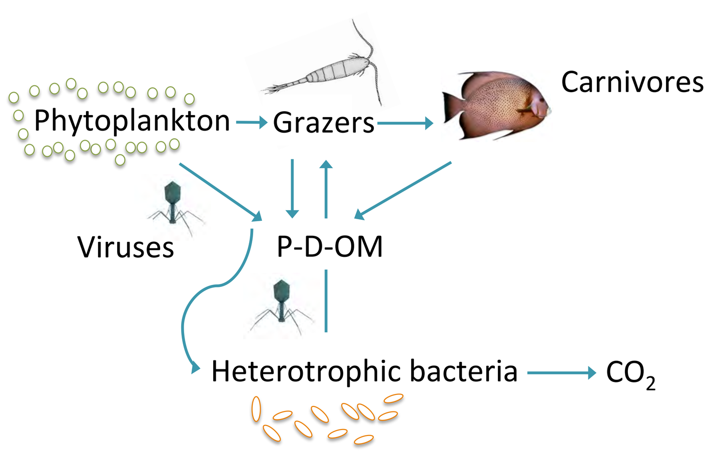
Marine viruses stimulate carbon flux to lower and higher trophic levels

Sheri A. Floge[1], Jesica D. Waller[2], David M. Fields[3] and Matthew B. Sullivan[4]

[1] Wake Forest University, [2]Maine Department of Marine Resources, [3]Bigelow Laboratory for Ocean Sciences, [4]The Ohio State University

BACKGROUND

- Phytoplankton play critical roles in biogeochemical cycles, including transforming atmospheric CO_2 into particulate matter that eventually sinks to the deep ocean (the biological carbon pump).
- The fate of carbon (C) within the oceans is controlled by individual interactions in a highly complex and interconnected marine food web.
- Independent analyses of global datasets revealed a strong positive link between viruses and C flux to the deep ocean[1, 2].
- Mechanisms underlying virus-enhanced C transfer to particulate organic matter (POM) are unknown.



Food web demonstrating the "viral shunt"[3].

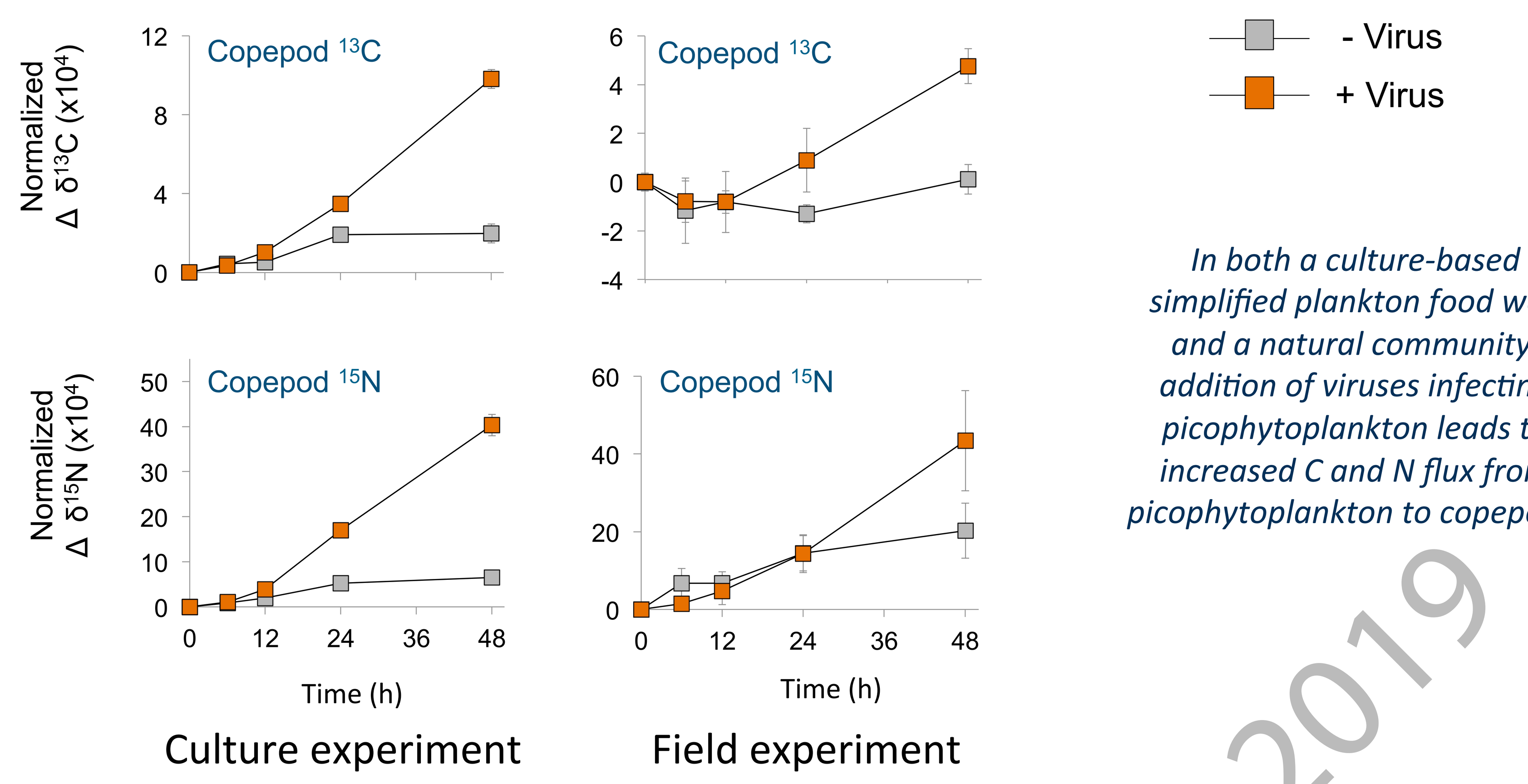
Viral shunt paradigm. Viruses enhance C and nutrient recycling and reduce upward trophic transfer and export of carbon

OBJECTIVES

Quantify viral impacts on trophic transfer of carbon (C) and nitrogen (N) using a combination of stable isotope labeling (^{13}C, ^{15}N), fluorescence activated cell sorting (FACS) and isotope ratio mass spectrometry (IRMS) in a mock plankton community and a coastal ocean ecosystem.

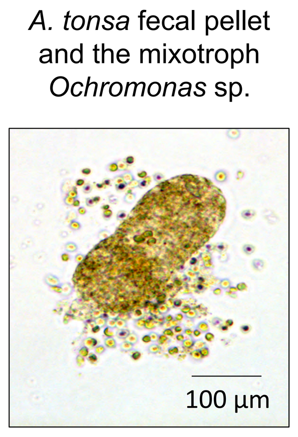
RESULTS

1. VIRUSES ENHANCE C and N FLUX TO ZOOPLANKTON

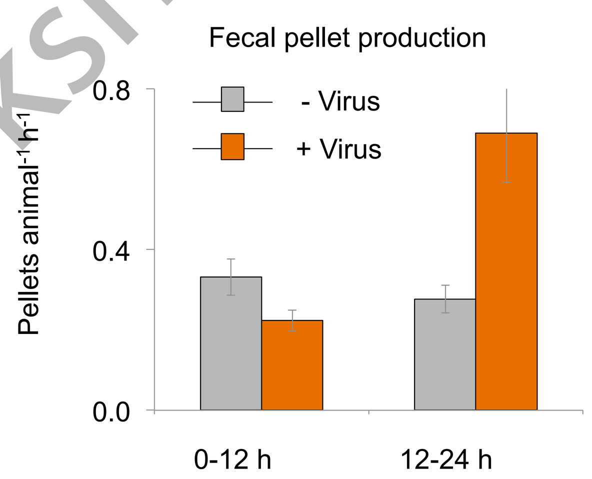


In both a culture-based simplified plankton food web and a natural community, addition of viruses infecting picophytoplankton leads to increased C and N flux from picophytoplankton to copepods.

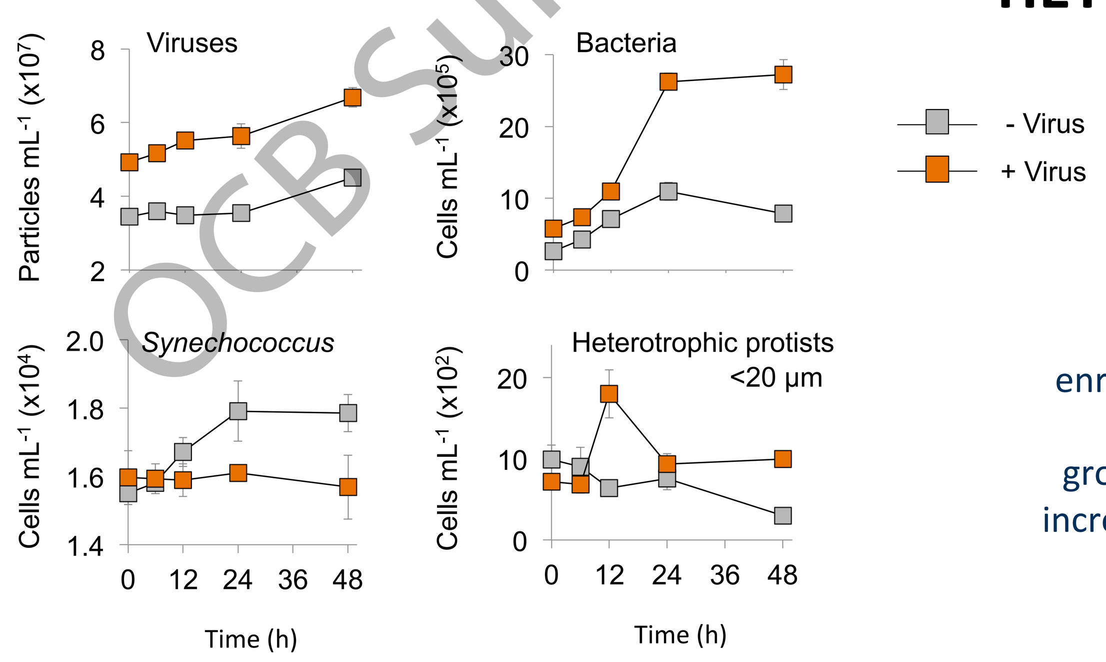
2. VIRUSES ENHANCE COPEPOD FECAL PELLET PRODUCTION



Acartia tonsa fecal pellet production was >2x higher in the presence of viruses infecting the picophytoplankton Ostreococcus tauri.



3. VIRUSES FACILITATE GROWTH OF BACTERIA AND HETEROTROPHIC PROTISTS



Field experiments revealed that enrichment of a natural virus community resulted in reduced *Synechococcus* growth, increased bacterial growth, and increased growth of heterotrophic protists feeding upon *Synechococcus*.

CONCLUSIONS

- Marine viruses enhance C transfer from picophytoplankton to higher trophic levels (copepods).
- Marine viruses also stimulate DOC release and bacterial growth (C transfer to lower trophic levels).
- Picophytoplankton viruses stimulate zooplankton feeding and fecal pellet formation, thereby *potentially* facilitating C export to the deep sea.

FUTURE DIRECTIONS

Intact, virus-infected cells release a range of dissolved organic substances, including chemoattractants, but little is known about the composition and impact of viral-derived organic compounds on microbial interaction dynamics.

Current work includes use of:

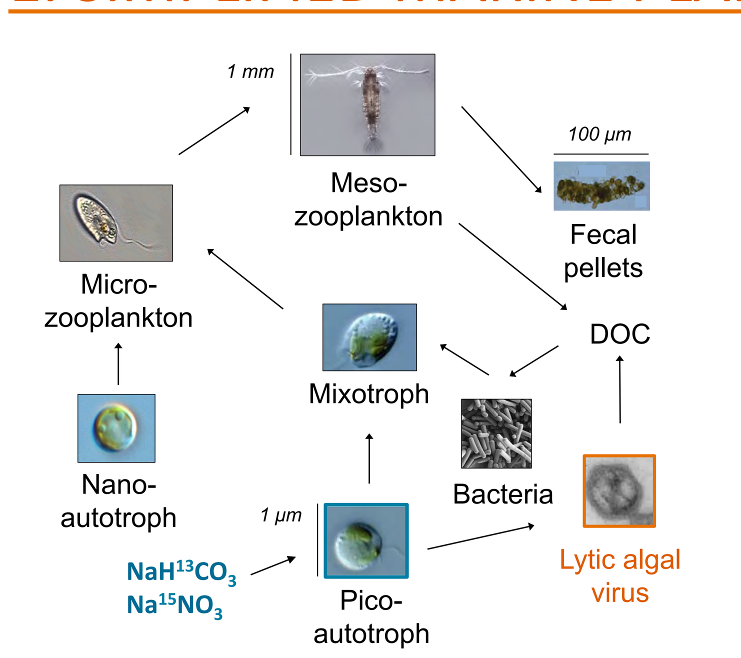
- Microfluidics to quantify the chemotactic response of various marine microbes to dissolved organic matter (DOM) released from intact, virus-infected picophytoplankton.
- Chip-based microbial food webs to characterize viral impacts on micron-scale predator-prey interactions.
- Untargeted stable isotope enabled mass spectrometry to identify novel viral-derived chemoattractants.

We observed viral-stimulated formation of aggregates >500 µm.

- Quantifying viral impacts on aggregate formation is essential to understanding C export.

METHODS:

1. SIMPLIFIED MARINE PLANKTON FOOD WEB

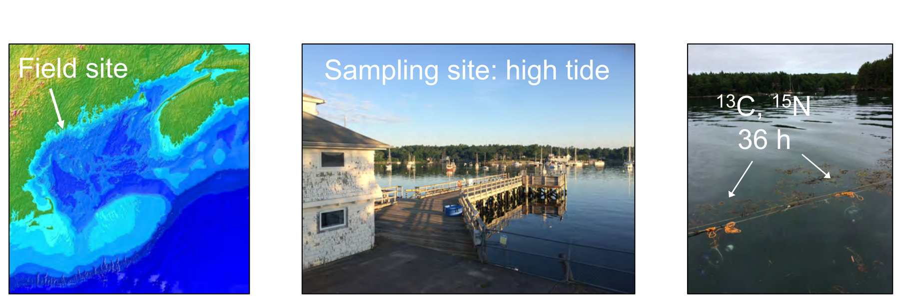


Arrows represent possible C flux pathways

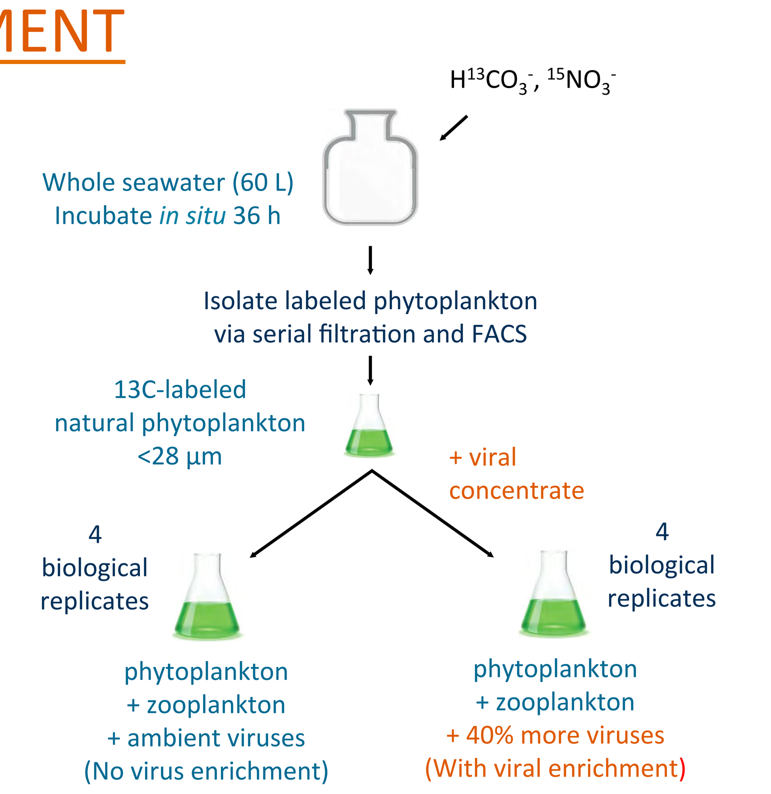
Eight orders of magnitude difference in organism C content and volume from bottom to top of food web

- The picophytoplankton, *Ostreococcus tauri*, (RCC4221) was grown with ^{13}C-labeled sodium bicarbonate and ^{15}N-labeled sodium nitrate for 48 h.
- The cells were then washed and resuspended in stable-isotope free seawater, enabling us to trace the flow of C and N from the picophytoplankton to other members of the plankton community.
- **One treatment contained the lytic virus OtV5, that infects only the *Ostreococcus tauri* picophytoplankton; one treatment contained no OtV5.**
- Five biological replicates per treatment.
- Other model organisms added: the nanoautotroph *Isochrysis galbana* (CCMP1323), the mixotroph *Ochromonas sp*. (CCMP1391), the microzooplankton *Oxyrrhis marina* (CCMP3375), and the calenoid copepod *Acartia tonsa*.
- Heterotrophic bacteria in the model community were derived from the copepods and non-axenic plankton cultures.
- Samples were collected at 0, 6, 12, 24, 48 h for virus and organism abundance, ^{13}C-DIC, ^{13}C-DOC, ^{13}C-POC, ^{15}N-PON, and copepod ^{13}C and ^{15}N.

2. COASTAL ATLANTIC OCEAN FIELD EXPERIMENT



- Field experiments conducted in July 2016, Boothbay, Maine, during a *Synechococcus* bloom.
- Whole seawater was incubated *in situ* for 36 h with $NaH^{13}CO_3$ and $Na^{15}NO_3$
- ^{13}C, ^{15}N-labeled phytoplankton <28 nm were isolated via fluorescence activated cell sorting (FACS) and re-mixed at *in situ* concentrations with a natural plankton assemblage <210 µm.
- One treatment received 40% more viruses (isolated and concentrated from field site).
- Both treatments received adult, female *Acartia tonsa*, isolated from field site, as the apex predator in the food web.
- Samples were collected at 0, 6, 12, 24, 48 h for virus and organism abundance, ^{13}C-DIC, ^{13}C-DOC, ^{13}C-POC, ^{15}N-PON, and copepod ^{13}C and ^{15}N.



ACKNOWLEDGEMENTS

This work was supported by National Science Foundation Biological Oceanography (award #1829905), the A. O. Beckman Foundation (Postdoctoral Fellowship Program), and the Gordon and Betty Moore Foundation (GBMF #3790, 3305). We also thank Bigelow Analytical Services and the J. J. MacIsaac Center for Aquatic Cytometry for facilities support.

REFERENCES

1. Guidi *et al.* 2016. Plankton networks driving carbon export in the oligotrophic ocean. Nature 532: 465-473.
2. Brum *et al.* 2015. Patterns and ecological drivers of ocean viral communities. Science 348: 1261498.
3. Wilhelm, S. W., and C. A. Suttle. 1999. Viruses and nutrient cycles in the sea. Bioscience 49: 781-788.