A study using amplicon sequencing of the viral mcp gene of dinoRNAVs to analyze their dynamics in the reef-buliding coral *Porites c.f. lobata* at three reef zones around Moorea, French Polynesia

Website: https://www.bco-dmo.org/dataset/906617
Data Type: Other Field Results
Version: 1
Version Date: 2023-09-27

Project
» Collaborative Research: Viral Reefscapes: The Role of Viruses in Coral Reef Health, Disease, and Biogeochemical Cycling (Moorea Virus Project)

Contributors	Affiliation	Role
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Abstract
These data are from a study that used amplicon sequencing of the viral major capsid protein (mcp) gene of positive-sense single-stranded RNA viruses known to infect symbiotic dinoflagellates ('dinoRNAVs') to analyze their dynamics in the reef-building coral, Porites lobata. The investigators repeatedly sampled 54 colonies across three environmentally distinct reef zones (fringing reef, back reef, and forereef) around the island of Moorea, French Polynesia over a three-year period and spanning a reef-wide thermal stress event. Temperature was measured in each reefzone to quantify differences in temperature according to reef zone and during the thermal stress event. Images were also taken of all colonies at each sample point in order to analyze colony health and partial mortality. By the end of the sampling period, 28% (5/18) of corals in the fringing reef experienced partial mortality versus 78% (14/18) of corals in the forereef. Over 90% (50/54) of colonies had detectable dinoRNAV infections. Reef zone influenced the composition and richness of viral mcp amino acid types ('aminotypes'), with the fringing reef containing the highest aminotype richness. The reef-wide thermal stress event significantly increased aminotype dispersion, and this pattern was strongest in the colonies that experienced partial mortality. Amplicon sequencing was also used to amplify the D1–D2 region of the 28 S large subunit (LSU) nuclear ribosomal RNA gene and to ultimately identify the dominant lineages of Symbiodinaiceae (the hosts of dinoRNAVs) in all P. lobata colonies. All colonies were dominated by Cladocopium C15 symbionts. These findings demonstrate that dinoRNAV infections respond to environmental fluctuations experienced in situ on reefs.

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Coverage

Spatial Extent: **N**:-17.4721 **E**:-149.8 **S**:-17.5084 **W**:-149.853
Temporal Extent: 2017-09-12 - 2020-10-28

Dataset Description

This dataset is comprised of three components:

(1) **temperature data measured at each reef site** - available in the csv data file
"906617_v1_temperature_moorea_reefs.csv"

(2) **images of Porites lobata colonies taken at each site** - available in the PDF "POR colony health
images.pdf"

(3) **dinoRNAV mcp gene amplicon libraries and Symbiodiniaceae LSU gene amplicon libraries** -
available in the National Center for Biotechnology Information Sequence Read Archive under accession
numbers PRJNA928208 and PRJNA930706, respectively. Parameters used to analyze the viral data are
described in Zenodo at https://zenodo.org/record/7552892#.Y_PAa-zMK3I (doi: 10.5281/zenodo.7552892)

Results of this study have been published in Howe-Kerr et al., 2023. (doi: 10.1038/s43705-023-00227-7).

Methods & Sampling

Sample collection for 'omics analyses (both Symbiodiniaceae LSU and dinoRNAV MCP):
Fifty-four colonies of *Porites lobata* were tagged on the north shore of Moorea, French Polynesia, spanning
nine sites that encompassed three reef zones (fringing, back, and forereef; n = 6 colonies/site, n = 3 sites/reef
zone). Each tagged colony was sampled in August 2018 (dry season), March 2019 (wet season), August 2019
(dry), and October 2020 (dry). Samples could not be collected in March 2020 due to the COVID-19 pandemic.
Tissue samples were collected for amplicon sequencing; for each *P. lobata* colony, ~3 to 6 small fragments (1
square centimeter (cm²)) of skeleton/tissue were sampled across the colony surface using bone cutters,
placed in a sterile Whirl-Pak® (Nasco), and then preserved in DNA/RNA shield (ZymoResearch, Irvine,
California, USA). Samples in the DNA/RNA shield were kept on ice until returning to shore, at which point they
were vortexed for 25 minutes at full speed with 5 ceramic beads and 1.35 grams (g) garnet matrix (MP
Biomedicals) and then frozen at -40° Celsius (C). These samples were wrapped in aluminum foil and stored at -
40°C until further processing. DNA and RNA were extracted from the coral samples, which included mucus,
tissue, and skeleton, using enzyme digestions and a ZymoBIOMICs DNA/RNA Kit (ZymoResearch, Irvine,
California, USA, following Grupstra et al., 2022).

Symbiodiniaceae LSU:
To identify dominant Symbiodiniaceae lineages, the D1-D2 region of the 28S large subunit (LSU) nuclear
ribosomal RNA gene was amplified from coral holobiont DNA. At the Oregon State University Center for
Qualitative Life Sciences (CQLS), PCR reactions were conducted using the primers LSU1F_illu and LSU1R_illu
with attached Miseq Adapters; cycles were as follows: 95°C for 3 minutes, and then 15 cycles of 95°C for 30
seconds, 56°C for 30 seconds, and 72°C for 30 seconds, and finally, 72°C for 4 minutes. PCR clean-up was
completed using Agencourt AMPure XP Magnetic Beads. PCR reactions to incorporate Illumina indexing primers
were conducted as follows: 95°C for 3 minutes, and then 20 cycles of 95°C for 30 seconds, 56°C for 30
seconds, and 72°C for 30 seconds, and finally 72°C for 4 minutes. The resulting PCR product was purified with
Agencourt AMPure XP Magnetic Beads, quantified via qPCR using the KAPA library quantification kit (Roche
Sequencing Solutions, Pleasanton, CA), pooled in equal molar amounts, and sequenced on the Illumina MiSeq
platform with PE300 chemistry.

Amplicons of the LSU gene were processed in RStudio (version 1.1.456) through the DADA2 pipeline (version
1.11.0), which generates unique sequences at single-nucleotide resolution (amplicon sequence variants or
'ASVs') (Grupstra et al., 2022). Forward and reverse paired reads were truncated at 210 and 160 base pairs,
respectively, based on quality plots. Reads with a total expected error of one or more, or contained Ns, were
discarded. ASVs were inferred from unique reads, paired reads were merged, ASVs that did not match a target
length of 286 ± 5 were removed, and chimeras were detected and removed. This DADA2 curated table of
ASVs were then further collapsed using the LULU package (Frøslev et al., 2017), which merges potentially
erroneous ASVs based on sequence similarity and co-occurrence patterns (default parameters of 84%
similarity, 90% co-occurrence used here; see https://zenodo.org/record/7552892#.Y_PAa-zMK3I). Taxonomy

was assigned to curated ASVs based on BLAST (blastn) searches to NCBI's nr/nt database, and ASVs that had best matches to non-Symbiodiniaceae were discarded.

dinoRNAV MCP:
The dinoRNAV major capsid protein (mcp) gene was amplified from cDNA (generated from coral holobiont RNA), using a nested PCR protocol with primers from Montalvo-Proaño et al. (2017). Cleaned and normalized libraries were sequenced on the Illumina MiSeq platform using PE300 v3 chemistry at the Oregon State University Center for Qualitative Life Sciences (CQLS). Sequencing and bioinformatic analyses were conducted following Grupstra et al., (2022) using the program vAMPirus (v1.0.1, Veglia et al. 2021). Briefly, after adapter removal, quality filtering, primer removal, read merging, and length filtering, amplicon sequence variants (ASVs) were generated and chimeras removed using VSEARCH with the UNOISE3 algorithm (Rognes et al., 2016; Edgar et al., 2016). Parameters and program information for each of these steps can be found in the vAMPirus config file included as a Supplementary File ("HoweKerr_etal_2023_vAMPirus.config") in Howe-Kerr et al. (2023) and all non-read files used to run the analyses and generate the results can be found at the vAMPirus Zenodo (https://doi.org/10.5281/zenodo.7552892). To collapse some of the diversity associated with the high mutation rate of ssRNA viruses, ASVs were then translated and aligned into unique amino acid types ('aminotypes') using VirtualRibosome (v2.0, Wernersson et al., 2006) and CD-HIT (v.4.8.1, Fu et al., 2012).

Temperature:
Water temperatures were measured every two hours year-round at each site for the duration of the study using a HOBO® temperature logger.

Colony images:
Photographs of each colony were taken at each sampling point and used in visual assessments to determine if a colony remained apparently healthy or experienced partial mortality over the course of the study; a third category ('ambiguous') was used to describe colonies for which health trajectory could not be determined based on the images available. Colonies were considered to have experienced partial mortality if there was a clear loss of live tissue surface area between August 2018 and October 2020; colonies were considered to have remained apparently healthy if there was an increase in live tissue in images taken over the course of the assessed period or if there was no clear change in live tissue surface area. Final health determination was based on assessments by three separate observers, who assessed the images separately and blindly without knowing what reef zone or site a set of images was collected from. Differences in coral health among reef types and sites were assessed with Chi-squared tests.

Known issues or problems:
Due to logistical issues, HOBO® temperature logger data are not available for October 2020.

Data Processing Description

Temperature data processing:
Raw temperature values at the start and end of each HOBO® logger launch were removed (since these were temperature recordings that occurred before or after deployment to the reef).

Colony image processing:
Colony images are grouped by Reef Type, Site, and Colony ID; all images of the same colony over time are depicted on the same page.

BCO-DMO Processing Description

- Created a table out of the site locations (lats/lons) provided in the parameters section of the metadata; saved as a CSV file named "site_coordinates.csv".
- Imported original temperature file "Hobodata_temperature_filt.xlsx" into the BCO-DMO system, along with "site_coordinates.csv".
- Joined latitude and longitude columns from the site coordinates file to the temperature data file by matching on site_number and reef_zone.
- Converted the date/time column to ISO 8601 format in the local time zone (GMT-10).
- Created a date/time column in ISO 8601 format in the UTC time zone.
- Removed the "row_number" column (unnecessary for data reuse).
- Saved the final temperature file as "906617_v1_temperature_moorea_reefs.csv".

Data Files

File
Temperature measured at each site by HOBO loggers filename: 906617_v1_temperature_moorea_reefs.csv (Comma Separated Values (.csv), 7.57 MB) MD5:13a5d2b95eae99df05ebcb562db1b375 Water temperature data associated with dataset ID 906617, version 1.

Supplemental Files

File
Porites lobata colony images at sampling points filename: POR colony health images.pdf (Portable Document Format (.pdf), 26.67 MB) MD5:90fe12233721f510d96044cbffc5bb03 Images associated with dataset 906617, version 1. This PDF file contains images of focal Porites lobata colonies, taken at each sampling point, and used to determine whether colonies experienced partial mortality during the study duration.

Related Publications

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. https://doi.org/10.1101/081257
Software

Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nature Communications, 8(1). https://doi.org/10.1038/s41467-017-01312-x
Methods

Fu, L., Niu, B., Zhu, Z., Wu, S., & Li, W. (2012). CD-HIT: accelerated for clustering the next-generation sequencing data. Bioinformatics, 28(23), 3150–3152. doi:10.1093/bioinformatics/bts565
Software

Grupstra, C. G. B., Howe-Kerr, L. I., Veglia, A. J., Bryant, R. L., Coy, S. R., Blackwelder, P. L., & Correa, A. M. S. (2022). Thermal stress triggers productive viral infection of a key coral reef symbiont. The ISME Journal, 16(5), 1430–1441. https://doi.org/10.1038/s41396-022-01194-y
Methods

Howe-Kerr, L. I., Grupstra, C. G. B., Rabbitt, K. M., Conetta, D., Coy, S. R., Klinges, J. G., Maher, R. L., McConnell, K. M., Meiling, S. S., Messyasz, A., Schmeltzer, E. R., Seabrook, S., Sims, J. A., Veglia, A. J., Thurber, A. R., Thurber, R. L. V., & Correa, A. M. S. (2023). Viruses of a key coral symbiont exhibit temperature-driven productivity across a reefscape. ISME Communications, 3(1). https://doi.org/10.1038/s43705-023-00227-7
Results

Howe-Kerr, L., Veglia, A., & Correa, A. (2023). vAMPirus Analysis - Viruses of a key coral symbiont exhibit temperature-driven productivity across a reefscape (Version 1.0.0). Zenodo. https://doi.org/10.5281/ZENODO.7552892
Methods

Montalvo-Proaño, J., Buerger, P., Weynberg, K. D., & van Oppen, M. J. H. (2017). A PCR-Based Assay Targeting the Major Capsid Protein Gene of a Dinorna-Like ssRNA Virus That Infects Coral Photosymbionts. Frontiers in Microbiology, 8. https://doi.org/10.3389/fmicb.2017.01665
Methods

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. PeerJ, 4, e2584. Portico. https://doi.org/10.7717/peerj.2584

Software

Veglia, A., Vicéns, R. E. R., Grupstra, C., Howe-Kerr, L., & Correa, A. (2021). vAMPirus: An automated, comprehensive virus amplicon sequencing analysis program (Version v1.0.1) [Computer software]. Zenodo. https://doi.org/10.5281/ZENODO.4549851

Software

Wernersson, R. (2006). Virtual Ribosome--a comprehensive DNA translation tool with support for integration of sequence feature annotation. Nucleic Acids Research, 34(Web Server), W385–W388. https://doi.org/[10.1093/nar/gkl252](10.1093/nar/gkl252)

Software

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Related Datasets

IsRelatedTo

Rice University. Symbiodiniaceae, Porites lobata Symbiodiniaceae LSU amplicon sequencing 2018-2020 Moorea, French Polynesia. 2023/02. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA930706. NCBI:BioProject: PRJNA930706.

Rice University. dinoRNA virus, Porites lobata Symbiodiniaceae infecting RNA viruses. 2023/01. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA928208. NCBI:BioProject: PRJNA928208.

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Parameters

Parameter	Description	Units
site_number	Site ID number, refers to the site location within reef zone	unitless
reef_zone	Reef type: "fringe" = Fringing reef (closest to shore) ;"back" = Back reef (lagoon); "fore" = Forereef (furthest from shore)	unitless
Latitude	Latitude of the reef site; negative values = South	decimal degrees
Longitude	Longitude of the reef site; negative values = West	decimal degrees
temperature_reading_number	ID number that indicates a unique reading; readings occurred every two hours	unitless
ISO_DateTime_Local	Date and time of the temperature reading in the local time zone (GMT-10); in ISO 8601 format	unitless
ISO_DateTime_UTC	Date and time of the temperature reading in UTC; in ISO 8601 format	unitless
temp_celsius	Temperature reading	degrees Celsius

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
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	GoPro and Olympus Tough camera
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	HOBO® temperature logger (Onset brands, # UA-002-64)
Generic Instrument Name	Temperature Logger
Generic Instrument Description	Records temperature data over a period of time.

Project Information

Collaborative Research: Viral Reefscapes: The Role of Viruses in Coral Reef Health, Disease, and Biogeochemical Cycling (Moorea Virus Project)

Coverage: Moorea, French Polynesia, Pacific 17 S 150 W

Ecologically and economically, coral reefs are among the most valuable ecosystems on Earth. These habitats are estimated to harbor up to nine million species, contribute ~30 billion US dollars annually to the global economy, and are tropical epicenters of biogeochemical cycling. Global (climate change) and local (nutrient pollution and overfishing) stressors are drivers of coral reef decline that can disrupt the symbiotic associations among corals and resident microbial communities, including dinoflagellate algae, bacteria, and viruses. Viruses interact with all living cellular organisms, are abundant in oceans, and integral to marine ecosystem functioning. This project will be the first to quantify the variability of viral infection in corals across different reef habitats and across time. This will increase our understanding of the total diversity of coral viruses and illuminate the full suite of factors that trigger viral outbreaks on reefs. At the same time the project will evaluate how carbon and nitrogen cycling are altered on coral reefs as a result of global and local stressors that trigger viral infection. This project will ultimately broaden our understanding of the impacts of viruses on reefs beyond their role as putative disease agents. Results of the project will be communicated broadly in scientific arenas, in K-12, undergraduate, and graduate education and training programs, and to the general public through video and multimedia productions, as well as outreach events. 2-D Reef Replicas from our field sites across Moorea will be constructed, allowing children and adults in the US and French Polynesia to 'become' marine scientists and use quadrats, transect tapes, and identification guides to quantify metrics of reef change. Three graduate students will be involved in all aspects of the research and an effort will be made to recruit and support minority students. All datasets will be made freely available to the public and newly developed methods from this project will serve as an important set of springboard tools and baselines for future lines of inquiry into the processes that influence reef health.

Coral reefs, found in nutrient-poor shallow waters, are biodiversity and productivity hotspots that provide substantial ecological and societal benefits. Corals energetically subsidize these oligotrophic ecosystems by releasing significant amounts of mucus (an organic carbon and nitrogen-rich matrix) into the surrounding seawater. Viral production in reef waters can be a significant portion of total reef carbon cycling, accounting for ~10% of gross benthic carbon fixation in reef ecosystems. Viruses are also ~10 times more abundant on coral surfaces than in the water column meaning that viral infection experienced by corals during stress likely results is an increase in carbon and perhaps nitrogen flux to the water column. Thus phages and eukaryotic viruses may be responsible for shifting reef health and function directly via coral and symbiont infection and by altering biogeochemical cycling in host colonies and the adjacent reef system. The main goal of this project is to experimentally interrogate and then model the links among viral infections, declines in coral and reef health, and associated shifts in biogeochemical cycling in reef ecosystems. Lab and field experiments will be conducted at the Moorea Coral Reef LTER to characterize the spatiotemporal dynamics of viruses within two dominant reef-building coral species that differ in their susceptibility to abiotic stress. A novel viral infection and induction approach will be coupled with stable isotopic pulse-chase experiments to quantify and track carbon and nitrogen flux out of coral holobionts (host and microbial symbionts) and into dissolved and particulate pools. In these experiments, virus, bacteria, and symbiont abundance, diversity, and function will be measured simultaneously with the health and activity of the host. Pulse-chase techniques, as well as flux- and niche-based modeling, will result in a holistic understanding of how corals and associated viruses impact reef energy budgets and the ramifications of carbon and nitrogen flux for reef communities. Ultimately, this project will quantify and describe an integrated mechanism by which environmental stressors alter viral, microbial, and coral diversity and, consequently, ecosystem function.

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1635798
NSF Division of Ocean Sciences (NSF OCE)	OCE-1635913
Gulf Research Program of the National Academies of Sciences, Engineering, and Medicine (GRP)	Early-Career Research Fellowship #2000009651