B. elegans cold water coral lab culture: particulate data

Project
» Collaborative Research: Refining the use of scleractinian cold-water coral skeleton-bound d15N as a proxy for marine N cycling (Coral-bound N)

Contributors	Affiliation	Role
Gothmann, Anne M.	Saint Olaf College	Co-Principal Investigator
Granger, Julie	University of Connecticut (UConn)	Co-Principal Investigator
Prokopenko, Maria	Pomona College (Pomona)	Co-Principal Investigator
Mottram, Josie	Saint Olaf College	Student
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
particulate data collected near Friday Harbor, WA.

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Methods & Sampling

Corals were collected by divers using blunt-tipped diving knives to remove corals from vertical rock walls at 10-20 m depths. A subset of the corals were immediately frozen for determination of N isotope ratios of tissue and skeleton. Another subset of corals were shipped live overnight to St. Olaf College for the culture experiments.

For the culture experiment, corals were divided into four groups that were each fed *Artemia* nauplii with a different known d15N. The coral tissue was sampled at discrete intervals over the course of the experiment as described below.

For the starvation experiment, corals were split into two group, starved and unstarved. The starved group was fed once every two weeks and the unstarved corals were fed twice a week. The coral tissue was sampled at discrete intervals throughouth the experiment as described below.

Once separated from the skeleton, coral tissue was lyophilized and analyzed using a Costech Elemental Analyzer Isotope Ratio Mass Spectrometer.

Once separated from the coral tissue, the coral skeletons were rinsed and ultrasonicated two times in Milli-Q water, then ultrasonicated in 1% sodium hypochlorite in 20 minute intervals until no tissue remained on the skeleton. The skeletons were then prepared following the methods of Wang et al (2014). The skeleton was ground to a powder using a mortar and pestle, then rinsed with sodium hypochlorite to remove any remaining tissue. The skeletal materials were then dissolved with 4N hydrochloric acid, then oxidized to nitrate by

autoclaving in a basic potassium persulfate solution. Skeletal material was oxidized in tandem with standards of glutamine reference material USGS-40 and USGS-41. The samples were then analyzed by Gas Chromatography- Isotope Ratio Mass Spectrometry using the denitrifier method (Sigman et al., 2001). In brief, the denitrifier method uses the denitfrying bacteria *Pseudomonas chlororaphis* f. sp. *aureofaciens* to convert nitrate to nitrous oxide. *P. aureofaciens* was grown in media amended with 10mM nitrate in stoppered glass bottles for 7-10 days before being harvested and resuspended in nitrate free media. Three milliliters of resuspended bacteria was allocated to 20mL headspace vials which were sparged with dinitrogen gas for 6 hours. Nitrate sample solutions were injected into vials (target of 20nmol nitrogen for seawater samples and 7nmol for skeletal matrix samples) and incubated overnight to allow for the complete conversation of nitrate to nitrous oxide. The nitrous oxide was extracted and purified using a Thermo Gas Bench II with a GC Pal autosampler and dual cold traps and analyzed on a Thermo Advantage continuous flow isotope ratio mass spectrometer. Analyzes were referenced to N2O injected from a pure gas cylinder and standardized through comparison potassium nitrate reference materials International Atomic Energy Agency Nitrate (IAEA-N3) and the isotopic nitrate reference material from the United States Geological Survey 34 (USGS-34).

Artemia nauplii samples were stored frozen then lyophilized prior to analysis on the Elemental Analyzer Isotope Ratio Mass Spectrometer.

Nitrate samples were collected with Van-Doren Sampler and filtered with pre-combusted glass fiber filters (GF/F, 0.7uM nominal pore size). The nitrate concentrations were determined using reduction to nitrous oxide in hot vanadium III solution followed by chemiluminescence detection of nitrous oxide on a Teledyne chemiluminescence NOx analyzer Model T200. The nitrogen and oxygen isotopes of nitrate were analyzed with the denitrifier method on an IRMS (described above).

Suspended particulate organic matter was collected with a Van-Doren Sampler and then collected on pre-combusted GF/F. The filters were lyophilized prior to analysis on an EA-IRMS.

Net tow material was collected with plankton nets with mesh sizes ranging from 80uM, 120uM, and 150uM. The net tow material was filtered and collected on a pre-combusted GF/F which was lyophilized prior to analysis on the EA.

Hydrologic depth profiles were characterized with a CastAway-CTD profiler.

Data Processing Description

Coral tissue and particulate matter was analyzed in tandem with the glutamine standards USGS-40 and USGS-41. These standards were used to correct the data from the EA-IRMS.

Coral skeleton material was oxidized in tandem with USGS-40 and USGS-41 which was used to correct for any oxidation blank. While IAEA-N3 and USGS-34 were used as standard material to correct the nitrate isotope data collected off the IRMS.

Data corrections were performed in Excel. The data reported here is averages of multiple runs when applicable. The uploaded data indicates when these are analytical replicates or sample replicates, all have n ≥ 2.

BCO-DMO Processing Description

Coral tissue and particulate matter was analyzed in tandem with the glutamine standards USGS-40 and USGS-41. These standards were used to correct the data from the EA-IRMS.

Coral skeleton material was oxidized in tandem with USGS-40 and USGS-41 which was used to correct for any oxidation blank. While IAEA-N3 and USGS-34 were used as standard material to correct the nitrate isotope data collected off the IRMS.

Data corrections were performed in Excel. The data reported here is averages of multiple runs when applicable. The uploaded data indicates when these are analytical replicates or sample replicates, all have n ≥ 2.

Related Publications

Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., & Böhlke, J. K. (2001). A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. Analytical Chemistry, 73(17), 4145–4153. doi:10.1021/ac010088e
Methods

Parameters

Parameters for this dataset have not yet been identified

Project Information

Collaborative Research: Refining the use of scleractinian cold-water coral skeleton-bound d15N as a proxy for marine N cycling (Coral-bound N)

Coverage: Global ocean

NSF abstract:

Refining the use of scleractinian cold-water coral skeleton-bound d15N as a proxy for marine N cycling

Recent studies show that cold-water corals and their skeletons provide valuable information about the marine nitrogen (N) cycle. This information can shed light on the processes that both drive and respond to changes in Earth's climate. Cold-water corals are found across the global ocean and can be dated with decadal precision, offering spatial and temporal records of the N cycle in the past. In addition, a single skeleton can be used to reconstruct both surface and deep ocean composition. Despite the promise of cold-water corals, we don't fully understand how they record changes in the marine N cycle. We must strengthen this understanding before we use cold-water corals to produce reliable records of marine N cycling across space and time, across different coral species, and under different lifestyle and feeding patterns. This project examines how the isotopic composition of organic N trapped in coral skeletons is linked to marine N cycle properties. The study includes a series of lab experiments, measurements of live corals sampled from the natural environment, and measurements of coral skeletal material from different ocean regions and depth horizons archived in museums. The project involves undergraduates at St. Olaf College, Pomona College and Mt. San Antonio College, one of the largest community colleges in Southern California. These students will conduct the research with scientists and peers in collaborating labs. Participation in the project will build student research skills and scientific knowledge for advanced study and prepare students for the scientific workforce. The project will also develop educational materials, including YouTube videos, to promote interest in marine science and awareness of how climate change influences global oceans. These educational materials will be created in collaboration with high school students from underrepresented groups.

The main tool used to investigate marine N cycle history is the isotope composition of particulate organic nitrogen (δ15N-PON) exported from the euphotic zone, which can be accessed using sedimentary archives such as foraminiferal tests, anoxic sediments and soft corals. Recently, the δ15N of organic N trapped within asymbiotic scleractinian cold-water coral (CWC) skeletons has been shown to record the δ15N-PON exported from the surface ocean (Wang et al. 2014; Wang et al. 2017). In order to reliably apply CWC δ15N as a proxy, however, we must explain a ~8.5‰ offset between the δ15N of organic nitrogen within the CWC skeleton and the exported δ15N-PON in regions of coral growth (Wang et al. 2014). The nature of the δ15N offset must be accounted for to be confident that CWC records marine N cycle history consistently across space and time, across different coral species, and for corals with different lifestyle conditions. Through coral culture experiments, measurements of live corals samples from the natural environment, and archives of corals skeletal material from different ocean regions and depth horizons, this research will test whether the offset

arises from: (1) a biosynthetic isotope offset between CWC tissue and skeleton, (2) an unusual trophic transfer between CWC tissue and diet, and/or (3) coral feeding on material with elevated δ15N relative to exported δ15N-PON. This work will also provide estimates of N turnover time in CWC, which are scant, and will inform trophic ecology of CWC.

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-1949119

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