Amino acid isotopes from mussels along the California margin from 2009-2013 (Amino Acid Sediment 15N project)

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Project
» The Use of Nitrogen Isotopes of Amino Acids To Understand Marine Sedimentary 15N Records (Amino Acid Sediment 15N)

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Abstract

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Coverage

Spatial Extent: **N**:42.9567 **E**:-116.4835 **S**:31.9339 **W**:-125.0088
Temporal Extent: 2009 - 2013

Dataset Description

N, C compound-specific amino acid isotopes from mussels along CA margin: new approach for paleo-isoscape reconstructions.

Methods & Sampling

Sampling

Mussels were collected in the winter (Dec – Feb) of 2009–2010. Sites were chosen to be approximately evenly distributed along the CA coastline, with ,80 km geographic separation between each

sampling site. Our main goal here was to sample mussels from a wide geographic range across the CCS, although for observing finer scale local or regional variations, a finer-scale sampling strategy would like be required. Typically 5 individual mussels were collected from each site, all between 30–40 mm maximum shell length, which were immediately placed on dry ice until further preparation. The adductor muscle of each individual was dissected for analysis. This tissue was selected because isotopic values in muscle tissue have shown relatively long turnover times; based on past growth data, mussels of this size would be expected to

integrate approximately annual variability in suspended food source isotopic values for each location sampled.

The dissected adductor tissue was carefully separated from other tissue types, rinsed with deionized water, refrozen, and then freeze-dried for 48 hrs. Lipids were removed using petroleum ether in a Dionex Accelerated Solvent Extractor (Bannockburn, IL). Finally, in preparation for CSI-AA, composite samples were made from a subset of 13 collection sites. For each location chosen for CSI-AA (based on the bulk d15N record), 160.05 mg of lyophilized tissue was weighed and combined for each individual mussel (n = 5).

Elemental and Bulk Isotopic Analyses

Stable carbon (d13C) and nitrogen (d15N) isotope ratios were determined via elemental analyzer isotope ratio mass spectrometry (EA-IRMS) at the University of California, Santa Cruz, Stable Isotope Laboratory (UCSC-SIL; http://emerald.ucsc.edu/~silab/). Approximately 1 mg of each dry isolated DOM sample was weighed into tin capsules (Costec, 5 x 9 mm) for analysis. EA-IRMS analysis was conducted using a Carlo Erba CHNS-O EA1108-elemental analyzer interfaced via a ConFlo III device with a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific). Standards, EA-IRMS protocols, and correction routines followed standard UCSC-SIL protocols. Analytical uncertainties of n=3 replicate measurements of isotopic standards ranged from ± 0.05 to 0.1‰ for both d13C and d15N. Carbon to nitrogen elemental ratios were similarly determined by elemental analysis. The presented ratios are atomic ratios $(C/N)_a$ normalized to the mass of C and N, but have been abbreviated as C/N throughout.

Compound-specific amino acid Isotopic 13C Analyses

Individual AA d13C values were measured as trifluoroacetyl isopropyl ester (TFA-IP) AA derivatives, after acid hydrolysis. Samples were hydrolyzed by adding 2.5 mg homogenized composite muscle tissue to 1 ml of 6 N HCl, and heating for 20 h at 110 deg C under nitrogen. After drying, AA isopropyl esters were prepared with a 1:5 mixture of AcCl:2-propanol (110 deg C, 60 min) and then acylated using a 1:3 mixture of di chloro - methane (DCM) and trifluoroacetic anhydride (TFAA) (100 deg C, 15 min). Derivatized AAs were dissolved in DCM to a final ratio of approximately 2.5 mg of original tissue to 250 ul DCM for injection on the gas chromatograph IRMS (GC-IRMS) system.

Isotopic analysis was conducted on a Thermo Trace GC Ultra with inline oxidation and reduction furnaces, coupled to a ThermoFinnigan Delta Plus XP IRMS, equipped with a CTC Analytics autosampler. Derivatives (1 ul) were injected (250 deg C constant temperature) onto an Agilent DB-5 column (50 m × 0.32 mm ID × 0.52 um film thickness), with a He carrier flow rate of 2 ml min−1 (constant flow). Separations were achieved with a 4-ramp oven program: 52 deg C, 2 min hold; ramp 1 = 15 deg C min−1 to 75 deg C, hold for 2 min; ramp 2 = 4 deg C min−1 to 185 deg C, hold for 2 min; ramp 3 = 4 deg C min−1 to 200 deg C; ramp 4 = 30 deg C min−1 to 240 deg C, hold for 5 min.

This method allowed for d13C determination of the following AAs in mussel tissue: non-essential AAs alanine (Ala), aspartic acid + asparagine (Asp), glutamic acid + glutamine (Glu), glycine (Gly), proline (Pro), serine (Ser), and tyrosine Tyr); and essential AAs leucine (Leu), isoleucine (Ile), valine (Val), phenylalanine (Phe), and threonine (Thr). Acid hydrolysis destroys tryptophan and cystine, so these were not detected, and it also deaminates\ asparagine to aspartic acid, and glutamine to glutamic acid. While the abbreviations Glx and Asx\ are sometimes used to denote the combined Gln+Glu and Asn+Asp peaks, in order to correspond better with extant CSI-AA literature, we have elected to simply use Asp and Glu abbreviations, as defined above.

All samples were analyzed on the GC-IRMS in triplicate, and measured AA d13C values were corrected for the C added during derivatization, following the approach of Silfer et al. (1991). Reproducibility for tissue samples was typically less than <0.3‰ (n = 3). The average mean deviation for all tissue sample replicates was 0.4‰.

Compound-specific amino acid Isotopic 15N Analyses

Amino acid d15N values were measured as Trifluoroacetyl isopropyl ester (TFA-IP) AA derivatives, following protocols described in detail elsewhere (e.g.,. Briefly, samples were hydrolyzed (6 N HCl, 20 hr at 110uC) under nitrogen, and TFA derivatives subsequently prepared from free AA using a modified version of the protocol described by Silfer (Silfer et al. 1991): isopropyl esters were made with a 1:5 mixture of Acetyl Chloride (AcCl):2-propanol (110uC, 60 minutes), and then acylated using a 1:3 mixture of Dichloromethane:Trifluroacetyl acetate (DCM:TFAA) (100uC, 15 minutes). Derivatized AAs were dissolved in DCM to a final ratio of approximately 4 mg of original tissue to 250 ml DCM. After derivatization, samples were analyzed by a Varian gaschromatograph coupled to a Finnegan Delta-Plus isotope ratiomass spectrometer (GC-IRMS). AAs were separated using a 50 m, 0.32 ID Hewlett Packard Ultra-1 column with 1 mm film thickness. Under our analytical conditions, d15N values could be reproducibly measured for alanine (Ala), aspartic acid + asparagine (Asp), glutamic acid + glutamine (Glu), leucine (Leu), isoleucine (Ile), proline (Pro),

valine (Val), glycine (Gly), lysine (Lys), serine (Ser), phenylalanine (Phe), threonine (Thr), and tyrosine (Tyr) (Fig. S4). Most AAs were measured with a standard error of ,1.0% (based on n = 4 injections), and the average mean deviations for individual AA d15N measurements across all tissue sample replicates was 0.5%.

Data Processing Description

Thermo-Finnigan Isodat software and Microsoft Excel 2013.

BCO-DMO Processing:
- modified parameter names to conform with BCO-DMO naming conventions;
- replaced missing data/blanks with "nd" ("no data")

Data Files

File
mussel_data.csv (Comma Separated Values (.csv), 9.68 KB) MD5:be12f8b7565891addc577773149b5b0d Primary data file for dataset ID 713831

Parameters

Parameter	Description	Units
Site	Site where sampling occurred	unitless
Identifier	Sample ID	unitless
Habitat_Type	Description of habitat	unitless
n	Number of samples analyzed	count
Latitude_degMin	Latitude in degree minutes	Degree minutes
Longitude_degMin	Longitude in degree minutes	Degree minutes
lat	Latitude in decimal degrees	Decimal degrees
lon	Longitude in decimal degrss	Decimal degrees
Bulk_d15N	15N/14N isotopic ratio	per mil

Bulk_d15N_stdev	Standard deviation of isotopic ratio	per mil
Bulk_d13C	13C/12C isotopic ratio	per mil
Bulk_d13C_stdev	Standard deviation of isotopic ratio	per mil
Average_Trophic	Average d15N values of [Alanine, Aspartic Acid, Glutamic Acid, Leucine, Isoleucine, Proline, Valine]	per mil
Average_Source	Average d15N values of [Glycine, Lysine, Phenylalanine, Serine, Tyrosine]	per mil
Trophic_Position	Trophic Position; { [(d15N Glutamic Acid - d15N Phenylalanine) - 3.4] / 7.6 } + 1	unitless
Ala	Alanine 15N or 13C value	per mil
Ala_stdev	Alanine standard deviation	per mil
Asx	Asn+Asp 15N or 13C peak values	per mil
Asx_stdev	Asx standard deviation	per mil
Glx	Gln+Glu 15N or 13C peak values	per mil
Glx_stdev	Glx standard deviation	per mil
Gly	Glycine 15N or 13C value	per mil
Gly_stdev	Glycine standard deviation	per mil
Ile	Isoleucine 15N or 13 C value	per mil
Ile_stdev	Isoleucine standard deviation	per mil
Leu	Leucine 15N or 13C value	per mil
Leu_stdev	Leucine standard deviation	per mil

Lys	Lysine 15N or 13C value	per mil
Lys_stdev	Lysine standard deviation	per mil
Phe	Phenylalanine 15N or 13C value	per mil
Phe_stdev	Phenylalanine standard deviation	per mil
Pro	Proline 15N or 13C value	per mil
Pro_stdev	Proline standard deviation	per mil
Ser	Serine 15N or 13C value	per mil
Ser_stdev	Serine standard deviation	per mil
Thr	Threonine 15N or 13C value	per mil
Thr_stdev	Threonine standard deviation	per mil
Tyr	Tyrosine 15N or 13C value	per mil
Tyr_stdev	Tyrosine standard deviation	per mil
Val	Valine 15N or 13C value	per mil
Val_stdev	Valine standard deviation	per mil
element	Isotopic element; Nitrogen AA or Carbon AA	unitless

Instruments

Dataset-specific Instrument Name	Carlo Erba CHNS-O EA1108-elemental analyzer
Generic Instrument Name	Elemental Analyzer
Dataset-specific Description	Interfaced via a ConFlo III device with a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific)
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	Trace GC, interfaced via a ConFlo III and GCC device
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Used with with a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific)
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	hermoFinnigan Delta Plus XP isotope ratio mass spectrometer (Thermo Fisher Scientific)
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	Used with Trace GC, interfaced via a ConFlo III and GCC device
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).

Deployments

McCarthy_2009

Website	https://www.bco-dmo.org/deployment/713862
Platform	shoreside Calif_shore
Start Date	2009-12-01
End Date	2010-02-28
Description	Sampling took place in California coast littoral waters from San Diego to the Oregon border

Project Information

The Use of Nitrogen Isotopes of Amino Acids To Understand Marine Sedimentary 15N Records (Amino Acid Sediment 15N)

Coverage: California Margin , Santa Barbara Basin , CA current system, Eastern Tropical Pacific

The bioavailability of nutrients plays a crucial role in oceanic biological productivity, the carbon cycle, and climate change. The global ocean inventory of nitrogen (N) is determined by the balance of N-fixation (sources) and denitrification (sinks). In this three-year project, a researcher from the University of California, Santa Cruz, will focus on developing compound-specific N isotope (d15N) analysis of amino acids as a new tool for understanding N source and transformation of organic matter in paleo-reservoirs. The offsets in the isotopic ratios of individual amino acid groups may yield information about trophic transfer, heterotrophic microbial reworking, and autotrophic versus heterotrophic sources. By measuring and comparing the bulk and amino acid d15N in size-fractioned samples from plankton tows, sediments traps, and multi-cores in oxic and suboxic depositional environments, the researcher will: (1) Provide a proxy of the d15N of average exported photoautotrophic organic matter; and (2) Provide a new level of detail into sedimentary organic N degradation and preservation.

Broader impacts:
This project will improve understanding of the fundamental underpinnings and behaviors of d15N amino acid patterns and how they behave in contrasting sedimentary environments, while also developing a potential paleoceanographic proxy. Funding will support a graduate student and undergraduate research at the institution. The researcher will also conduct community outreach in the form of a workshop/tutorial on the proxy development.

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1131816