Virus and prokaryote abundances from experiments conducted with samples collected at a hydrothermal vent site by ROV SuBastian during R/V Falkor (too) expedition FKt230627 along the East Pacific Rise in July of 2023

Website: https://www.bco-dmo.org/dataset/936224
Version: 1
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Project
» The Underworld of Hydrothermal Vents (Vent Underworld)

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Abstract
We used the virus-dilution technique to quantify lytic virus production at nine sites across vent, sub-vent, and non-vent habitats along the East Pacific Rise during expedition FKt230627 aboard R/V Falkor(too). This technique leverages the density-dependent nature of viral infection, preventing new infections by dilution. Samples were collected via ROV SuBastian, sequentially filtered to remove larger particles, and concentrated using tangential-flow filtration. Duplicate incubations were set up with prokaryotic concentrate and virus-free water, pressurized to 250 bar, and incubated in the dark at in situ temperatures for 30 hours. Subsamples were taken every six hours for enumeration of prokaryotes and viruses via flow cytometry. Parallel experiments were conducted at surface pressure to assess the impact of pressure on virus production rates. The methodology ensures observed increases in viral abundance are due to pre-existing infections. The experiments were conducted aboard R/V Falkor(too) between 5-22 July 2023 by Tinkara Tinta and Nicole Krause. Prokaryotes and viruses were enumerated flow-cytometrically back in the lab by Christian Winter.

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Coverage

Location: East Pacific Rise (Hydrothermal vent site Tica, ~2515m depth)
Spatial Extent: **N**:9.840335 **E**:-104.291555 **S**:9.839953 **W**:-104.291922
Temporal Extent: 2023-07-06 - 2023-07-23

Methods & Sampling

We employed the virus-dilution technique to quantify lytic virus production at nine sampling sites across three distinct habitats (vent, sub-vent, non-vent) along the East Pacific Rise (9°N 50'N and 104°W) in the Tica vent area during expedition FKt230627 aboard R/V Falkor(too). The virus-dilution technique leverages the density-dependent nature of viral infection. In this approach, viruses are largely removed from the sample, and the remaining prokaryotic cells are incubated in virus-free sample water. Due to the extreme dilution of viruses in the sample, new viral infections are prevented during the incubation period. Consequently, any observed increase in viral abundance is attributable to viral infection processes that were already active prior to sample collection (Köstner et al. 2017).

Samples were collected using a suction-sampler operated onboard the remotely-operated vehicle (ROV) SuBastian. Immediately after the ROV was brought on board, a total of 4 L per sample were transferred into two 2L Nalgene flasks subsequently used for setting-up the experimental incubations by sequential filtration. First, the sample was filtered over membrane filters with a pore-size of 3 µm (Isopore, TSTP04700, Millipore) to remove larger particles. Subsequently, prokaryotic cells within the filtrate of this first filtration step were concentrated using a tangential-flow filtration device with a pore-size of 0.22 µm (Vivaflow 200, PES membrane, Sartorius) until the volume of the retentate approached ~200 mL. The retentate from this second filtration step was subjected to another tangential-flow ultrafiltration device with a molecular weight cut-off of 100 kDa (Vivaflow 200, PES membrane, Sartorius) to remove the viruses, yielding a virus-free ultrafiltrate to be used as culture medium in the experimental incubations.

For each sample, duplicate experimental incubations were prepared by mixing 3 mL of prokaryotic concentrate with 47 mL of virus-free water from the same sample, yielding a final volume of 50 mL per incubation. Immediately afterward, 1.5 mL subsamples were taken to enumerate prokaryotes and viruses (see below). Subsequently, for each duplicate incubation, five 2.5 mL glass vials were filled with the prepared dilution of prokaryotic concentrate in virus-free filtrate, tightly sealed, and placed into pressure chambers (Klose et al. 2015, one chamber per duplicate incubation). The experiments were incubated at 250 bar pressure (approximately 2500 m depth), in the dark, and at in situ temperature for 30 hours. Every ~6 hours, the chambers were depressurized to remove one glass vial per duplicate incubation to collect 1.5 mL of water for subsequent enumeration of prokaryotes and viruses (see below). Immediately after subsampling, the chambers containing the remaining glass vials were re-pressurized to 250 bar and incubated in the dark and at in situ temperature until the next subsampling or until the experiments concluded after 30 hours. To assess the impact of pressure on virus production rates, virus-dilution experiments were also conducted at surface pressure conditions at specific sampling sites.

Samples for the enumeration of prokaryotic cells and viruses were immediately fixed with glutaraldehyde at a final concentration of 0.5% at room temperature for 10 minutes, flash-frozen in liquid nitrogen, and stored at -80°C until analysis by flow cytometry (Brussaard et al. 2010). Upon thawing samples were stained with SYBR Green I for 10 minutes at room temperature for prokaryotes or at 80°C for viruses. The samples were then analyzed using a FACSAria III flow cytometer (Becton Dickinson), and prokaryotes and viruses were enumerated on cytograms of side scatter versus green fluorescence (Brussaard et al. 2010).

Data Processing Description

Submitted data consist of temporal developments of prokaryotic and viral abundances (both in $Nx10^4$ mL^{-1}) within the experimental incubations.

BCO-DMO Processing Description

BCO-DMO Data Manager Processing Notes:
* Data from source file BCODMOData.xls Sheet 1 were imported into the BCO-DMO data system with values "/N" identified as missing data identifiers. This table was attached to this dataset as 936224_v1_virus-production-hydrothermal-vent.csv

** Missing data in the BCO-DMO system displays as a blank (null values) by default and will vary depending upon the file format downloaded (blank in csv files, NaN in .mat matlab files, etc).

* Parameters (column names) were renamed to comply with BCO-DMO naming conventions. See https://www.bco-dmo.org/page/bco-dmo-data-processing-conventions

* Date format converted to ISO 8601 date format
* Time format converted to ISO 8601 time format
* timestamp with timezone column added with UTC time (ISO_DateTime_UTC column) from local data and times provided as UTC-6 (local time zone specified by data provider).

* columns "prokaryotes" and "viruses" were rounded to two decimal places to match the displayed format shown in Excel.

Problem Description

Occasionally, glass vial seals were not perfect (manufacturing issue).

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Related Publications

Brussaard, C. P. D., Payet, J. P., Winter, C., & Weinbauer, M. G. (2010). Quantification of aquatic viruses by flow cytometry. Manual of Aquatic Viral Ecology, 102–109. https://doi.org/10.4319/mave.2010.978-0-9845591-0-7.102
Methods

Klose, J., Polz, M. F., Wagner, M., Schimak, M. P., Gollner, S., & Bright, M. (2015). Endosymbionts escape dead hydrothermal vent tubeworms to enrich the free-living population. Proceedings of the National Academy of Sciences, 112(36), 11300–11305. https://doi.org/10.1073/pnas.1501160112
Methods

Köstner, N., Scharnreitner, L., Jürgens, K., Labrenz, M., Herndl, G. J., & Winter, C. (2017). High viral abundance as a consequence of low viral decay in the Baltic Sea redoxcline. PLOS ONE, 12(6), e0178467. https://doi.org/10.1371/journal.pone.0178467
Methods

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Parameters

Parameter	Description	Units
dive	Dive number of ROV	unitlesss
sample_id	Identification code of sample as collected by the ROV.	unitlesss
subsample_id	ID of the sample (subsample of ROV sample_id)	unitlesss
habitat	Habitat. vent=vent fluid; sub ven=sampled from within the vent using a hose; non vent=deep sea not within direct influence of vents	unitlesss
latitude	latitude	decimal degrees
longitude	longitude	decimal degrees
depth	meters below surface	meters (m)
temperature	in situ water temperature	unknown
incubated_at_250bar	Whether incubated at 250 bar pressure (yes/no)	unitless
date_local	date (local) of day aboard ship (UTC-6)	unitless
time_local	time (local) of day aboard ship (UTC-6)	unitless
ISO_DateTime_UTC	timestamp with timezone in ISO 8601 format (UTC)	unitless
prokaryotes	prokaryotic abundance	Nx10^4 mL^-1
viruses	viral abundance	Nx10^4 mL^-1

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Instruments

Dataset-specific Instrument Name	FACS Aria III flow cytometer
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	FACS Aria III flow cytometer: The instrument was used to enumerate prokarytes and viruses after staining with the dsDNA-specific stain SYBR Green I.
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Dataset-specific Instrument Name	
Generic Instrument Name	Pressure Vessel
Dataset-specific Description	customized pressure vessel: Pressure vessels are custom items (see Klose et al. 2015; www.pnas.org/cgi/doi/10.1073/pnas.1501160112).
Generic Instrument Description	A pressure vessel is a container designed to hold gases or liquids at a pressure substantially different from the ambient pressure. Construction methods and materials may be chosen to suit the pressure application, and will depend on the size of the vessel, the contents, working pressure, mass constraints, and the number of items required. Examples include glassware, autoclaves, compressed gas cylinders, compressors (including refrigeration), vacuum chambers and custom designed laboratory vessels.

Dataset-specific Instrument Name	Waters 515 HPLC Pump
Generic Instrument Name	Pump
Dataset-specific Description	Waters 515 HPLC Pump: Glass vials were placed into pressure vessels and pressurized using a Waters 515 HPLC Pump up to 250 bar.
Generic Instrument Description	A pump is a device that moves fluids (liquids or gases), or sometimes slurries, by mechanical action. Pumps can be classified into three major groups according to the method they use to move the fluid: direct lift, displacement, and gravity pumps

Dataset-specific Instrument Name	
Generic Instrument Name	ROV SuBastian
Dataset-specific Description	ROV SuBastian and its Multi-Chamber Suction Sampler: Water samples were collected with ROV SuBastian suction sampler at the vent sites.
Generic Instrument Description	ROV SuBastian is operated from the research vessel Falkor and the R/V Falkor(too). The ROV is outfitted with a suite of sensors and scientific equipment to support scientific data and sample collection, as well as interactive research, experimentation, and technology development. More information available at https://schmidtocean.org/technology/robotic-platforms/4500-m-remotely-op...

Dataset-specific Instrument Name	Vivaflow 200 tangential-flow filtration device
Generic Instrument Name	water filtration device
Dataset-specific Description	Vivaflow 200 tangential-flow filtration device: self-contained tangential-flow filter (0.22 µm poresize and 100 kDa molecular weight cut-off) used to obtain prokaryotic concentrates and virus-free ultrafiltrate
Generic Instrument Description	A manufactured device which is used to remove contaminants from water impeding the flow of particles or solutes.

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Project Information

The Underworld of Hydrothermal Vents (Vent Underworld)

Website: https://schmidtocean.org/cruise/underworld-of-hydrothermal-vents/

Coverage: East Pacific Rise

Since the discovery of deep-sea hydrothermal vents in 1977, scientists realize that life exists above and below the seafloor. The extent to which they are interconnected, however, remains poorly understood. We propose to characterize subsurface biosphere diversity, from viruses to animals, at deep-sea hydrothermal vents to elucidate the nature and extent of connectivity between the surficial and subsurface biospheres. We will test the hypotheses that 1) eukaryotic life is also an integral component of the subseafloor biosphere; 2) the subseafloor is inhabited by both cosmopolitan and endemic protists and fungi; and 3) the subseafloor habitats harbor larvae -and perhaps adult life stages- of vent endemic animals. This research will transform our understanding of the ecology/evolution of subseafloor habitats and our thinking about animal recruitment at deep-sea vents. Consistent with SOI's mission, we will expand our knowledge of limits of eukaryotic life. During a three-week cruise with R/V Falkor and ROV SuBastian to "East Pacific Rise" vents at the 9º50'N region, we will use in situ and lab experiments, molecular identification with amplicon sequencing, Illumina NextSeq 500 technology, 2bRAD population genetic analyses, and RT qPCR for functional analyses. Our data management plan includes deposition of metadata/samples to long-term repositories. Outreach includes inquiry-learning telepresence programs for children from USA and Europe.

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Funding

Funding Source	Award
Schmidt Ocean Institute (SOI)	FKt230627

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