Stable carbon isotope data for thirteen individual amino acids from twelve species of eukaryotic microalgae and four species of eukaryotic microalgae

Website: https://www.bco-dmo.org/dataset/905161
Data Type: experimental
Version: 1
Version Date: 2023-07-25

Project
» Collaborative Research: Sources and transformations of export production: A novel 50-year record of pelagic-benthic coupling from coral and plankton bioarchives (GoME Copepod Coral Export)

Contributors	Affiliation	Role
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Abstract
Using controlled cultures, this project characterized the amino acid carbon isotope fingerprints — a multivariate metric of amino acid carbon isotope values — of twelve different species of eukayrotic microalgae from four major Classes of eukaryotic microalgae: diatoms, dinoflagellates, raphidophytes, and prasinophytes (three species per Class). Phytoplankton cultures were generated from established laboratory culture lines in the URI microalgal libraries and the National Center for Marine Algae and Microbiota (NCMA; formerly CCMP). This dataset includes stable carbon isotope data for thirteen individual amino acids from all twelve species of eukaryotic microalgae grown at 20° Celsius (C) and four species of eukaryotic microalgae (one from each of the four Classes) raised at 15°C, 20°C, and 25°C. Cultures were grown in triplicate for each species and temperature treatment under highly constrained growth conditions. These amino acid carbon isotope data were used to identify primary producers at the base of food webs supporting consumers in two contrasting systems from published literature: 1) penguins feeding in a diatom-based food web (McMahon et al. 2015 Ecology and Evolution 5:1278–1290) and 2) mixotrophic corals receiving amino acids directly from autotrophic endosymbiotic dinoflagellates and indirectly from water column diatoms, prasinophytes, and cyanobacteria, likely via heterotrophic feeding on zooplankton (Fox et al. 2019 Functional Ecology 33:2203-2214). The increased taxonomic specificity of CSIA-AA (Compound-Specific Isotope Analysis of Amino Acids) fingerprints developed here will greatly improve future efforts to reconstruct the contribution of diverse eukaryotic microalgae to the sources and cycling of organic matter in food web dynamics and biogeochemical cycling studies.

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Coverage

Temporal Extent: 2019 - 2019

Methods & Sampling

This study was conducted at the University of Rhode Island (URI) Phytoplankton Culture Laboratory and Ocean Ecogeochemistry Isotope Laboratory.

A library of three species from each of four major eukaryotic microalgae classes - diatoms (Class Bacillariophyceae), dinoflagellates (Class Dinophyceae), raphidophytes (Class Raphidophyceae), and prasinophytes (Class Mamiellophyceae) - was generated from established laboratory culture lines in the URI microalgal libraries and the National Center for Marine Algae and Microbiota (NCMA; formerly CCMP). Cultures were grown in triplicate in either f/2 or L1 media created using 0.22-micrometer (µm) filtered, autoclaved Narragansett Bay, Rhode Island seawater. All seawater was collected at the same time and location to ensure consistent water conditions for all cultures. Cultures were grown in climate-controlled incubators under a light intensity of 55 ± 10 micromoles photons per square meter per second (µmol photons m-2 s-1) on a 12-hours:12-hours light:dark cycle. One species from each of the four Classes was grown in triplicate under three temperature treatments: 15° Celsius (C), 20°C, and 25°C. A microplate reader was used to obtain growth rates to target biomass collection. The Supplemental File "Stahl_et_al_2023_L&O_BCO-DMO_Supplemental_DataSet.pdf" contains the identification and laboratory culture conditions for the four major groups of eukaryotic microalgae.

Once cultures reached sufficient density to obtain ~5 milligrams (mg) of dry weight needed for amino acid isotope analysis, cultures were gently vacuum filtered onto either 5 µm PETE membrane filters (Sterlitech), 2 µm PETE membrane filters (Sterlitech), or 0.22 µm PES membrane filters (Millipore Express PLUS) depending on the size of the species being filtered. Filtered biomass was frozen at -20°C, freeze dried for 72 hours, and homogenized prior to isotope analysis. Dried, homogenized samples were acid hydrolyzed in 6 N hydrochloric acid at 110°C for 20 hours, filtered through a 0.45 µm nylon syringe filter (Restek), and evaporated to dryness under a gentle stream of N_2. Five µl of nor-leucine (Sigma-Aldrich) with a known δ13C value was added to each sample and standard as an internal calibration. Acid hydrolyzed samples were derivatized to N-trifluoroacetic acid isopropyl esters and the carbon isotope values of 13 individual amino acids were separated and analyzed on a BPX5 column (60 meters length, 0.32 millimeters internal diameter (ID), 1 µm film thickness) in a Thermo Trace 1310 gas chromatograph (GC) and analyzed on a Finnegan MAT Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) interfaced to the GC through a GC-IsoLink II and reduction furnace (1000°C) at the University of Rhode Island, Graduate School of Oceanography. Standardization of runs was achieved using intermittent pulses of a CO_2 reference gas of known isotopic value. Amino acid standards of known isotopic value were derivatized concurrently with samples and analyzed bracketing each sample. All samples were analyzed minimally in triplicate along with the amino acid mixed standard and a cyanobacteria working lab standard. Normalized δ13CAAnorm values were calculated using the following equation: δ13CAAnorm = δ13CAA - δ13CAAmean

Data Processing Description

All statistical analyses were performed in R version 3.6.3 with RStudio interface version 1.1.456.

BCO-DMO Processing Description

- imported original file named "Stahl_et_al_2023_L&O_BCO-DMO_DataSet.xlsx" into the BCO-DMO system;
- renamed fields to comply with BCO-DMO naming conventions;
- named the final data file "905161_v1_pp_amino_acid_c_isotopes.csv";
- converted original file named "Stahl_et_al_2023_L&O_BCO-DMO_Supplemental_DataSet.xlsx" into PDF format and attached as a Supplemental File.

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Data Files

File
905161_v1_pp_amino_acid_c_isotopes.csv (Comma Separated Values (.csv), 7.78 KB) MD5:be81c6ff158e40f6e3d753bfe8266b47 Primary data file for dataset ID 905161, version 1.

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Supplemental Files

File
Stahl_et_al_2023_L&O_BCO-DMO_Supplemental_DataSet.pdf (Portable Document Format (.pdf), 193.37 KB) MD5:c126574e394a1678144692ed2ba1ad0b Supplemental file for dataset ID 905161, version 1. Contains the identification and laboratory culture conditions for four major groups of eukaryotic microalgae: diatoms, dinoflagellates, raphidophytes, and prasinophytes, including taxonomy, size range, distribution, collection location, and culture growth conditions (media, temperature, salinity, and light intensity and duration). Column names, descriptions, units: Group = Taxonomic group of eukaryotic microalgae and Taxonomic Class. Genus Species = Genus and species of eukaryotic microalgae. Size Range = Typical cell size range of this species of eukaryotic microalgae (micrometers (µm)). Ocean Distribution = Typical natural geographic distribution of this species of eukaryotic microalgae. Collection Region = Specific location of collection of the strain of eukaryotic microalgae used in this study. Media = Cell growth media type from National Center for Marine Algae and Microbiota. Temp = Incubation temperature for that culture of eukaryotic microalgae (degrees Celsius). Salinity = Salinity of water for that culture of eukaryotic microalgae (PSU). Light Intensity = Light intensity experienced by that culture of eukaryotic microalgae (microEinsteins (µE)). Light-Dark duration = Light and dark exposure experienced by that culture of eukaryotic microalgae (hours).

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Related Publications

Fox, M. D., Elliott Smith, E. A., Smith, J. E., & Newsome, S. D. (2019). Trophic plasticity in a common reef-building coral: Insights from δ13C analysis of essential amino acids. Functional Ecology, 33(11), 2203–2214. Portico. https://doi.org/10.1111/1365-2435.13441
Methods

McMahon, K. W., Polito, M. J., Abel, S., McCarthy, M. D., & Thorrold, S. R. (2015). Carbon and nitrogen isotope fractionation of amino acids in an avian marine predator, the gentoo penguin (Pygoscelis papua). Ecology and Evolution, 5(6), 1278–1290. Portico. https://doi.org/10.1002/ece3.1437
Methods

Stahl, A. (2021). Identifying novel isotopic tracers of marine primary producers to study food web carbon cycles. Open Access Master's Theses. Paper 1936. https://digitalcommons.uri.edu/theses/1936. DOI: 10.23860/thesis-stahl-angela-2021. https://doi.org/10.23860/thesis-Stahl-Angela-2021
Results

Stahl, A. R., Rynearson, T. A., & McMahon, K. W. (2023). Amino acid carbon isotope fingerprints are unique among eukaryotic microalgal taxonomic groups. Limnology and Oceanography, 68(6), 1331–1345. Portico. https://doi.org/10.1002/lno.12350

Parameters

Parameter	Description	Units
SampleID	Unique ID for each species of eukaryotic microalgae. Integers reflect separate species and decimal numbers reflect replicate number of that species.	unitless
Group	Taxonomic group of eukaryotic microalgae	unitless
Genus_Species	Genus and species of eukaryotic microalgae	unitless
Temperature	Incubation temperature for that culture of eukaryotic microalgae	degrees Celsius
Gly	Carbon isotope value of Glycine measured in this culture of eukaryotic microalgae	per mil
Ser	Carbon isotope value of Serine measured in this culture of eukaryotic microalgae	per mil
Ala	Carbon isotope value of Alanine measured in this culture of eukaryotic microalgae	per mil
Asx	Carbon isotope value of Aspartic acid and Arginine measured in this culture of eukaryotic microalgae	per mil
Glx	Carbon isotope value of Glutamic acid and Glutamine measured in this culture of eukaryotic microalgae	per mil
Pro	Carbon isotope value of Proline measured in this culture of eukaryotic microalgae	per mil
Thr	Carbon isotope value of THreonine measured in this culture of eukaryotic microalgae	per mil
Ile	Carbon isotope value of Isoleucine measured in this culture of eukaryotic microalgae	per mil
Lys	Carbon isotope value of Lysine measured in this culture of eukaryotic microalgae	per mil
Met	Carbon isotope value of Methionine measured in this culture of eukaryotic microalgae	per mil
Val	Carbon isotope value of Valine measured in this culture of eukaryotic microalgae	per mil

Phe	Carbon isotope value of Phenylalanine measured in this culture of eukaryotic microalgae	per mil
Leu	Carbon isotope value of Leucine measured in this culture of eukaryotic microalgae	per mil

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Instruments

Dataset-specific Instrument Name	Thermo Trace 1310 gas chromatograph (GC)
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Thermo Trace 1310 gas chromatograph (GC) and analyzed on a Finnegan MAT Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) interfaced to the GC through a GC-IsoLink II and reduction furnace (1000°C) used to separate and analyze the carbon isotope values of 13 individual amino acids from each sample and standard.
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Finnegan MAT Delta V Plus Isotope Ratio Mass Spectrometer (IRMS)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Thermo Trace 1310 gas chromatograph (GC) and analyzed on a Finnegan MAT Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) interfaced to the GC through a GC-IsoLink II and reduction furnace (1000°C) used to separate and analyze the carbon isotope values of 13 individual amino acids from each sample and standard.
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	SpectraMax M5 Series microplate reader (Molecular Devices)
Generic Instrument Name	plate reader
Dataset-specific Description	Microplate reader (SpectraMax M5 Series, Molecular Devices) - used to obtain growth rates to target biomass collection approximately during exponential growth or early in the stationary phase.
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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Project Information

Collaborative Research: Sources and transformations of export production: A novel 50-year record of pelagic-benthic coupling from coral and plankton bioarchives (GoME Copepod Coral Export)

Coverage: Jordan Basin, Gulf of Maine (43 to 44.25N, 68.5 to 66.5W)

NSF Award Abstract:
Changes in ocean life, the environment, and the climate can influence the timing and composition of biological material that sinks to the sea floor. As this material sinks it is consumed by bottom-dwelling organisms such as deep-sea corals. Similar to tree rings, corals preserve a history of growth embedded in their skeletons, which can be analyzed using a new technique called microgeochemistry. This project is compiling a historic dataset from deep-sea corals spanning 50 years in the Gulf of Maine to understand how biological material sinking to the bottom has changed with time. Results from the coral analysis are being compared with archival samples of small planktonic crustaceans, copepods, to better understand the connection between productivity in the surface waters and the geochemical record in the coral tissue. A complementary modeling approach is identifying environmental and climatic drivers of decadal-scale oceanographic change with the sources and transformations of organic matter that connect the surface and the deep ocean. This cross-disciplinary project is unifying transformational research with broader impacts focused on science education and outreach that broaden the understanding of the links between climate, oceanography, and marine ecosystem response using a 50-year historical context. Two open-access, media-enhanced, and National curriculum standards-aligned educational lessons plans are being developed through partnerships with a science documentary filmmaker, K-12 teachers from RI and ME, and the PBS LearningMedia Program. The topics of these lesson plans are: 1) Deep-sea exploration: A window into the past and future, and 2) Changing food webs on a changing planet. The project's educational goals include training of three graduate students, career development of five early career researchers, and research experiences for undergraduates from underrepresented groups in STEM. The multi-faceted research and education effort is addressing a question described as highest priority in the Ocean Sciences by the National Research Council: How are ocean biogeochemical and physical processes linked to today's climate and its variability?

Pelagic-benthic coupling regulates ocean production and food web dynamics, biogeochemical cycling, and climate feedback mechanisms through the export of surface production to the ocean interior. Yet access to long-term data sets of export production are scarce and urgently needed to test assumptions about 1) the sources and transformations of organic matter through different food web pathways, and 2) the variability of these processes across climatic, oceanographic, and ecological changes through time. The proposed work is testing key hypotheses about bottom-up mechanisms that link decadal-scale oceanographic changes in hydrography and biogeochemical cycling with phytoplankton community composition, zooplankton abundance and trophic dynamics, and the resulting composition of export production. Complementary approaches are generating multiple and independent 50+ year, annually resolved time series of phytoplankton community composition, zooplankton trophic dynamics, and export composition. Coral tissue and archived zooplankton samples are being analyzed using pioneering molecular geochemistry approaches to assess changes in diet related variation in primary production. Deep-sea corals are being collected using a remotely operated vehicle (ROV), and zooplankton are available through archival samples from a Gulf of Maine long-term monitoring program managed by NOAA. The stable isotope data are being integrated with additional data from existing long-standing ocean monitoring programs and incorporated into a unifying modeling approach to identify unique ecosystem states and their environmental drivers. The project is focused on Jordan Basin in the Gulf of Maine, which has a long history of oceanographic study and is experiencing significant changes due to climate warming, making it an ideal natural laboratory for testing hypotheses on drivers of change in the composition of exported organic matter, and the relative importance of primary (e.g., phyto-detritus) vs. secondary production (e.g., copepod fecal pellets), and large vs. small pelagic plankton dynamics.

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-2049307

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