Dynamics of the Apostichopus californicus-associated flavivirus under suboxic conditions and organic matter amendment in mesocosm experiment

Website: https://www.bco-dmo.org/dataset/984803
Data Type: Other Field Results, experimental
Version: 1
Version Date: 2025-09-20

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

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Abstract
Flaviviruses cause some of the most detrimental vertebrate diseases, yet little is known of their impacts on invertebrates. 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. 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. This primary dataset contains per-specimen measurements including experimental treatments, microbial productivity (animal surface and mesocosm water), body wall lipid/protein content, animal mass loss, tissue-specific PcaFV viral loads at initial and final timepoints, and collection metadata. The supplemental file provides time-series measurements of dissolved oxygen and temperature in mesocosms, indexed by tank number, treatment, and timestamp.

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Coverage

Location: Southeast Alaska - Thimbleberry Bay near Sitka, Alaska
Spatial Extent: **Lat**:57.031861 **Lon**:-135.250972
Temporal Extent: 2021-11-09 - 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. Logger data is included as a supplemental file attached to this dataset. Loggers began collecting data on 9 November 2021.

Quantitative reverse transcriptase PCR (qRT-PCR) using primers PcaFV_NS5_F3 (5'- CCA GCC ATG GAT GAG TAA TG-3') and PcaFV_NS5_R3 (5'- GCT GAA CTG CTC CTG AAA CC-3'), and probe PcaFV_NS5_Pr3 (5'-[FAM]CAC GAA TGT ACG GCA ACG GAC G[TAMRA]-3') was used to determine PcaFV load within individual tissue RNA extracts. qRT-PCR reactions were performed in duplicate for each specimen and compared against duplicate reactions of oligonucleotide standards spanning the amplicon region (from 10^2 to 10^7 copies μl^{-1}) and using 4 negative controls (nuclease free H_2O only). PCR reactions (20 µL) contained 1 X Luna Universal Probe One-Step Reaction Mix (New England Biolabs), 1X Luna WarmStart RT Enzyme Mix (New England Biolabs), 8 µmol each of primers and probes, and 1 µL of template RNA. Reactions were thermal cycled in an ABI StepOne qPCR machine. Reactions were initially held at 55°C for 10 min, followed by an initial denaturation step at 95°C for 1 min. Reactions were then subject to 45 cycles of denature at 95°C and anneal at 56°C, with fluorescence data collected after the annealing step. Linearity of standards was checked during downstream analysis of qPCR data.

Relative microbial activity was measured in each mesocosm and from the surface of 2 specimens per mesocosm by bromodeoxyuridine (BrDU) incorporation. Five 2 mL water samples were retrieved from each mesocosm by pipette and placed into 2 ml cryovials. Water samples (5 replicates) were collected from the surface of each of two haphazardly chosen specimens by pressing the pipette tip against the dorsal epidermis and slowly retrieving sample, which was then placed into 2 mL cryovials. Water samples were inoculated with 0.04 nmol BrDU and incubated in the mesocosm for 8 h. Incubations were then retrieved, and flash frozen in liquid N_2.

A standard for comparison between BrDU blots was made by amending 50 mL of water collected from an aquarium with 0.04 nmol BrDU. A single sheet of chromatography paper was placed in a Tupperware container soaked with 6X SSC Buffer with a nylon transfer membrane on top and allowed to soak for 10 minutes. The nylon transfer filter was then mounted in a slot blot manifold. Samples (0.5 mL) were pipetted into slot wells, where each membrane included 6 standards, then vacuum filtered through the membrane. The membrane was placed sample side down onto a stack of 3 sheets of chromatography paper with the lysis solution, then

immediately turned sample side up and incubated for 10 min. This was repeated in the same fashion for neutralization solution. The membrane was then placed sample-side down on a piece chromatography paper soaked in FixDenat for 15 s, followed by 30 min incubation on the FixDenat stack. The membrane was placed between two sheets of chromatography paper and baked at 80°C for ~1 h.

The dried membrane was placed in a plastic tray, 25 mL Blocking Solution was added, and the apparatus incubated on a rotating incubator at 30 rev min^{-1} for 1 h. After this time, the blocking solution was poured off and 6 mL of Antibody Incubation Solution was added to the tray, which was placed on a rotating incubator at 30 rev min^{-1} for 3 h. The antibody incubation solution was poured off and 25 mL wash solution was added, which was incubated on rotating incubator for 5 min. The wash step was repeated. After washing, the wash solution was poured off, and 25 mL of maleic acid buffer was added and incubated on rotating incubator for 5 min. This maleic acid buffer treatment then was repeated. The nylon transfer membrane was placed in a plastic tray, sample side up, to which 4 mL of the Supersignal West Femto was added and incubated for 2 minutes. The membrane was visualized in a BioRad ChemiDoc system at high sensitivity and 4 x 4 binning with an exposure time of 600 s.

Protein content was measured in body wall tissues using RIPA buffer extraction and Bradford reagent spectrophotometry. Two biopsy punch (5 mm) tissue samples were harvested from frozen animal carcasses and placed into 2 mL microcentrifuge tubes. RIPA buffer (2 mL; 150mM NaCl, 10 mM Tris, 1 mM EDTA, 1% Triton X-100, 0.01% sodium dodecyl sulfate, and 0.01% sodium deoxycholate) was added to each sample, and samples were homogenized in a mortar and pestle for 2 min or until the tissue was completely homogenized. Samples were incubated at room temperature in a shaking incubator for 2 h. Following this, tubes were centrifuged at 16,000 x g at 4°C for 20 min, and supernatant removed into a new microcentrifuge tube. Protein quantity was determined against a bovine serum albumin standard. Bradford reagent (1500 µL) was added to 30 µL sample, allowed to incubate at room temperature for 15 min, then absorbance at 595 nm measured on a spectrophotometer.

Lipid content was assessed. Two biopsy punch (5 mm) tissue samples were harvested from animal carcass dorsal body wall and placed into 15 mL centrifuge tubes. Tissue samples were weighed, amended with 12 mL chloroform:methanol, then homogenized in a mortar and pestle until the tissue had completely disintegrated and resembled a paste. The homogenates were centrifuged at 3,000 x g for 10 min in a benchtop centrifuge and the solvent fraction removed into fresh centrifuge tubes. Three microcentrifuge tubes per sample were weighed, and then 2 mL of the solvent fraction from each tissue sample homogenate was placed into the tubes. Tubes were then placed into a rotating vacuum desiccator until they were dry. Sample tubes were then re-weighed, and mass difference calculated on aggregate for all replicate tubes per sample as a percentage of wet weight.

BCO-DMO Processing Description

- Imported "PcaFV_Ancillary.txt" into the BCO-DMO system
- Converted "Date of Collection" to ISO 8601 format YYYY-MM-DD
- Renamed "Date_of_Collection" to "Date_of_Animal_Sampling" and added "Date_of_Specimen_Collection" to differentiate those dates
- Removed Latitude and Longitude since all samples were from same location, noted in the methods, and dataset is experimental data
- Removed special characters from parameter names in compliance with BCO-DMO guidelines
- Exported file as "984803_v1_exp_data_pcafv_loads.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 2025-09-20.

Apostichopus californicus (urn:lsid:marinespecies.org:taxname:529363)

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Data Files

File
984803_v1_exp_data_pcafv_loads.csv (Comma Separated Values (.csv), 5.39 KB) MD5:3fa5d6ada13246e1ab906dee594e3a43
Primary data file for dataset ID 984803, version 1

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Supplemental Files

File
PcaFV_O2.txt (Plain Text, 715.50 KB) MD5:b74e4514d2332bec70645240d4df2e3c
Oxygen concentration and temperature in mesocosm experiments, including Tank No, Treatment, Date, Time, DO (mg/L), Temp (oC), Latitude and Longitude of lab location

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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) **16S rRNA V4 sequence metadata from surface swabs from Apostichopus californicus-associated flavivirus experiment under suboxic conditions and organic matter amendment.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-10-07 doi:10.26008/1912/bco-dmo.984835.1 [view at BCO-DMO]
Relationship Description: Data collected from samples gathered for the same experiment.

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Parameters

Parameter	Description	Units
Specimen_No	Specimen Number	unitless
Treatment	Experimental Treatment Type: Control (1 and 2), Fucose/Rhamnose, Glucose, N-Acetylglucosamine, N2, Peptone	unitless
Animal_Surf_Productivity	Relative microbial productivity on animal surface	unitless
Mesocosm_Water_Productivity	Relative microbial productivity in plankton in mesocosm	unitless
Tissue_Lipid_pct	Body Wall Lipid content	percent
Tissue_Protein_Concentration	Body Wall Protein content	ug/mL
Animal_Mass_loss	Animal weight loss from start of experiment to end of experiment	percent per day
Tube_Foot_PcaFV_Load_Day_0	PcaFV Load in Tube Feet Tissues at T = 0	copies / ng RNA
Tube_Foot_PcaFV_Load_Day_7	PcaFV Load in Tube Feet Tissues at T = 7	copies / ng RNA
Resp_Tree_PcaFV_Load_Day_7	PcaFV Load in Respiratory Tree Tissues at T = 7	copies / ng RNA
Gonad_PcaFV_Load_Day_7	PcaFV Load in Gonad Tissues at T = 7	copies / ng RNA
Intestine_PcaFV_Load_Day_7	PcaFV Load in Intestinal Tissues at T = 7	copies / ng RNA
Date_of_Animal_Sampling	Date of Initial Animal Sampling	unitless
Date_of_Specimen_Collection	Date of Specimen Collection (Thimbleberry Bay)	unitless

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Instruments

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	Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Following this, tubes were centrifuged at 16,000 x g at 4°C for 20 min, and supernatant removed into a new microcentrifuge tube.
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	Mortar and pestle
Generic Instrument Name	Homogenizer
Dataset-specific Description	RIPA buffer (2 mL; 150mM NaCl, 10 mM Tris, 1 mM EDTA, 1% Triton X-100, 0.01% sodium doecyl sulfate, and 0.01% sodium deoxycholate) was added to each sample, and samples were homogenized in a mortar and pestle for 2 min or until the tissue was completely homogenized.
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	Continuous 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	ABI StepOne qPCR
Generic Instrument Name	qPCR Thermal Cycler
Dataset-specific Description	Reactions were thermal cycled in an ABI StepOne qPCR machine.
Generic Instrument Description	An instrument for quantitative polymerase chain reaction (qPCR), also known as real-time polymerase chain reaction (Real-Time PCR).

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 (57.031861N, 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).
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

Dataset-specific Instrument Name	Shaking incubator
Generic Instrument Name	Shaker
Dataset-specific Description	RIPA buffer (2 mL; 150mM NaCl, 10 mM Tris, 1 mM EDTA, 1% Triton X-100, 0.01% sodium doecyl sulfate, and 0.01% sodium deoxycholate) was added to each sample, and samples were homogenized in a mortar and pestle for 2 min or until the tissue was completely homogenized. Samples were incubated at room temperature in a shaking incubator for 2 h.
Generic Instrument Description	A Shaker is a piece of lab equipment used to mix, blend, or to agitate substances in tube(s) or flask(s) by shaking them, which is mainly used in the fields of chemistry and biology. A shaker contains an oscillating board which is used to place the flasks, beakers, test tubes, etc.

Dataset-specific Instrument Name	Spectrophotometer
Generic Instrument Name	Spectrometer
Dataset-specific Description	Bradford reagent (1500 µL) was added to 30 µL sample, allowed to incubate at room temperature for 15 min, then absorbance at 595 nm measured on a spectrophotometer.
Generic Instrument Description	A spectrometer is an optical instrument used to measure properties of light over a specific portion of the electromagnetic spectrum.

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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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