

Viruses as regulators of nutrient cycles in aquatic environments

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ABSTRACT

Viruses are abundant and dynamic members of marine environments. The persistence of viral communities in aquatic systems requires the daily destruction of a significant proportion of the bacterial and phytoplankton populations. While the destruction of host cells by viruses has several implications, one of the most important effects may be the role viruses play as regulators of nutrient cycles. Over the last several years we have obtained estimates of viral turnover rates and viral production for a variety of environments. We have used these estimates to infer the remobilization of nutrients in marine systems attributable to viral lysis. For example, viral lysis of bacterioplankton in the western Gulf of Mexico was estimated to liberate 0.12 to 0.55 $\mu\text{g C L}^{-1} \text{d}^{-1}$ in offshore waters and 0.72 to 5.2 $\mu\text{g C L}^{-1} \text{d}^{-1}$ in coastal waters. Similarly, virally mediated carbon release in the Strait of Georgia, British Columbia, ranged from 1.0 to 8.3 $\mu\text{g C L}^{-1} \text{d}^{-1}$, with the highest estimates associated with strong tidal mixing. Viruses also play an important role in the remobilization of organic nutrients and trace elements. For example, in the Strait of Georgia viral lysis was estimated to result in the remobilization of 0.3 to 1.7 $\mu\text{g L}^{-1} \text{d}^{-1}$ of organic nitrogen, 0.03 to 0.14 $\mu\text{g L}^{-1} \text{d}^{-1}$ of organic phosphorus and 0.06 to 0.33 $\text{ng L}^{-1} \text{d}^{-1}$ of organically complexed iron. The information presented demonstrates the importance of including viral processes in models of marine carbon and nutrient fluxes.

Introduction

Viruses are pervasive in marine environments and cause rates of microbial mortality equal to grazing [4, 12]. Although viruses have been known to be present in aquatic environments for many years, only recently have microbial ecologists begun to include viruses in models of microbial dynamics. Over the past decade, increasing focus on mechanisms of carbon and nutrient regeneration in marine systems has led to the need for better information on the role of planktonic viruses in these processes. Direct measurements of nutrient regeneration in microbial communities *via* this process remain difficult. However, laboratory experiments with viral-host systems [6, 8] as well as field studies directed at modeling this process [14] have provided convincing evidence that virally-mediated nutrient recycling may be important in some systems. In this paper we provide a synopsis of the available information on the virally-mediated nutrient release from bacterioplankton in contrasting regimes of the western Gulf of Mexico and the Strait of Georgia.

Microbial Biosystems: New Frontiers

Proceedings of the 8th International Symposium on Microbial Ecology

Bell CR, Brylinsky M, Johnson-Green P (ed)

Atlantic Canada Society for Microbial Ecology, Halifax, Canada, 1999.

Approaches to studying the impact of viruses on aquatic nutrient cycles

Laboratory studies have used a combination of viral-host systems, estimates of viral production and destruction rates from *in situ* measurements, and data on cellular elemental quotas to estimate the remobilization of nutrients by viral lysis. It has been suggested that viruses infecting marine bacterioplankton, cyanobacteria and eukaryotic plankton destroy up to 50 % of their host communities on a daily basis [5]. This leads to the release of large amounts of carbon and nutrient elements (e.g., organic nitrogen, phosphorus and iron) into the surrounding environment (Figure 1). While much of this material would be immediately available to bacterioplankton and the microbial loop, other components are potentially less biologically labile and would require the actions of exogenous enzymes, grazers or solar radiation. Moreover, lysis of bacterioplankton by viruses will produce nutrient elements in organic complexes, differing in nature from the inorganic nutrients released by grazers in these systems.

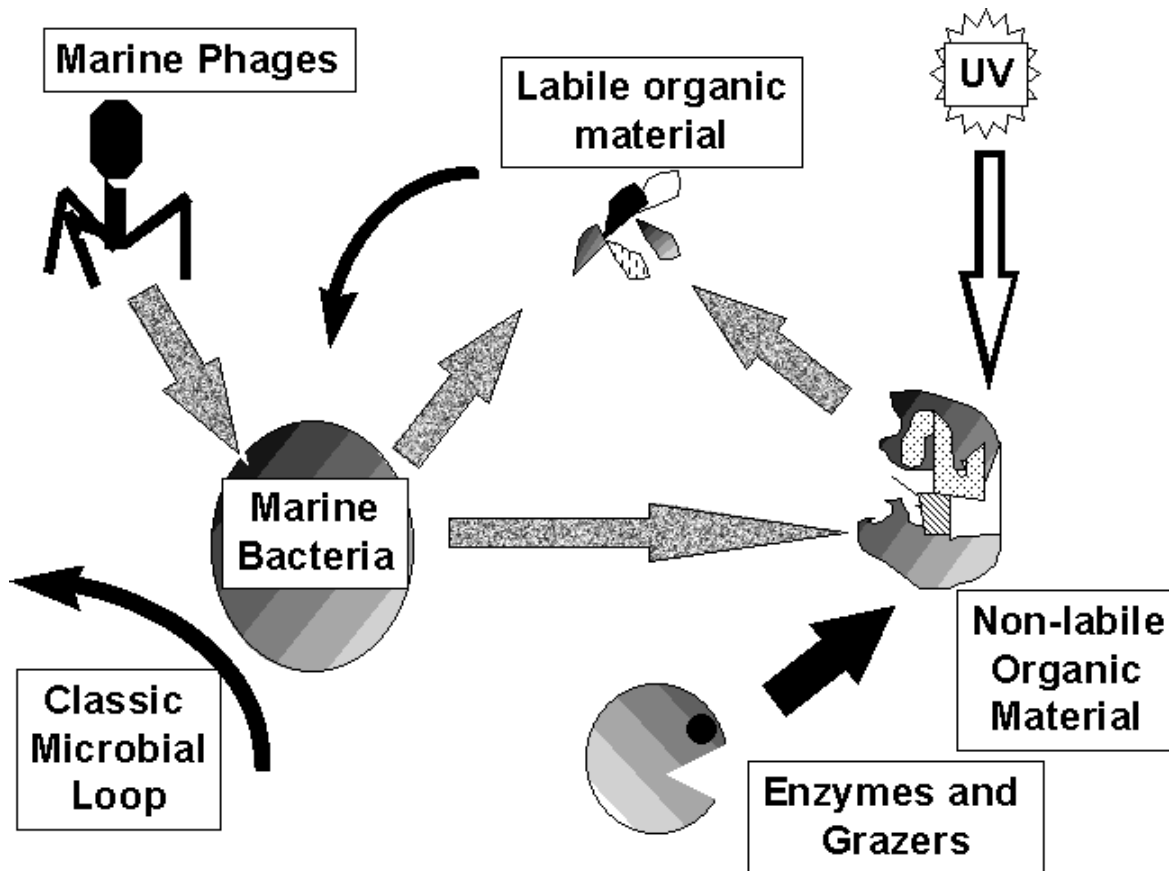


Fig. 1. The remobilization of dissolved organic material by viral lysis in marine systems. Viruses influence marine nutrient cycles by producing labile, organically complexed carbon and nutrient elements (e.g. N, P, and Fe) which are immediately available back to the microbial loop. As well, viral lysis will produce a proportion of these elements in forms that are available to the microbial loop on longer time scales and require the action of ultraviolet radiation (UV), grazers or endogenous exozymes to make them more labile.

Laboratory studies on virally mediated nutrient remobilization

The release of elements from planktonic organisms during virally mediated lysis has been studied in the laboratory with several virus-host systems. The alga, *Aureococcus anophagefferens*, forms extensive blooms (up to 10^9 cells L^{-1}) along the northeast coast of the United States [3]. Field observations Sieburth et al. (1988) and laboratory studies [9] have shown that viruses are potentially a major source of mortality for this alga. Gobler et al. (1997) illustrated that viral lysis of cultures of *A. anophagefferens* is accompanied by a large release of dissolved organic carbon (DOC), reaching nearly 160 % of levels in uninfected control cultures. Calculations based on these data suggest that lysis of a typical bloom of this alga would increase DOC levels by about 40 mM. This represents a large input of labile organic carbon that would increase the size of the ambient (and largely refractory) DOC pool by as much as 29% over a period of a few days. Their experiments demonstrated that viral lysis of phytoplankton can result in both a sudden and large release of dissolved organic carbon, and a rapid increase in bacterial growth rates and productivity fueled by virally-mediated nutrient releases.

Radiotracer experiments with *A. anophagefferens* [6] also demonstrated the release of phosphorus, selenium and iron as the result of viral lysis. Some elements (P, Fe) were rapidly returned back to particulate forms, indicating uptake by bacteria. Similarly, analysis of particulate nitrogen showed that there was no net release of N to the dissolved phase during the lysis period, indicating that N released as the result of viral lysis was also rapidly incorporated by bacteria. The virally-mediated loss of C to the dissolved phase was in marked contrast to the behavior of N, P and Fe in these experiments, indicating substantial fractionation between released C and other nutrients after lysis. This fractionation was also reflected in solar-radiation-exposed versus unexposed lysates, where more carbon was remobilized to the particulate form in the unexposed samples. Recent evidence [1, 2] suggests that DOM in surface waters may become more biorefractory after sunlight exposure, possibly due to competition between bacteria and photolysis for highly labile particles. However, while organic carbon may be lost as DIC, organic and inorganic nutrients (e.g., iron) may be more efficiently recycled after UV exposure [6]. Similarly, culture studies by Middleboe et al. [8] demonstrated a tight coupling between phosphorus released by viral lysis of bacteria and bacterial growth.

Modeling virally-mediated nutrient release in marine systems

We have recently modeled daily release rates of carbon and other nutrients as the result of viral lysis of bacterioplankton. Studies have been completed in the western Gulf of Mexico, a relatively stable and steady-state environment [14], as well as in the turbulently mixed environment of Discovery Passage in the northern Strait of Georgia, British Columbia. The potential role that viruses play in nutrient regeneration in these contrasting environments was determined from turnover rates of viruses and their inferred effect on bacterioplankton mortality. This information, combined with estimates of burst size during viral lysis, bacterial growth rate and bacterial elemental quotas was used to determine the release rates of various elements (Table 1a and 1b).

Strait of Georgia sites are divided into stratified and tidally mixed stations. Viral production rates in the Strait of Georgia were determined by a dilution technique combined with epifluorescence counts of viral particles in surface waters. Nutrient release rates due to

host cell lysis are based on estimates of burst size and viral production rates. Bacterial carbon demand ($\mu\text{g C L}^{-1} \text{d}^{-1}$) was determined from growth rates based on incorporation of tritiated thymidine, cellular carbon quotas of $23.3 \text{ fg cell}^{-1}$ and a growth efficiency of 50%.

Table 1a. Release of carbon and nutrient elements by viral lysis of bacterioplankton in the Gulf of Mexico (from [14]).

GULF OF MEXICO				
	STATION B	STATION C	STATION E	STATION F
Viral Turnover Rate ($10^3 \text{ particles ml}^{-1} \text{h}^{-1}$)	3.7 (0.7)	5.8 (0.6)	121.7 (3.7)	270.8 (2.1)
Carbon released ($\mu\text{g L}^{-1} \text{d}^{-1}$)	0.12 - 0.15	0.28 - 0.55	3.4 - 5.2	0.72 - 0.96
Percentage of Bacterial Carbon Demand	5.3 - 6.6%	3.6 - 7.2%	19.7 - 30.1%	4.2 - 5.5%
Nitrogen Remobilized ($\mu\text{g L}^{-1} \text{d}^{-1}$)	0.02 - 0.03	0.05 - 0.11	0.65 - 1.0	0.14 - 0.79

Stations B and C are offshore (400 & 300 km) and E and F are nearshore (75 & 60 km). All Gulf of Mexico stations exhibited stable pycnoclines. Viral turnover rates were determined from the decay rate of viral particles in surface deployments.

Table 1b. Release of carbon and nutrient elements by viral lysis of bacterioplankton in the Strait of Georgia.

STRAIT OF GEORGIA							
	STRATIFIED SITES			EBBING / MIXED SITES			
	G1	G3	G4	H1	H3	H4	H5
Viral Production Rate ($10^6 \text{ particles ml}^{-1} \text{h}^{-1}$)	3.3 (0.5)	3.1 (0.8)	2.2 (0.7)	6.2 (1.5)	17.9 (0.7)	4.3 (1.1)	7.9 (0.7)
Carbon Released ($\mu\text{g L}^{-1} \text{d}^{-1}$)	1.51	1.45	1.03	2.91	8.25	2.00	3.66
Percentage of Bacterial Carbon Demand	83%	95%	51%	1148%	452%	143%	142%
<u>Nutrients Remobilized</u>							
Nitrogen ($\mu\text{g L}^{-1} \text{d}^{-1}$)	0.36	0.35	0.25	0.70	1.98	0.48	0.88
Phosphorus ($\mu\text{g L}^{-1} \text{d}^{-1}$)	0.03	0.03	0.02	0.06	0.18	0.04	0.08
Iron ($\text{pg L}^{-1} \text{d}^{-1}$)	0.07	0.07	0.05	0.14	0.39	0.09	0.17

The release of other nutrients by viral lysis was determined from cell quotas of 5.6 fg cell⁻¹ for nitrogen [11], 0.5 fg cell⁻¹ for phosphorus [7] and 1.1 ag cell⁻¹ for iron [13].

In the thermally stabilized water column of the western Gulf of Mexico, viral production rates ranged from *ca.* 10³ ml⁻¹ h⁻¹ in oligotrophic offshore waters to *ca.* 10⁵ ml⁻¹ h⁻¹ in mesotrophic nearshore waters. These production rates of viruses would lead to bacterial destruction rates and subsequent carbon remobilization rates that could supply *ca.* 5 to 7 % of the bacterial carbon demand in offshore environments, and up to 30 % of the bacterial carbon demand at nearshore stations. Similarly, at two stratified stations in the mesotrophic Strait of Georgia, inferred carbon remobilization rates could supply *ca.* 80-95 % of the bacterial carbon demand (51 % at Station G4, where waters were moving into the passage). However, at stations where the water column was well mixed as the result of tidally driven currents, estimates of viral production rates imply that bacterial lysis released carbon at rates *ca.* 140 to >1000 % higher than the bacterial carbon demand. This, in combination with extremely low chlorophyll *a* values in these regions (data not shown) suggests that viral lysis may be the key mechanism supplying DOM to heterotrophic bacterioplankton in this non-steady-state environment.

Conclusions

It has become apparent over the last decade that viruses play critical roles in the recycling and remobilization of carbon and nutrients in marine systems. The results reviewed here demonstrate that viruses can contribute to, as well as control, marine nutrient cycles. Although we are able to estimate the quantities of nutrients that are released during viral lysis, the character, bioavailability and residence time of these components requires further study. The development of novel techniques for tracking dissolved organic materials as well as the use of radiotracers to track various elements during viral lysis will hopefully provide further resolution to this issue.

Acknowledgements

The authors thank AM Chan, DR Garza, DA Hutchins, WH Jeffrey, SM Short, and MG Weinbauer for assistance in gathering this information and discussions on this topic, and R Benner for preprints of publications. Research supported by grants from the U.S. National Science Foundation (OCE-9415602) and Natural Science and Engineering Research Council of Canada to C.A.S.

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