Bulk and AA d15N values for ala, glu, and phe from phytoplantkon and grazers grown in lab chemostats

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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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Dataset Description

Bulk and AA d15N values for ala, glu, and phe from phytoplantkon and grazers grown in lab chemostats. These data were also published in Table 2 of:
Décima, M., M. R. Landry, C. J. Bradley, and M. L. Fogel. 2017. Alanine d^{15}N trophic fractionation in heterotrophic protists Limnol. Oceanogr., doi:10.1002/lno.10567

The details of the experimental conditions were published in Table 1 of the above publicaiton, and are also available in PDF.

Methods & Sampling

Trophic changes in d15N of AAs from phytoplankton to protistan and metazoan plankton consumers were examined in six chemostat set-ups, including five two-stage experiments and one 3-stage experiment (Décima et al. 2017).

All experiments were conducted in a temperature-controlled room (16 or 18 degrees Celsius) with the algal prey in 2.2-L stage 1 reactors (dilution rate = 0.5 d-1) receiving continuous nutrient input from a 20-L reservoir under continuous light (~120 umol photon m-2 s-1). Seawater collected from the Scripps pier (La Jolla, CA) was autoclaved and filtered for all media preparations, using f/2 Guillard concentrations for all nutrients except NO-3 or PO3- 4, which were varied to produce conditions of N- (Exps. 1 and 4-6) or P-limitation (Exps. 2), while Exp. 3 had no imposed limitation. Flow between the nutrient reservoir and the chemostat culture reactors was continuously pumped through platinum-cured silicone tubes using a Masterflex L/S model 7519-05 peristaltic pump equipped with individually adjustable cassettes for each tube.

For the two-stage experiments, consumers in the stage 2 reactors were grown on the phytoplankton from stage 1 outflow under continuous light or continuous dark conditions to either allow (light) or suppress (dark) phytoplankton uptake of the nutrients regenerated by consumer excretion. The reactors were monitored daily

for 12-15 days to ensure constant conditions, thus minimizing predator: prey isotopic mismatch, and samples were taken for cell abundances and biovolumes using an Elzone counter and epifluorescence microscopy. Samples for CSIA-AA were collected at the end of the experiments on pre-combusted 47-mm GFF filters, stored at -80 C, and dried at 60 C for at least 24 h before analyses.

For the 3 stage experiment, the second and third stage reactors had 3.6 L volumes. In the third reactor, metazoan consumers were grown on protistan zooplankton flowing from the second reactor, in turn growing on phytoplankton from the first reactor. Experiments were conducted for 14 days, after which the first and second stages were harvested by filtering the contents onto pre-combusted 47-mm GFFs. Metazoan consumers and <200-um particles in the third-stage reactor were collected as above.

Samples were hydrolyzed and purified AAs derivitized according to established protocols (Popp et al. 2007; Hannides et al. 2009; Décima et al. 2017). All samples were injected (splitless injector) onto a *forte* BPx5 capillary column (60 m x 0.32 mm x 1.0-um 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 per min. Once at 125 C, the temperature was increased at a rate of 3 C per min to 160 C and then to 190 C at a rate of 4 C per min. The final temperature of 300 C was reached by ramping to 275 C at 6 C per min and then 15 C per min afterward. Samples were analyzed in triplicate and normalized to the known δ15N values of a suite of 14 AAs analyzed before and after each set of 3 samples.

References:
Décima, M., M. R. Landry, C. J. Bradley, and M. L. Fogel. 2017. Alanine d15N trophic fractionation in heterotrophic protists Limnol. Oceanogr., doi:10.1002/lno.10567

Gutiérrez-Rodríguez, A., Décima, M., Popp, B. N., and Landry, M. R. 2014. Isotopic invisibility of protozoan trophic steps in marine food webs. Limnol. Oceanogr. 59: 1590-1598.

Hannides, C. C. S., B. N. Popp, M. R. Landry, and B. S. Graham. 2009. Quantification of zooplankton trophic position in the North Pacific Subtropical Gyre using stable nitrogen isotopes. Limnol. Oceanogr. 54: 50-61.

Landry, M.R. and M.R. Décima. 2017. Protistan microzooplankton and the trophic position of tuna: Quantifying the trophic link between micro- and mesozooplankton in marine food webs. ICES J. Mar. Sci. doi:10.1093/icesjms/fsx006

Popp, B. N., B. S. Graham, R. J. Olson, C. C. S. Hannides, M. J. Lott, G. A. Lopez-Ibarra, F. Galvan-Magana, and B. Fry. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids, p. 173-190. *In* T. E. Dawson and R. T. W. Siegwolf [eds.], Stable isotopes as indicators of ecological change. Terrestrial Ecology Series. Elsevier Academic Press.

Data Processing Description

Amino acid data were processed using R software to correct sample values using a standard curve of known versus measured δ15N values. The standard deviations of measured δ15N values of standard AAs ranged from 0.1 to 1.7‰. Statistical tests using AA data were processed using Matlab 2013a.

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';
- converted Table 1 to PDF.

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Data Files

File
AA_d15N.csv (Comma Separated Values (.csv), 1.50 KB) MD5:9dec22eeed85dd8bb7385d248bb6c405 Primary data file for dataset ID 699904

Parameters

Parameter	Description	Units
experiment	Experiment number. See details on experimental conditions (PDF)	unitless
organism	Name of the organism	unitless
stage	Stage of the experiment	unitless
bulk	bulk material d15N	per mil (‰)
bulk_std	Standard deviation (of 2 replicates) of bulk	per mil (‰)
Ala1	Alanine d15N of replicate 1	per mil (‰)
Glu1	Glutamic acid d15N of replicate 1	per mil (‰)
Phe1	Phenylalanine d15N of replicate 1	per mil (‰)
Ala1_std	Standard deviation (of 3 machine injections) of Ala1	per mil (‰)
Glu1_std	Standard deviation (of 3 machine injections) of Glu1	per mil (‰)
Phe1_std	Standard deviation (of 3 machine injections) of Phe1	per mil (‰)
Ala2	Alanine d15N of replicate 2	per mil (‰)
Glu2	Glutamic acid d15N of replicate 2	per mil (‰)
Phe2	Phenylalanine d15N of replicate 2	per mil (‰)
Ala2_std	Standard deviation (of 3 machine injections) of Ala2	per mil (‰)
Glu2_std	Standard deviation (of 3 machine injections) of Glu2	per mil (‰)
Phe2_std	Standard deviation (of 3 machine injections) of Phe2	per mil (‰)

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Instruments

Dataset-specific Instrument Name	epifluorescence microscopy
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	The reactors were monitored daily for 12-15 days to ensure constant conditions, thus minimizing predator: prey isotopic mismatch, and samples were taken for cell abundances and biovolumes using an Elzone counter and epifluorescence microscopy.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Trace GC gas chromatograph, GC 1310 gas chromatograph
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	For experiments 1-4, we used a Delta V Plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980C), reduction furnace (650C), and liquid nitrogen cold trap as described in Hannides et al. (2009). For experiments 5-6 we used 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 (1000C) 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	For experiments 1-4, we used a Delta V Plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980C), reduction furnace (650C), and liquid nitrogen cold trap as described in Hannides et al. (2009). For experiments 5-6 we used 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 (1000C) and liquid nitrogen cold trap.
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.

Dataset-specific Instrument Name	Elzone counter
Generic Instrument Name	Particle Size Analyzer
Dataset-specific Description	The reactors were monitored daily for 12-15 days to ensure constant conditions, thus minimizing predator: prey isotopic mismatch, and samples were taken for cell abundances and biovolumes using an Elzone counter and epifluorescence microscopy.
Generic Instrument Description	Particle size analysis, particle size measurement, or simply particle sizing is the collective name of the technical procedures, or laboratory techniques which determines the size range, and/or the average, or mean size of the particles in a powder or liquid sample.

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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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