Chemotaxis of Vibrio alginolyticus towards live phage-infected/control Synechococcus cells (VIC project)

Website: https://www.bco-dmo.org/dataset/913619
Data Type: model results, experimental
Version: 1
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Project
» Collaborative Research: Viral induced chemotaxis mediating cross-trophic microbial interactions and carbon flux (VIC)

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Abstract
This data set summarises the chemotactic response of model heterotrophic bacteria, Vibrio alginolyticus towards phage-infected cyanobacteria Synechococcus cells/exudates respectively. Phage treatments are fully described in: https://doi.org/10.1038/s43705-022-00169-6. Six microfluidic experiments were conducted each on different days: three with phage infection and three control, uninfected experiments. Both uninfected and phage-infected experiments were performed identically, with the substitution of phage addition for an equivalent volume of SN media in the uninfected experiments. All infection experiments were performed within a week using the same phage stock, with Synechococcus WH8102 as the host. Non-motile host cells were loaded into a microfluidic chamber with the model heterotrophic bacteria V. alginolyticus. Analysis of the cell spatial distribution over time revealed a strong sustained accumulation of chemotactic bacteria towards phage-infected cyanobacteria, and no measurable accumulation in the control condition. This provides the first direct experimental evidence of chemotaxis of heterotrophic bacteria towards cyanobacteria.

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Coverage

Temporal Extent: 2021-10 - 2023-04

Dataset Description

Data Representing the Net Chemotactic Movement of *V. alginolyticus* (see the primary data file

associated with this dataset)

These data describe the net chemotactic movement of *V. alginolyticus* in response to axenic *Synechococcus* WH8102 cells under two conditions:

1) Uninfected *Synechococcus* WH8102 cells (UI)

2) *Synechococcus* WH8102 cells infected with the cyanophage S-SSM5 (INF)

V. alginolyticus chemotaxis was evaluated by microscopic imaging in phase contrast using a Zeiss AxioObserver inverted microscope (10x, 0.25NA objective) paired with a CMOS camera (Grasshopper 3, Teledyne FLIR). Images were collected at a frame rate of 1 frame per second for 3,600 seconds. All data were processed using custom MATLAB scripts (version 2021b). The processing summary that results in this data table is:

1) Locate particles using a bandpass filter/peak finding algorithm (available: https://site.physics.georgetown.edu/matlab/)

2) Bin particles according to x-position to examine distribution across the field of view

For each condition (UI and INF), three biological replicates (1,2,3) were conducted using cells sampled from independent cultures of *Synechococcus* WH8102 at two timepoints: 4- and 8-hours post-infection (HPI). For a total of 12 chemotaxis experiments (these 12 experiments are represented in the 12 tabs of the raw position file V.alg_WH8102cells_PosData_JM.xlsx).

Chemotactic responses (accumulation index) are described by the values given in this dataset for each replicate experiment. The accumulation index is calculated based on bacterial cell positions in every image over the chemotaxis time series (3,600s) and is defined by the equation described in the supplemental file *V.alg_WH8102_accumulation_index_details.pdf*.

The accumulation index describes directional chemotaxis of the *V. alginolyticus* population in response to intact *Synechococcus* WH8102 cells. The analysis found that *V. alginolyticus* exhibited a stronger chemotactic bias towards *Synechococcus* WH8102 when they were infected with the cyanophage S-SSM5 which provides support to the overall hypothesis of the project that viral infection of picophytoplankton may result in attraction of heterotrophic microbes thereby altering carbon flux.

Raw Data Representation of the Chemotactic Movement of *V. alginolyticus* (see supplemental file V.alg_WH8102cells_PosData_JM.xlsx)

V. alginolyticus chemotaxis was evaluated by microscopic imaging in phase contrast using a Zeiss AxioObserver inverted microscope (10x, 0.25NA objective) paired with a CMOS camera (Grasshopper 3, Teledyne FLIR). Images were collected at a frame rate of 1 frame per second for 3,600 seconds. All data were processed using custom MATLAB scripts (version 2021b). The processing summary is:

1) Locate particles using a bandpass filter/peak finding algorithm (available: https://site.physics.georgetown.edu/matlab/).

2) Bin particles according to x-position to examine distribution across the field of view.

Within each spreadsheet/tab in the supplemental file *V.alg_WH8102cells_PosData_JM.xlsx*, the first column indicates the timepoint during the chemotaxis experiment (0 to 60 minutes) at which the counts were made. These positional data were collected from each microscopy image taken at a frame rate of 1 frame per second for 3,600 seconds (60 minutes) and were used to calculate the accumulation index as described above. The first column in each spreadsheet denotes the chemotaxis timepoint. Each subsequent column represents a physical width within the microfluidic channel and are used to bin the cell count data. Each microfluidic channel is ~1000µm wide and this distance is divided into 30 bins. The midpoint, or center, of each bin is given in the first row of each column ('x =').

As stated, these data were used to calculate the accumulation index and inform directional motility bias of *V. alginolyticus* in response to *Synechococcus* cells under uninfected control and cyanophage-infected conditions.

Methods & Sampling

Experimental Culture Details:

Vibrio alginolyticus (YM4; wild-type) from -80℃ stock were grown overnight in Marine 2216 media (Difco) by incubating at 30℃ and shaking at 600 revolutions per minute (RPM). The overnight culture was diluted 100-fold into fresh pre-warmed 2216 media and grown for three hours (30℃, shaking at 600 RPM) to O.D. ≈ 0.2. 2 ml of culture was then washed and resuspended (1,500 RCF for 5 min) in 0.5ml of artificial seawater (ASW).

Artificial seawater (ASW) was prepared following the NCMA ESAW Medium recipe, which was adapted from Harrison et al.and modified by Berges, and filtered through a 0.2um filter immediately prior to use.

Axenic Synechococcus WH8102 (CCMP 490 2370) were grown in SN media, prepared with ASW, in sterile 40 ml polystyrene culture flasks at 22°C on a 14 h : 10 h light-dark cycle at 50 µmol photons m−2 s −1. Culture growth was tracked using a SpectraMax ID3 plate reader (Molecular Devices) and cell counts were measured using a CytoFLEX flow cytometer (Beckman Coulter).

Microfluidic Device Details:

A simple three-inlet gradient generation microfluidic device was used to produce the chemical gradients. Microfluidic devices were made using standard soft lithography techniques. Polydimethylsiloxane (Dow Corning SYLGARD 184) channels were cast on photoresist (Microchem) molds fabricated via photolithography and plasma bonded to standard glass slides. Gradient generation channels were designed with three inlets (width 0.5mm) carrying the chemostimulus solution, cell suspension and ASW media, respectively. Prior to use, the chambers were pretreated with a 0.5% BSA solution to mitigate cell adhesion. The three solutions were flow stratified for a minimum of 2min using a syringe pump (Harvard Apparatus), whereby flow rates were adjusted to maintain a 4:1:4 ratio of the stream widths. Upon halting the flow, a monotonic chemotaxis profile was established through diffusion. A chemostimulus gradient develops in the channel via diffusion, and the chemotactic response of the cell population was observed over time. Imaging was performed with phase-contrast microscopy (4×; Zeiss AxioObserver) at 1 fps over the course of ~10 min using a CMOS camera (Grasshopper S, Teledyne FLIR). An example of the gradient generator used can be found in: https://doi.org/10.7554/eLife.85348

Experiment Details:

Six microfluidic experiments were conducted each on different days: three with phage infection and three control, uninfected experiments. Both uninfected and phage-infected experiments were performed identically, with the substitution of phage addition for an equivalent volume of SN media in the uninfected experiments. All infection experiments were performed within a week using the same phage stock, with the infectious phage titer of the stock determined to be 1.4x10^8 pfu/ml via plaque assay method with Synechococcus WH8102 as the host. One day prior to experiments, exponential phase Synechococcus cultures were diluted with fresh SN media to a cell concentration of 7-9x10^5 cells/ml.

On the day of experiments, at the onset of the light cycle, culture concentrations were adjusted to 1x10^6 cells/ml as necessary with fresh SN media. A volume of phage stock (or SN media for uninfected controls) was added to achieve a target multiplicity of infection (MOI) of 3. Cultures were then mixed for one min every five min for 45min in 50ml polypropylene conical tubes, then centrifuged at 2,000 RCF for 15~min at 20C. Supernatants were decanted and cells were resuspended in an equal volume of fresh ASW and transferred to sterile 50ml polystyrene tubes. At approximately two and six hours post infections(T=3 and T=7hpi), live cell concentration was assessed by flow cytometry and 10ml of culture was collected for chemotaxis assays. Sub-samples were fixed in 0.5% glutaraldehyde for cell and phage quantification. Chemotaxis assays were carried out as described above, with the live Synechoccocus cells in place of the chemoattractant solution.

Flow Cytometry Data Details

This data is captured in Supplemental File fcm.countdata_synwh8102_s-ssm5.csv.

Flow cytometry data quantifying *Synechococcus* cell concentrations and S-SSM5 cyanophage particle concentrations in each biological replicate culture. Samples were collected from cultures, fixed in 0.5% glutaraldehyde, and flash frozen in LN2 before being stored at -80°C. Samples were thawed on ice prior to being quantified.

The Sample Name column indicates the time at which samples were collected (e.g., 1hpi = 1-hour post-infection). Samples named ‘SN’, ‘SW’, or ‘TE Blank’ correspond to blanks (negative controls) performed with

SN media, 0.2µm filtered natural seawater, and TE buffer. The Sample Type column indicates whether *Synechococcus* cells or S-SSM5 cyanophage were quantified. *Synechococcus* were detected by chlorophyll-a autofluorescence using a 488nm laser with emission detected using a 690/50nm filter. A violet laser (405nm) was used to detect side scatter of particles (405/10nm emission filter). Cyanophages were stained with SYBR Green I which was also excited using a 488nm laser with emission detected using a 525/40nm filter. Size of particles was determined using the same violet side scatter parameters as above. The condition and replicate number are both indicated in the Treatment column.

Raw counts are given in the Count column with blank subtracted counts shown in the Blank Adjusted Count column. The acquisition time, volume of sample run, and the calculated concentration of either Synechococcus or cyanophage are given in the following columns, respectively. 'NA' indicates that data were not acquired for those specific samples. Particle concentrations were determined by calculating the number of gated particles per volume of sample analyzed, then accounting for the sample dilution.

The flow cytometry data support the chemotaxis experiments by providing data to show that uninfected *Synechococcus* cultures maintained their cell concentrations over the course of each experiment and that no cyanophage were detected in these cultures. Similarly, cyanophage-infected *Synechococcus* cultures displayed cell loss characteristic of viral lysis and increases in cyanophage concentration which combined are indicative of a productive infection. These data support that the observed directional motility biases (or lack thereof) in chemotaxis of *V. alginolyticus* were indeed in response to either uninfected and cyanophage-infected *Synechococcus*.

Data Processing Description

Data processing step summary:

1. Locate particles using a bandpass filter/peak finding algorithm (available: https://site.physics.georgetown.edu/matlab/)
2. Bin particles according to x-position to examine distribution across the field of view

All data were processed using custom MATLAB scripts (version 2021b). These MATLAB scripts are captured in the Supplemental File, Filtrate_Chemotaxis_Analysis.zip.

BCO-DMO Curation Notes

Spaces in column names have been replaced with underscores ("_").

All chemotaxis data values have been rounded to maximum precision (14).

Time values have been rounded to a precision of 4.

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Data Files

File	
913619_v1_V_alginolyticus_WH8102_cells_betatable.csv	(Comma Separated Values (.csv), 1.20 MB) MD5:166b6768cce98bcdead915cc357a62e7
Primary data file for dataset ID 913619, version 1	

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Supplemental Files

File
Chemotaxis Analysis Scripts filename: Filtrate_Chemotaxis_Analysis.zip (MATLAB Programming Script (.m), 8.95 KB) MD5:c95a81fdfa46f53b94038aeb7c2f57cd Each file contains more description of usage, input, and output in the header of each m-file. Below is a brief description of the purpose of each function. --- "Chemotaxis_Analysis.m" contains setup and parameters, you should only need to change numbers/details here. --- "cntrd.m" contains function: cntrd(im,mx,sz,interactive) PURPOSE: calculates the centroid of bright spots to sub-pixel accuracy. Inspired by Grier & Crocker's feature for IDL, but greatly simplified and optimized for matlab. --- pkfnd.m contains function: pkfnd(im,th,sz) PURPOSE: finds local maxima in an image to pixel level accuracy. This provides a rough guess of particle centers to be used by cntrd.m. Inspired by the lmx subroutine of Grier and Crocker's feature.pro --- "bpass.m" contains function: bpass(image_array,lnoise,lobject,threshold) PURPOSE: Implements a real-space bandpass filter that suppresses pixel noise and long-wavelength image variations while retaining information of a characteristic size. ---
fcm.countdata_synwh8102_s-ssm5.csv (Comma Separated Values (.csv), 21.60 KB) MD5:a8c60696ef53e8c846eb9b2548218f1a
V.alg_WH8102_accumulation_index_details.pdf (Portable Document Format (.pdf), 121.65 KB) MD5:1ddb4d1f5ffa8963aef942c5b53fdf5b Accumulation index (chemotactic response) equation and details.
V.alg_WH8102cells_PosData_JM.xlsx (Microsoft Excel, 6.30 MB) MD5:76310db1051ade037facecf667918243 Each spreadsheet in this dataset contains the raw count data of V. alginolyticus cells inside the microfluidic device, with respect to their position in the channel, acquired from each chemotaxis experiment. As noted by their titles, spreadsheets are separated by the condition, replicate number, and timepoint at which Synechococcus cells were collected from cultures.

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Related Publications

Henshaw, R. J., Moon, J., Stehnach, M. R., Bowen, B. P., Kosina, S. M., Northen, T. R., Guasto, J. S., & Floge, S. A. (2023). Early viral infection of cyanobacteria drives bacterial chemotaxis in the oceans. https://doi.org/10.1101/2023.10.24.563588
Results

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Related Datasets

IsRelatedTo

Henshaw, R. J. (2023) **Chemotaxis of Vibrio alginolyticus to control/phage-infected Synechococcus exudates from 2020-2021 (VIC project).** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-12-19 doi:10.26008/1912/bco-dmo.885574.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Time	Time point when count of cells at a given location was taken.	minutes
INF1_4HPI	Net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 during replicate 1 at 4 hours past the infection time.	unitless
INF2_4HPI	Net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 during replicate 2 at 4 hours past the infection time.	unitless
INF3_4HPI	Net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 during replicate 3 at 4 hours past the infection time.	unitless
INF_4HPI_AVG	Average net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 across replicates at 4 hours past the infection time.	unitless
INF_4HPI_SEM	Standard error of net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 across replicates at 4 hours past the infection time.	unitless
INF1_8HPI	Net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 during replicate 1 at 8 hours past the infection time.	unitless
INF2_8HPI	Net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 during replicate 2 at 8 hours past the infection time.	unitless
INF3_8HPI	Net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 during replicate 3 at 8 hours past the infection time.	unitless
INF_8HPI_AVG	Average net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 across replicates at 8 hours past the infection time.	unitless
INF_8HPI_SEM	Standard error of net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 across replicates at 8 hours past the infection time.	unitless
UI1_4HPI	Net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells during replicate 1 at 4 hours past the infection time.	unitless

UI2_4HPI	Net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells during replicate 2 at 4 hours past the infection time.	unitless
UI3_4HPI	Net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells during replicate 3 at 4 hours past the infection time.	unitless
UI_4HPI_AVG	Average net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 across replicates at 4 hours past the infection time.	unitless
UI_4HPI_SEM	Standard error of net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells across replicates at 4 hours past the infection time.	unitless
UI1_8HPI	Net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells during replicate 1 at 8 hours past the infection time.	unitless
UI2_8HPI	Net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells during replicate 2 at 8 hours past the infection time.	unitless
UI3_8HPI	Net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells during replicate 3 at 8 hours past the infection time.	unitless
UI_8HPI_AVG	Average net chemotactic movement value of V. alginolytcus in response to Synechococcus WH8102 cells infected with the cyanophage S-SSM5 across replicates at 8 hours past the infection time.	unitless
UI_8HPI_SEM	Standard error of net chemotactic movement value of V. alginolytcus in response to uninfected Synechococcus WH8102 cells across replicates at 8 hours past the infection time.	unitless

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Instruments

Dataset-specific Instrument Name	CMOS camera (Grasshopper, Teledyne FLIR)
Generic Instrument Name	Camera
Dataset-specific Description	Imaging was performed with phase-contrast microscopy (4×; Zeiss AxioObserver) at 1 fps over the course of ~10 min using a CMOS camera (Grasshopper S, Teledyne FLIR).
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	CytoFLEX flow cytometer (Beckman Coulter)
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Culture growth was tracked using a SpectraMax ID3 plate reader (Molecular Devices) and cell counts were measured using a CytoFLEX flow cytometer (Beckman Coulter).
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. Description from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm

Dataset-specific Instrument Name	Zeiss AxioObserver Inverted microscope
Generic Instrument Name	Inverted Microscope
Dataset-specific Description	Imaging was performed with phase-contrast microscopy (4×; Zeiss AxioObserver) at 1 fps over the course of ~10 min using a CMOS camera (Grasshopper S, Teledyne FLIR).
Generic Instrument Description	An inverted microscope is a microscope with its light source and condenser on the top, above the stage pointing down, while the objectives and turret are below the stage pointing up. It was invented in 1850 by J. Lawrence Smith, a faculty member of Tulane University (then named the Medical College of Louisiana). Inverted microscopes are useful for observing living cells or organisms at the bottom of a large container (e.g. a tissue culture flask) under more natural conditions than on a glass slide, as is the case with a conventional microscope. Inverted microscopes are also used in micromanipulation applications where space above the specimen is required for manipulator mechanisms and the microtools they hold, and in metallurgical applications where polished samples can be placed on top of the stage and viewed from underneath using reflecting objectives. The stage on an inverted microscope is usually fixed, and focus is adjusted by moving the objective lens along a vertical axis to bring it closer to or further from the specimen. The focus mechanism typically has a dual concentric knob for coarse and fine adjustment. Depending on the size of the microscope, four to six objective lenses of different magnifications may be fitted to a rotating turret known as a nosepiece. These microscopes may also be fitted with accessories for fitting still and video cameras, fluorescence illumination, confocal scanning and many other applications.

Dataset-specific Instrument Name	SpectraMax ID3 plate reader (Molecular Devices)
Generic Instrument Name	plate reader
Dataset-specific Description	Culture growth was tracked using a SpectraMax ID3 plate reader (Molecular Devices) and cell counts were measured using a CytoFLEX flow cytometer (Beckman Coulter).
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Harvard Apparatus syringe pump
Generic Instrument Name	Pump
Dataset-specific Description	The three solutions were flow stratified for a minimum of 2min using a syringe pump (Harvard Apparatus), whereby flow rates were adjusted to maintain a 4:1:4 ratio of the stream widths. Upon halting the flow, a monotonic chemotaxis profile was established through diffusion.
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

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Project Information

Collaborative Research: Viral induced chemotaxis mediating cross-trophic microbial interactions and carbon flux (VIC)

Coverage: Culture-based work

NSF Award Abstract:
Drifting photosynthetic microbes in surface ocean waters carry out nearly half of global carbon (C) fixation, both supporting the marine food web and reducing atmospheric carbon dioxide (CO_2) levels. The fate of C in ocean ecosystems is controlled by myriad individual interactions within a highly interconnected planktonic food web, the sheer complexity of which has hindered predictive understanding of global C cycling. Chemical cues govern microbial interactions, and during infection, marine viruses manipulate the metabolism of phytoplankton and bacteria, facilitating the release of dissolved organic matter from infected cells. This research aims to determine how viral metabolic reprogramming of and organic matter release from intact, infected phytoplankton influences microbial interactions and C cycling. The interdisciplinary, collaborative nature of the

project will enable direct training of two postdoctoral researchers, one graduate student, and undergraduate students in viral ecology, microfluidics, and metabolomics. An educational outreach program that engages middle school students in hands-on, high speed imaging of microbes will be expanded, and the project will culminate in a three-day workshop to advance the application of microfluidic devices and mass spectrometry analyses in microbial ecology.

The overarching hypothesis behind this research is that viral infection alters the chemical landscape of intact, infected picophytoplankton cells, attracting neighboring chemotactic bacteria and protistan zooplankton, and altering C flux pathways. To test this idea, a series of linked multi-scale laboratory-based experiments will be run to 1) Characterize the response of diverse model marine microbes to dissolved organic matter (DOM) released from intact, virus-infected picophytoplankton using microfluidics-based chemotaxis assays, 2) Identify key viral-derived DOM compounds eliciting chemotactic responses using stable isotope labeling, metabolomics analyses, and chemotaxis assays, and 3) Quantify micron-scale cross-trophic encounter dynamics and evaluate their impact on bulk-scale C cycling using liter-scale measurements of C dynamics linked to high spatiotemporal resolution live imaging of microbial food webs. The ultimate goal of the project is to develop a mechanistic understanding of the role of intact, virus-infected cells in oceanic C cycling.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829905
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829827

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