16S rRNA V4 sequence metadata from surface swabs from Apostichopus californicus-associated flavivirus experiment under suboxic conditions and organic matter amendment

Website: https://www.bco-dmo.org/dataset/984835
Data Type: Other Field Results, experimental
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
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Abstract
Microbial activities at the animal-water interface are hypothesized to influence viral replication and possibly contribute to pathology of echinoderm wasting diseases due to hypoxic stress. We assessed the impacts of enhanced microbial production and suboxic stress on Apostichopus californicus (urn:lsid:marinespecies.org:taxname:529363) associated flavivirus (PcaFV) load in a mesocosm experiment. This dataset contains 16S rRNA V4 amplicon sequencing metadata for this experiment. Organic matter amendment and suboxic stress resulted in lower PcaFV load, which also correlated negatively with animal mass loss and microbial activity at the animal-water interface. These data suggest that PcaFV replication and persistence was best supported in healthier specimens. Our results do not support the hypothesis that suboxic stress or microbial activity promote PcaFV replication, but rather that PcaFV appears to be a neutral or beneficial symbiont of Apostichopus californicus.

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Coverage

Location: Thimbleberry Bay (57.031861N, 135.250972W), near Sitka, Alaska
Spatial Extent: **Lat**:57.031861 **Lon**:-135.250972
Temporal Extent: 2021-11-11 - 2021-11-18

Methods & Sampling

Forty-two specimens of *Apostichopus californicus* (urn:lsid:marinespecies.org:taxname:529363) were collected by SCUBA divers in Thimbleberry Bay (57.031861N, 135.250972W), near Sitka, Alaska on 10 November 2021 and transported together in plastic tubs to the lab at the University of Alaska Southeast (Japonski Island, Sitka). Specimens were immediately weighed, photographed, and placed into individual mesh containers within 7 x 1200 L outdoor mesocosms (6 specimens per mesocosm) filled with seawater from the nearby Sitka Channel. Two mesocosms served as controls (no amendment), 4 mesocosms were subject to daily organic matter (20 µM) amendment, and 1 mesocosm was continuously sparged with N_2 (Airgas, medical grade; the other 6 mesocosms were bubbled continuously with air). Seawater was subject to 50% volume water change daily and specimens were not fed during captivity. Mesocosms were covered while not sampled (i.e., they were light limited). We selected two organic matter substrates (glucose and peptone) based on their ability to stimulate microbial activity in prior work in addition to two common constituents of dissolved organic matter in coastal environments (N-acetylglucosamine and fucose + rhamnose). We monitored dissolved oxygen levels in each mesocosm using continuous submersible HOBO loggers.

Surface swabs of each individual were collected daily by rubbing a Puritan polyester sterile swab over a ~ 1 cm^2 area of epidermis. Swabs were cut using clean scissors to remove the polyester tip and placed into 2 mL cryovials containing RNALater. Tube feet samples were collected from each specimen daily using disposable plastic forceps, and feet were placed immediately into cryovials containing RNALater. A 5mm biopsy punch of the dorsal body wall was collected from half of the individuals in each mesocosm at 0, 1, 3, and 6 d, which were then preserved in RNALater. All specimens were photographed and weighed daily. All RNALater preserved samples were frozen at -80°C and transported in liquid N_2 to the laboratory at Cornell University. Animal carcasses were frozen at -20°C and transported on blue ice to Cornell.

Surface swabs were collected from 42 animals over the course of 7 d (sampled on t = 0 d, 1 d, 3 d, 5 d, and 7 d) and frozen at -20°C until further processing. DNA was extracted from frozen swabs using Zymo Quick-DNA Fungal/Bacterial kits (Zymo Research) as per the manufacturer's protocol. Bacterial communities in sample extracts were identified using dual-barcoded PCR (polymerase chain reaction) amplification and sequencing of the V4 region of the 16S rRNA gene. Each 40 µL PCR reaction comprised 1X PCR master mix (One-Taq Quick-Load 2x Master Mix with Standard; New England Biolabs), 0.125 µM of each barcoded primer (515f; 5'-GTG **Y**CA GCM GCC GCG GTA A-3' and 806r; 5'-GGA CTA C**N**V GGG TWT CTA AT-3'), and 2 uL of template (swab extract). 16S rRNA amplicons were pooled at equimolar concentrations using SequalPrep Normalization Plate kit (Invitrogen) and sequenced on one lane of Illumina MiSeq (2 x 250 paired end) at the Cornell University Biotechnology Research Center. 16S rRNA amplicon sequences were submitted to NCBI (BioProject accession number PRJNA947521, see Related Datasets).

BCO-DMO Processing Description

- Imported "MIMARKS.survey.host-associated.5.0_Sitka_Sea_Cucumber_16S.xlsx" into the BCO-DMO system
- Converted "collection_date" to ISO 8601 format YYYY-MM-DD
- Removed parameters with no values
- Removed special characters from parameter names
- Split "lat_lon" parameter into "Latitude" and "Longitude"
- Replaced lat and lon values with values provided by submitter for collection location
- Renamed "collection_date" to "swab_collection_date"
- Created a new column, "specimen_collection_date" with values of 2021-11-10, the date all specimens were collected

- Filtered out all rows with "host_subject_id" values that included "BLANK", "Blank", or "Basket", upon submitter request
- Added corresponding BioSample identifiers (provided by submitter) to the dataset
- Added corresponding AphiaIDs and LSIDs for relevant species
- Renamed "misc_param" and "host_taxid" to "replicate_ID" and "host_NCBI_taxid"
- Exported file as "984835_v1_swab_sequence_accessions.csv"

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Data Files

File
984835_v1_swab_sequence_accessions.csv (Comma Separated Values (.csv), 160.36 KB) MD5:d0ce959cb8edc3c47d8873fbd0589deb Primary data file for dataset ID 984835, version 1

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Related Publications

Crandell, J. G., Altera, A. K., DeRito, C. M., Hebert, K. P., Lim, E. G., Markis, J., Philipp, K. H., Rede, J. E., Schwartz, M., Vilanova-Cuevas, B., Wang, E., & Hewson, I. (2023). Dynamics of the Apostichopus californicus-associated flavivirus under suboxic conditions and organic matter amendment. Frontiers in Marine Science, 10. https://doi.org/10.3389/fmars.2023.1295276
Results

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Related Datasets

IsRelatedTo

Crandell, J., Altera, A., DeRito, C., Hebert, K., Lim, E. G., Markis, J., Philipp, K. H., Rede, J., Schwartz, M., Vilanova-Cuevas, B., Wang, E., Hewson, I. (2025) **Dynamics of the Apostichopus californicus-associated flavivirus under suboxic conditions and organic matter amendment in mesocosm experiment.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-09-20 doi:10.26008/1912/bco-dmo.984803.1 [view at BCO-DMO]
Relationship Description: Data collected from samples gathered for the same experiment.

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

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Parameters

Parameter	Description	Units
sample_name	Sequencing library name	unitless

bioproject_accession	NCBI BioProject Accession Number	unitless
biosample	NCBI BioSample Accession Number	unitless
organism	Microorganism name for accession	unitless
specimen_collection_date	Date of Specimen Collection (Thimbleberry Bay)	unitless
swab_collection_date	Date of surface swab; Surface swabs were collected from 42 animals over the course of 7 d (sampled on t = 0 d, 1 d, 3 d, 5 d, and 7 d) starting on 2021-11-11	unitless
Latitude	Latitude of specimen collection, positive is North	decimal degrees
Longitude	Longitude of specimen collection, negative is West	decimal degrees
env_broad_scale	Biome of sample	unitless
env_local_scale	Marine biome	unitless
env_medium	Intertidal	unitless
geo_loc_name	Geographic Location	unitless
host	Host Organism	unitless
host_subject_id	Unique identifier by which each sequence can be identified	unitless
replicate_ID	Sample replicate ID	unitless
host_tissue_sampled	Type of Tissue of original sample	unitless
host_NCBI_taxid	NCBI Taxonomy of the Host	unitless
AphiaID	Unique identifier for the listed taxon in the Aphia database	unitless
LSID	Life Science Identifier (LSID) for the listed taxon	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	16S rRNA amplicons were pooled at equimolar concentrations using SequalPrep Normalization Plate kit (Invitrogen) and sequenced on one lane of Illumina MiSeq (2 x 250 paired end) at the Cornell University Biotechnology Research Center.
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	Specimens were immediately weighed, photographed, and placed into individual mesh containers within 7 x 1200 L outdoor mesocosms (6 specimens per mesocosm) filled with seawater from the nearby Sitka Channel.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Submersible HOBO logger
Generic Instrument Name	Oxygen Sensor
Dataset-specific Description	We monitored dissolved oxygen levels in each mesocosm using continuous submersible HOBO loggers.
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	Scale
Generic Instrument Name	scale or balance
Dataset-specific Description	Specimens were immediately weighed, photographed, and placed into individual mesh containers within 7 x 1200 L outdoor mesocosms (6 specimens per mesocosm) filled with seawater from the nearby Sitka Channel.
Generic Instrument Description	Devices that determine the mass or weight of a sample.

Dataset-specific Instrument Name	SCUBA
Generic Instrument Name	Self-Contained Underwater Breathing Apparatus
Dataset-specific Description	Forty-two specimens of Apostichopus californicus were collected by SCUBA divers in Thimbleberry Bay (57.0297N, 135.2283W), near Sitka, Alaska on 10 November 2021 and transported together in plastic tubs to the lab at the University of Alaska Southeast (Japonski Island, Sitka).
Generic Instrument Description	The self-contained underwater breathing apparatus or scuba diving system is the result of technological developments and innovations that began almost 300 years ago. Scuba diving is the most extensively used system for breathing underwater by recreational divers throughout the world and in various forms is also widely used to perform underwater work for military, scientific, and commercial purposes. Reference: https://oceanexplorer.noaa.gov/technology/technical/technical.html

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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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