Prevalence of PcaFV in Apostichopus californicus specimens collected in Southeast Alaska from Jun to Nov 2021

Website: https://www.bco-dmo.org/dataset/984849
Data Type: Other Field Results
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Project
» Exploring the role of boundary layer microbial remineralization in flavivirus-host dynamics (Holothurian Flaviviruses)

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Abstract
Understanding the prevalence and load of aquatic invertebrate flaviviruses is essential to estimating risk to fisheries. We surveyed the prevalence of the Apostichopus californicus (urn:lsid:marinespecies.org:taxname:529363) associated flavivirus (PcaFV) using two approaches: Loop-mediated isothermal amplification (LAMP) and quantitative reverse transcriptase PCR. We targeted this survey around populations of A. californicus: specimens collected from Sitka Sound, Sitka, Alaska (Baranof Island), Southeast Alaska, and Nanoose, British Columbia, Canada. We found that LAMP detected PcaFV in 21% of tested specimens, and qRT-PCR in 88% of tested specimens. The mean load of PcaFV in copies per ng RNA extracted was significantly higher in specimens that were positive by LAMP compared to those in which LAMP did not detect PcaFV. This dataset contains PcaFV prevalence determined by RT-LAMP and qRT-PCR, as well as associated collection metadata.

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Coverage

Location: Southeast Alaska, Sitka
Spatial Extent: **N**:58.3769 **E**:-124.1411 **S**:49.2907 **W**:-135.4042
Temporal Extent: 2021-06-29 - 2021-11-11

Methods & Sampling

Sea cucumbers were collected at 3 locations (near Juneau, Alaska, near Sitka, Alaska, and Nanoose, British Columbia) to assess the presence and load of PcaFV and other aquatic invertebrate flaviviruses.

Twenty individual *Apostichopus californicus* specimens were collected by SCUBA divers during routine fisheries surveys by Alaska Department of Fish and Game from near southeastern Alaska (Juneau) in June/July 2021. Specimens were immediately placed into a refrigerated hold on the ship in zip-lock bags before transport to the lab in Juneau later in the day. There, specimens were frozen at -20°C and couriered overnight to Cornell University (Ithaca, NY, USA) where they were frozen at -80°C on arrival.

Thirty-two specimens of *Apostichopus californicus* were collected by SCUBA divers in Sitka Sound (57.1273 N, 135.4042 W), 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 sampled for tube feet by pulling them off and placing into RNALater, before freezing at -20^{o}C for transport to the lab at the University of Alaska Southeast.

Five specimens of *Apostichopus californicus* were collected from Nanoose, British Columbia, Canada in September 2021 and transported on wet ice to the lab at Cornell University for further analysis.

RT-LAMP was performed in duplicate in 25 µL reactions containing 1X WarmStart Colorimetric RT-LAMP Master Mix (New England Biolabs), 0.5 µL of fluorescent dye, 2.5 µL primers designed using the New England BioLabs primer tool around a portion of the PcaFV NS5 amplicon (GenBank accession MT949664), and 5 µL of extracted RNA. The primer master mix comprised 16 µL of the 100uM PcaFV_NS5_FIP and PcaFV-NS5_BIP primers, 2 µL of 100uM PcaFV_NS5_F3 and PcaFV_NS5_B3 primers, 4 µL of the 100uM PcaFV_NS5_LoopF and PcaFV_NS5_Loop B primers, as well as 56 µL of nuclease-free water (diH_2O). Reactions were performed in optically clear quantitative PCR tubes (Applied Biosystems). Negative controls comprised 5 µL of diH_2O, while positive controls used 5 µL of PcaFV synthetic gene fragment. The tube strips were incubated in a water bath at 65°C for 30 min. After incubation, tubes were removed from the bath and scored based on color change. Positive detections were scored when the reaction appeared yellow and negative detections were scored when the reaction appeared red. Positive controls were consistently yellow, and negative controls were consistently red. When positive controls had not turned yellow, reactions were further incubated for 15 min in the bath, and then reassessed. Possible detections were scored when the reactions turned orange.

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.

BCO-DMO Processing Description

- Imported "PcaFV_NaturalLoads_LAMP.xlsx" into Excel

- Formatted "PcaFV Quantity (qRTPCR)" to show all numbers for 30 digits and formatted date field as YYYY-MM-DD and exported to "PcaFV_NaturalLoads_LAMP.csv"
- Imported "PcaFV_NaturalLoads_LAMP.csv" into the BCO-DMO system
- Formatted the lat and lon to remove the letters and convert to a numeric field (adding a negative for W values
- Renamed parameter names to comply with BCO-DMO parameter naming guidelines
- Replaced "Stellawagan Bank, Sitka, Alaska" location value with "Sitka Sound, Sitka, Alaska", upon submitter request
- Replaced "2015-07-05" with "2021-07-05", upon submitter request
- Exported file as "984849_v1_pcafv_natural_loads.csv"

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Data Files

File
984849_v1_pcafv_natural_loads.csv (Comma Separated Values (.csv), 6.30 KB) MD5:f3ff15903da689493cd2681e9676090f Primary data file for dataset ID 984849, 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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Parameters

Parameter	Description	Units
Sample_Name	Name of specimen	unitless
Sample_Identity	Species sampled	unitless
Geographic_Location	Geographic Location	unitless
Tissue_Type	Tissue Type	unitless
PcaFV_Quantity_qRTPCR	Quantity of PcaFV determined by quantitative reverse transcriptase PCR (qRT-PCR) targeting PcaFV	copies ng^{-1} RNA
LAMP_Detection	Result of LAMP detection based on colorimetric change	unitless
Date_of_Collection	Date of specimen collection	unitless
Latitude	Latitude of specimen collection, positive is North	decimal degrees
Longitude	Longitude of specimen collection, negative is West	decimal degrees

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Instruments

Dataset-specific Instrument Name	ABI Step One Real Time PCR machine
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	SCUBA
Generic Instrument Name	Self-Contained Underwater Breathing Apparatus
Dataset-specific Description	Twenty individual Apostichopus californicus specimens were collected by SCUBA divers during routine fisheries surveys by Alaska Department of Fish and Game from near southeastern Alaska (Juneau) in June/July 2021. Thirty-two specimens of Apostichopus californicus were collected by SCUBA divers in Sitka Sound (57.1273 N, 135.4042 W), 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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