Sample log for 16S and 18S rDNA amplicons sequenced with 454 technology collected on the R/V Atlantic Explorer BATS site in 2012 (DimBio NABE project)

Website: https://www.bco-dmo.org/dataset/565711
Version: 2015-09-08
Version Date: 2015-09-18

Project
» Functional diversity of marine eukaryotic phytoplankton and their contributions to the C and N cycling (DimBio NABE)

Program
» Dimensions of Biodiversity (Dimensions of Biodiversity)

Contributors	Affiliation	Role
Allen, Andrew E.	J. Craig Venter Institute (JCVI)	Principal Investigator
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

This is a log of the rDNA samples collected in February and August, 2012 in the Bermuda Atlantic Time Series (BATS) area.

Once the data are submitted to GenBank, accession numbers will be provided.

Related Dataset:

BATS_Feb_Aug_2012_diversity_rDNA_BLAST

Processed sequences from this data set can be found at https://scripps.ucsd.edu/labs/aallen/data/ under microbial diversity data sets.

Methods & Sampling

Oceanographic sampling: Samples were collected in February and August 2012 at the BATS site on the RV/Atlantic Explorer. Water was collected with Niskin bottles mounted on a CTD rosette with integrated chlorophyll a fluorometer. Samples were collected from the mixed layer, chlorophyll max and mesopelagic. 200 L of seawater was passed through a 20 mm nytex mesh into 50L carboys cleaned with 0.1% bleach and distilled water. The 20 mm-filtered seawater was serially filtered through 3.0, 0.8, and 0.1 mm filters (Millipore), with rapid transfer of filters to storage buffer and -80oC as described in Rusch et al. (2007). Nucleic acids were extracted as previously described (Rusch et al 2007).

16S/18S small subunit ribosomal rDNA transcript sequencing: Approximately 500 bp of the v3v5 region of 16S rDNA gene was amplified using the nearly universal eubacterial primers 341F (5'-CCTACGGGNGGCWGCAG-3') (Lane et al., 1985) and 926R (5'-CCGTCAATTCMTTTRAGT-3') (Herlemann et al., 2011). Approximately 500 bp of the v4 region of the eukaryotic 18S rDNA transcript was amplified with the nearly universal primers TAReuk454FWD1 (5′-CCAGCASCYGCGGTAATTCC-3′) and TAReukREV3 (5′-ACTTTCGTTCTTGATYRA-3′) (Stoek et al., 2010). Primers were adapted for 454 sequencing by addition of FLX Titanium adapters (A adapter sequence: 5′ CCATCTCATCCCTGCGTGTCTCCGACTCAG 3′; B adapter sequence: 5′ CCTATCCCCTGTGTGCCTTGGCAGTCTCAG 3′) and incorporation of 10bp multiplex identifier (MID) barcodes to facilitate multiplexing.

Life Technologies AccuPrime PCR system was used to amplify the DNA. 1 µl of each sample (35-100 ng) was used in a 20 µl PCR reaction, which contained 1X AccuPrime Buffer II, .75 units of AccuPrime Taq High Fidelity, and a final primer concentration of 200 nM. A no template negative control for cDNA synthesis was used as a negative control for subsequent PCRs reactions. Cycling conditions included an initial denaturation at 95°C for 2 minutes, 30 cycles of 95°C for 20 seconds, 56°C for 30 seconds, 72°C for 5 minutes using a Life Technologies ProFlex PCR system. 2 µl of the samples and 5 µL of the negative control were run on a 1% agarose gel at 105 V for 35 minutes. Reactions were cleaned using Ampure XP beads (Beckman Coulter, Brea CA). The final products were resuspended in 25 µL of Qiagen elution buffer and 2.5 µL was uses for visualization on an agarose gel. 1 µL was used to quantify the final product using LifeTechnologies' PicoGreen Quant-IT assay. 20 ng and 30 ng of each 16S or 18S amplicon respectively were pooled separately for sequencing.

Library QC, emPCR, enrichment and 454 sequencing were performed by following the vendor's standard protocols (Roche Diagnostics) with some modifications. Specifically, qPCR was used to accurately estimate the number of molecules needed for emPCR using KAPA Biosystems Library Quantification Kit. Automation (BioMek FX) was used to "break" the emulsions after emPCR and butanol was used to enable easier sample handling during the breaking process. The REM e (Robotic Enrichment Module) from Roche was used to automate the bead enrichment process in the pipeline. Generally, between 10,000 and 40,000 16S and 18S sequences were recovered and analyzed for each of the samples.]

References:

Herlemann D P R, Labrenz M, Jürgens K, Bertilsson S, Waniek J J, Andersson A F (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. The ISME J 5: 1571-1579

Lane D J, Pace B, Olsen G J, Stahl D A, Sogin M L, Pace N R (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc of the Nat Acad of Sci USA. 82: 6955-6959

Stoeck T, Bass D, Nebel M, Christen, R, Jones M D M, Breiner H W, Richards T A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology 19: 21-31.

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, Yooseph S et al (2007). The Sorcerer II global ocean sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. PloS Biology 5: 398-431.

Data Processing Description

Taxonomic annotation was performed via BLAST search against the SILVA database (Quast et al., 2013)

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner F O (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41: D590-6

BCO-DMO Processing:

- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard
- reformatted date from m/d/yyyy to yyyy-mm-dd
- corrected spelling (mesopelagic, season)
- removed trailing blanks
- replaced embedded blanks with underscore

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Data Files

File
sample_log.csv (Comma Separated Values (.csv), 5.70 KB) MD5:de59215d752f40881ddb70c4d4b4aa8e Primary data file for dataset ID 565711

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Parameters

Parameter	Description	Units
sample	sample identification	unitless
cruise_id	cruise identification	unitless
season	season sample taken	unitless
mon_year	month and year of sample aquisition	unitless
date	sampling date; format yyyy-mm-dd	unitless
time	sampling time; format HHMM	unitless
water_mass	description of water mass	unitless
depth	depth	meters
technology	sequencing technology	unitless
primer_F	forward primer used	unitless
primer_F_seq	forward primer sequence	unitless
primer_R	reverse primer used	unitless
primer_R_seq	reverse primer sequence	unitless
sample_id	sample identification	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	CTD - profiler
Generic Instrument Description	The Conductivity, Temperature, Depth (CTD) unit is an integrated instrument package designed to measure the conductivity, temperature, and pressure (depth) of the water column. The instrument is lowered via cable through the water column. It permits scientists to observe the physical properties in real-time via a conducting cable, which is typically connected to a CTD to a deck unit and computer on a ship. The CTD is often configured with additional optional sensors including fluorometers, transmissometers and/or radiometers. It is often combined with a Rosette of water sampling bottles (e.g. Niskin, GO-FLO) for collecting discrete water samples during the cast. This term applies to profiling CTDs. For fixed CTDs, see https://www.bco-dmo.org/instrument/869934.

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	
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	
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Life Technologies AccuPrime PCR system
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

AE1203

Website	https://www.bco-dmo.org/deployment/567244
Platform	R/V Atlantic Explorer
Start Date	2012-02-21
End Date	2012-02-25

AE1220

Website	https://www.bco-dmo.org/deployment/567245
Platform	R/V Atlantic Explorer
Start Date	2012-08-07
End Date	2012-08-10

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Project Information

Functional diversity of marine eukaryotic phytoplankton and their contributions to the C and N cycling (DimBio NABE)

Coverage: North Atlantic Ocean, transects from southwest to northeast

This project will investigate the taxonomic, genetic and functional diversity of eukaryotic phytoplankton at two North Atlantic sites (subarctic and subtropical) in two seasons. The PIs will use diagnostic microarrays for community analysis based on functional genes (both DNA and RNA) and next generation sequencing (i.e., transcriptomics using 454 technology) to identify the players, both in terms of community composition and activity, and to explore the functional diversity of the natural assemblage. In order to identify which groups are active in C and N assimilation and which N source is being utilized by the different size and functional groups, both filter-separated and flow cytometry-sorted samples will be used to 1) measure 13C primary production and 15N assimilation by incubations with isotope tracers, 2) measure the natural stable N isotope signatures of different taxonomic groups and 3) link the molecular diversity to the functional diversity in C and N transformations. Using flow cytometry linked to mass spectrometry, these investigators have found an unexpectedly strong differentiation in the form of N assimilated by prokaryotes and eukaryotes, with eukaryotes being more dynamic.

This project will investigate the taxonomic, genetic and functional diversity of eukaryotic phytoplankton and to link this diversity and assemblage composition to the carbon and nitrogen biogeochemistry of the surface ocean. Taxonomic diversity will be investigated by identifying the components of the phytoplankton assemblages using molecular, chemical and microscope methods. Genetic diversity will be explored at several levels, including direct sequencing of clone libraries of key functional genes and metatranscriptomic sequencing and microarray analysis of size fractionated/sorted phytoplankton assemblages. Using natural abundance and tracer stable isotope methods, genetic and taxonomic diversity will be linked to functional diversity in C and N assimilation in size- fractionated and taxon-sorted populations.

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Program Information

Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503446

Coverage: global

(adapted from the NSF Synopsis of Program)
Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to understand the roles of biodiversity in critical ecological and evolutionary processes.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1136477

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