

## Virus-Host pairing based on EAF

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## Abstract

Viruses impact aquatic biogeochemistry via host cell lysis and rewiring host metabolic processes. However, their functional role in aquatic dark carbon cycling is mostly unexplored. Here, we established a method to identify active viruses targeting primary producers (chemoautotrophs), elucidate viral-mediated processes impacting dark carbon cycling, and approximate the rate of viral-induced carbon turnover using  $^{13}\text{C}$ -DNA stable isotopic probing combined with metagenomics. Water samples were taken 10 meters below the surface of Siders Pond, a salt-stratified meromictic pond in Falmouth, Massachusetts. At 10 meters of depth the water is anoxic and sulfidic, making Siders Pond ideal for studying microbial interactions within an euxinic environment. Parallel incubations were conducted on the samples with equimolar levels of  $^{12}\text{C}$ - and  $^{13}\text{C}$ -DIC. Treatments were then filtered through a  $0.2\mu\text{m}$  filter (cell-enriched retentate) followed by a  $0.02\mu\text{m}$  filter (viral-enriched retentate) and extracted for metagenomic analysis. Calculating the difference in DNA density between the  $^{12}\text{C}$ - and  $^{13}\text{C}$ -fractions, we were able to identify the metagenomic assembled genomes of active chemoautotrophic community members as well as the viral sequences of viral populations targeting them. Most of the viral sequences belonged to the class Caudoviricetes with  $> 75\%$  of sequences being novel at the Order and Family levels. Further, viral populations were found to contain auxiliary metabolic genes associated with cofactors/vitamins, amino acids, carbohydrates, and energy metabolisms. Our results demonstrate the important roles viruses play in dark carbon cycling and the potential for this methodology to analyze host-viral interactions and biogeochemical cycling within other aquatic ecosystems.

*Keywords:* metagenome, bioinformatics, prophage, phage, prokaryotic host

Word count: X

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## Methods

We report how we determined our sample size, all data exclusions (if any), all manipulations, and all measures in the study.

## Participants

## Material

## Procedure

## Data analysis

We used R (Version 4.3.2; R Core Team, 2023) and the R-packages *papaja* (Version 0.1.2; Aust & Barth, 2023), and *tinylabels* (Version 0.2.4; Barth, 2023) for all our analyses.

## Results

## Discussion

## References

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