

Natural abundance of ^{15}N and ^{18}O measured in samples collected over the continental shelf west of the Antarctic Peninsula on cruise LMG1801 from January to February 2018

Website: <https://www.bco-dmo.org/dataset/842944>

Data Type: Cruise Results

Version: 1

Version Date: 2021-04-08

Project

» [Collaborative Research: Chemoautotrophy in Antarctic Bacterioplankton Communities Supported by the Oxidation of Urea-derived Nitrogen](#) (Oxidation of Urea N)

Contributors	Affiliation	Role
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Abstract

This dataset reports the natural abundance of ^{15}N and ^{18}O measured in samples collected over the continental shelf west of the Antarctic Peninsula on cruise LMG1801 from January to February 2018.

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Coverage

Spatial Extent: N:-64.03196 E:-64.03196 S:-69.25615 W:-78.20207

Temporal Extent: 2018-01-05 - 2018-02-04

Acquisition Description

Sample Collection. Samples were collected on the Antarctic continental shelf and slope west of the Antarctic Peninsula within the PAL-LTER sampling domain (<http://pal.lternet.edu/>) during summer (cruise

dates 30 Dec 2017 through 12 Feb 2018; sampling dates 5 Jan to 4 Feb 2018) from the ARSV Laurence M Gould (LMG 1801, PAL-LTER cruise 26, DOI: [10.7284/907858](https://doi.org/10.7284/907858)). Sampling focused on three or 4 depths at each station chosen to represent the Antarctic Surface Water (ASW, 0 -34 m depth), the Winter Water (WW, the water column temperature minimum, generally between 35 and 174 m) the Circumpolar Deep Water (CDW, 175-1000 m) and slope water (SLOPE, >1000 m, generally ~10 m above the bottom at deep stations on the slope, 2500-3048m). Water samples were collected from Niskin bottles (General Oceanics Inc., Miami, FL, USA) into opaque 2 L HDPE plastic bottles or into aged, acid-washed, sample-rinsed 250 ml polycarbonate bottles (Nalge) completely filled (~270 mL) directly from Niskin bottles as soon as possible after the rosette was secured on deck. Subsequent processing took place in an adjacent laboratory.

Samples for DNA analysis were taken from the 2 L opaque HDPE bottles and were filtered under pressure through 0.22 µm pore size Sterivex GVWP filters (EMD Millipore, Billerica, MA, USA) using a peristaltic pump. Residual seawater was expelled from the filter using a syringe filled with air, then ~1.8 ml of lysis buffer (0.75 M sucrose, 40 mM EDTA, 50 mM Tris, pH 8.3) was added to the filter capsule, which was capped and placed in a -20 °C freezer. The frozen samples were aggregated into Ziploc Freezer Bags and transferred to a -80 °C freezer for the remainder of the cruise and for shipping to the laboratory.

Two samples of the Sterivex filtrate (40 mL each into new 50 mL disposable centrifuge tubes, VWR, rinsed 3x with sample) were frozen immediately at -20 °C, then aggregated into Ziploc Freezer Bags and transferred to a -80 °C freezer for the remainder of the cruise and for shipping to the laboratory. These were used for subsequent determination of 1) urea concentration and 2) the natural abundance of ^{15}N in the nitrite plus nitrate pools ($^{15}\text{NO}_x$ hereinafter). An additional sample of the Sterivex filtrate was stored in a polycarbonate bottle at 4 °C for subsequent onboard determination of ammonia concentration by the Holmes et al (1999) o-phthaldialdehyde method and nitrite concentration by the diazo-coupling method (Strickland and Parsons 1972). Technical difficulties encountered during onboard analysis resulted in the loss of ammonium and nitrite data for some samples.

Samples for DNA and chemical analyses were shipped on dry ice from Punta Arenas, Chile to the Hollibaugh laboratory at the University of Georgia. Upon arrival they were stored in a -80 °C freezer until analyzed. Samples for ^{15}N analysis were shipped on dry ice from Punta Arenas, Chile to the Popp laboratory at the University of Hawaii. Upon arrival they were stored in a -40 °C freezer until analyzed.

Nitrogen oxidation rates. Oxidation rates of N supplied as ammonium, nitrite, urea and putrescine (1,4-diaminobutane) were measured in ~48 h incubations using ^{15}N -labeled substrates (>98 at% ^{15}N , Cambridge Isotope Laboratories, Tewksbury, MA, USA) added within ~1 hr of sample collection to yield ~44 nM amendments (Santoro et al., 2010; Beman et al., 2012). Labeled substrates were added to duplicate bottles that were placed in cardboard boxes and incubated in the dark in a Percival incubator (Perry, IA, USA). Incubation temperature was recorded at 5-minute time steps with HOBO TidBit data loggers (Onset Computer Corp., Bourne MA, Figure 1) placed in bottles of filtered seawater incubated in cardboard boxes identical to those used for experiments (see the "[Incubator Temperature.xlsx](#)" Supplemental File). Incubations were terminated after ~48 hr by decanting 40 mL subsamples from each bottle into new, sample rinsed, 50 mL polypropylene centrifuge tubes that were immediately frozen at -80 °C. Water in these tubes was used for subsequent analysis of $^{15}\text{NO}_x$. The natural abundance of ^{15}N in NO_x was taken as the initial (time = 0) value for calculating the amount of ^{15}N oxidized to nitrate or nitrite during the incubations.

Chemical analyses. Urea content was determined by the diacetyl monoxime method (Rahmatullah and Boyde 1980, Mulvenna and Savidge 1992). Subsamples from samples that were also used to determine oxidation of ^{15}N supplied as putrescine were sent to Dr. X. Mou's laboratory at Kent State University where they were analyzed to determine polyamine and DFAA content as described previously (Lu et al 2014).

^{15}N in nitrite plus nitrate. The $^{15}\text{NO}_x$ in samples was measured using the 'denitrifier method' (Sigman et al., 2001) with *Pseudomonas aureofaciens* as described in Popp et al. (1995), Dore et al. (1998) and Beman et al. (2011). The nitrous oxide produced was analyzed using a Gas Bench II coupled to a MAT 252 mass spectrometer following the recommendations of Casciotti et al. (2002). Typically nineteen samples

plus one sample duplicate was analyzed along with duplicate reference materials USGS 32, USGS 34 and USGS 35 (or NIST 3), which were used to normalize the measured $d^{15}\text{N}$ values to AIR. In addition, a laboratory reference solution made from analytical grade NaNO_3 with $d^{15}\text{N}$ value (-52.2‰) that was known through extensive characterization using NIST/USGS reference materials was also analyzed in duplicate with each batch of 19 samples.

We calculated oxidation rates from $d^{15}\text{N}$ enrichment of the NO_x pool in the bottles at the ends of the incubations compared to the initial value in the unamended seawater sample ("natural abundance"). We assumed that the $d^{15}\text{N}$ of naturally occurring ammonia, urea and putrescine is the same as that of N in bulk organic matter, and that the $d^{15}\text{N}$ value of nitrite in our samples is -30 ‰ as reported by Smart et al. (2015). Samples with low or no activity sometimes yielded negative rates because the $d^{15}\text{NO}_x$ "natural abundance" value for that sample was greater than the $d^{15}\text{NO}_x$ value of amended sample. We analyzed control samples consisting of filtered seawater taken at the beginning of the cruise, amended with $^{15}\text{NO}_2$, then immediately frozen at -80 °C, or "time zero" samples from time course experiments performed throughout the cruise, to determine the contribution of autooxidation or isotope exchange to the apparent rate of nitrite oxidation. These samples were treated with sulfamic acid to remove unreacted $^{15}\text{NO}_2$ (Granger and Sigman 2009). The analysis indicated that about 7.9% of the ^{15}N supplied as NO_2 had been converted to $^{15}\text{NO}_3$ by the time we analyzed the samples. We also performed an independent chemical analysis of nitrite and nitrate (Strickland and Parsons 1972) in the (nominally) 0.125 mM $^{15}\text{NO}_2$ working stock solution a few months after the cruise. This analysis indicated that about 14.3% of the of the nitrite plus nitrate in the stock was nitrate. Because this stock had been handled and shipped separately from the cruise samples, we used 7.9% as the best estimate of the amount of ^{15}N label converted to nitrate. We have incorporated corrections for this reaction into rates calculated from field data.

We ran time-course incubations with samples from 2 or 3 depths at 34 stations to verify that oxidation rates did not change significantly during incubations, for example, due to substrate depletion or changes in the population of ammonia oxidizers. These experiments were set up in 250 ml polycarbonate bottles as above. Two bottles were sampled at each time point over time courses of 72 to 96 h. These data are presented in [Table 1](#) (PDF; see Supplemental Files). AO rates determined from the slope of linear regressions of the data from a given sample were compared to rates determined from samples taken at the 48 hr time point. We performed analogous experiments to examine the effect on N oxidation rates of variation in incubation temperature and in substrate concentration.

Summary Statistics for $d^{15}\text{NO}_x$ (mean, median, maximum, minimum, and standard error of $d^{15}\text{NO}_x$ measurements grouped by the water mass depth ranges) are provided in the Supplemental File [d15NOx_Summary_Statistics.JPG](#).

Precision. Analytical uncertainty in $d^{15}\text{N}$ values was determined from duplicate analyses of USGS reference materials, our laboratory reference solution and samples analyzed in duplicate and ranged from 0.36‰ to 0.56‰ ([Table 1](#) (PDF) Supplemental File). All rate measurements were also performed in duplicate (biological replicates) and their uncertainty is also presented in Table 1. Accuracy was determined based on isotope analysis of the laboratory reference solution, which was not used to normalize the isotopic results of samples and was found to be 0.42‰ (at% ^{15}N = 0.00019, n = 56).

Rate calculations. We integrated the data we collected to calculate oxidation rates of N supplied as ammonia and urea as described in Popp et al. (1995), Dore et al. (1998) and Beman et al. (2011). We used ammonium concentration data from shipboard analyses. Nitrite + nitrate concentrations were determined by PAL-LTER personnel and were obtained from their database. Urea concentrations were measured on samples shipped frozen to the University of Georgia (Hollibaugh lab). Chemical data needed for rate calculations were not available for some samples so we substituted water mass averages determined from other samples taken on the cruise.

We determined the limits of detection and precision of nutrient analyses as follows. The precision of nitrate plus nitrite analyses run by LTER personnel (<https://oceaninformatics.ucsd.edu/datazoo/catalogs/pallter/datasets/27>) was reported to be 100 nM. The precision and limit of detection of putrescine (polyamine) analysis is given in Lu et al. (2018) as 1 nM. We

determined the precision of ammonium, urea and nitrite analyses as the mean standard deviation of replicate (2 or 3) analyses of a given sample. They are: ammonium, 65 nM; urea, 10 nM; and nitrite, 70 nM. The limits of detection were taken as 1.96 times the precision of the relevant measurements.

We ran Monte Carlo simulations to estimate the precision and the limits of detection of rate measurements. The models incorporated the estimates of precision given above and the means of the measured *in situ* concentrations of the reactants, the mean *in situ* concentration of NO_x (from PAL-LTER data), the precision of the measured d¹⁵NO_x in the experiments at the beginning (natural abundance) and end of the incubations. We ran 10,000 trials using random numbers generated with population means and standard deviations (assuming normally distributed variance and produced using an Excel spreadsheet in the EasyFit[®] app)) equivalent to the test values. The standard deviation of the 10,000 rates calculated in this manner was taken as an overall estimate of the precision of the rates we report. The results of these models are summarized in Supplemental Table 3. Our estimates of the precision of the rate measurements (oxidation of ¹⁵N supplied as ammonium urea, putrescine or nitrite to ¹⁵NO_x, nmol L⁻¹ d⁻¹) are: ammonia, 2.18; urea 0.31; putrescine, 0.51; nitrite, 4.6, for relative standard deviations (RSD; ((standard deviation/mean)*100)) of: 15.3%; 11.3%; 8.2% and 32%, respectively, of the calculated rates. Model runs, which also tested the sensitivity of the calculated rates to the accuracy of estimates of some variables, are summarized in Supplemental [Table 3](#) (.xlsx file), which also includes the equations used to calculate rates.

Processing Description

BCO-DMO Processing:

- renamed fields to comply with BCO-DMO naming conventions;
- 2021-03-16: replaced with data file received on 2021-03-14; converted latitude and longitude values to negative; formatted date/time field to ISO8601 format.
- 2021-04-08: replaced data file with copy received on 2021-03-18.

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Related Publications

Beman, J. M., Chow, C.-E., King, A. L., Feng, Y., Fuhrman, J. A., Andersson, A., ... Hutchins, D. A. (2011). Global declines in oceanic nitrification rates as a consequence of ocean acidification. *Proceedings of the National Academy of Sciences*, 108(1), 208–213. doi:[10.1073/pnas.1011053108](https://doi.org/10.1073/pnas.1011053108)
Methods

Casciotti, K. L., Sigman, D. M., Hastings, M. G., Böhlke, J. K., & Hilkert, A. (2002). Measurement of the Oxygen Isotopic Composition of Nitrate in Seawater and Freshwater Using the Denitrifier Method. *Analytical Chemistry*, 74(19), 4905–4912. doi:[10.1021/ac020113w](https://doi.org/10.1021/ac020113w)
Methods

Dore, J. E., Popp, B. N., Karl, D. M., & Sansone, F. J. (1998). A large source of atmospheric nitrous oxide from subtropical North Pacific surface waters. *Nature*, 396(6706), 63–66. doi:[10.1038/23921](https://doi.org/10.1038/23921)
Methods

Granger, J., & Sigman, D. M. (2009). Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method. *Rapid Communications in Mass Spectrometry*, 23(23), 3753–3762. doi:[10.1002/rcm.4307](https://doi.org/10.1002/rcm.4307)
Methods

Holmes, R. M., Aminot, A., Kérouel, R., Hooker, B. A., & Peterson, B. J. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences*, 56(10), 1801–1808. doi:[10.1139/f99-128](https://doi.org/10.1139/f99-128)
Methods

Lu, X., Zou, L., Clevinger, C., Liu, Q., Hollibaugh, J. T., & Mou, X. (2014). Temporal dynamics and depth variations of dissolved free amino acids and polyamines in coastal seawater determined by high-performance liquid chromatography. *Marine Chemistry*, 163, 36–44. doi:[10.1016/j.marchem.2014.04.004](https://doi.org/10.1016/j.marchem.2014.04.004)
Methods

Michael Beman, J., Popp, B. N., & Alford, S. E. (2012). Quantification of ammonia oxidation rates and ammonia-oxidizing archaea and bacteria at high resolution in the Gulf of California and eastern tropical North Pacific Ocean. *Limnology and Oceanography*, 57(3), 711–726. doi:[10.4319/lo.2012.57.3.0711](https://doi.org/10.4319/lo.2012.57.3.0711)
Methods

Mulvenna, P. F., & Savidge, G. (1992). A modified manual method for the determination of urea in seawater using diacetylmonoxime reagent. *Estuarine, Coastal and Shelf Science*, 34(5), 429–438. doi:[10.1016/s0272-7714\(05\)80115-5](https://doi.org/10.1016/s0272-7714(05)80115-5) [https://doi.org/10.1016/S0272-7714\(05\)80115-5](https://doi.org/10.1016/S0272-7714(05)80115-5)
Methods

Popp, B. N., Sansone, F. J., Rust, T. M., & Merritt, D. A. (1995). Determination of Concentration and Carbon Isotopic Composition of Dissolved Methane in Sediments and Nearshore Waters. *Analytical Chemistry*, 67(2), 405–411. doi:[10.1021/ac00098a028](https://doi.org/10.1021/ac00098a028)
Methods

Rahmatullah, M., & Boyde, T. R. C. (1980). Improvements in the determination of urea using diacetyl monoxime; methods with and without deproteinisation. *Clinica Chimica Acta*, 107(1-2), 3–9. doi:[10.1016/0009-8981\(80\)90407-6](https://doi.org/10.1016/0009-8981(80)90407-6)
Methods

Santoro, A. E., Casciotti, K. L., & Francis, C. A. (2010). Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. *Environmental Microbiology*, 12(7), 1989–2006. doi:[10.1111/j.1462-2920.2010.02205.x](https://doi.org/10.1111/j.1462-2920.2010.02205.x)
Methods

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](https://doi.org/10.1021/ac010088e)
Methods

Smart, S. M., Fawcett, S. E., Thomalla, S. J., Weigand, M. A., Reason, C. J. C., & Sigman, D. M. (2015). Isotopic evidence for nitrification in the Antarctic winter mixed layer. *Global Biogeochemical Cycles*, 29(4), 427–445. doi:[10.1002/2014gb005013](https://doi.org/10.1002/2014gb005013) <https://doi.org/10.1002/2014GB005013>
Methods

Strickland, J. D. H. and Parsons, T. R. (1972). *A Practical Hand Book of Seawater Analysis*. Fisheries Research Board of Canada Bulletin 157, 2nd Edition, 310 p.
Methods

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Related Datasets

IsRelatedTo

Hollibaugh, J. T., Wallsgrove, N. J., Allen, T., Popp, B. N. (2021) **Results of measurements of the oxidation of ^{15}N supplied as ammonia, urea, putrescine or nitrite in samples collected from continental shelf waters west of the Antarctic Peninsula on cruise LMG1801 from January to February 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-03-18 doi:[10.26008/1912/bco-dmo.840629.1](https://doi.org/10.26008/1912/bco-dmo.840629.1) [[view at BCO-DMO](#)]

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Parameters

Parameter	Description	Units
Event_Log_Number	Sequential numbers keyed to the bridge log of activities	unitless
Cast_Start_Time_GMT	Date and time of day for beginning CTD cast = sample collection; 24-hour clock; formatted to ISO8601 standard (UTC/GMT): YYYY-MM-DDThh:mmZ	unitless
Latitude	Latitude in decimal degrees (negative values = South)	degrees North
Longitude	Longitude in decimal degrees (negative values = West)	degrees East
Station_Description	PAL-LTER category for the station	unitless
LTER_Grid_Station	Station location on the PAL-LTER sampling grid (http://pal.lternet.edu)	unitless
Sample_Depth	Depth sampled in meters	meters (m)
d15N	d15N value of the NO ₂ + NO ₃ in the sample as determined by the denitrifier method, units of parts per thousand (o/oo or per mil). Replicate determinations are retained but were averaged for use in calculating oxidation rates.	parts per thousand (o/oo or per mil)
d18O	d18O value of the NO ₂ + NO ₃ in the sample as determined by the denitrifier method, units of parts per thousand (o/oo or per mil).	parts per thousand (o/oo or per mil)
Analytical_Batch	Samples were run in batches of 20 samples with 10 standards, this column identifies the batch that included a specific sample	unitless

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Instruments

Dataset-specific Instrument Name	Niskin bottles (General Oceanics Inc., Miami, FL, USA)
Generic Instrument Name	Niskin bottle
Generic Instrument Description	A Niskin bottle (a next generation water sampler based on the Nansen bottle) is a cylindrical, non-metallic water collection device with stoppers at both ends. The bottles can be attached individually on a hydrowire or deployed in 12, 24, or 36 bottle Rosette systems mounted on a frame and combined with a CTD. Niskin bottles are used to collect discrete water samples for a range of measurements including pigments, nutrients, plankton, etc.

Dataset-specific Instrument Name	Gas Bench II coupled to a MAT 252 mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
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	Percival incubator
Generic Instrument Name	In-situ incubator
Generic Instrument Description	A device on shipboard or in the laboratory that holds water samples under controlled conditions of temperature and possibly illumination.

Dataset-specific Instrument Name	HOBO TidBit data loggers
Generic Instrument Name	Temperature Logger
Generic Instrument Description	Records temperature data over a period of time.

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Deployments

LMG1801

Website	https://www.bco-dmo.org/deployment/839984
Platform	ARSV Laurence M. Gould
Start Date	2017-12-30
End Date	2018-02-12
Description	Additional cruise information is available from the Rolling Deck to Repository (R2R): https://www.rvdata.us/search/cruise/LMG1801 Cruise DOI: 10.7284/907858

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Project Information

Collaborative Research: Chemoautotrophy in Antarctic Bacterioplankton Communities Supported by the Oxidation of Urea-derived Nitrogen (Oxidation of Urea N)

Coverage: Coastal, shelf and slope waters off the West Antarctic Peninsula, PAL-LTER sampling grid, Lawrence M Gould cruise 18-01

NSF Award Abstract:

Part 1: The project addresses fundamental questions regarding the role of nitrification (the conversion of ammonium to nitrate by a two-step process involving two different guilds of microorganisms: ammonia- and nitrite-oxidizers) in the Antarctic marine ecosystem. Specifically, the project seeks to evaluate the contribution of primary production supported by the energy in nitrogen compounds to the overall supply of organic carbon to the food web of the Southern Ocean. Previous measurements indicate that nitrification could contribute about 9% to primary production supporting the Antarctic food web on an annual basis, but those measurements did not include the additional production associated with nitrite oxidation. Additionally, the project will aim to determine the significance of the contribution of other sources of nitrogen, (specifically organic nitrogen and urea released by other organisms) to nitrification because these contributions may not be assessed by standard protocols. Such work will aid in better understanding the basis of the energy for the Antarctic marine ecosystems on an annual basis as well as better aid in understanding the energetics of the ecosystem in times and places where primary production based on light energy is limited (i.e. during the polar night or under sea ice cover).

This project will result in training a postdoctoral researcher and provide undergraduate students opportunities to gain hand-on experience with research on microbial geochemistry. The Palmer Long Term Ecological Research (LTER) activities have focused largely on the interaction between ocean climate and the marine food web affecting top predators. Relatively little effort has been devoted to studying processes related to the microbial geochemistry of nitrogen cycling, yet these are a major themes at other LTER sites. This work will contribute substantially to understanding an important aspect of nitrogen cycling and bacterioplankton production in the study area. The team will be working synergistically and be participating fully in the education and outreach efforts of the Palmer LTER, including making highlights of the findings available for posting to their project web site and participating in any special efforts they have in the area of outreach.

Part 2: The proposed work will quantify oxidation rates of ^{15}N supplied as ammonium, urea and nitrite, allowing the estimation of the contribution of urea-derived N and complete nitrification (ammonia to nitrate) to chemoautotrophy and bacterioplankton production in Antarctic coastal waters. The project will compare these estimates to direct measurements of the incorporation of ^{14}C into organic matter in the dark for an independent estimate of chemoautotrophy. The team aims to collect samples spanning the water column: from surface water (~ 10 m), winter water (50-100 m) and circumpolar deep water (> 150 m); on a cruise surveying the continental shelf and slope west of the Antarctic Peninsula in the austral summer of 2018. Other samples will be taken to measure the concentrations of nitrate, nitrite, ammonia and urea, for qPCR analysis of the abundance of relevant microorganisms, and for studies of related processes. The project will rely on collaboration with the existing Palmer LTER to ensure that ancillary data (bacterioplankton abundance and production, chlorophyll, physical and chemical variables) will be available. The synergistic activities of this project along with the LTER activities will provide a unique opportunity to assess chemoautotrophy in context of the overall ecosystem's dynamics- including both primary and secondary production processes.

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Funding

Funding Source	Award
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	OPP-1643466
NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP)	OPP-1643345

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