Relative abundances of different Syndiniales groups from surface water samples collected at the Martha's Vineyard Coastal Observatory (MVCO) monthly or bimonthly between 2013 and 2021

Website: https://www.bco-dmo.org/dataset/897547
Data Type: Other Field Results
Version: 1
Version Date: 2023-06-14

Project
» Trojan Horses in the Marine Realm: Protist Parasite-host Dynamics in Coastal Waters (Coastal Parasites)

Contributors	Affiliation	Role
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Abstract
The diversity, persistence, and relative abundance of Syndiniales parasite taxa at the Martha's Vineyard Coastal Observatory (MVCO) were examined in surface water samples collected approximately monthly or bimonthly between 2013 and 2021. V4 amplicons from extracted DNA were amplified, sequenced (MiSeq), and taxonomically identified. The relative abundances of different Syndiniales groups and clades were determined and compared to identify the dominant taxa, when they occurred, and how they differed from studies in other marine regions.

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Coverage

Spatial Extent: **Lat**:41.325 **Lon**:-70.5667
Temporal Extent: 2013-02-14 - 2021-12-20

Methods & Sampling

Water samples were collected monthly or bimonthly (September 2019 to October 2020) at the Martha's Vineyard Coastal Observatory (MVCO) from about 2 meters below the surface using bucket casts or a CTD deployed from R/V Tioga. Samples were transferred to a carboy in a cooler for transport back to the laboratory. Water was processed within 2 hours of collection. One liter of whole seawater was collected onto 47-millimeter (mm) 0.2-micrometer (µm) Millpore Isopore filters and stored at -20 degrees Celsius (°C) for DNA extraction and amplicon sequencing. Nucleic acids were extracted from half of a 47mm filter that was cut into small pieces using scissors and cleaned with alcohol in between samples. The lysis method followed a hot detergent plus bead disruption protocol (Gast et al., 2004). Filter pieces were placed in a sterile 2-milliliter (ml) microfuge tube and 2 x lysis buffer was added, along with approximately 50 microliters (µl) of beads. The

tubes were vortexed for 30 seconds and then placed at 65°C for 5 minutes, followed by two cycles of vortex and heat incubation. Sodium chloride and CTAB were added, and the samples were incubated at 70°C for 10 minutes. Following extraction with an equal volume of chloroform, the aqueous phase was removed and precipitated overnight at -20°C with 0.6 volume of isopropanol. The recovered DNA pellet was resuspended in 20 µl of sterile water.

Amplification of the V4 region of the 18S ribosomal RNA gene was accomplished using the primers 574V4F (5' [TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG]CGGTAAYTCCAGCTCYV) and 1132V4R (5' [GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG]CCGTCAATTHCTTYAART), described in Hugerth et al. (2014) and modified to include 5' adapter sequences (indicated by square brackets). PCR reactions were performed in triplicate for each sample using 1 µl template DNA, 1.25 units AmpliTaq DNA polymerase, 2 millimoles (mM) MgCl2, 2 µl 2.5 µM dNTPs, and 2.5 µl 10X reaction buffer (25 µl total volume) with the conditions: 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 90 seconds; 72°C for 5 minutes; 4°C hold. No template negative controls were included. Each sample reaction was examined to confirm the correct product size of approximately 500 base pairs (bp). Triplicate reactions were pooled and sent to the RI-INBRE Molecular Informatics Core for library preparation and Illumina MiSeq (250 bp paired end; 500 cycle kit V2) sequencing.

Amplicon data collected in this work was combined with other MVCO time series amplicon data to examine the temporal variation in Syndiniales types. The prior sequencing effort covered samples collected between 2013-2019 and 2020-2021, and used the same primer set reported here. Trimmed and demultiplexed raw reads were imported into qiime2 and the forward reads were analyzed for high quality (Q value 30 over 90% of read), removal of chimeric sequences, identification of amplicon sequence variants (ASVs) at 100% similarity, removal of ASVs that occurred in fewer than 2 samples, and assignment of taxonomy using PR2 database.

Data Processing Description

Data Processing:
qiime2 was used to process the amplicon sequence data.

Demultiplex

qiime tools import --type 'SampleData[PairedEndSequencesWithQuality]' --input-path <manifest file name> --output-path <name.qza> --input-format PairedEndFastqManifestPhred33V2

qiime tools import --type 'SampleData[SequencesWithQuality]' --input-path <manifest file name> --output-path <name.qza> --input-format SingleEndFastqManifestPhred33V2

To evaluate the sequences qiime demux summarize --i-data name.qza --o-visualization name.qzv

Denoise, dereplicate, quality filter & remove chimeras

qiime dada2 denoise-single --i-demultiplexed-seqs <.qza file from above> --p-trim-left 10 --p-trunc-len 290 --p-chimera-method pooled --o-table <name.qza> --o-representative-sequences <name.qza> --o-denoising-stats <name.qza>

Remove singletons

qiime feature-table filter-features --i-table <name of table generated in prior step> --p-min-frequency 2 --o-filtered-table <new table name .qza>

qiime feature-table filter-seqs --i-data <name of rep seq file created in prior step> --i-table <name of filtered table> --o-filtered-data <new repseq file .qza>

Cluster sequences based upon identity

qiime vsearch cluster-features-de-novo --i-table <name.qza> (filtered table) --i-sequences <name.qza> (filtered rep-seqs file) --p-perc-identity 1.0 --o-clustered-table <name.qza> (this is a biom table that has abundances of each cluster) --o-clustered-sequences <name.qza> (this is a new rep-set of sequences - your ASVs)

Assign taxonomy

qiime feature-classifier classify-consensus-vsearch --i-query <name.qza> (this is the rep-set of sequences just created by cluster) --i-reference-reads pr2_4.12.0_18S.qza (this is the PR2 reference set of sequences) --i-reference-taxonomy pr2_4.12.0_18S_tax.qza (this is the corresponding PR2 taxonomy) --p-maxaccepts 1 --o-classification <name.qza> (this is your rep-set sequences and their taxonomy)

BCO-DMO Processing:
- added columns for the latitude and longitude of the sampling locations;
- converted the date field to YYYY-MM-DD format.

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Data Files

File	
mvco_syndiniales_counts.csv	(Comma Separated Values (.csv), 947.93 KB) MD5:818b17ac0f9e5f6be8f117dbe74bad70
Primary data file for dataset ID 897547	

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Supplemental Files

File	
MVCO_water_amt.csv	(Comma Separated Values (.csv), 479 bytes) MD5:9507d1db7ea4f392cf29437923a11134
Supplemental file for dataset IDs 897547 and 897734. Contains the volumes of water filtered for each sample. Column names and descriptions: Date = date in YYYY-MM-DD format. Host hybridization amount of water filtered in Liters. Dinospore hybridzation amount of water filtered in Liters. DNA extraction amount water filtered in Liters.	

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Related Publications

Gast, R. J., Dennett, M. R., & Caron, D. A. (2004). Characterization of Protistan Assemblages in the Ross Sea, Antarctica, by Denaturing Gradient Gel Electrophoresis. Applied and Environmental Microbiology, 70(4), 2028–2037. https://doi.org/10.1128/aem.70.4.2028-2037.2004 https://doi.org/10.1128/AEM.70.4.2028-2037.2004
Methods

Hugerth, L. W., Muller, E. E. L., Hu, Y. O. O., Lebrun, L. A. M., Roume, H., Lundin, D., … Andersson, A. F. (2014). Systematic Design of 18S rRNA Gene Primers for Determining Eukaryotic Diversity in Microbial Consortia. PLoS ONE, 9(4), e95567. doi:10.1371/journal.pone.0095567
Methods

Sehein, T., Gast, R., Pachiadaki, M., Guillou, L., & Edgcomb, V. (2021). Group II Syndiniales ALV01 CARD-FISH v1. https://doi.org/10.17504/protocols.io.bsxmnfk6
Methods

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Related Datasets

IsRelatedTo

Gast, R. J. (2023) **Group II Syndiniales infected host and dinospore counts determined from CARD-FISH hybridization carried out on samples collected at the Martha's Vineyard Coastal Observatory (MVCO) monthly or bimonthly from September 2019 to October 2020.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-06-21 doi:10.26008/1912/bco-dmo.897734.1 [view at BCO-DMO]

Sosik, H. M., Crockford, E. T., & Peacock, E. (2022). Dissolved inorganic nutrients from the Martha's Vineyard Coastal Observatory (MVCO), including 4 macro-nutrients from water column bottle samples, ongoing since 2003 (NES-LTER since 2017) [Data set]. Environmental Data Initiative. https://doi.org/10.6073/PASTA/CA34BE7554DDC67C9FA0F8DEA01F375B https://doi.org/10.6073/pasta/ca34be7554ddc67c9fa0f8dea01f375b

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Parameters

Parameter	Description	Units
latitude	latitude of Martha's Vineyard Coastal Observatory (MVCO); positive values = North	decimal degrees
longitude	longitude of Martha's Vineyard Coastal Observatory (MVCO); negative values = West	decimal degrees
taxon	taxon	unitless
date	date when water samples were collected	unitless
counts	amplicon sequence variant (ASV) count	unitless

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Instruments

Dataset-specific Instrument Name	bucket
Generic Instrument Name	bucket
Dataset-specific Description	Samples were collected from about 2 meters below the surface using bucket casts or a CTD.
Generic Instrument Description	A bucket used to collect surface sea water samples.

Dataset-specific Instrument Name	CTD
Generic Instrument Name	CTD - profiler
Dataset-specific Description	Samples were collected from about 2 meters below the surface using bucket casts or a CTD.
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	PCR
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)

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Project Information

Trojan Horses in the Marine Realm: Protist Parasite-host Dynamics in Coastal Waters (Coastal Parasites)

Coverage: Salt Pond, Falmouth, MA

NSF Award Abstract:
The ecological importance of parasitic dinoflagellates has been recognized for some time, particularly during epidemic outbreaks that cause mass mortality of their hosts, damage to aquaculture, and render commercially valuable Crustacea unpalatable. The dominate parasitic dinoflagellate group found in international global ocean surveys is referred to as MALV II Syndiniales. In the planktonic environment, the MALV II Syndiniales group not only exerts top-down controls on their prey populations, but based on their apparent ubiquity and abundance, they likely shape the pools of nutrients in marine water columns. Data on cultured samples reveals this hyper-diverse group can infect a wide range of protist hosts, as well as copepods, and fish larvae. Gaps in knowledge of the specificity and dynamics of the host-parasite interactions contribute to difficulties in estimating the impacts on the coastal ecosystems. In this project, researchers combine novel methods in microscopy, genomics, and chemistry to track host-parasite dynamics at a coastal site over an annual cycle followed by

modeling to assess the impacts on microbial ecosystem dynamics. The researchers will engage undergraduate and high school students in field and laboratory research activities. In addition, support for a graduate student is included along with plans to disseminate the research results more broadly through publications and presentations.

Syndiniales parasitism is a widespread, albeit under-studied symbiotic interaction in the marine environment and little is known about regulation of protist populations by these parasites. In spite of their cosmopolitan distribution in the global ocean and their apparent abundance in molecular datasets of protist marker genes, little is known about the ecology of these parasites and almost no genomic data exists for them. In this project, the researchers combine high-resolution sampling, water chemistry (including nutrients) analyses, molecular marker gene analyses, fluorescence in situ hybridization, single cell genomics, and modeling to produce the first focused assessment of host-MALVII parasite dynamics and ecology at the community level in a coastal marine ecosystem. The researchers will evaluate temporal dynamics of host and parasite diversity and will examine temporal variation in levels of infection of the protist community and host-parasite specificity using high-resolution sampling in Salt Pond, Falmouth, MA, and in situ hybridization microscopy. Molecular approaches include amplicon tag high throughput sequencing, leveraging the emerging third generation sequencing technology, Oxford Nanopore's MinION to elucidate host-parasite identities. The researchers will also apply advances in single-cell genome sequencing to inform on strain-specific genome content, including the molecular mechanisms underpinning protist parasitism. Contributions to pools of particulate and dissolved organic matter will be estimated for several of the most commonly infected host taxa in Salt Pond using laboratory experiments, providing the first set of values for modeling impacts of Syndiniales parasitism on pools of organic and inorganic nutrients.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1851012

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