Transcriptome and viral metagenome data from Diadema antillarum collected at St. Thomas (USVI) and Saba (Caribbean Netherlands) in Apr 2022

Website: https://www.bco-dmo.org/dataset/985619
Data Type: Other Field Results
Version: 1
Version Date: 2025-10-07

Project
» Exploring the role of boundary layer microbial remineralization in flavivirus-host dynamics (Holothurian Flaviviruses)

Contributors	Affiliation	Role
Hewson, Ian	Cornell University (Cornell)	Principal Investigator
Brandt, Marilyn	University of the Virgin Islands Center for Marine and Environmental Studies (UVI)	Scientist
Breitbart, Mya	University of South Florida (USF)	Scientist
Henson, Michael	Northern Illinois University	Scientist
Hylkema, Alwin	Van Hall Larenstein University of Applied Sciences	Scientist
Gittens, Samuel	University of the Virgin Islands Center for Marine and Environmental Studies (UVI)	Student
Souza, Matthew	University of the Virgin Islands Center for Marine and Environmental Studies (UVI)	Student
Vilanova-Cuevas, Brayan	Cornell University (Cornell)	Student
Budd, Kayla	University of the Virgin Islands Center for Marine and Environmental Studies (UVI)	Technician
DeRito, Christopher	Cornell University (Cornell)	Technician
Sevier, Moriah	University of the Virgin Islands Center for Marine and Environmental Studies (UVI)	Technician
Von Hoene, Sarah	University of the Virgin Islands Center for Marine and Environmental Studies (UVI)	Technician
Mickle, Audrey	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
A mass mortality of the tropical echinoderm Diadema antillarum (urn:lsid:marinespecies.org:taxname:124332) occurred beginning in January 2022 and continuing to at least July 2022. We sought to identify flaviviruses associated with this condition by performing viral metagenomics and tissue transcriptomics. We prepared these libraries from grossly normal, abnormal and reference specimens collected from St Thomas, U.S. Virgin Islands (USVI), and Saba, Caribbean Netherlands. This dataset includes the accession information for the sequences performed and archived at the National Center for Biotechnology Information (NCBI) Sequence Read Archive. While we were unsuccessful in recovering flaviviruses from tissues of these organisms, we were able to detect additional pathogenic agents (Philaster apodigitiformis; Ciliophora) from transcriptomes.

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Coverage

Location: St Thomas, US Virgin Islands and Saba, Caribbean Netherlands, intertidal
Spatial Extent: **N**:18.3451 **E**:-63.2181 **S**:17.616 **W**:-64.694
Temporal Extent: 2022-04-07 - 2022-04-21

Methods & Sampling

Collection

Specimens of *Diadema antillarum* (urn:lsid:marinespecies.org:taxname:124332) were collected by snorkel and transported to the lab in seawater buckets. The specimens were photographed, and vivisected into tissue components (body wall, spine, gonad, digestive tract and coelomic fluid), which were then frozen in liquid N2 for transport to the laboratory at Cornell University.

Transcriptomic Sequencing

Transcriptomes from coelomic fluid of grossly normal (n = 2) and abnormal (n = 2) individuals from an affected site and of grossly normal individuals from a reference site (n = 2) were prepared alongside grossly normal (n = 2) aboral body wall tissues from a reference site to examine expressed genes and microorganisms associated with infection. RNA was extracted from coelomic fluid (300 µl) and body wall tissues (200 mg) using the Zymo Quick-RNA Tissue/Insect Kit using on-column deoxyribonuclease I digestion per the manufacturer's protocols. Transcriptome libraries were prepared from purified RNA extracts using the Zymo RiboFree kit and sequenced on a single run of Illumina MiSeq using a Nextera DNA Library Prep Kit at the Biotechnology Resource Center at Cornell University. All sequences have been deposited at National Center for Biotechnology Information (NCBI) Sequence Read Archive under Bioproject PRJNA900371 and SRA accession numbers SRR22260770 to SRR22260777.

Viral Metagenomic Sequencing

Small subsamples of each specimen were prepared for viral metagenomic sequencing broadly following the approach of Ng et al. (2011). Briefly, tissues were homogenized in sterile, 0.02 µm filtered phosphate buffered saline by beating using Zymo ZR Basher Beads (part number S6012-50) in a Biospec Instruments homogenizer for 1 min at maximum speed. The homogenates were centrifuged at 3,000 × g for 1 min to remove large cell debris, before filtering the supernatant through 0.2 µm pore size polyethersulfone syringe filters. Filtered homogenates were treated with 5U Turbo DNase (Invitrogen; part number AM2238), 5U Benzonase nuclease (Sigma Aldrich; part number E1014) and 20U RNase ONE (Promega; part number M4261) for 2 hours at 37 °C to digest extracellular and non-ribosome-bound nucleic acids. Following incubation, RNA was extracted from subsamples of purified virus-sized material using the Zymo Viral RNA kit (part number R1034) before storing RNA at −80 °C until further processing.

RNA in viral extracts was amplified using the SeqPlex RNA kit (Sigma Aldrich; part number SEQXE-10RXN, n = 11 libraries). Subsequent specimens were amplified using the TransPlex kit (WTA2; Sigma Aldrich; part number WTA2-10RXN, n = 3 libraries), which was used in prior RNA viral metagenomic work. Amplification products were purified using the Zymo DNA Clean and Concentrator -5 (part number D4004), quantified by Pico Green fluorescence (Invitrogen; part number P11496), and submitted for sequencing at the Cornell Biotechnology Resource Center. Libraries were prepared for sequencing using the Nextera Flex kit (Illumina; part number 20018704) and sequenced on the Illumina MiSeq platform using the 500 bp Nano kit. Sequences from all libraries were archived at NCBI under Bioproject PRJNA1117494 and SRA accessions SRR29258987 to SRR29258999.

BCO-DMO Curation Notes

- Imported "Virome_Transcriptome_Metadata.txt" into the BCO-DMO system
- Converted "Date" to ISO 8601 YYYY-MM-DD format
- Removed the N and W indicators, in accordance with BCO-DMO style for the "Latitude" and "Longitude", making the W values negative
- Renamed NCBI identifier fields to include more standard and descriptive titles
- Exported file as "985619_v1_virome_transcriptome.csv"

Scientific names in the data were checked using World Register of Marine Species (WoRMS) Taxon Match. All scientific names in the data are valid and accepted names as of 2025-10-01.

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Data Files

File	
985619_v1_virome_transcriptome.csv	(Comma Separated Values (.csv), 6.86 KB) MD5:8ebd493a0762ee990e484104c758e1e7
Primary data file for dataset ID 985619, version 1	

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Related Publications

Hewson, I., Brandt, M., Budd, K., Breitbart, M., DeRito, C., Gittens Jr, S., Henson, M. W., Hylkema, A., Sevier, M., Souza, M., Vilanova-Cuevas, B., & Von Hoene, S. (2024). Viral metagenomic investigation of two Caribbean echinoderms, Diadema antillarum (Echinoidea) and Holothuria floridana (Holothuria). PeerJ, 12, e18321. Portico. https://doi.org/10.7717/peerj.18321
Results

Hewson, I., Ritchie, I. T., Evans, J. S., Altera, A., Behringer, D., Bowman, E., Brandt, M., Budd, K. A., Camacho, R. A., Cornwell, T. O., Countway, P. D., Croquer, A., Delgado, G. A., DeRito, C., Duermit-Moreau, E., Francis-Floyd, R., Gittens, S., Henderson, L., Hylkema, A., … Breitbart, M. (2023). A scuticociliate causes mass mortality of Diadema antillarum in the Caribbean Sea. Science Advances, 9(16). https://doi.org/10.1126/sciadv.adg3200
Results

Ng, T. F. F., Wheeler, E., Greig, D., Waltzek, T. B., Gulland, F., & Breitbart, M. (2011). Metagenomic identification of a novel anellovirus in Pacific harbor seal (Phoca vitulina richardsii) lung samples and its detection in samples from multiple years. Journal of General Virology, 92(6), 1318–1323. https://doi.org/10.1099/vir.0.029678-0
Methods

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Related Datasets

IsRelatedTo

Hewson, I., Brandt, M., Budd, K., Breitbart, M., DeRito, C., Gittens, S., Henson, M., Hylkema, A., Sevier, M., Souza, M., Vilanova-Cuevas, B., Von Hoene, S. (2025) **Viral metagenomic investigation of the Caribbean echinoderm Holothuria floridana collected in Marathon, Florida on 25 Aug 2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-10-07 doi:10.26008/1912/bco-dmo.985655.1 [view at BCO-DMO]
Relationship Description: Data are referenced in the same results publication.

References

Cornell University. Surface-associated micobiome analysis (16S rRNA gene sequencing) of the sea cucumber, Apostichopus californicus, under suboxic conditions and organic matter enrichment. 2023/03. 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/PRJNA947521. NCBI:BioProject: PRJNA947521. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA947521

Cornell University. Viral metagenomic investigation of two Caribbean echinoderms. 2024/05. 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/PRJNA1117494. NCBI:BioProject: PRJNA1117494. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1117494

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Parameters

Parameter	Description	Units
SRA_accession	NCBI Run accession in the Sequence Read Archive (SRA)	unitless
SRA_study	NCBI study accession in the Sequence Read Archive (SRA)	unitless
bioproject_accession	NCBI BioProject identifier	unitless
biosample_accession	NCBI BioSample accession number	unitless
sample_name	Sample Name of Initial Sample	unitless
library_ID	Library Name of Sequence Data	unitless
title	NCBI Identifier Description	unitless
library_strategy	Sequencing library strategy: RNA-Seq	unitless
library_source	Source of sequencing library: Transcriptome or Metagenome	unitless
library_selection	How libraries were prepared for sequencing: size selected or random	unitless
library_layout	Description of sequencing reads: paired	unitless
platform	Sequencing Platform	unitless
instrument_model	Sequencing Instrument Used	unitless

design_description	Brief description of how libraries were prepared from source material	unitless
filetype	Sequence file format	unitless
Latitude	Latitude the specimens were collected, North is positive	decimal degrees
Longitude	Longitude the specimens were collected, West is negative	decimal degrees
Date_specimen_collected	Date the specimens were collected	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Transcriptome libraries were prepared from purified RNA extracts using the Zymo RiboFree kit and sequenced on a single run of Illumina MiSeq using a Nextera DNA Library Prep Kit at the Biotechnology Resource Center at Cornell University.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Photographed
Generic Instrument Name	Camera
Dataset-specific Description	The specimens were photographed, and vivisected into tissue components (body wall, spine, gonad, digestive tract and coelomic fluid), which were then frozen in liquid N2 for transport to the laboratory at Cornell University.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	The homogenates were centrifuged at 3,000 × g for 1 min to remove large cell debris, before filtering the supernatant through 0.2 µm pore size polyethersulfone syringe filters.
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	Snorkel
Generic Instrument Name	Diving Mask and Snorkel
Dataset-specific Description	Specimens were collected by snorkel and transported to the lab in seawater buckets.
Generic Instrument Description	A diving mask (also half mask, dive mask or scuba mask) is an item of diving equipment that allows underwater divers, including, scuba divers, free-divers, and snorkelers to see clearly underwater. Snorkel: A breathing apparatus for swimmers and surface divers that allows swimming or continuous use of a face mask without lifting the head to breathe, consisting of a tube that curves out of the mouth and extends above the surface of the water.

Dataset-specific Instrument Name	Biospec Instruments homogenizer
Generic Instrument Name	Homogenizer
Dataset-specific Description	Briefly, tissues were homogenized in sterile, 0.02 µm filtered phosphate buffered saline by beating using Zymo ZR Basher Beads (part number S6012-50) in a Biospec Instruments homogenizer for 1 min at maximum speed.
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Incubator
Generic Instrument Name	Incubator
Dataset-specific Description	Filtered homogenates were treated with 5U Turbo DNase (Invitrogen; part number AM2238), 5U Benzonase nuclease (Sigma Aldrich; part number E1014) and 20U RNase ONE (Promega; part number M4261) for 2 hours at 37 °C to digest extracellular and non-ribosome-bound nucleic acids. Following incubation, RNA was extracted from subsamples of purified virus-sized material using the Zymo Viral RNA kit (part number R1034) before storing RNA at −80 °C until further processing.
Generic Instrument Description	A device in which environmental conditions (light, photoperiod, temperature, humidity, etc.) can be controlled. Note: we have more specific terms for shipboard incubators (https://www.bco-dmo.org/instrument/629001) and in-situ incubators (https://www.bco-dmo.org/instrument/494).

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Project Information

Exploring the role of boundary layer microbial remineralization in flavivirus-host dynamics (Holothurian Flaviviruses)

Coverage: Northeastern Pacific Ocean

NSF Award Abstract:

Marine diseases pose considerable risks to invertebrates, such as sea cucumbers, in the face of changing ocean conditions. While many invertebrate diseases are driven by pathogens, the interplay between animal biology and environmental conditions often mediates the outcome of the pathogen-host relationship. Sea cucumbers are ecologically and economically important animals that occur in a wide range of marine habitats. This project aims to decipher how the interaction between the biology of sea cucumbers, environmental conditions, and a newly-discovered type of virus, seemingly innocuous under typical conditions, may lead to lethal disease in giant Pacific sea cucumbers in the U.S. West Coast. The study includes surveys in coastal regions in southeast Alaska, Washington, and California as well as laboratory experiments manipulating seawater oxygen concentrations, temperature, and simulated microalgal blooms. The project engages community scientists, fishers, high school students, and indigenous groups, and supports training of one graduate and several undergraduate students. A workshop that brings together scientists across marine ecology, disease, and veterinary disciplines is planned to prepare a handbook of best practices in marine disease investigation.

Metagenomic and community-level sequencing efforts have revealed an astonishing diversity of viruses associated with grossly normal marine invertebrates. The vast majority of detected viruses likely represents asymptomatic infections under typical conditions but may generate pathology in hosts under changing environmental conditions. This project investigates the ecology of a group of enveloped positive sense single-stranded RNA viruses (flaviviruses) that this research team has recently discovered in the giant California sea cucumber Apostichopus californicus by addressing three hypotheses: 1) Aquatic insect-only Flaviviruses (aiFVs) do not cause gross pathology under typical conditions; 2) aiFVs proliferate and generate clinical and gross pathology under suboxic stress; and 3) Periodic increases in primary production and mean temperature excursions cause aiFV proliferation and subsequently exacerbate holothurian disease process. The study comprises a restricted survey of aiFV diversity via amplicon sequencing and their prevalence within and between populations, development of an antibody-based approach for aiFV detection, and examination of aiFV behavior in concert with host transcription and veterinary pathology. The study includes field surveys and in laboratory manipulative experiments.

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-2049225

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