Oxygen, carbon, and 13C values from the deep-sea incubator (DSI) and parallel laboratory incubations with mesopelagic water from the same region and depth on May 12, 2023

Website: https://www.bco-dmo.org/dataset/995790
Version: 1
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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 oxygen, carbon, and 13C values from syringes used in incubations with mesopelagic water (~300 meters depth) either in situ using a deep-sea incubator, or in the laboratory in parallel incubations. The organic substrate was the particulate organic carbon fraction (retained by a 0.2-micrometer polycarbonate filter) of 13C-labeled algae from two cultures: the green alga Tetraselmis sp. and the diatom Thalassiosira weissflogii. The syringes were incubated for four days in order to measure organic carbon decay.

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Coverage

Location: North Atlantic continental shelf break
Spatial Extent: **Lat**:36.788 **Lon**:-74.629
Temporal Extent: 2023-05-12 - 2023-05-16

Methods & Sampling

Cruise/Deployment Information:
Field samples were obtained using R/V Fay Slover (Ocean & Earth Sciences, Old Dominion University).
Deployment date of Deep-Sea Incubator: 2023-05-12
Retrieval date of Deep-Sea Incubator: 2023-05-16
Location: Continental shelf break off Virginia Beach, Virginia, USA
Chief scientist: Alexander B. Bochdansky, Ocean & Earth Sciences, Old Dominion University, abochdan@odu.edu

In situ experiments with the DSI:
The deep-sea incubator (DSI) consists of 24 gas-tight glass syringes and a mechanism that fills the syringes to 42 milliliters (ml) with ambient water set on a timer with a 1-hour delay. This gives the DSI more than sufficient time to settle to the target depth and adjust to the in-situ temperature. The anchor depth was 600 meters (m) and the tether from the anchor to the DSI was 300 m resulting in a deployment depth of 300 m. The syringes

were pre-filled with 2 ml of labeled algal phytodetritus from batch cultures of *Thalassiosira weissflogii* and *Tetraselmis* sp. The cultures were grown on a f/2+ (*T. weissflogii*) or f/2 (*Tetraselmis*) medium enriched with 13C-sodium bicarbonate, frozen at the late exponential stage, and then filtered onto a 0.2-micrometer (um) pore size polycarbonate filter. The particulate material (POC) was resuspended in a 50 ml centrifuge tube filled with the same volume of water as the original culture volume with artificial seawater. The filtrate was used as a source of 13C-labeled dissolved organic carbon (DOC). POC was dislodged from the filter by vigorous vortex mixing and shaking of the centrifuge tube. DOC was the filtrate. Two milliliters of the slurry of POC or the DOC filtrate were then injected into each syringe using an automatic pipette. Controls were poisoned with mercuric chloride (HgCl2). The four treatments were as follows:

1.) 3C labeled POC from *Thalassiosira weissflogii* (4 replicates)
2.) 13C labeled DOC from *Thalassiosira weissflogii* (4 replicates)
3.) 13C labeled POC from *Tetraselmis* sp. (4 replicates)
4.) 13C labeled DOC from *Tetraselmis* sp. (4 replicates)
5.) 13C labeled POC from *Thalassiosira weissflogii* with HgCl2 (duplicates)
6.) 13C labeled DOC from *Thalassiosira weissflogii* with HgCl2 (duplicates)
7.) 13C labeled POC from *Tetraselmis* sp. with HgCl2 (duplicates)
8.) 13C labeled DOC from *Tetraselmis* sp. with HgCl2 (duplicates)

The incubation started on May 12, 2023 at 13:09. The syringes were processed (tfinal) on May 16, 2023 (i.e., 4.25 days later).

Parallel laboratory incubations:
The laboratory incubations were set up in parallel using the exact same protocol as with the field incubations except they are filled with seawater from close to the same depth (300 m) as the deep-sea incubator collected with Niskin bottles. The water was returned to lab laboratory in a temperature controlled cool chest (11-12 degrees Celsius (C)) and experiments were started approximately 6.5 hours and after the syringes were filled with labeled material. They were placed in a cold room at 12 degrees C in the dark until retrieval.

The incubation interval for the laboratory experiments was 4 days.

The temperature of the DSI was unknown until retrieval. Because of the highly variable bathymetry at the deployment site (at the continental shelf break), the DSI landed in slightly deeper water than anticipated, which resulted in an average temperature of 8.2 degrees C (range 6.5 – 10.3 degrees C) according to an attached temperature logger.

Oxygen was measured by inserting a Unisense microprobe (a polargraphic oxygen sensor that is drawn out into a capillary) approximately 2 centimeters (cm) into the syringe immediately after removing the Luer-lock valve. A saturated oxygen standard and a NaOH-ascorbic acid standard (zero oxygen) at the same salinity and concentration were used as standards. A two-point calibration suffices as the polarographic sensor signal is strictly linear.

Five ml from each syringe was filtered onto a muffled GF/F filter and frozen until later processing. The filters were dried at 50 degrees C, acidified under HCl fumes to remove inorganic carbon, dried again and sent to the Stable Isotope Facility at UC Davis. There, the filters were encapsulated in tin capsules and processed in an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS). Only the 13C and bulk particulate organic carbon values (12C + 13C) values were used for this analysis.

BCO-DMO Processing Description

- Imported original file "PO13C FIGURES for DSI drop 12May2023 Version 2.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 "995790_v1_dsi_o2_c_13c.csv".

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Parameters

Parameter	Description	Units
Sample_ID	Unique sample ID	unitless
Description	TW is Thalassiosira weissflogii, Tetra is Tetrasellmis sp., POC is particulate organic carbon, T0 and Tfin are times zero and times final of the experiment, respectively	unitless
Time_point	t0 beginning of experiment, tfin end of the experiment	unitless
Date	Date at which the individual syringes were processed	unitless
Live_dead	Live: no preserved; dead: preserved with HgCl2	unitless
Reading_Oxygen_units	Reading of polarographic oxygen sensor	picoAmps
Reading_Oxygen_saturation_average	Average reading of polarographic oxygen sensor at oxygen saturation	picoAmps
Reading_zero_oxygen	Reading of polarographic oxygen sensor at zero oxygen saturation	picoAmps
Total_C_ug	IRMS measurement of carbon on filter	milligrams (mg)
Total_C_ug_L	Carbon of sample per liter	milligrams per liter (mg/L)
Elapsed_time	Incubation period	days
Conc_13C	13C-carbon concentration of the sample (IRMS)	milligrams per liter (mg/L)

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Instruments

Dataset-specific Instrument Name	EA – IRMS at UC Davis
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	13C and 15N isotopes using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to either an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK) or a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK)
Generic Instrument Description	Instruments that quantify carbon, nitrogen and sometimes other elements by combusting the sample at very high temperature and assaying the resulting gaseous oxides. Usually used for samples including organic material.

Dataset-specific Instrument Name	deep sea incubator (DSI)
Generic Instrument Name	In-situ incubator
Dataset-specific Description	The deep sea incubator (DSI) consists of 24 gas-tight glass syringes and a mechanism that fills the syringes to 42 ml with ambient water set on a timer with a 1-hour delay.
Generic Instrument Description	A device on a ship or in the laboratory that holds water samples under controlled conditions of temperature and possibly illumination.

Dataset-specific Instrument Name	Isoprime VisION IRMS or PDZ Europa 20-20
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	13C and 15N isotopes using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to either an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK) or a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK)
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

Dataset-specific Instrument Name	Niskin bottles
Generic Instrument Name	Niskin bottle
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	temperature logger
Generic Instrument Name	Temperature Logger
Generic Instrument Description	Records temperature data over a period of time.

Dataset-specific Instrument Name	Unisense polarographic oxygen sensor type: MicroRespiration System
Generic Instrument Name	Unisense oxygen microsensor
Dataset-specific Description	Oxygen was measured by inserting a Unisense microprobe.
Generic Instrument Description	The Unisence oxygen microsensor is a miniaturized Clark-type dissolved oxygen instrument, including glass micro-sensors with minute tips (diameters ranging from 1 to 800 um). A gold sensing cathode is polarized against an internal reference and, driven by external partial pressure, oxygen from the environment penetrates through the sensor tip membrane and is reduced at the sensing cathode surface. A picoammeter converts the resulting reduction current to a signal. The sensor also includes a polarized guard cathode, which scavenges oxygen in the electrolyte, thus minimizing zero-current and pre-polarization time. See more on the manufacturer's website: https://www.unisense.com/

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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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