Iron concentrations of phage from experiments of iron-labelled E. coli infected with T4 and T5 bacteriophage, 2018 and 2019.

Website: https://www.bco-dmo.org/dataset/757485
Data Type: experimental
Version: 1
Version Date: 2019-02-27

Project
» EAGER: Iron-Virus Interactions in the Ocean (Fe-Virus)

Contributors	Affiliation	Role
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Abstract
This data was collected as part of a study investigating the source of iron to bacteriophage (phage for short, or viruses that infect and kill bacteria) progeny. Evidence from a phage that infects E. coli shows iron incorporated into the tail fiber structure. This study aims at identifying whether the source of the iron is environmental or bacterially derived. E. coli bacterial cultures were grown in minimal media spiked with 10 µM 57FeSO4 then infected with phage T4 or T5. The phages were purified by methods of centrifugation, filtration, density-dependent ultracentrifugation, and dialyzing. The resulting phage fractions were quantified by SYBR epifluorescence microscopy and metal concentrations were measured on an ELEMENT XR ICP-MS.

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Coverage

Temporal Extent: 2018-06-22 - 2019-02-07

Dataset Description

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Methods & Sampling

All materials were cleaned by soaking overnight with heating (1.5% Citrad®, by Decon Labs, Inc) in deionized water, rinsed in RO water, and soaked in 10% TMG HCl (Fisher) in ultrapure water for 30 days, then rinsed with ultrapure water, let dry in an AirClean 400 work station overnight, and double-bagged in polyethylene bags (Mellett et al. 2018). M9 minimal media for bacterial cultures was made using ultrapure water (18.2 MΩ cm), containing final concentrations of 33.7 mM Na2HPO4·2H2O (Sigma-Aldrich ≥99.0% Titration), 22 mM KH2PO4 (ACS Reagent ≥99% purity), 8.56 mM NaCl (Certified ACS ≥99.0% purity), 18.7 mM NH4Cl (Fisher Scientific ≥99.0% purity FCC), 0.1 M MgSO4 (Sigma-Aldrich ≥99.99% trace metal purity), 0.1 M CaCl2 (Alfa Aesar from Fisher Scientific 99.99% metals basis), 1 µg/ml Thiamine HCl (Fisher Scientific 99% purity), and 0.5% Glucose (Fisher Scientific 99% purity) in 1 L of Milli-Q (Kutter and Sulakvelidze 2004, Table 1; see Supplemental Documents below).

M9 minimal media was spiked with 57FeSO4 (final concentration: 10 µM), then filtered through a 0.02 µm Whatman Anotop syringe filter that had been rinsed with Milli-Q; the first few drops of media were discarded (Sample 2). Some un-spiked media was reserved for bacterial pellet rinses (Sample 1). A volume of 20 mL media was inoculated with a frozen culture of the E. coli strain ZK126 (Betty Kutter, Evergreen State College) which was grown over eight generations exclusively on M9 minimal media with 57FeSO4. The culture was placed into a polyethylene bag and vented to avoid contamination, then incubated while shaking overnight at 37 °C to reach late-logarithmic growth. The following morning, three 45 mL aliquots of 10 µM 57FeSO4 spiked and 0.02 µm filtered media samples (one 45 mL blank sample remained un-inoculated with E. coli) were weighed into 250 mL acid-cleaned polycarbonate flasks. Each of the three aliquots were inoculated with 10% of the overnight bacterial culture. The three bacterial cultures as well as the blank samples were placed in a vented polyethylene bag and incubated while shaking at 37 °C. Once the culture reached mid-logarithmic growth, as indicated by an absorbance (OD600) measured on a spectrophotometer between 0.200-0.500 (Figure 1), the cultures were divided into 20 mL aliquots (Figure 2, see Supplemental Documents, below).

To rinse excess 57Fe label from the surface of the bacterial cells, the aliquoted cultures were transferred to a 50 mL falcon tube and centrifuged at a speed of 6500 x g. The bacterial cells pelleted to the base of the tube, and the supernatant was discarded. A volume of 10 mL of fresh Fe-less (not spiked with 57FeSO4) 0.02 µm filtered media was added and vortexed for 1 minute. The rinsed bacterial cells were again centrifuged 6500 x g for 5 minutes to pellet, and the cell rinsing was repeated three times. The final bacterial pellet was re-suspended in 20 mL of Fe-less media. Samples designated A, B, and C were T4 phage lysates, samples D, E, and F remained uninfected by phage and served as bacterial control cells that were burst open by treatment with chloroform, and samples G, H, and I were blanks of Fe-less media. For all rounds (Round 1, 2, 3, 4, and 5 samples A, B, and C (Figure 3) were infected with 5 µl of T4 bacteriophage (Betty Kutter, Evergreen State College) at a titer of 1.3 x 1011 phage/ml. For Rounds 2, 3, 4, and 5, samples M, N, and O were infected with 5 µl of T5 bacteriophage (ATCC® 11303-B5™) at a titer of 8.2 x 1011 phage/ml. Samples D, E, and F (bacterial controls) as well as G, H, and I (blanks) remained uninfected and instead were stored at 4 °C overnight to be used as phage-free lysis controls. Phage-infected cultures A, B, and C were left shaking at 37 °C overnight.

On the next day of the experiment, the uninfected bacterial controls D, E, and F were treated with 20% chloroform (Fisher Scientific, Mobile phase for HPLC applications ≥99.8% purity) and vortexed for one minute to burst open the bacterial cells without viral lysis. The necessity for this viral lysis-free control is to account for colloids from within the bacterial cells that contain 57Fe and would purify with T4 phage. For Round 5 all phages (A, B, C, M, N, O) and blanks (G, H, I) were also treated with 20% chloroform and vortexed for one minute. All the samples were then centrifuged in 50 mL Falcon tubes at a speed of 9500 x g for 5 minutes to pellet the remaining bacterial debris. The supernatant, which contained the T4 phage progeny for samples A, B, and C and the T5 phage progeny for samples M, N, and O, was then filtered using a 0.22 µm Sterivex PVDF syringe filter (EMD Millipore), with a Milli-Q pre-rinse and the first few drops of sample discarded. The filtrate was the fraction containing phage and any soluble or colloidal 57Fe within the dissolved size fraction (<0.22 µm). The subsequent filtration of a subsample of the dissolved fraction through a 0.02 µm Whatman Anotop syringe filter (with a Milli-Q pre-rinse and the first few drops of sample discarded) was collected for the soluble fraction (<0.02 µm). The difference between the dissolved and the soluble fractions are used to calculate iron within the colloidal fraction (0.2 µm-0.02 µm).

The phage within the dissolved fraction were further purified using a sucrose cushion, which is a density-dependent technique used to concentrate and purify phage by precipitating viral particles below a dense layer of sucrose (Hurwitz et al. 2013). To do so, a 2.5 ml layer of 38% sucrose (Fisher Scientific) in SM buffer (100 mM NaCl, 8 mM MgSO4, 50 mM Tris-HCl in Milli-Q, pH 7.5 and 0.02 µm filtered) was added to the bottom of the ultracentrifuge tube (Beckman Coulter), followed by 1 ml of sample and 10.5 ml of SM Buffer by carefully tilting the tube so as not to disturb the dense sucrose layer. The samples were spun in a Beckman Coulter SW40Ti swinging bucket ultracentrifuge, for 3 hours and 15 minutes at 175,000 x g (37,200 rpm). The sucrose and

SM buffer layers were then discarded, and the tubes were dried in a laminar flow clean hood (Air Clean) for 20 minutes. The pelleted phages from samples A-C and M-O, including any potential bacterial colloids of the same density as the phage as accounted for in samples D-F, were then resuspended in 1 mL of SM Buffer.

All the samples were dialyzed using Float-A-Lyzer 100 kDa dialysis devices (Fisher Scientific) in 1 L of dialysis buffer (10 mM NaCl, 50 mM Tris-Cl pH 8.0, 10 mM MgCl2) for a total of 6 buffer changes over 4 days. Bacterial and viral counts were performed throughout for samples using SYBR nucleic acid stain under epifluorescence microscopy (Noble & Furman 1998).

Metal concentrations were quantified using Element XR ICP-MS (Thermo) after 50-fold dilution with 5% nitric (Fisher Scientific, Optima) containing 10 ppb rhodium as internal standard, and using external standard calibration curves. Blank values after rhodium correction are listed in Table 2 (see Supplemental Documents below).

Data Processing Description

BCO-DMO Processing:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions (replaced spaces with underscores, removed puncutation, placed letters before numbers)
- reformatted year from mdyy to yyyy-mm-dd
- replaced blanks, na, and nda with "nd" for "no data"
- removed trailing comma and space from 'Fe-less (un-spiked) 0.02 um filtered M9 Minimal Media, ' in DESCRIPTION
- replaced mu symbol with u in data (DESCRIPTION column) (311 occurrences)

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Data Files

File	
Fe_label_Ecoli_T4_T5.csv	(Comma Separated Values (.csv), 126.12 KB) MD5:8a357014359dff88917fae1f2a8291b7
Primary data file for dataset ID 757485	

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Supplemental Files

File
Figure 1: Growth curve for E. coli ZK126 filename: Figure1.PNG (Portable Network Graphics (.png), 34.65 KB) MD5:20bd130515d75a048de0f4a5d71ddc3c Growth curve for E. coli ZK126 measured on a spectrophotometer. Mid-logarithmic growth (indicated by shaded area) was an OD600= 0.200-0.500.
Figure 2: Schematic of E. coli cell preparation filename: Figure2.PNG (Portable Network Graphics (.png), 129.69 KB) MD5:31844f4dbd1b22673e4254ab3606cbbb Figure 2: Schematic of E. coli cell preparation
Figure 3: Schematic of 57Fe-labeling experiment for E. coli phage T4 & T5 filename: Figure3.PNG (Plain Text, 151.65 KB) MD5:6d9e34c0779ad1bf2177a5369ac09a05 Figure 3: Flowchart of the Tracing 57Fe to E. coli phages T4 & T5 Experimental method. First, cell cultures were grown and aliquoted into triplicate samples of each treatment: T4 infected (A, B, C), T5 infected (M, N, O), Bacterial Control (D, E, F), and Blank Control (G, H, I). Cell cultures were rinsed to remove excess 57Fe, then lysed by phage T4 infection, phage T5 infection, or chloroform for the bacterial lysis control. The lysates are then purified for the phage by centrifugation, filtration, sucrose ultracentrifugation, and dialysis.
Table 1: M9 Minimal Media composition filename: Table1.PNG (Portable Network Graphics (.png), 72.58 KB) MD5:4c3b736bea304289bc90aeeecff73aab Table 1: M9 minimal media composition from Kutter and Sulakvelidze (2004). A 1 L stock of 20X M9 Salts and 0.5 L of all stock solutions were weighed separately in acid-cleaned Nalgene fluorinated HDPE bottles and brought to desired concentrations using Milli-Q water (18.2 MΩ cm). A 1 L volume of liquid culture was prepared by adding all sterile stock solution components to 912.5 mL of Milli-Q water in an acid-cleaned HDPE bottle on a balance. *FeSO4 added as 57FeSO4 spike separately after all other components combined. Completed M9 minimal media was 0.02 µm filtered prior to use.
Table 2: Blank values after Rhodium correction as measured on Element XR ICP-MS filename: Table2.PNG (Portable Network Graphics (.png), 33.46 KB) MD5:c04badc61edad91d5a3846e2d5224039 Table 2: Analytical blank values for 56Fe and 57Fe after rhodium correction as measured on ELEMENT XR ICP-MS. “Expt” refers to experimental and analytical dataset; e.g. Expt 2.1 is experiment 2, first analytical dataset, while Expt 2.2 is experiment 2, second analytical dataset. The number of blank measurements used to calculate the average and standard deviation for each round is “n”.

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Related Publications

Gledhill, M. (2012). The organic complexation of iron in the marine environment: a review. Frontiers in Microbiology, 3. doi:10.3389/fmicb.2012.00069
Methods

Hurwitz, B. L., Deng, L., Poulos, B. T., & Sullivan, M. B. (2012). Evaluation of methods to concentrate and purify ocean virus communities through comparative, replicated metagenomics. Environmental Microbiology, 15(5), 1428–1440. doi:10.1111/j.1462-2920.2012.02836.x
Methods

Kutter, E., & Sulakvelidze, A. (2004). Bacteriophages: biology and applications. CRC Press. https://isbnsearch.org/isbn/9780203491751
Methods

Mellett, T., Brown, M.T., Chappell, P.D., Duckham, C., Fitzsimmons, J.N., Till, C.P., Sherrell, R.M., Maldonado, M.T., and Buck, K.N. (2017). The biogeochemical cycling of iron, copper, nickel, cadmium, manganese, cobalt, lead, and scandium in a California Current experimental study. Limnology and Oceanography, 63(S1), S425–S447. doi:10.1002/lno.10751
Methods

Noble, R., & Fuhrman, J. (1998). Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquatic Microbial Ecology, 14, 113–118. doi:10.3354/ame014113
Methods

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Parameters

Parameter	Description	Units
date_utc	UTC date when media was filtered and the experiment initiated	unitless
expt_round	round of experiment and sample analysis on the XR ICP-MS	unitless
sample	sample identifier: 1 for Fe-less (un-spiked) 0.02 um filtered M9 Minimal Media; 2 for 10 uM 57FeSO4 spiked 0.02 um filtered M9 minimal media; or 3 for 0.02 um filtered SM Buffer. Each stage of the experiment was designated sequentially as follows: 20's for pelleted E. coli cultures grown to mid-logarithmic phase and rinsed three times in Fe-less media (no 57Fe spike) by centrifugation and re-suspension; 30's for supernatant of pelleted bacteria (20's); 40's for unfiltered centrifuged supernatant of bacterial culture infected with phage overnight; 50's for 0.22 um filtered dissolved fraction; 60's for 0.02 um filtered soluble fraction; 70's for bacterial samples treated with chloroform; 80's for the supernatant (sample layer) above the sucrose cushion following ultracentrifugation; 90's for sucrose cushion pellet re-suspended in 0.02 um filtered SM buffer; DIA for dialyzed samples post-dialysis over 6 buffer tank changes; and B for dialysis buffer: B1 pre-dialysis; B1-2 post-initial dialysis; B3-2 post-third dialysis buffer tank change; and B6-2 post- final dialysis buffer tank change. Samples were treated in triplicate: A; B; C for E. coli phage T4 samples; D; E; F for chloroform-lysed bacterial control samples; and G; H; I for Blanks; and M; N; O for E. coli phage T5 samples.	unitless
description	description of phage purification step and type of sample	unitless
Fe_56_nM	Concentration of 56Fe as determined by XR ICP-MS	nanoMolar
Fe_57_nM	Concentration of 57Fe as determined by XR ICP-MS	nanoMolar
volume	sample volume	liters
Fe_56_nmol	56Fe concentration as determined by XR ICP-MS	nanomoles
Fe_57_nmol	57Fe concentration as determined by XR ICP-MS	nanomoles
Cu_63_nmol	63Cu concentration as determined by XR ICP-MS	nanomoles
Zn_66_nmol	66Zn concentration as determined by XR ICP-MS	nanomoles
Ni_60_nmol	60Ni concentration as determined by XR ICP-MS	nanomoles

Pb_208_nmol	208Pb concentration as determined by XR ICP-MS	nanomoles
bact_cells_ml	SYBR epifluorescence bacterial counts	cells/milliliter
phage_VPL_ml	phage cell concentration	virus-like particles/milliliter
Fe_57_atoms_per_phage	57Fe content measured by XR ICP- MS; converted to moles; multiplied by Avogadro's constant (6.022 x 1023 atoms/mol); and divided by number of phage in the sample	atoms
notes_expt	comments about samples	unitless

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Instruments

Dataset-specific Instrument Name	Beckman Coulter SW40Ti swinging bucket ultracentrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Used to concentrate cells and separate bacteria from phage.
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	ELEMENT XR High Resolution Inductively Coupled Plasma Mass Spectrometer
Generic Instrument Name	Inductively Coupled Plasma Mass Spectrometer
Dataset-specific Description	Used to measure metal concentrations.
Generic Instrument Description	An ICP Mass Spec is an instrument that passes nebulized samples into an inductively-coupled gas plasma (8-10000 K) where they are atomized and ionized. Ions of specific mass-to-charge ratios are quantified in a quadrupole mass spectrometer.

Dataset-specific Instrument Name	
Generic Instrument Name	Spectrophotometer
Dataset-specific Description	Used to measure bacterial cell concentrations.
Generic Instrument Description	An instrument used to measure the relative absorption of electromagnetic radiation of different wavelengths in the near infra-red, visible and ultraviolet wavebands by samples.

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Project Information

EAGER: Iron-Virus Interactions in the Ocean (Fe-Virus)

Iron is an essential micronutrient for phytoplankton that is required for photosynthesis and respiration. Insufficient iron has been shown to limit phytoplankton growth in large regions of the surface ocean, and correspondingly, iron cycling is directly linked to carbon cycling in much of the marine environment. Nearly all iron in seawater (>99%) exists as complexes with organic molecules called ligands, which govern the concentration of iron dissolved in the water and the bioavailability of that iron to phytoplankton. However, despite the importance of iron-binding organic ligands, their sources and identities are largely unknown. Viruses, the majority of which are phages (viruses that infect bacteria), are extremely abundant in seawater and are in the same size fraction as dissolved iron. Recent evidence that non-marine phages contain iron as part of their structures has led to the proposal that marine phages may represent a previously overlooked class of organic iron-binding ligands. This project is determining the contribution of marine phages to dissolved iron pools and culture phage-host systems in the laboratory to determine if phages utilize bacterial iron-uptake receptors for infection in the manner of a Trojan horse. As the first study to examine the biogeochemical impact of trace elements contained within the structure of highly abundant marine phage particles, successful completion of the proposed research will be transformative for biological and chemical oceanography and have far-reaching implications for other fields, including human health where iron availability plays an important role in microbial pathogenesis. This project contributes to the multidisciplinary training of a graduate student and postdoctoral researcher. Research results will be disseminated through scientific publications and presentations, and the public will be educated about linkages between viruses and ocean chemistry via a hands-on exhibit for the annual St. Petersburg Science Festival.

Building upon evidence from non-marine model systems demonstrating the presence of iron ions in phage tail proteins and phage utilization of cell surface receptors for siderophore-bound iron, this project combines field and laboratory-based experiments to test the following three hypotheses regarding iron-virus interactions in the oceans: (1) Iron incorporated into phage tails originates from bacterial cell reserves, reducing the amount of iron available for remineralization upon lysis; (2) Phages constitute important iron-binding ligands in the oceans, accounting for a substantial portion of organically complexed colloidal dissolved iron; (3) Marine phages compete with siderophore-bound iron for uptake receptors on the bacterial cell surface and use iron in their tails as a Trojan horse for infection. Initial calculations predict that phages could account for up to 70% of the colloidal fraction of organically complexed dissolved iron in the surface ocean; therefore, this project is critical for advancing knowledge of trace-metal cycling as well as phage-host interactions. Additionally, if a portion of the cellular iron thought to be released from bacterial cells for remineralization following lysis is already incorporated into phage tails, then these findings will have significant implications for oceanic biogeochemical models. Through a combination of laboratory-based culture experiments and field sample measurements, this project could reveal the identity of a ubiquitous component of colloidal organic iron-binding ligands, modify the estimates of iron concentrations and species released through viral lysis, and potentially identify a novel receptor type for marine phage that may compete with the acquisition of siderophore-bound iron by host bacteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1722761

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