Trial A test of the dissolution method for estimates of the 15N2 atom% of incubations

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Project
» EAGER: Collaborative Research: Detection limit in marine nitrogen fixation measurements - Constraints of rates from the mesopelagic ocean (EAGER NitFix)

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Abstract
The “dissolution” method to measure N2 fixation rates with 15N2 gas tracer involves the preparation of 15N2-enriched water that is then added to each incubation bottle. Investigators typically measure the 15N2 atom% of the 15N2-enriched inoculum by MIMS, and extrapolate the 15N2 atom% in the incubations based on the inoculum value. Here, we demonstrate that such extrapolation yields inaccurate estimates of the 15N2 atom% of incubations. The latter should be measured directly.

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Dataset Description

Trial A test of the dissolution method

Methods & Sampling

Inocula of 15N2-enriched water were prepared according to either of two protocols outlined by Klawonn et al. (2015). In a first Trial A, respective 1.9 mL of 15N2 gas aliquots (Cambridge Isotope Laboratories, Lot #I-21065) were injected into crimped-sealed 120 mL glass serum vials filled with deionized water. To dissolve the 15N2 bubble, each of the two serum vials was vortexed for 5 minutes. Two subsamples of each inoculum were dispensed into Exetainers™ with a peristaltic pump for analysis on the MIMS. An aliquot of each inoculum (5 % vol/vol) was then dispensed in replicate 160 mL serum incubation bottles containing air-equilibrated deionized water (Trials A1-A4), which were then crimped-sealed. Following homogenization, triplicate subsamples of each incubation were collected in Exetainers™ for MIMS analysis. The 15N atom % of the inocula and of the corresponding incubations were measured by MIMS at the University of Connecticut (Bay Instruments) and computed as follows:

Equation 4:

$$MIMS\ A_{N_2}\ (\%) = \left[\frac{\frac{m}{z}_{30} + 0.5 * \frac{m}{z}_{29}}{\frac{m}{z}_{28} + \frac{m}{z}_{29} + \frac{m}{z}_{30}}\right] \times 100$$

In both trials, the concentration of N2 isotopologues (m/z 28, 29, and 30) in each of the 15N2-enriched inocula was then extrapolated from the ionization efficiency of N isotopologues in air-equilibrated seawater. We define the ionization efficiency as the ratio of the isotopologue ion current measured by MIMS relative to its concentration in air-equilibrated seawater (ASW):

Equation S2:

$$Ionization\ efficiency\ of\ \ 28N_2 = \frac{m}{z}28\ ion\ current_{\ ASW} \div [\ 28N]_{ASW}$$

For instance, at a temperature of 25ºC and salinity of 35 psu, the solubility coefficients of Hamme and Emerson (2004) predict a N2 concentration of 388.9 µmol kg-1. The fraction of 15N in N2 (i.e., 15N/(14N+15N)) for air-equilibrated seawater is 0.003663 (Mariotti, 1983), such that the expected fractions of 28N2, 29N2, and 30N2 derived from their binomial probability distributions are as follows:

$$28N_2 = \left[1 - \frac{15N}{14N + 15N}\right]^2 x\ 100$$

= 99.2687 % Equation S3a

$$29N_2 = 2\ x\ \left[\frac{15N}{14N + 15N}\, x\, (1 - \frac{15N}{14N + 15N})\right] x\ 100$$

= 0.7299 % Equation S3b

$$30N_2 = \left[\frac{15N}{14N + 15N}\right]^2 x\ 100$$

= 0.0013 % Equation S3c

Accordingly, air-equilibrated concentrations of 28N2, 29N2, and 30N2 at this temperature and salinity are 386.0, 2.8, and 0.005 µmol kg-1, respectively. The ionization efficiency of the isotopologues is then equal to the ion current of m/z 28 recorded for ASW divided by the corresponding 28N2 concentration (Eq. S2). We used the ionization efficiency of m/z 28 in ASW to derive the N2 isotopologue concentrations in the inocula from their respective MIMS ion currents. We did not derive distinct ionization efficiencies from the ion current-to-concentration of m/z 29 and 30 in ASW, as these isotopologues are poorly resolved by the MIMS at natural abundance. Thus, we are assuming that the ionization efficiency of m/z 29 and 30 isotopologues is roughly similar to that of m/z 28 (i.e., that ionization isotope effects are negligible for our purposes). The initial expected AN2 of the “incubations” was then calculated using a linear mixing model with N2 isotopologue concentrations in ambient and enriched seawater

Data Processing Description

BCO-DMO Processing Notes:
- table was extracted from original spreadsheet.
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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Data Files

File
trial_a.csv (Comma Separated Values (.csv), 6.16 KB) MD5:2298e8da89522e950fdb4b6cf8296398 Primary data file for dataset ID 778126

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Supplemental Files

File
equation 4 EAGER_NitFix filename: equation_4.jpg (JPEG Image (.jpg), 24.62 KB) MD5:25f19b478991407bd347bd9221c4857b The 15N atom % of the inocula and of the corresponding incubations were measured by MIMS at the University of Connecticut (Bay Instruments) and computed using this equation.
equation S2 Eager_NitFix filename: equation_s2.jpg (JPEG Image (.jpg), 23.66 KB) MD5:af88aa623a8e948669b26dbeb4dbaeaf Equation for the definition of the ionization efficiency as the ratio of the isotopologue ion current measured by MIMS relative to its concentration in air-equilibrated seawater (ASW).
equation s3a Eager_NitFix filename: equation_s3a.jpg (JPEG Image (.jpg), 9.58 KB) MD5:fdc41b085863b05134fbb25f6cc25c54 Equation for the binomial probability distribution for 28N2.
equation s3b Eager_NitFix filename: equation_s3b.jpg (JPEG Image (.jpg), 22.12 KB) MD5:d7200880be28043d090d28bebeda9d12 Equation for the binomial probability distribution for 29N2.
equation s3c Eager_NitFix filename: equation_s3c.jpg (JPEG Image (.jpg), 14.71 KB) MD5:04c7942d3273c43e9c10e3ed2b41b0fd Equation for the binomial probability distribution for 30N2.

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Related Publications

White, A. E., Granger, J., Selden, C., Gradoville, M. R., Potts, L., Bourbonnais, A., Fulweiler, R. W., Knapp, A. N., Mohr, W., Moisander, P. H., Tobias, C. R., Caffin, M., Wilson, S. T., Benavides, M., Bonnet, S., Mulholland, M. R., & Chang, B. X. (2020). A critical review of the 15N2 tracer method to measure diazotrophic production in pelagic ecosystems. Limnology and Oceanography: Methods, 18(4), 129–147. Wiley. https://doi.org/10.1002/lom3.10353
Results

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Parameters

Parameter	Description	Units
Sample	sample	unitless
Baro_Press	barometric pressue	unknown
Time_of_analysis	time of analysis	unitless
m_z_28	mass-to-charge	unitless
m_z_29	mass-to-charge	unitless
m_z_30	mass-to-charge	unitless
m_z_32	mass-to-charge	unitless
m_z_40	mass-to-charge	unitless
N2_Ar	N2/Ar ratio	unitless
ratio_28_29	28/29 ratio	unitless
ratio_28_30	28/30 ratio	unitless
meas_at_pcnt	measured atom percent	unitless
avg_measured_a_pcnt	average measured atom percent	unitless

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Instruments

Dataset-specific Instrument Name	Isotope Ratio Mass Spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	continuous flow Delta V Isotope Ratio Mass Spectrometer (Smith et al. 2015), and continuous flow-GV Isoprime IRMS (Charoenpong et al., 2014)
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	Membrane Inlet Mass Spectrometer
Generic Instrument Name	Membrane Inlet Mass Spectrometer
Dataset-specific Description	Membrane Inlet Mass Spectrometer (Bay Instruments)
Generic Instrument Description	Membrane-introduction mass spectrometry (MIMS) is a method of introducing analytes into the mass spectrometer's vacuum chamber via a semipermeable membrane.

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Project Information

EAGER: Collaborative Research: Detection limit in marine nitrogen fixation measurements - Constraints of rates from the mesopelagic ocean (EAGER NitFix)

Coverage: North Atlantic Ocean, Pacific Ocean

NSF Award Abstract:
The availability of nitrogen is required to support the growth and production of organisms living in the surface of our global ocean. This element can be scarce. To alleviate this scarcity, a special class of bacteria and archaea, called nitrogen fixers, can derive the nitrogen needed for growth from nitrogen gas. This project would carefully examine one specific method for measuring nitrogen fixation that has been used recently to suggest the occurrence of small amounts of nitrogen fixation in subsurface ocean waters. If these reports are verified, then a revision of our understanding of the marine nitrogen cycle may be needed. The Ocean Carbon and Biogeochemistry program will be used as a platform to develop community consensus for best practices in nitrogen fixation measurements and detection of diversity, activity, and abundances of the organisms responsible. In addition, a session will be organized in a future national/international conference to communicate with the broader scientific community while developing these best practices.

The goal of this study is to conduct a thorough examination of potential experimental and analytical errors inherent to the 15N2-tracer nitrogen fixation method, in tandem with comprehensive molecular measurements, in mesopelagic ocean waters. Samples will be collected and experimental work conducted on a cruise transect in the North Atlantic Ocean, followed by analytical work in the laboratory. The specific aims of this study are to (1) determine the minimum quantifiable rates of 15N2 fixation based on incubations of mesopelagic waters via characterization of sources of experimental and analytical error, and (2) seek evidence of presence and expression of nitrogen fixation genes via comprehensive molecular approaches on corresponding samples. The range of detectable rates and diazotroph activity from the measurements made in this study will be informative for the understanding of the importance of nitrogen fixation in the oceanic nitrogen budget.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1732246

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