Results of qPCR assessments of the abundance of genes related to nitrification, and measurements of ammonia, urea, nitrite and polyamine concentrations in samples collected on cruise LMG1801 on R/V Laurence M. Gould from January to February 2018

Website: https://www.bco-dmo.org/dataset/840475
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
Hollibaugh, James T.	University of Georgia (UGA)	Principal Investigator, Contact
Popp, Brian N.	University of Hawaii at Manoa (SOEST)	Co-Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
The dataset includes the result of qPCR assessments of the abundance of genes related to nitrification, and measurements of ammonia, urea, nitrite and polyamine concentrations.

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Coverage

Spatial Extent: **N**:-64.032 **E**:-64.032 **S**:-69.2562 **W**:-78.2021
Temporal Extent: 2018-01-05 - 2018-02-04

Methods & Sampling

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). 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 um 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 ° 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 ($^{15}NO_x$ 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.

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).

Analysis of gene abundance. DNA was recovered from Sterivex filters using a lysozyme and proteinase K digestion, followed by purification using a phenol–chloroform extraction following Tolar et al. (2013). Abundances of Archaea and Bacteria genes were determined by quantitative PCR (qPCR) used an iCycler CFX Connect Real-Time PCR Detection System (BioRad) using either SYBR Green I dye (BioRad, Hercules, CA, USA) or TaqMan (Applied Biosystems, Carlsbad, CA, USA) chemistries following published protocols (Kalanetra et al., 2009; Tolar et al., 2013) and the primers and probes listed in the attached Supplemental Files ("Primer_Specs.pdf" or "Primer_Specs.xlsx"). Reactions were set up in triplicate and analyzed against a range of standards (10^1–10^7 copies per ul) as described in Tolar et al. (2013). qPCR conditions for Archaea *amoA*, ammonia-oxidizing Betaproteobacteria *amoA* and Bacteria 16S rRNA (*rrs*) genes have been described previously (Kalanetra et al., 2009; Tolar et al., 2013). Thaumarchaeota *ureC* genes were quantified under the same conditions as *amoA*, with an annealing temperature of 53 °C (from Alonso-Sáez et al., 2012). Nitrospina *rrs* genes were quantified as in Mincer et al. (2007). Raw abundance data (copies per ul of DNA extract) were converted to concentrations of genes (copies per liter of seawater) using the volume filtered and the extract volume and assuming 100% extraction efficiency as in Tolar et al. (2013). The percent of total prokaryotes represented by Thaumarchaeota was calculated using *rrs* gene abundance (Bacteria plus Thaumarchaeota) determined by qPCR and corrected using an average of 1.8 Bacteria *rrs* genes per genome (Biers et al., 2009) and 1.0 Thaumarchaeota *rrs* gene per genome, as described previously (Kalanetra et al., 2009). Prokaryote abundance determined by qPCR correlated well with total prokaryote counts made with a flow cytometer by Palmer LTER personnel during LMG 18-01 (model II regression: n=78, $r^2 = 0.45$, $p<<0.0001$; slope = 0.65, 95% CL = 0.49 – 0.82; intercept = 0.36, 95% CL = 0.31 - 0.41 * 10^9 cells L^{-1}).

BCO-DMO Processing Description

- replaced 'NAN' with 'nd' as missing data identifier;
- renamed fields to comply with BCO-DMO naming conventions;
- corrected dates where month and day were reversed;
- converted cast start date/time field to ISO8601 format;
- 2021-03-17: revised/updated the Methods & Sampling section of the metadata;
- 2021-04-08: replaced data file with copy received on 2021-03-18.

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Data Files

File
qPCR.csv (Comma Separated Values (.csv), 14.02 KB) MD5:c033ffcb55a763e95dc067014de615fb Primary data file for dataset ID 840475

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Supplemental Files

File
Primer_Specs.pdf (Portable Document Format (.pdf), 330.25 KB) MD5:67b131a8eb73a274f9cf66ae82d8630c Specifications of the qPCR primers and reactions used to generate this dataset. This information is presented in a table in PDF format. Rows include: Forward primer, Reverse primer, Probe1, Reference(s), Cycling conditions, Number of plates run, r2 of qPCR standard curve (mean±SD), Primer Efficiency (mean±SD, %), Limit of Detection (103 copies L-1). Columns/rows and definitions: Gene target = Name of the gene being amplified, no units. Forward primer name = Name of the primer given in the reference describing it. Forward primer sequence =Sequence of the forward primer, in standard genetic notation. Reverse primer name = Name of the primer given in the reference describing it. Reverse primer sequence = Sequence of the reverse primer, in standard genetic notation. Probe name = Name of the probe given in the reference describing it, for TaqMan reactions only. Probe sequence = Probe sequence, in standard genetic notation. Primer Reference(s) = Publication describing the primer set. Cycling conditions = PCR conditions. Number of plates run = Number of 96 well plates run to quantify the target genes in all samples. r2 of standard curve (mean±SD) = Linearity of qPCR standard curve. Primer Efficiency (mean±SD, %) = Efficiency of the qPCR reaction calculated as (observed/expected) abundance of standards, %. Limit of Detection (103 copies L-1) = Limit of detection of the gene in environmental samples estimated from volume filtered, extract volume (assumes 100% extraction efficiency) and qPCR reaction sensitivity determined from standard curves.
Primer_Specs.xlsx (Octet Stream, 13.66 KB) MD5:ba53ba7c449e60e4f0f4e2918f009a72 Specifications of the qPCR primers and reactions used to generate this dataset. This information is presented in Excel (.xlsx) format. Rows include: Forward primer, Reverse primer, Probe1, Reference(s), Cycling conditions, Number of plates run, r2 of qPCR standard curve (mean±SD), Primer Efficiency (mean±SD, %), Limit of Detection (103 copies L-1). Additional column/row information is provided on the "Metadata" sheet within the Excel file.

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Related Publications

Alonso-Sáez, L., A. S. Waller, D. R. Mende, K. Bakker, H. Farnelid, P. L. Yager, C. Lovejoy, J.-É. Tremblay, M. Potvin, F. Heinrich, M. Estrada, L. Riemann, P. Bork, C. Pedrós-Alió, and S. Bertilsson. 2012. Role for urea in nitrification by polar marine Archaea. Proceedings of the National Academy of Sciences 109:17989-17994. DOI: 10.1073/pnas.1201914109
Methods

Biers, E. J., Sun, S., & Howard, E. C. (2009). Prokaryotic Genomes and Diversity in Surface Ocean Waters: Interrogating the Global Ocean Sampling Metagenome. Applied and Environmental Microbiology, 75(7), 2221–2229. doi:10.1128/aem.02118-08 https://doi.org/10.1128/AEM.02118-08
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
Methods

Kalanetra, K. M., Bano, N., & Hollibaugh, J. T. (2009). Ammonia-oxidizingArchaeain the Arctic Ocean and Antarctic coastal waters. Environmental Microbiology, 11(9), 2434–2445. doi:10.1111/j.1462-2920.2009.01974.x
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
Methods

Mincer, T. J., Church, M. J., Taylor, L. T., Preston, C., Karl, D. M., & DeLong, E. F. (2007). Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. Environmental Microbiology, 9(5), 1162–1175. doi:10.1111/j.1462-2920.2007.01239.x
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
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
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

Tolar, B. B., King, G. M., & Hollibaugh, J. T. (2013). An Analysis of Thaumarchaeota Populations from the Northern Gulf of Mexico. Frontiers in Microbiology, 4. doi:10.3389/fmicb.2013.00072
Methods

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Parameters

Parameter	Description	Units
Event_Log_Number	Sequential numbers keyed to the bridge log of activities	unitless
Cast_Start_ISO_DateTime_UTC	Date and time of CTD cast, sample collection; 24-hour clock; formatted to ISO8601 standard: YYYY-MM-DDThh:mmZ	unitless

Latitude	Latitude in decimal degrees	degrees North
Longitude	Longitude in decimal degrees	degrees East
Description	Station category	unitless
LTER_Grid_Station_Text	Station location on the PAL-LTER sampling grid (http://pal.lternet.edu)	unitless
Depth	Depth sampled in meters	meters (m)
Temperature	Water temperature from the CTD in Centigrade degrees	degrees Celsius
Salinity	Salinity calculated from water temperature and conductivity from the ship's CTD, practical salinity units	PSU
bact16S	Concentration of Bacteria 16S rRNA genes determined by qPCR in units of 106 copies L-1. "nd" = no data. Limit of detection estimated from volume filtered, DNA extract volume, template volume used per reaction and standard curves is 5 * 106 copies L-1, values	10^6 copies L-1
Thaum_16S	Concentration of Marine Group 1 Archaea (Thaumarchaeota) 16S rRNA genes determined by qPCR in units of 103 copies L-1. "nd" = no data. Limit of detection estimated from volume filtered, DNA extract volume, template volume used per reaction and standard curves is 2 * 103 copies L-1, values	10^3 copies L-1
WCA_amoA	Concentration of ammonia monooxygenase subunit A genes from the Water Column clade A Thaumarchaeota determined by qPCR in units of 103 copies L-1."nd" = no data. Limit of detection estimated from volume filtered, DNA extract volume, template volume used per reaction and standard curves is 1 * 103 copies L-1, values	10^3 copies L-1
WCB_amoA	Concentration of ammonia monooxygenase subunit A genes from the Water Column clade B Thaumarchaeota determined by qPCR in units of 103 copies L-1. "nd" = no data. Limit of detection estimated from volume filtered, DNA extract volume, template volume used per reaction and standard curves is 1 * 103 copies L-1, values	10^3 copies L-1
Thaumarchaeota_ureC	Concentration of Thaumarchaeota urease subunit C genes determined by qPCR in units of 103 copies L-1. "nd" = no data. Limit of detection estimated from volume filtered, DNA extract volume, template volume used per reaction and standard curves is 8 * 103 copies L-1, values	10^3 copies L-1
AOB_amoA	Concentration of ammonia monooxygenase subunit A genes from Beta-proteobacteria determined by qPCR in units of 103 copies L-1. "nd" = no data. Limit of detection estimated from volume filtered, DNA extract volume, template volume used per reaction and standard curves is 8 * 103 copies L-1, values	10^3 copies L-1
NOB_16S_rRNA	Concentration of Nitrospina 16S rRNA genes determined by qPCR in units of 103 copies L-1. "nd" = no data. Limit of detection estimated from volume filtered, DNA extract volume, template volume used per reaction and standard curves is 2 * 103 copies L-1, values	10^3 copies L-1
Ammonium	Concentration of ammonium in 10-9 mol L-1. "nd" = no data. Precision estimated from mean standard deviation of replicate analyses to be 65 nM, values	nanomolar (nM)

Urea	Concentration of urea in 10-9 mol L-1. "nd" = no data. Precision estimated from mean standard deviation of replicate analyses to be 10 nM, values	nanomolar (nM)
Nitrite	Concentration of nitrite in 10-9 mol L-1. "nd" = no data. Precision estimated from mean standard deviation of replicate analyses to be 70 nM, values	nanomolar (nM)
Putrescine	Concentration of putrescine (1,4 butanediamine) in units of 10-9 mol L-1. "nd" = no data. Limit of detection estimated from standard curves to be 0.1 nM, values	nanomolar (nM)
Cadaverine	Concentration ofcadaverine (1,5 pentanediamine) in 10-9 mol L-1. "nd" = no data. Limit of detection estimated from standard curves to be 0.1 nM, values	nanomolar (nM)
Norspermidine	Concentration of norspermidine (N,N'-bis(3-aminopropyl)-1,3-propanediamine) in 10-9 mol L-1. "nd" = no data. Limit of detection estimated from standard curves to be 0.1 nM, values	nanomolar (nM)
Spermidine	Concentration of spermidine (1,5,10 decanetriamine) in units of 10-9 mol L-1. "nd" = no data. Limit of detection estimated from standard curves to be 0.1 nM, values	nanomolar (nM)
Spermine	Concentration of spermine (N,N'-bis(3-aminopropyl)-1,4 butanediamine) in units of 10-9 mol L-1. "nd" = no data. Limit of detection estimated from standard curves to be 0.1 nM, values	nanomolar (nM)

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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	iCycler CFX Connect Real-Time PCR Detection System (BioRad)
Generic Instrument Name	qPCR Thermal Cycler
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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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 15N 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 14C 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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