T. rotula microbiome global sample

Website: https://www.bco-dmo.org/dataset/860347
Data Type: Other Field Results
Version: 1
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Project
» Connecting local, regional and global scales of gene flow in planktonic marine diatoms (Diatom Gene Flow)

Contributors	Affiliation	Role
Rynearson, Tatiana A.	University of Rhode Island (URI-GSO)	Principal Investigator
Ahern, Olivia	University of Rhode Island (URI-GSO)	Contact
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This dataset includes NCBI identifiers and descriptions of T. rotula microbiome global samples.

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Coverage

Spatial Extent: **N**:51.18 **E**:174.81 **S**:-41.3 **W**:-125.09
Temporal Extent: 2010-01-26 - 2010-11-19

Methods & Sampling

Single cells and chains of *T. rotula* were subjected to three serial washes in separate aliquots of 200 µl sterile seawater, with <2 µl seawater carried between washes. Individual cells or chains were then cultured with associated bacteria in 1ml f/20 in sterile polystyrene plates at ~*in situ* temperature and 90-120 µmol photons m^{-2} s^{-1} on a 12:12-h light:dark cycle. Isolates were grown for two weeks, or until the phytoplankton concentration reached ~1,000 cells mL^{-1}, whichever occurred sooner. Single cell isolates were filtered onto 0.2-µm filters (Millipore) and stored at -80 °C until DNA extraction. DNA was extracted from *T. rotula* isolates and bacterial co-cultures following Whittaker and Rynearson (2017).

Data Processing Description

The V3-V4 rDNA region of the 16S rRNA gene was amplified from all samples using a dual index approach in 20 µl reactions containing 1µl genomic DNA, 0.4 U of Taq (Lucigen) polymerase, and a final concentration of 200 µM dNTPs, 2 mM $MgCl_2$, and 0.5 µM of each primer (16S V3 Forward – CCTACGGGNGGCWSCAG and 16S V4 Reverse – GGACTACNVGGGTWTCTAAT). The PCR was run for 30 seconds at 98 °C, followed by 30-35 cycles at 98 °C for 10 seconds, 55 °C for 30 sec and 72 °C for 30 sec, with a final extension at 72 °C for 2 min. Triplicate 20 µl reactions were pooled and gel-purified for each sample.

Raw paired-end libraries were quality controlled using the ea-utils V.1.04.807 script fastq-mcf to a mate-pair quality mean of 25 with a 10 basepair (bp) sliding window and a minimum length of 150 bp. Forward and reverse reads were merged using the USEARCH V.8.1.1861 script fastq_mergepairs with a minimum overlap length of 10 bp and zero differences in the overlap region followed by clustering using Minimum Entropy Decomposition (MED) V.2.0 with standard settings. Chloroplasts were filtered using the QIIME V.1.9.1 script filter_fasta.py followed by an additional clustering step in MED with a minimum substantive abundance (-M) of 53 sequences required to form a single node. Chimeras were identified and removed using the USEARCH V.11.0.667 script uchime2_ref in sensitive mode against the Silva v128 database. Silva database v128 was used to assign taxonomy to OTUs and libraries were then rarefied using the script multiple_rarefactions_even_depth.py ten times to an even depth of 501 sequences per sample and OTU tables were merged using merge_otu_tables.py in QIIME V.1.9.1.

BCO-DMO Processing:
- flagged N/A as missing data identifier (replaced ;with nd);
- converted date format to YYYY-MM-DD
- renamed fields to conform with BCO-DMO naming conventions;
- removed commas from the Sample_Site column.

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Data Files

File	
t_rotula_microbiome_global.csv	(Comma Separated Values (.csv), 12.58 KB) MD5:f65f7f7ac6b9bd465a21395e913c2f44
Primary data file for dataset ID 860347	

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Related Publications

Ahern, O. M., Whittaker, K. A., Williams, T. C., Hunt, D. E., & Rynearson, T. A. (2021). Host genotype structures the microbiome of a globally dispersed marine phytoplankton. Proceedings of the National Academy of Sciences, 118(48). https://doi.org/10.1073/pnas.2105207118
Results

Whittaker, K. A., & Rynearson, T. A. (2017). Evidence for environmental and ecological selection in a microbe with no geographic limits to gene flow. Proceedings of the National Academy of Sciences, 114(10), 2651–2656. doi:10.1073/pnas.1612346114
Methods

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Parameters

Parameter	Description	Units
SRA_Library_Name	Sample ID	unitless
Desc	Library description	unitless
Sample_Site	Sample site	unitless
Sample_Name	Timepoint identifier	unitless
Sample_date	Date of collection; format: YYYY-MM-DD	unitless
Lat	Latitude	degrees North
Long	Longitude	degrees East
Chl_a	Chlorophyll a concentration	milligrams per liter (mg/L)
Salinity	Sea surface salinity	parts per thousand (ppt)
SST	Sea surface temperature	degrees Celsius
T_rotula_Cell_Abundance	T. rotula cell abundance at time of collection	cells per milliliter (cells/mL)
Study_Accession	NCBI SRA accession for the projct	unitless
Bioproject	NCBI BioProject ID number	unitless
SRA_Accession	NCBI SRA accession numbers	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq 2x250 bp PE reads
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Microscope - Optical
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	
Generic Instrument Name	Spectrophotometer
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
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)

Dataset-specific Instrument Name	YSI
Generic Instrument Name	Water Quality Multiprobe
Dataset-specific Description	A YSI instrument measured SST and salinity.
Generic Instrument Description	An instrument which measures multiple water quality parameters based on the sensor configuration.

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Project Information

Connecting local, regional and global scales of gene flow in planktonic marine diatoms (Diatom Gene Flow)

Coverage: global diatom samples; laboratory-based analyses

Description from NSF award abstract:
Diatoms are ubiquitous, unicellular, eukaryotes that generate about 40% of the organic carbon fixed annually in the sea. Interpretation of diatom species distributions and abundances in relation to environmental conditions has relied on two assumptions: (1) cells with identical morphologies represent the same species and (2) high potentials for dispersal and gene flow in passively drifting diatoms prevent local adaptation. Recent studies have challenged both assumptions, suggesting diatoms possess rich patterns of genetic and physiological variation both within and between species. Although there is emerging evidence of intra-specific population differentiation on local scales (~100km), it is commonly assumed that planktonic microbes are homogenously distributed on global scales (e.g. Fenchel and Finlay 2004). There is currently no data on diatoms to support this assumption. Aside from intriguing data on local scales, nothing is known about regional and global-scale population genetics and biogeography of diatoms.

The research proposed here will focus on the essential questions of if and how populations of planktonic diatoms are connected at local, regional and global scales. Connectivity among populations can influence a species" ecology, adaptive potential, evolutionary longevity and ultimately speciation potential. The proposed research will examine how local populations are connected to each other on regional scales and how regional dynamics connect to global-scale biogeographies using two model diatom species. rDNA sequence variation will be used to test whether broad species distributions observed in diatoms result from cryptic speciation. Within species, microsatellite markers will be used to identify genetically distinct populations, determine their relatedness to each other and examine spatial patterns of differentiation. The degree of physiological variation that accompanies genetic differentiation between populations will also be examined. Samples will be collected in a framework of existing oceanography and biodiversity programs, permitting genetic data to be interpreted in the context of larger, often long-term, studies. Because little is known about diatom biogeography, this work will begin to shed light on the connections between local and global population dynamics. Because the proposed research will represent the first large-scale sampling of diatom population genetics, it will also serve to generate many new hypotheses about the mechanisms that regulate ecological processes such as bloom formation over space and time and evolutionary processes such as the development of reproductive isolation and eventual speciation in planktonic organisms.

Related publications:
Rynearson, T.A., E.O. Lin and E.V. Armbrust. 2009. Metapopulation structure in the planktonic diatom *Ditylum brightwellii* (Bacillariophyceae). *Protist*, 160(1):111-121. doi:10.1016/j.protis.2008.10.003

Whittaker, K., Rignanese, D., Olson, R., Rynearson, T., 2012. Molecular subdivision of the marine diatom *Thalassiosira rotula* in relation to geographic distribution, genome size, and physiology. *BMC Evolutionary Biology,* 12:209. doi:10.1186/1471-2148-12-209

Boyd, P.W., Rynearson, T.A., Armstrong, E.A., Fu, F., Hayashi, K., Hu, Z., Hutchins, D.A., Kudela, R.M., Litchman, E., Mulholland, M.R., Passow, U., Strzepek, R.F., Whittaker, K.A., Yu, E., Thomas, M.K., 2013. Marine Phytoplankton Temperature versus Growth Responses from Polar to Tropical Waters - Outcome of a Scientific Community-Wide Study. *PLoS One*, 8(5), e63091. doi:10.1371/journal.pone.0063091

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0727227

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