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

Website: https://www.bco-dmo.org/dataset/897734
Data Type: Other Field Results
Version: 1
Version Date: 2023-06-21

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

Contributors	Affiliation	Role
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Abstract
This dataset includes Group II Syndiniales infected host and dinospore counts determined from CARD-FISH hybridization carried out on water samples collected at the Martha's Vineyard Coastal Observatory (MVCO) monthly or bimonthly from September 2019 to October 2020 from about 2 meters below the surface. Samples were collected using bucket casts or a CTD. The detailed hybridization method using the ALV01 (Group II) probe can be found at dx.doi.org/10.17504/protocols.io.bsxmnfk6.

Table of Contents

Coverage

Spatial Extent: **Lat**:41.325 **Lon**:-70.5667
Temporal Extent: 2019-09-16 - 2020-10-15

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. 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. For in situ hybridization, variable amounts of whole water were collected by gentle filtration onto 47mm 3 µm Millipore Isopore filters for detection of infected hosts, and onto 47mm 0.2 µm Millipore Isopore filters (black) for the detection of free dinospores (see Supplemental File "MVCO_water_amt.csv"). Filtration was stopped when about 20 milliliters (ml) of water

remained in the filter tower, and 20 ml of 8% formaldehyde (in filter sterile seawater) was added to fix the cells. After an hour, the solution was filtered, and the samples were rinsed three times with 20 ml of phosphate buffered saline (1x PBS). Filters were stored at -20°C until in situ hybridization.

The detailed hybridization method can be found at protocols.io at doi: 10.17504/protocols.io.bsxmnfk6. A one-eighth pie-shaped section was cut from each filter and placed on a glass slide. 20 µl of hybridization buffer (40% formamide) containing the horseradish peroxidase labeled probe (ALV01; 5'-GCC TGC CGT GAA CAC TCT-3'; Chambouvet el al. 2008) in a 9:1 ratio (final probe concentration 50 nanograms per microliter (ng µl-1)) was placed on the filter slice. Slides were put into 50ml centrifuge tubes containing a hybridization buffer saturated paper towel and incubated overnight at 37°C. Filter pieces were washed twice in 3 ml of wash solution at 46°C, then allowed to soak in 3 ml of TNT buffer at room temperature. Filters were placed on a new glass slide and the fluorescence signal was amplified using a tyramide signal amplification kit (Akoya Biosciences TSA Plus Fluorescein; NEL741001KT). After signal amplification, filters were washed twice in 3 ml of TNT buffer at 55°C to neutralize any residual fluorescein. Filter pieces were transferred to new glass slides and cellulose was stained using 20 µl of Calcofluor White for 7 minutes. Filter pieces were washed twice in 3 ml of water, then placed on new glass slides for counterstaining and mounting with a mixture of propidium iodide and Citiflour media (20 µl per slide). Hybridized filters were held overnight at 4°C, and then at -20°C for long-term storage.

Data Processing Description

Data Processing:
The filters were viewed using FITC, which allowed visualization of the CARD-FISH fluorescein stain and the red propidium iodide staining of the nuclei. Infected hosts were enumerated under 630X magnification by counting 30 random fields that covered the entire filter piece. Free dinospores were counted at 630X, again covering the whole filter piece in 30 random fields of view. The average number of cells per field was calculated and then multiplied by 8 to represent the whole filter area. This was then converted to cells per liter using correction factors calculated using a stage micrometer and the filtration region diameter.

Cells per ml = (avg COUNT per field)*8 / ml filtered) x CF

CF = area of funnel um^2/ area of microscope field um^2

For Zeiss S100 with 47 mm filter at 63X/40X, CF = $1.01736 \times 10^9/7.7644 \times 10^4$ (13103), 1.94638×10^5 (5226)

Calculation of the percent total infected protistan cells was accomplished using estimations of total eukaryote abundances from the Imaging Flow CytoBot (IFCB). The total number of cells was estimated as the average of all automated counts collected on the same day of sampling, or on a set of days near the day of sampling. IFCB counts are based upon an automated classifier, and objects binned as detritus or other non-plankton cells were excluded from the final estimates. All cell abundances were expressed as per liter.

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

[table of contents | back to top]

Data Files

File	
mvco_card-fish_results.csv	(Comma Separated Values (.csv), 988 bytes) MD5:6f196d89ae4e0521bc6ce0b19f4ccb31
Primary data file for dataset ID 897734.	

[table of contents | back to top]

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.

[table of contents | back to top]

Related Publications

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

[table of contents | back to top]

Related Datasets

IsRelatedTo

Gast, R. J. (2023) **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.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-06-14 doi:10.26008/1912/bco-dmo.897547.1 [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

[table of contents | back to top]

Parameters

Parameter	Description	Units
date	sample date	unitless
percent_infected_hosts	percent infected hosts (by GrpII Syndiniales)	unitless (percent)
GrpII_dinospores_per_L	GrpII dinospores per liter	per liter
avg_cells_per_L	average number of cells per liter	per liter
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

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	
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	Imaging Flow CytoBot (IFCB)
Generic Instrument Name	Imaging FlowCytobot
Dataset-specific Description	Calculation of the percent total infected protistan cells was accomplished using estimations of total eukaryote abundances from the Imaging Flow CytoBot (IFCB).
Generic Instrument Description	The Imaging FlowCytobot (IFCB) is an in-situ automated submersible imaging flow cytometer that generates images of particles in-flow taken from the aquatic environment. https://mclanelabs.com/imaging-flowcytobot/

Dataset-specific Instrument Name	Zeiss Axioskop S-100
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Hybridized filters were counted on a Zeiss Axioskop S-100 with epifluorescence.
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]