

CSIA 15N AA data from phytoplankton, microzooplankton, and *Calanus pacificus*.

Website: <https://www.bco-dmo.org/dataset/744468>

Data Type: Other Field Results

Version: 1

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Project

» [Resolving the trophic connection between protistan grazers and mesozooplankton in marine food webs using amino acid-specific stable isotope analyses](#) (CSIA-AA Mesozooplankton TP)

Contributors	Affiliation	Role
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Abstract

CSIA 15N AA data from phytoplankton, microzooplankton, and *Calanus pacificus*.

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Coverage

Spatial Extent: Lat:32.868814 Lon:-117.254046

Dataset Description

15N AA from phytoplankton, microzooplankton, and *Calanus pacificus*.

Methods & Sampling

Hydrolysis, derivatization and analyses of samples for CSIA – For Exps. 3-4, samples (1-2 mg dry weight) for CSIA-AA were added to 1 ml 6 N HPLC-grade HCl, then flushed with N₂, capped with a Teflon-lined cap, and hydrolyzed at 150°C for 70 min. The resulting hydrolysate was evaporated to dryness under N₂ at 55°C, redissolved in 1 ml 0.01 N HCl, purified by filtration (0.45-µm hydrophilic filter), washed with 1 ml of 0.01 N HCl, and further purified using cation-exchange chromatography with a 5-cm resin column (Dowex 50WX8-400) in a glass Pasteur pipette. AAs were eluted with 4 ml of 2 N NH₄OH and evaporated to dryness under a stream of N₂ at 80°C, then reacidified with 0.5 ml of 0.2 N HCl, flushed with N₂, heated to 110°C for 5 min and evaporated to dryness under N₂ at 55°C. Hydrolyzed samples were esterified with 2 ml of 4:1 isopropanol:acetyl chloride, flushed with N₂ and heated to 110°C for 60 min. After drying at 60°C under N₂, the samples were acylated by adding 1 ml of 3:1 methylene chloride:trifluoroacetic anhydride (TFAA) and heated to 100°C for 15 min. The derivatized AAs were further purified by solvent extraction following (Ueda et al. 1989). The acylated AA esters were evaporated at room temperature under N₂ and redissolved in 3 ml of 1:2 chloroform:P-buffer (KH₂PO₄ + Na₂HPO₄ in Milli-Q water, pH 7). Vigorous shaking ensured that the derivitized AAs were partitioned into chloroform while contaminants remained in the P-buffer. The solvents

were separated by centrifugation (10 min at 600 g), the chloroform was transferred to a clean vial, and the solvent extraction process repeated. Finally, to ensure derivatization, the acylation step was repeated. Samples were stored at -20°C in 3:1 methylene chloride:TFAA for up to 2 weeks until analysis. AA derivatives were then analyzed by isotope monitoring gas chromatography-mass spectrometry. We used a Delta V Plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (650°C), and liquid nitrogen cold trap. Internal reference compounds (aminoadipic acid and norleucine) of known nitrogen isotopic composition were co-injected with samples and used to normalize the measured $\delta^{15}\text{N}$ values of unknown AAs, and a suite of eight AAs with known isotopic composition was analyzed every 3 injections for additional quality control. At least three injections per sample were analyzed. For Exps. 5 and 6, CSIA-AA samples were hydrolyzed in 6N HCl at 110°C for 20 h. The hydrolysate was evaporated to dryness at temperature under a continuous stream of N₂ gas, then esterified with 4:1 isotopropanol:acetyl chloride at 110°C for 60 min, acylated in a 1:1 solution of methylene chloride:trifluoroacetic anhydride (DCM:TFAA) for 10 min at 110°C. Samples were stored at -20°C in 1:1 DCM:TFAA for up to 3 months before isotope analysis using a Delta V Plus mass spectrometer (Thermo Scientific) interfaced through a Conflo IV to a GC 1310 gas chromatograph coupled to a GC Isolink combustion-reduction furnace (1000°C) and liquid nitrogen cold trap. All samples were injected (splitless injector) onto a forte BPx5 capillary column (60 m x 0.32 mm x 1.0- μm film thickness) at an injector temperature of 250°C with a constant helium flow rate of 1.4 ml min⁻¹. The column was initially held at 50°C for 2 min and then increased to 125 °C at a rate of 15°C min⁻¹. Once at 125°C, the temperature was increased at a rate of 3°C min⁻¹ to 160°C and then to 190°C at a rate of 4°C min⁻¹. The final temperature of 300°C was reached by ramping to 275°C at 6°C min⁻¹ and then 15°C min⁻¹ afterward. Samples were analyzed in triplicate and normalized to the known $\delta^{15}\text{N}$ values of a suite of 14 AAs analyzed before and after each set of 3 samples.

Data Processing Description

Data were processed using R software to correct sample values using a standard curve of known versus measured $\delta^{15}\text{N}$ values. The standard deviations of measured $\delta^{15}\text{N}$ values of standard AAs ranged from 0.1 to 1.7‰. Analyses of replicate samples are noted in data table.

BCO-DMO Processing:

- modified parameter names to conform with BCO-DMO naming conventions;
- replaced spaces with underscores in organism and stage columns;
- replaced missing data with 'nd'

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Data Files

File
chemostat.csv (Comma Separated Values (.csv), 3.41 KB) MD5:c50d164eb068111191eaa3909f4f1872
Primary data file for dataset ID 744468

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Parameters

Parameter	Description	Units
Experiment	Experiment number	unitless
Organism	Species name or Particulate organic matter (POM)	unitless
stage	Refers to the experimental stage	unitless
bulk_d15N	15N of bulk material	per mil
Ala_d15N	15N of AA Alanine	per mil
Asp_d15N	15N of AA Aspartic acid	per mil
Glu_d15N	15N of AA Glutamic acid	per mil
IsoL_d15N	15N of AA IsoLeucine	per mil
Leu_d15N	15N of AA Leucine	per mil
Pro_d15N	15N of AA Proline	per mil
Val_d15N	15N of AA Valine	per mil
Gly_d15N	15N of AA Glycine	per mil
Lys_d15N	15N of AA Lysine	per mil
Phe_d15N	15N of AA Phenylalanine	per mil
Ser_d15N	15N of AA Serine	per mil
Thr_d15N	15N of AA Treonine	per mil
Note_Organism	Each note corresponds to the cell in this row in the Organism column	unitless
Note_bulk_d15N	Each note corresponds to the cell in this row in the bulk d15N column	unitless
Note_Gly_d15N	Each note corresponds to the cell in this row in the Gly d15N column	unitless

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Instruments

Dataset-specific Instrument Name	Trace GC gas chromatograph, GC 1310 gas chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Used with mass spectrometer
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	GC 1310
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Coupled to a GC Isolink combustion-reduction furnace (1000 deg C) and liquid nitrogen cold trap.
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	Delta V Plus
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Used for sampling
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Project Information

Resolving the trophic connection between protistan grazers and mesozooplankton in marine food webs using amino acid-specific stable isotope analyses (CSIA-AA Mesozooplankton TP)

Coverage: California Current, eastern Pacific Ocean

Description from NSF award abstract:

Energy dissipation and elemental cycling by protistan consumers in lower trophic levels of ocean food webs are of sufficient magnitude, based on global mean measures of the amount of primary production consumed, to strongly alter the efficiencies of material transfers to higher-level consumers and to export. We presently know very little about these microbial food web steps, how they vary regionally or temporally, or how they might be altered by climate change. Compound Specific Isotope Analysis of Amino Acids (CSIA-AA) offers an approach for advancing our understanding of microbial food web structure and trophic fluxes based on the trophic positions (TP) of mesozooplankton as temporal integrators of the fluxes from direct feeding on phytoplankton and indirect transfers via protistan microzooplankton. Preliminary laboratory experiments to test this idea have demonstrated that the standard application of the method, using labeled phenylalanine as the representative source AA for the primary producer baseline and labeled glutamic acid as the indicator AA for trophic enrichment, does not produce a measureable trophic-step signal for protistan grazers. However, the results have also shown that an alternative high-turnover AA, alanine, strongly enriches in protistan as well as metazoan consumers, and leads to substantially higher TP estimates of mesozooplankton in field-collected specimens than that based on labeled glutamic acid.

This research project will test the hypothesis that labeled alanine provides a quantifiable and consistent index of trophic enrichment for protistan steps in marine food webs. The research will involve three major elements. First, controlled laboratory experiments will be conducted with chemostat systems to compare 15N

enrichments of alanine to other AAs for a representative suite of ciliate and flagellate grazers feeding on phytoplankton, and to evaluate the two-step enrichment from phytoplankton via a protistan grazer to a suspension-feeding copepod. Second, field-collected mesozooplankton from four distinct ecological regions of the Pacific Ocean will be analyzed by CSIA-AA to test the transfer of alanine enrichment through a metazoan trophic step (comparing suspension feeding species to primary carnivores) and to assess how the TP index differs with trophic structure over a broad range of ecological conditions. Last, CSIA-AA assessments of TP for size-structured zooplankton will be integrated into inverse models of nitrogen flows in the four regions (equatorial Pacific, subtropical North Pacific, California Current and Costa Rica Dome) as a major constraint for resolving and comparing fluxes through the microbial food web over the range of ecological conditions. A properly calibrated CSIA-AA assessment of mesozooplankton trophic position will provide a new and valuable approach for regional intercomparisons of lower-level food web structure, for assessing temporal and spatial trends in climate change, for ocean ecosystem model validation, and for better understanding of lower food-web energetic constraints on ocean fisheries.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1260055

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