Ammonia-nitrite-nitrate assays from R/V Clifford A. Barnes cruises CB921, CB924, CB928, CB933, CB944 from the East Sound, Hood Canal; 2008-2010 (Marine Nitrogen Cycle project)

Website: https://www.bco-dmo.org/dataset/3452
Version: 24 March 2011
Version Date: 2011-03-24

Project
» Quantifying the role of Group I Crenarchaeota in the marine nitrogen cycle using cultures and environmental monitoring of ammonia oxidation, 16S rRNA genes and lipid biomarkers (Marine Nitrogen Cycle)

Contributors	Affiliation	Role
Ingalls, Anitra E.	University of Washington (UW)	Principal Investigator, Contact
Devol, Allan	University of Washington (UW)	Co-Principal Investigator
Stahl, David A.	University of Washington (UW)	Co-Principal Investigator
Gegg, Stephen R.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

Dataset Description

HC Data - Ammonia-nitrite-nitrate assays

Data Processing Description

OPA ammonia assay
Prepare all solutions in acid-washed (0.1% HCl), triple-rinsed (MilliQ H2O) brown HDPE bottles. Prepare stock solutions with freshly drawn ultra-pure deionized Milli-Q water. For the OPA solution use ethanol of the highest purity available.

SOLUTIONS:
Sodium sulfite solution: Dissolve 1 g sodium sulfite in 125 ml MilliQ water.

Borate buffer solution: Dissolve 40 g disodium tetraborate decahydrate in 1000 ml MilliQ water. If necessary, filter the solution to remove turbitity. The solution is stable.

OPA solution: Dissolve 2 g of standard grade o-phtaldialdehyde (P- 1378 Sigma) in 50 ml of ethanol in the dark.

Ammonium chloride stock solution (10mM): Dissolve 535 mg dried NH4Cl in 1000 ml of MilliQ water.

WORKING REAGENT:
In a HDPE bottle mix 1000 ml of borate buffer with 50 ml of OPA solution and 5 ml of sodium sulfite. This reagent is light-sensitive. Let the reagent age at least one day before use. Store dark for at least 2 month.

PROTOCOL:

Prepare 2 µM ammonium standard in a 250ml HDPE bottle: Add 40 µl of ammonium chloride stock solution (10 mM) to 200 ml Milli-Q water.

Prepare standard curve as follows:
Standard [nM] MilliQ water 2 µM NH4Cl
0 80 0
0 80 0
10 79.6 0.4
25 79 1
500 60 20
1000 40 40
2000 0 80

Fill 80 ml of water sample into a 250-ml HDPE bottle.
Add 20 ml of working reagent to all samples and standards.
Incubate for accurately 30 min at 65°C and cool to room temperature.
Analyze fluorescence intensity in Turner fluorometer with CDOM/Ammonia kit.

Reference:
Holmes, R. M., A. Aminot, R. Kerouel, B. A. Hooker and B. J. Peterson (1999). A simple and precise method for measuring ammonium and marine and freshwater ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 56(10): 1801-1809. Keroul & Aminot (1997) Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. Marine Chemistry 57: 265-275.

Nitrite Determination
REAGENTS:
Sulfanilamide reagent: Add 100 ml conc. HCl to 700ml MilliQ water
Add 10g Sulfanilamide, stir to dissolve.
Make up volume to 1000 ml
Store at 4?C in the dark, stable for several weeks.

Naphthylethylenediamine (NED) reagent: Dissolve 1 g of N-(1-Naphthyl)-ethylendiamin-dihydrochloride in 1000 ml of MilliQ water. Store at 4?C in the dark, stable for several weeks.

Nitrite standard stock solution (10 mM): Dissolve 0.690 g dried NaNO2 in 1000 ml MilliQ water.

PROCEDURE:
Prepare 2 µM nitrite standard in a 250ml HDPE bottle: Add 40 µl of nitrite standard stock solution (10 mM) to 200 ml artificial seawater (SCM salts).

Prepare nitrite standard curve as follows:
Standard [nM] SCM salts [ml] 2 µM Nitrite Standard [ml]
0 100 0
0 100 0
10 99.5 0.5
25 98.75 1.25
500 75 25
1000 50 50
2000 0 100

Fill 100 ml of sample water into 250ml HDPE bottles.
Add 2 ml of sulfanilamide reagent, swirl and let stand for 1 min.
Add 2 ml of NED reagent and swirl again.
Incubate for 10 to 15 min.
Read absorbance at 540nm in a 5cm cuvette

Grasshoff, Kremling, Erhard (1999) Methods of Seawater Analysis, 3 edn. Wiley-VCH

Nitrate Assay for Seawater
Deionized water and standards
For preparing standard and reagent solutions use deionized water purified by a distilling unit followed by the Millipore Synergy 185 Water System that produces water with 18 MΩ resistance.

REAGENTS:
2% (w/v) resorcinol solution: prepare fresh daily by dissolving 2.0 g of ACS reagent grade resorcinol crystals in 100 ml Milli-Q water.

Concentrated sulfuric acid
Nitrate stock standard solution (10 mM): Dissolve 0.85 g $NaNO_3$ in 1 L of MilliQ water. Store stock solutions in a 1L polyethylene bottle at 4 °C in a refrigerator.
Prepare working standard solutions from serial dilutions of stock solution with artificial sea water (SCM media – 26 g NaCl, 5 g $MgSO_4 \cdot 7H_2O$, 5 g $MgCl_2 \cdot 6H_2O$, 1.5 g $CaCl_2 \cdot 2H_2O$, 0.1 g KBr in 1 L Milli-Q water).

PROCEDURE:
Prepare 40 µM nitrate standard in a 250ml HDPE bottle by adding 400 µl of nitrate standard stock solution (10 mM) to 99.6 ml of artificial seawater (SCM media).

Prepare nitrate standard curve as follows:
Standard [µM] SCM salts [ml] 40 µM Nitrate Standard [ml]
0 20 0
0 20 0
0.25 19.875 0.125
0.5 19.75 0.25
10 15 5
20 10 10
40 0 20

Transfer 20.0 ml of a seawater sample or standard into a 250 ml brown PE bottle.
Add 2.4 ml of 2% resorcinol solution and swirl to mix the resorcinol with the sample.
Carefully add 20.0 ml of concentrated sulfuric acid, close the bottle with its lid and then gently swirl to mix the solution. Let the bottles stand for 30 min.
Place the bottles in a water bath until it reaches room temperature.
Measure the absorbance of the sample at 505 nm in a 5 cm cuvette against a blank with acidified seawater.

Reference:
Zhang, J.Z. & Fischer, C.J., 2006. A simplified resorcinol method for direct spectrophotometric determination of nitrate in seawater. Mar. Chem. 99, 220-226.

BCO-DMO Processing Notes
Generated from original .xls file "Ingalls_HC_Data for NODC.xls" contributed by Anitra Ingalls

BCO-DMO Edits
- Multiple sheet spreadsheet converted to individual, single sheet spreadsheets by cruise
- Cruise Id standardized to R2R Catalog
- Cruise Id and station metadata added to each data record
- Parameters edited to conform to BCO-DMO parameter naming convention
- "Date (euro)" removed
- Decimal data values padded to consistent decimal places as reported
- "nd" (no data) value inserted in blank cells

[table of contents | back to top]

Data Files

File
HC_Data.csv (Comma Separated Values (.csv), 18.56 KB) MD5:9d4625d87c0a19a6e600d5d09392c365 Primary data file for dataset ID 3452

[table of contents | back to top]

Parameters

Parameter	Description	Units
Cruise	Cruise Id	text
Station	Station Id	text
Date	Date (GMT)	YYYYMMDD
Time	Time (GMT)	HHMMSS
Longitude	Station longitude (West is negative)	decimal degrees
Latitude	Station latitude (South is negative)	decimal degrees
Depth	Depth	meters
Volume	Volume	liters(?)
NO2	NO2	microM
NO2_x_10	NO2 * 10	(tbd)
Temp	Temperature	Degrees Celsius
Sal	Salinity	PSU
O2	O2	mg/L
Chl	Chl	mg/m3
NO3minus	NO3-	microM
NH4	NH4	microM
NH4plus_x_10	NH4+ * 10	(tbd)
Sigma_t	Sigma-t	Kg/m3
Transm	Trans	percentage
PAR	PAR	percentage
Abs	Abs	(tbd)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	CTD Sea-Bird SBE 911plus
Generic Instrument Name	CTD Sea-Bird SBE 911plus
Generic Instrument Description	The Sea-Bird SBE 911 plus is a type of CTD instrument package for continuous measurement of conductivity, temperature and pressure. The SBE 911 plus includes the SBE 9plus Underwater Unit and the SBE 11plus Deck Unit (for real-time readout using conductive wire) for deployment from a vessel. The combination of the SBE 9 plus and SBE 11 plus is called a SBE 911 plus. The SBE 9 plus uses Sea-Bird's standard modular temperature and conductivity sensors (SBE 3 plus and SBE 4). The SBE 9 plus CTD can be configured with up to eight auxiliary sensors to measure other parameters including dissolved oxygen, pH, turbidity, fluorescence, light (PAR), light transmission, etc.). more information from Sea-Bird Electronics

[table of contents | back to top]

Deployments

CB921

Website	https://www.bco-dmo.org/deployment/58653
Platform	R/V Clifford A. Barnes
Start Date	2008-08-18
End Date	2008-08-21
Description	NOTE: CTD data list cruise id as CAB920 R2R Catalog lists cruise id as CB921 with Anitra Inglas as the Chief Sci Using the R2R Cruise Id

CB924

Website	https://www.bco-dmo.org/deployment/58654
Platform	R/V Clifford A. Barnes
Start Date	2008-10-06
End Date	2008-10-08
Description	Using the R2R Cruise Id

CB928

Website	https://www.bco-dmo.org/deployment/58655
Platform	R/V Clifford A. Barnes
Start Date	2008-12-08
End Date	2008-12-11
Description	NOTE: CTD data list cruise id as CAB927 R2R Catalog lists cruise id as CB928 with Anitra Inglas as the Chief Sci Using the R2R Cruise Id

CB933

Website	https://www.bco-dmo.org/deployment/58656
Platform	R/V Clifford A. Barnes
Start Date	2009-05-11
End Date	2009-05-15
Description	Using the R2R Cruise Id

CB944

Website	https://www.bco-dmo.org/deployment/58657
Platform	R/V Clifford A. Barnes
Start Date	2010-07-06
End Date	2010-07-08
Description	Using the R2R Cruise Id

[table of contents | back to top]

Project Information

Quantifying the role of Group I Crenarchaeota in the marine nitrogen cycle using cultures and environmental monitoring of ammonia oxidation, 16S rRNA genes and lipid biomarkers (Marine Nitrogen Cycle)

Coverage: Hood Canal, Puget Sound, Washington

Project Summary
Recent advances in molecular microbial ecology have overturned canonical paradigms of the marine nitrogen cycle. Estimates of global nitrogen fixation are regularly revised upward, the non-traditional bacterial denitrification pathway known as anammox is now thought to be responsible for a significant portion of global denitrification, and the discovery of ammonia-oxidizing *Archaea* necessitates a reevaluation of the contribution of traditional nitrifying bacteria to the global nitrogen cycle. While environmental gene sequencing and geochemical studies were critical to these discoveries, much of our understanding could not have been gained without the aid of studies on representative organisms in pure culture. Since their discovery in 1992, the ecological role of mesophilic marine *Archaea* has remained a mystery due in large part to the lack of a cultured representative.

We now have a mesophilic marine *Crenarchaea* in culture along with several lines of evidence that this and many other pelagic marine *Crenarchaea* oxidize ammonia to obtain the energy needed to sustain autotrophic carbon fixation. The distribution of marine *Crenarchaea* and their genes encoding ammonia-oxidizing enzymes, suggests that these organisms are responsible for the oxidation of a significant portion of the ocean's reduced nitrogen pools.

Here we propose to begin to better understand the physiological capabilities, distribution and quantitative significance of ammonia-oxidizing *Crenarchaea*. Our group is uniquely positioned to launch a comprehensive set of studies that will use cutting edge techniques to answer the following questions:
1) What factors control the rate and efficiency of *Archaeal* ammonia-oxidation?

2) What is the relative role of *Bacteria* and *Archaea* in ammonia-oxidation in the marine environment?

3) How can biomarkers be used to detect and assess the physiological status of living ammonia-oxidizing *Bacteria* and *Archaea*?

Our study uniquely combines culture work, molecular biology, organic geochemistry and field investigations into one of the first studies of the role of marine *Crenarchaea* in the biogeochemical cycling of nitrogen.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0623174

[table of contents | back to top]