community; in the second, live cells and nonliving organic matter (both POC and DOC) from *T. weissflogii* were added; and in the third, nonliving organic matter from three species was used. In each experiment, at least one treatment was set up the same way to allow direct comparisons from experiment to experiment, as mesopelagic water was collected at different times of the year and might include different microbial communities. To simulate decay, we used model material (live cells, POC, and DOC) in a state where they could be easily mixed to obtain representative subsamples and at low concentrations so that the addition of organic material would not lead to the depletion of oxygen. Carbon-14-labeled algal cultures (*E. huxleyi* CCMP374, *T. weissflogii* CCMP374 or *Tetraselmis* sp. UTEX SP22) in the early stationary phases were first gently filtered onto 0.8 micrometer (µm) polycarbonate filters (Isopore) to remove remaining inorganic ^{14}C and then resuspended in 0.2-µm-filtered artificial seawater. The preparation of the POC and DOC fractions was similar to the methods described by Cabrera-Brufau et al. (2021). The resuspended cells were frozen at -80 degrees Celsius (°C) for at least one hour to kill the cells and break them up to release DOC. Before the experiments, a sample of this culture was thawed and vacuum-filtered through a 0.2 µm filter, an operational cutoff for the separation of POC and DOC. The $PO^{14}C$ fraction on the filter was resuspended in filtered artificial seawater of the same volume, while the filtrate became the $DO^{14}C$ fraction. For the live treatments, *T. weissflogii* cultures were gently filtered (using 0.8 µm filters and low vacuum, < 10 millibars (mbar)), washed and resuspended in unlabeled seawater, and added to the experimental flasks immediately without further processing.

The mesopelagic water was collected at three different times from the same site at a depth of 300 meters (m), 115 kilometers (km) offshore of Virginia Beach (36.788° N, 74.629° W) using four 5-liter (L) Niskin bottles. The water temperature at the collection depth was consistently 12 ± 0.5 °C, regardless of the season. The water was gently transferred (i.e., with minimum turbulence and air bubbles) from the Niskin bottles through a hose into two 20-L plastic bladder tanks, which were immediately placed into a temperature-controlled cooler at either 8 °C (experiment 1) or 12 °C (experiments 2 and 3). Upon arrival at the lab and on the same day of collection, the water was moved to a dark temperature-controlled room in which the experiments were performed and set to either 8 °C (experiment 1) or 12 °C (experiments 2 and 3). The algal treatments were added the day after the water collection. All experimental treatments were conducted in triplicates. Thirteen milliliters (ml) of either the live algal suspension, $PO^{14}C$, or $DO^{14}C$ were added to 1.7 L of mesopelagic water in each 4-L aspirator flask. Unlabeled cultures of *T. weissflogii* grown under the same conditions were filtered onto muffled GF/F filters and analyzed for particulate carbon with a Europa 20-20 isotope ratio mass spectrometer to determine the total carbon content of the cultures at the time of harvest. Since all cultures were grown under the same conditions over many division cycles, these cells can be considered uniformly labeled. Consequently, the relative amount of added carbon can be calculated from the apportionment of disintegrations per minute (dpm) values. Samples were collected through a tube and a plastic ball valve connected to the aspirator flask to measure $PO^{14}C$, $DO^{14}C$, ATP, prokaryote abundance, and biochemical fractions. We also monitored the mesopelagic microbial community over three weeks without the addition of substrates, in a separate collection taken from the same site and depth, to confirm that both ATP and cell abundances changed relatively little during this time period.

Sample Collection:
Before sampling at each time point, the water in the flasks was swirled until well mixed through without excessive shaking. Preliminary experiments with similar additions of unlabeled substrates, in which we

measured oxygen concentrations using a polarographic oxygen sensor, revealed that the oxygen concentrations remained near saturation (data not shown). The geometry of the flasks was such that a large surface area of the water was exposed to air, and the agitation before sampling additionally mixed oxygen into the incubation water. Aggregates that could have created microzones of low oxygen were also not observed during the experiments. From each flask, 10–30 ml of water was purged from the valves, and then 30–40 ml of samples were collected into 50-ml Falcon centrifuge tubes. The protocol and rationale for sampling the carbon pools followed Bochdansky et al. (2010). From the collected water, four 5-ml samples were immediately vacuum-filtered onto GF/F filters. The first three filters were placed into plastic pony vials for $PO^{14}C$ analysis. The fourth filter (for ATP) was placed into a cryovial that contained 1 ml of phosphoric acid-benzalkonium chloride extractant (P-BAC), left at room temperature for 20–30 minutes for extraction, and subsequently kept in a -80 °C freezer until analysis (Bochdansky et al. 2021). Three 0.5-ml allotments of the filtrate were placed into separate pony vials for analysis of the $DO^{14}C$ fractions. The vials containing the POC and DOC fractions were acidified overnight with 0.25 ml of 0.2 N perchloric acid to eliminate inorganic carbon (Bochdansky et al. 2010). Four ml of scintillation cocktail (Bio-Safe II, Research Products International) was added to the vials the next day, which were then capped, inverted to ensure homogeneity, and analyzed on a liquid scintillation counter (LSC) (Perkin Elmer Tri-Carb model 3110) using a 20-minute count setting per vial to ensure sufficient time for accurate readings at low activity. Three blanks with only scintillation cocktail were run at every other time point; their values were averaged and subtracted from the sample values. The remainder of the liquid samples kept in the Falcon tubes were fixed for cell counts. Buffered 0.2-µm-filtered 37% formaldehyde was added to each sample at a final concentration of 0.2% and left overnight. The next day, subsamples of 5 ml were filtered onto black polycarbonate membranes (Isopore GTBP) and stored in a -80 °C freezer until analysis under the epifluorescence microscope. Depending on the experiment, one or two filters were prepared for each flask at each time point.

ATP Analysis:
The analysis of ATP samples followed the procedure of Bochdansky et al. (2021), which was modified from Holm-Hansen and Booth (1966). From each cryovial, 10 microliters (µl) of sample was placed into triplicate scintillation vials along with 3 ml of ~18.2 MΩ ultrapure water (Barnstaed) and 50 µl of CellTiter-Glo 2.0 (Promega, protocol modified as in Bochdansky et al. 2021). A standard solution made with 50 µl of an ATP standard of 16.4 nanomolar (nM) concentration was added along with the sample, water, and CellTiter-Glo to a fourth vial. The standards were interspersed with the samples during counting on the LSC to track the slow (i.e., over many hours) decay in luminescence over time, which allowed for a precise calculation of the ATP values.

Cell Counts:
The polycarbonate filter membranes were embedded in a mounting medium that contained 4',6-diamidino-2-phenylindole dihydrochloride (Fluoroshield with DAPI, F6057, Sigma-Aldrich) to detect double-stranded DNA using an Olympus BX51 epifluorescence microscope with a 100x immersion oil objective, 2x loupe, and 10x ocular lenses to obtain a 2000x total magnification (Hobbie et al. 1977; Porter and Feig 1980). Protist grazers were identified by their conspicuous round or oval nuclei that were larger than prokaryote cells.

Carbon decay models and statistics:
Visually, the trajectory of carbon decay over time appeared to consist of at least two phases. Therefore, piecewise regressions were used with the natural-log-transformed percentages of remaining $PO^{14}C$ and $DO^{14}C$ over time. We calculated exponential decay rate constants for each phase as the slopes of the linear regressions with the natural-log-transformed data. Analysis of Covariance (ANCOVA) homogeneity of slope tests were used on natural-log-transformed values to assess significance between treatments, variables, and biochemical fractions.

To capture a more highly resolved change in decay rates through the course of the experiments, we calculated first-order kinetics for each pair of time points (equation 1):

$$D = \ln(dpm_{t1}/dpm_{t2})\ (t_2-t_1)-1, \text{ (equation 1)}$$

where D is the instantaneous rate of decay (d-1), dpm_{t1} and dpm_{t2} are the initial and final values, respectively, of ^{14}C in the POC or DOC fractions, and t_1 and t_2 are the time points of the paired samples (d).

Organism Identifiers:
Scientific Name (Life Science Identifier [LSID])

Thalassiosira weissflogii (urn:lsid:marinespecies.org:taxname:163513)
Tetraselmis sp. (urn:lsid:marinespecies.org:taxname:134526)

Emiliania huxleyi (urn:lsid:marinespecies.org:taxname:115104)

Data Processing Description

Analysis of the data (reported Craft & Bochdansky, 2025) was performed using the Statistics and Machine Learning Toolbox in MATLAB (The MathWorks, Inc.) for the piecewise regressions and the Analysis of Covariance (ANCOVA) homogeneity of slopes tests, and a custom randomization program was also written in MATLAB to obtain p-values without the requirement of normality and homoscedasticity in the distributions of the residuals. Both sets of results are reported.

BCO-DMO Processing Description

- Imported original file "Mesopelagic Experiment Data 1Apr2025.csv" into the BCO-DMO processing system.
- Treated "NaN" as a missing data value (missing data are empty/blank in the final CSV file).
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved the final file as "995456_v1_bulk14c_atp_abundances.csv".

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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
Methods

Bochdansky, A., Bollens, S., Rollwagen-Bollens, G., & Gibson, A. (2010). Effect of the heterotrophic dinoflagellate Oxyrrhis marina and the copepod Acartia tonsa on vertical carbon flux in and around thin layers of the phytoflagellate Isochrysis galbana. Marine Ecology Progress Series, 402, 179–196. https://doi.org/10.3354/meps08428
Methods

Cabrera-Brufau, M., Arin, L., Sala, M. M., Cermeño, P., & Marrasé, C. (2021). Diatom Dominance Enhances Resistance of Phytoplanktonic POM to Mesopelagic Microbial Decomposition. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.683354
Methods

Craft, N. J., & Bochdansky, A. B. (2025). Organic carbon decay mediated by a mesopelagic microbial community: The relevance of carbon pools and broad biochemical composition. Limnology and Oceanography, 70(3), 634–649. Portico. https://doi.org/10.1002/lno.12796
Results

Hobbie, J. E., Daley, R. J., & Jasper, S. (1977). Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology, 33(5), 1225–1228. https://doi.org/10.1128/aem.33.5.1225-1228.1977
Methods

Holm-Hansen, O., & Booth, C. R. (1966). THE MEASUREMENT OF ADENOSINE TRIPHOSPHATE IN THE OCEAN AND ITS ECOLOGICAL SIGNIFICANCE1. Limnology and Oceanography, 11(4), 510–519. Portico. https://doi.org/10.4319/lo.1966.11.4.0510
Methods

Porter, K. G., & Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography, 25(5), 943–948. doi:10.4319/lo.1980.25.5.0943
Methods

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Related Datasets

IsRelatedTo

Bochdansky, A. B. (2026) **Biochemical decay rates from mesopelagic decay experiments with Thalassiosira weissflogii, Emiliania huxleyii, and Tetraselmis sp.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-03-31 http://lod.bco-dmo.org/id/dataset/995681 [view at BCO-DMO]
Relationship Description: These three related datasets are all from the same mesopleagic decay experiments.

Bochdansky, A. B. (2026) **Biochemical percentages (protein, polysaccharides, and lipids) from mesopelagic decay experiments with Thalassiosira weissflogii, Emiliania huxleyii, and Tetraselmis sp.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2026-03-27 http://lod.bco-dmo.org/id/dataset/995521 [view at BCO-DMO]
Relationship Description: These three related datasets are all from the same mesopleagic decay experiments.

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Parameters

Parameter	Description	Units
Experiment	Experiment number 1-3	unitless
Algal_substrate	One of three algal sources of carbon: Tw = Thalassiosira weissflogii, Eh = Emiliania huxleyi, Ts = Tetraselmis sp.	unitless
Carbon_pool	One of three carbon pools: Live = live algae, POC = particulate organic carbon, DOC = dissolved organic carbon	unitless
Elapsed_days	Number of days elapsed	days
Pcnt_14C_flask1	Percentage of labeled 14C remaining in the first replicate flask	percent (%)
Pcnt_14C_flask2	Percentage of labeled 14C remaining in the second replicate flask	percent (%)
Pcnt_14C_flask3	Percentage of labeled 14C remaining in the third replicate flask	percent (%)
Decay_1	Instantaneous rate of decay in the first replicate	per day (d-1)
Decay_2	Instantaneous rate of decay in the second replicate	per day (d-1)
Decay_3	Instantaneous rate of decay in the third replicate	per day (d-1)
ATP_flask1	Adenosine triphosphate concentration in replicate flask 1	nM (nanomolar)
ATP_flask2	Adenosine triphosphate concentration in replicate flask 2	nM (nanomolar)

ATP_flask3	Adenosine triphosphate concentration in replicate flask 3	nM (nanomolar)
Prok_flask1_filter1	Microscopic counts of prokaryotes in replicate flask 1 filter #1	cells per milliliter (cells ml-1)
Prok_flask2_filter1	Microscopic counts of prokaryotes in replicate flask 2 filter #1	cells per milliliter (cells ml-1)
Prok_flask3_filter1	Microscopic counts of prokaryotes in replicate flask 3 filter #1	cells per milliliter (cells ml-1)
Prok_flask1_filter2	Microscopic counts of prokaryotes in replicate flask 1 filter #2	cells per milliliter (cells ml-1)
Prok_flask2_filter2	Microscopic counts of prokaryotes in replicate flask 2 filter #2	cells per milliliter (cells ml-1)
Prok_flask3_filter2	Microscopic counts of prokaryotes in replicate flask 3 filter #2	cells per milliliter (cells ml-1)
Prot_flask1	Microscopic counts of protists in replicate flask 1	cells per milliliter (cells ml-1)
Prot_flask2	Microscopic counts of protists in replicate flask 2	cells per milliliter (cells ml-1)
Prot_flask3	Microscopic counts of protists in replicate flask 3	cells per milliliter (cells ml-1)

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Instruments

Dataset-specific Instrument Name	Olympus BX51 epifluorescence microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Used for cell counts with DAPI staining at 2000x magnification to enumerate prokaryotes and protist grazers on polycarbonate filters
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Europa 20-20 isotope ratio mass spectrometer
Generic Instrument Name	PDZ Europa 20-20 isotope ratio mass spectrometer
Dataset-specific Description	Used for the analysis of bulk carbon
Generic Instrument Description	The PDZ Europa 20-20 is a dedicated continuous flow isotope ratio mass spectrometer for hyphenated stable isotope analyses able to measure 15N, 13C, 18O, and 34S in a host of applications. The analyzer has been purposely designed to measure 2H by continuous flow methodology and is also suitable to analyze the light stable isotopes in all the commonly measured gases: H2, N2, NO, N2O, O2, CO, CO2, SO, and SO2.

Dataset-specific Instrument Name	Perkin Elmer Tri-Carb model 3110
Generic Instrument Name	PerkinElmer Tri-Carb 3110TR low activity liquid scintillation analyzer
Dataset-specific Description	Used for the analysis of 14C
Generic Instrument Description	The PerkinElmer Tri-Carb 3110TR is a benchtop liquid scintillation analyzer for detecting small amounts of alpha, beta, and gamma radioactivity. It features a Multichannel Analyzer with an effective resolution of 1/10 keV and an extended dynamic quench range. Sample capacity is either 408 standard 20 mL vials, or 720 small 4 or 7 mL vials. The instrument includes a barium-133 low-energy external standard source centered under the sample vial which eliminates the effects of volume variations. It has an energy range of 0-2000 keV and an operating ambient temperature range of 15-35 degrees Celsius.

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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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