

Viral release of iron and its bioavailability to marine plankton

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Abstract

The biological availability of Fe has been demonstrated to strongly influence both primary and secondary production in pelagic as well as coastal upwelling high-nutrient low-chlorophyll (HNLC) regimes. Although nearly all of the dissolved Fe in marine surface waters is thought to be complexed by organic ligands, the character and origin of these Fe-organic complexes remains a mystery. Here we report that the activity of naturally occurring viral populations in an HNLC coastal upwelling system can regenerate sufficient concentrations of dissolved Fe to support the growth of the native phytoplankton community. When combined with studies that have demonstrated that Fe in virus-mediated lysates of heterotrophic bacteria and cyanobacteria is highly bioavailable to model marine plankton, our data demonstrate that viral activity in this marine system (and potentially others) is critical to the recycling of organically complexed Fe that supports as much as 90% of primary production in HNLC surface waters.

Mesoscale fertilization experiments over the past decade have conclusively shown iron (Fe) to be a limiting nutrient of primary production in high-nutrient, low-chlorophyll (HNLC) regions of the open ocean (e.g., Boyd et al. 2000). Additionally, shipboard experiments have shown that Fe limitation may also control primary productivity in coastal upwelling areas (Hutchins et al. 1998; Bruland et al. 2001). In total, recent models have suggested that roughly half of the world's oceans may be Fe limited (Moore et al. 2002).

Dissolved Fe in marine systems has been demonstrated to be almost completely bound to strong organic ligands (Rue and Bruland 1995) that influence the biological availability of this Fe to marine plankton (Hutchins et al. 1999a). Although various Fe chelates, including siderophores and grazing byproducts, have been shown to act as sources of Fe to marine plankton (Wilhelm and Trick 1994; Butler 1998; Hutchins et al. 1999b), there remains, to date, no field evidence of the source (and supply rates) of the Fe-organic compounds that marine plankton assimilate during in situ growth. In HNLC areas, the recycling of Fe plays an important role: new Fe inputs to the system can only support

4–20% of the total primary production observed (Hutchins 1995).

Although their existence has been known for many years, marine viruses have recently been recognized as important factors that influence microbial communities. In marine surface waters, viral abundance is generally $>10^9$ L⁻¹, ~1 order of magnitude higher than the typical bacterial abundance (Fuhrman 1999). Estimates of virus-mediated lysis of planktonic bacteria range from 20% to 50% of the population d⁻¹ (Fuhrman 1999; Wilhelm and Suttle 1999). During viral lysis, the host cells are transformed from a series of uniform particles to a gradient of dissolved (<0.20 μm) through particulate (>0.20 μm) materials, and this can result in the potential release of significant quantities of organically complexed nutrients (Wilhelm and Suttle 2000). In their study using the pelagophyte *Aureococcus anophagefferens*, Gobler et al. (1997) demonstrated this idea through an examination of Fe released from this cell to bacteria as well as to the model organism *Thalassiosira pseudonana*. Although this work established the idea in principle, heterotrophic bacteria and cyanobacteria are thought to be the dominant targets of viruses in most marine surface waters (Fuhrman 1999). Given this high virus-induced mortality, as well as the high abundance of bacteria and cyanobacteria in many HNLC environments, we set out to investigate the role that the virus-mediated Fe recycling might play in these systems. Specifically, we hypothesized that viral activity may provide a significant proportion of the recycled Fe that is critical to community maintenance in HNLC systems and that the character of this Fe might influence its bioavailability to marine plankton.

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Material and methods

Production and fractionation of viral lysates—The fractionation of Fe in lysis products has been determined for the cyanobacterium *Synechococcus* sp. WH7803 grown in A⁺ medium (Wilhelm and Trick 1995) and the heterotrophic *Vibrio natriegens* PWH3a grown in carbon supplemented enriched seawater/artificial water (Berges et al. 2001), with all media containing added Si (12.5 $\mu\text{mol L}^{-1}$ final concentration) and ^{55}Fe (4.7 $\mu\text{mol L}^{-1}$ final Fe concentration). All cultures used in these studies were originally axenic, although minimal bacterial contamination was noted in some of the *Synechococcus* sp. WH7803 cultures. Cultures were grown at 25°C at an illumination of 65–85 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in acid-washed polycarbonate tubes. Cells were cultured for ~12 d (for *Synechococcus* sp. WH7803) or ~4 d (for *V. natriegens*), collected by filtration on 0.2- μm nominal pore-size polycarbonate filters (for *Synechococcus* sp. WH7803) or by centrifugation (for *V. natriegens*) and treated with Ti(III)-citrate-ethylene diaminetetraacetic acid (EDTA; Hudson and Morel 1989) to remove surface-associated ^{55}Fe . The cells were resuspended in A⁺ medium with Si (12.5 $\mu\text{mol L}^{-1}$) and Fe (31 nmol L^{-1}). For each treatment, 100 μl of phage in Fe-free medium was added to lyse the cells: cyanophage SYN-M3 (~3.5 $\times 10^7$ total particles) for *Synechococcus* sp. WH7803 and bacteriophage P1 (~2.0 $\times 10^8$ total particles) for *V. natriegens*. Controls received no phage. The cells were incubated for ~24 h (for *V. natriegens*) or ca 6 d (for *Synechococcus* sp. WH7803), to allow for infection and lysis.

Fe fractionation was initially examined in virus-mediated cellular lysates by filtration through 0.2- μm nominal pore-size polycarbonate filters. The ^{55}Fe in the filtrate was analyzed by liquid scintillation counting using a Wallac Tri-Lux scintillation counter with a ^{55}Fe quench correction program and Wallac SuperMix scintillation cocktail. Subsequent filtrate was further size fractionated using Millipore Amicon Centriprep centrifugal filter devices (3 and 30 kDa nominal cutoff, corresponding to pore sizes of ~1.8 and 4.3 nm, respectively; pore size values were provided by the manufacturer) using the manufacturer's recommended protocol.

Lysate uptake studies—All lysates used in uptake experiments were generated as in the fractionation experiments described above. The uptake of EDTA-Fe was determined using A⁺ medium with 80.5 $\mu\text{mol L}^{-1}$ EDTA and $^{55}\text{FeCl}_3$ (31 nmol L^{-1} total Fe concentration; 0.82 nmol L^{-1} $^{55}\text{FeCl}_3$). The uptake of inorganic Fe was measured from A⁺ medium supplemented with $^{55}\text{FeCl}_3$ but no added EDTA (31 nmol L^{-1} total Fe concentration; 0.82 nmol L^{-1} $^{55}\text{FeCl}_3$). Lysates of *Synechococcus* sp. WH7803 and *V. natriegens* for uptake studies were gently filtered through 0.2- μm nominal pore-size polycarbonate filters, to remove any unlysed cells. Aliquots (30 ml) of the filtrates were then transferred to acid clean 40-ml polycarbonate Oak Ridge tubes. Cultures (1 ml) of model marine organisms (the cyanobacterium *Synechococcus* sp. PCC 7002, the heterotrophic bacteria *Vibrio harveyi*, or the diatom *T. pseudonana*) were added to the filtrates. The cultures were then incubated under appropriate conditions for each model organism. At $t = 0$ and 24 h, 2-ml

samples of each culture were filtered through 0.2- μm nitrocellulose filters, and the extracellular Fe was removed as described above. The filters were placed into 4-ml scintillation vials and dissolved for 1 h with 500 μl of ethyl acetate. Scintillation fluid was then added, and the samples were analyzed for ^{55}Fe . Cell abundances (for normalization of uptake rates) were determined by Acridine Orange staining (Hobbie et al. 1977) or autofluorescence on a Leica DMRXA epifluorescent microscope. To allow for a comparison between our model organisms, Fe uptake rates were normalized to the cellular carbon content using previously determined cellular quotas of 23.3 fg for heterotrophic bacteria, 210 fg for cyanobacteria, and 5.94 pg (picograms) for diatoms (Waterbury et al. 1986; Lee and Fuhrman 1987; Montagnes et al. 1994).

Viral production estimates in the eastern subtropical Pacific Ocean—Viral production rates in the subtropical equatorial eastern Pacific Ocean off the coast of Peru were estimated as described by Wilhelm et al. (2002). This region has been well characterized in several recent studies (Hutchins et al. 2002; Eldridge et al. 2004). Surface seawater (~5 m depth) was cleanly collected with a PTFE Teflon diaphragm pump and PFA Teflon tubing connected to a PVC "fish" that was lowered off the ship outside of the wake (Bruland et al. 2001). Seawater (300 ml) was filtered under gentle vacuum through 0.22- μm nominal pore-size polycarbonate filters (Millipore). To prevent bacteria from settling on the filter, a retentate volume of >50 ml was maintained throughout filtration by addition of virus-free ultrafiltered (<30 kDa) seawater produced as ultrafiltrate from an Amicon M12 System equipped with an acid-cleaned S10Y30 cartridge. Additionally, bacteria were continuously resuspended with an acid-cleaned transfer pipette during filtration. The final volume of the retained cell suspension was brought to 300 ml through the addition of ultrafiltered water. As such, the concentration of naturally occurring viruses was reduced to ~4–40% of the initial concentration. Aliquots (100 ml) of the retained cell suspension were transferred into three acid-cleaned 250-ml polycarbonate flasks, which were incubated in the dark at in situ temperature. Subsamples (5 ml) were taken at $t = 0$ from whole water (before filtration) and from each bottle and then every 2–3 h for up to 12 h from each incubation bottle, preserved in 2.5% glutaraldehyde (final concentration), and stored in a refrigerator (4°C) until they were processed. The abundance of viruses and bacteria in samples were determined in the laboratory by staining samples with either SYBR Green I (Noble and Fuhrman 1998) or Acridine Orange staining (Hobbie et al. 1977) before direct enumeration by epifluorescence microscopy.

The production rate of viruses at each station was determined from regressions of samples in independent, replicate ($n = 3$) bottles for each station, as described above. To determine virus-induced mortality rates, a burst size of 25 viruses per lytic event was assumed (Wilhelm et al. 1998). This information was used to estimate the rate of virus-mediated mortality of bacteria (cells $\text{L}^{-1} \text{ d}^{-1}$) as

$$\begin{aligned} & \text{Virus-induced bacterial mortality} \\ & = \text{viral production/burst size} \end{aligned}$$

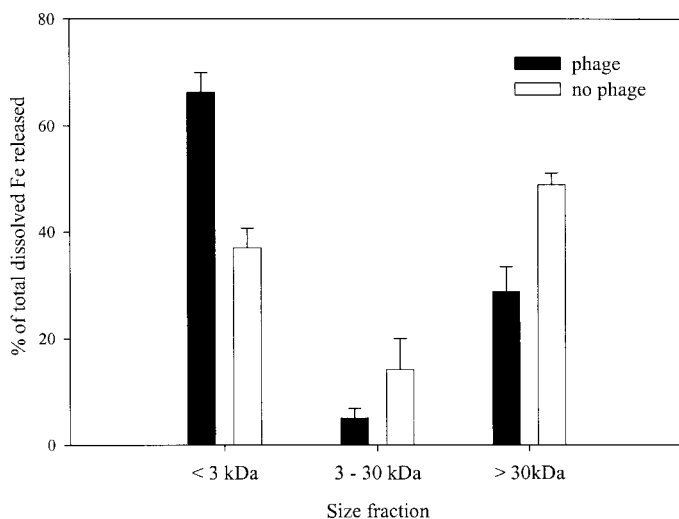


Fig. 1. Release of ⁵⁵Fe by heterotrophic bacteria. The size fractionation of dissolved released ⁵⁵Fe from *V. natriegens* was measured ($n = 3$, \pm SD) both with and without added bacteriophage. In the cultures with phage, the greatest percentage of ⁵⁵Fe was present in the smallest (<3 kDa) size class. The cultures that had no added phage released more ⁵⁵Fe into the larger size fractions.

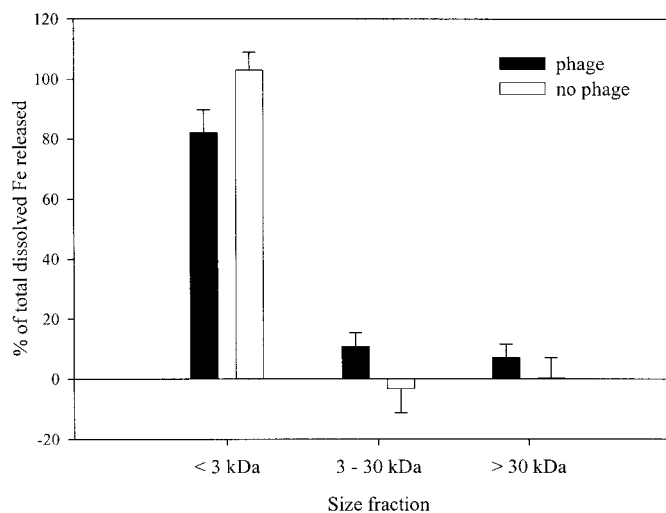


Fig. 2. Release of ⁵⁵Fe by cyanobacteria. The size fractionation of dissolved released ⁵⁵Fe from *Synechococcus* sp. WH7803 was measured ($n = 3$, \pm SD) both with and without added cyanophage. All of the ⁵⁵Fe released in absence of phage was found in the smallest (<3 kDa) size fraction. Although most of the ⁵⁵Fe released with added phage was also found in the smallest fraction, a significant proportion was in the larger classes.

To infer the effects on the regeneration of Fe, we assumed cellular Fe quotas for marine bacterioplankton of 1.1 ag (atograms) per cell (Tortell et al. 1996), and calculated Fe remobilized by viral lysis from

Fe remobilization

$$= \text{virus-induced bacterial mortality} \times 1.1 \text{ ag}$$

Fe uptake by natural communities—Fe assimilation rates for the natural marine assemblages were estimated at two stations in the Pacific Ocean (90°33.7'W, 8°41.5'N and 73°24.9'W, 16°27.3'S). Surface seawater was cleanly collected in acid-washed 2.7-L polycarbonate bottles. Saturating amounts of ⁵⁵Fe (2 nmol L⁻¹, as ⁵⁵FeCl₃ in 0.5 mol L⁻¹ HCl, ~42 mCi mg⁻¹; New England Nuclear) were added to replicate bottles, which were then incubated for 48 h at in situ temperatures and light levels. After incubation, the water was filtered in parallel through 0.2- and 1.0- μ m nominal pore-size polycarbonate filters that were then washed with Ti(III)-citrate-EDTA, as described above, to remove surface-associated ⁵⁵Fe. Assimilated ⁵⁵Fe was measured by scintillation counting, as described above, with the Wallac Tri-Lux scintillation counter.

Results

Fe fractionation in virus-mediated lysates—The lysis of *V. natriegens* laboratory cultures released a significantly greater percentage (24.9 \pm 2.9%) of intracellular Fe into the dissolved (<0.22 μ m) size class than was released from the unlysed controls (10.6 \pm 2.1%; $p < 0.01$). Differences were observed between lysed and unlysed cultures during fractionation of the dissolved Fe (Fig. 1). Dissolved Fe released from virus-lysed cells was predominantly (66.3 \pm 3.7%) in the <3-kDa fraction, with a smaller amount (28.8 \pm 4.7%)

in the ≥ 30 -kDa fraction. A lesser percentage (37.0 \pm 3.7%) of the Fe from the unlysed cells was found in the <3-kDa fraction, and a greater percentage (48.9 \pm 2.2%) was found in the ≥ 30 -kDa fraction.

After lysis of *Synechococcus* sp. WH7803, the majority (81.6 \pm 5.1%) of the original intracellular Fe was released into the dissolved phase (Fig. 2). This is in contrast to the unlysed controls, where 33.5 \pm 1.1% was released into the dissolved phase in the absence of viruses. All of the dissolved Fe (103.0 \pm 6.0%) released by the unlysed cells was found in the <3-kDa fraction, whereas significantly less ($p < 0.05$) of the dissolved Fe released by virus lysis was present in this fraction (82.1 \pm 7.7%). There were no significant differences seen in the other size fractions.

Assimilation rates of Fe from lysates—Fe present in the lysate of *V. natriegens* cultures was differentially bioavailable to the different model organisms over a period of 24 h (Fig. 3). The bacterium *V. harveyi* assimilated ⁵⁵Fe from the lysates at a rate of 3.1 (\pm 1.0) $\times 10^{-2}$ mol Fe (g C)⁻¹ (Fig. 4). This was significantly higher than the rate of ⁵⁵Fe assimilation from lysate for *Synechococcus* sp. PCC 7002 (8.7 \pm 0.7 $\times 10^{-4}$ mol Fe (g C)⁻¹; $p < 0.01$) and *T. pseudonana* (9.8 \pm 1.8 $\times 10^{-4}$ mol Fe (g C)⁻¹; $p < 0.01$). In all cases, ⁵⁵Fe in the dissolved phase of the *V. natriegens* lysates was assimilated at a statistically ($p < 0.05$) greater rate than either inorganic ⁵⁵Fe or ⁵⁵Fe-EDTA.

Fe released from lysis of *Synechococcus* sp. WH7803 was assimilated more rapidly by *V. harveyi* (2.6 \pm 0.3 $\times 10^{-5}$ mol Fe (g C)⁻¹) than by *Synechococcus* sp. PCC7002 (1.3 \pm 0.2 $\times 10^{-5}$ mol Fe [g C]⁻¹; $p < 0.01$) and *T. pseudonana* (6.9 \pm 6.3 $\times 10^{-6}$ mol Fe [g C]⁻¹; $p < 0.01$; Fig. 4). Although this Fe was also more readily assimilated than Fe from the inorganic or EDTA-complexed treatments by the

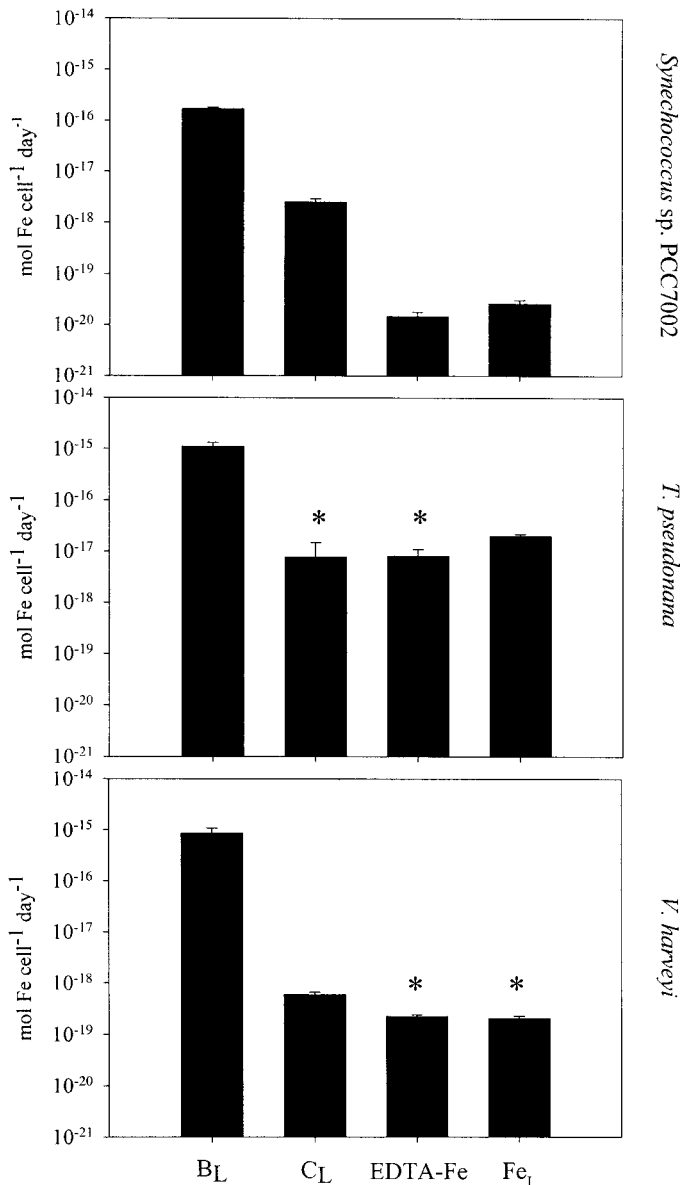


Fig. 3. Uptake of various Fe sources (mol Fe cell⁻¹ d⁻¹). The uptake of Fe from bacterial lysate (BL), cyanobacterial lysate (CL), EDTA bound Fe (EDTA-Fe), and inorganic Fe (Fe_i) by laboratory cultures of *T. pseudonana*, *V. harveyi*, and *Synechococcus* sp. PCC7002 was determined. Fe from the bacterial lysate had the greatest bioavailability to all three organisms tested. Values marked with an asterisk were not significantly different from one another. All other values had significant differences ($p < 0.05$).

prokaryotes, there was no significant difference between the rates of assimilation by *T. pseudonana* of Fe from the EDTA-complex treatments.

Virus production and estimated Fe remobilization rates—The effect of viral activity on Fe recycling in situ was determined at a station in the Humboldt Current and at three stations in the Peruvian upwelling (Table 1). Viral abundance at these stations ranged ~2–6 × 10⁷ ml⁻¹, whereas bacterial abundance ranged 5–18 × 10⁶ ml⁻¹. Virus-induced mortality

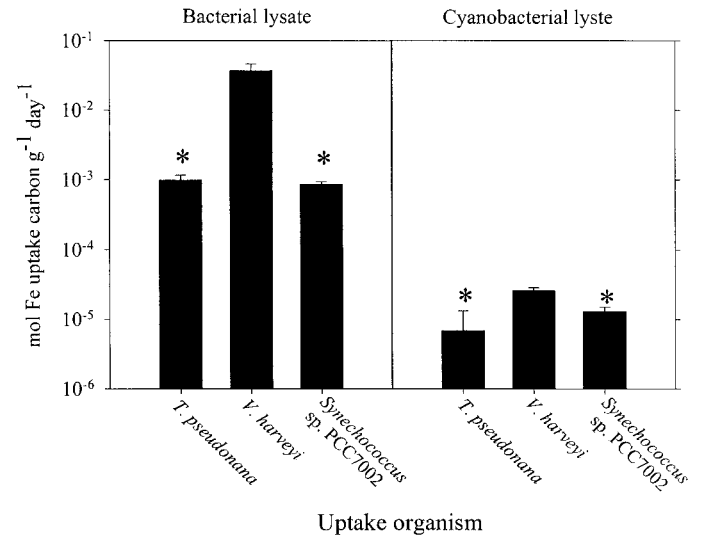


Fig. 4. Uptake of Fe released by viral lysis (mol Fe [g carbon]⁻¹ d⁻¹). The uptake of Fe by laboratory cultures of *T. pseudonana*, *V. harveyi*, and *Synechococcus* sp. PCC7002 was measured over 24 h to compare uptake rates among the three organisms. *V. harveyi* took up Fe from bacterial lysate at a rate more than an order of magnitude greater than either *T. pseudonana* or *Synechococcus* sp. PCC7002. Values marked with an asterisk were not significantly different from one another. All other values had significant differences ($p < 0.05$).

in these regions was consistent with results from previous studies (Fuhrman 1999; Wilhelm and Suttle 2000), ranging ~5.3–35.8% of the bacterial population lysed on a daily basis, given a burst size of 100 viruses per lytic event. We used an estimated burst size of 25 viruses per lytic event, because this appears to be typical of those found in environments similar to that of the current study. In the Gulf of Mexico, burst sizes were estimated to be 10–20 viruses per lytic event in offshore waters and 30–60 viruses per event in nearshore, mesotrophic waters (Wilhelm et al. 1998). These estimates, in conjunction with our ~25% partitioning into the dissolved phase, suggest that viral activity would result in a recycled dissolved Fe flux ranging 19.2–75.7 pmol L⁻¹ d⁻¹ (Table 1).

Fe uptake by natural communities—Saturated Fe uptake rates were measured at two stations in the Pacific Ocean. Fe assimilation rates over 48 h for phytoplankton (>1.0 μm size class) ranged from 19.5 (±7.8) to 30.6 (±1.5) pmol L⁻¹ d⁻¹. Bacterioplankton (0.2–1.0 μm size class) uptake rates ranged from 160 (±17) to 181 (±28) pmol L⁻¹ d⁻¹.

Discussion

The results of the present study demonstrate that the virus-mediated lysis of cyanobacteria and heterotrophic bacteria releases intracellular Fe into the dissolved size class at a greater rate than it is released from cells in the absence of phages. Although this is not surprising, the results of our size-fractionation experiments demonstrated that some of this virus-released Fe partitions into a different size class relative to Fe released from unlysed cells. Moreover, the re-

Table 1. Virus-mediated recycling of dissolved organic nutrients. Fe release rates from virus-lysed cells were determined from the inferred destruction rates of bacterioplankton, a burst size of 25 viruses per lytic event, and an estimated bacterial cellular quota for Fe (Tortell et al. 1996). Fe partitioning into the dissolved phase was estimated under the assumption of 25% partitioning after viral lysis (see text for details).

Date	09 Sep 00	12 Sep 00	21 Sep 00	23 Sep 00
Location	84°40.3'W, 4°10.7'S	80°13.1'W, 7°7.6'S	75°21.1'W, 15°23.4'S	73°24.9'W, 16°27.3'S
Bacterial abundance ($\times 10^6$ particles ml ⁻¹)	9.78	18.2	ND	5.39
Viral abundance ($\times 10^6$ particles ml ⁻¹)	27.8	60.7	ND	20.0
Viral production rate ($\times 10^6$ particles ml ⁻¹ h ⁻¹)	4.67	4.04	16.01	8.03
Total Fe remobilization (ng L ⁻¹ d ⁻¹)	4.92	4.28	16.92	8.48
Dissolved Fe remobilization (ng L ⁻¹ d ⁻¹)	1.23	1.07	4.23	2.12
Virally generated dissolved Fe flux (pmol L ⁻¹ d ⁻¹)	22.0	19.2	75.7	38.0

ND, not determined.

sults of our Fe-assimilation experiments demonstrated that Fe released via lysis is highly bioavailable to model marine planktonic organisms: Fe in bacterial lysates and, in most cases, cyanobacterial lysates, was more rapidly assimilated than either inorganic Fe or EDTA-Fe by our model organisms. When coupled with in situ measurements of viral production (and associated host lysis), these experiments suggest that the virus-mediated Fe release may provide an important fraction of the total bioavailable Fe in pelagic marine systems.

Fe release, fractionation, and uptake—Viral lysis of laboratory plankton cultures demonstrated that the size class of Fe released by virus-mediated lysis was dependent on the host organism. In our experiments, the lysis of cyanobacteria released a greater proportion of total intracellular Fe into the dissolved size class, relative to the lysis of heterotrophic bacteria. In many ways, this is not surprising, given that these prokaryotes are quite different both structurally and in terms of where the highest concentrations of Fe are located within the cells. Moreover, the high cell densities reached by heterotrophic bacteria in culture probably enhances aggregation. As such, the actual fate of Fe during the lysis of these prokaryotes in nature undoubtedly differs. In their studies with *A. anophagefferens*, Gobler et al. (1997) found that only 5% of the Fe was released into the dissolved phase during viral lysis. In their cultures, a marked increase in bacterial abundance concurrent with lysis may have combined with the release of high-molecular-weight materials to scavenge any free Fe.

Dissolved Fe (<0.2 μ m) released from lysis of *V. natriegens* was predominantly in the <3-kDa size class, although approximately one third was found to be associated with larger components. For unlysed control cells (cultures not exposed to a virus), approximately one half of the dissolved Fe partitioned into the >30-kDa size class, with the majority of the remainder in the <3-kDa class. Dissolved Fe released by the virus-mediated lysis of *Synechococcus* sp. WH7803 was also predominantly in the <3-kDa class, with a small but significant ($p < 0.05$) proportion found in the larger size

classes. In contrast, Fe released from unlysed *Synechococcus* sp. WH7803 cells was found only in the <3-kDa class.

Many studies make operational distinctions on the basis of the ability to fractionate samples—in our own case, we used cutoffs of 0.2 μ m and 30 and 3 kDa. It remains difficult to draw comparisons between the size fractionation of Fe observed in these lysis experiments and previously observed in situ Fe fractionation, because there are currently no universally recognized size standards for classifying “Fe-complexes” in the ocean. Estimates of “dissolved” Fe concentrations in near-surface ocean waters range from 95% in the “soluble” fraction (<200 kDa; Nishioka et al. 2001) to 90% in the “colloidal” size class (0.02–0.4 μ m; Wu et al. 2001).

The data above provide direct evidence that different approaches to Fe fractionation in natural systems may result in samples that contain Fe of different origins (and, as we will show, bioavailabilities), because such comparisons using different size fractions (or methods) may yield inconsistent results. For example, a previous study (Wang and Dei 2003) demonstrated that the cyanobacteria *Synechococcus bacillarus* (CCMP 1333) and *Trichodesmium* sp. were able to take up low-molecular-weight Fe (<1 kDa) at a faster rate than “colloidal” Fe (>1 kDa–0.2 μ m). However, Mioni et al. (2003) recently demonstrated that removal of the >0.2- μ m size class markedly reduced the bioavailability of Fe to a halotolerant Fe reporter strain (*Pseudomonas putida* Fe-Lux) in a freshwater system (Lake Erie).

Fe released by the virus-mediated lysis of plankton showed varying levels of bioavailability to our model organisms. When assimilation rates were normalized to estimates of cellular carbon (g), the Fe released from lysis of *V. natriegens* was significantly ($p < 0.05$) more bioavailable than Fe from autotrophic *Synechococcus* sp. WH 7803. This was consistent for the three model organisms that we studied. Fe contained in the bacterial lysate was assimilated by *V. harveyi* at a rate almost 3 orders of magnitude greater than Fe from cyanobacterial lysis. Both lysates were significantly ($p < 0.05$) more bioavailable than either inorganic Fe or EDTA-Fe during our incubations (24 h).

The Fe from the lysed bacterium was also more readily

assimilated by *T. pseudonana*, our model marine diatom. The uptake of Fe from the bacterial lysate by *T. pseudonana* occurred at a rate more than 2 orders of magnitude faster than the uptake rate of Fe from the cyanobacterial lysate. There was no significant difference in the uptake rate of Fe from cyanobacterial lysates, compared with EDTA-⁵⁵Fe, by *T. pseudonana*. Inorganic ⁵⁵Fe was slightly but significantly ($p < 0.05$) more bioavailable than either the cyanobacterial lysate or the EDTA-Fe during these short-term experiments (although laboratory observations over longer periods have demonstrated that EDTA is required for the cells to grow in this synthetic medium). These uptake experiments showed that prokaryotic and eukaryotic organisms can assimilate Fe released from the lysis of both heterotrophic and autotrophic marine plankton but may preferentially assimilate different forms.

The resolution of the marine Fe cycle remains complicated by our inability to determine how organic complexation with individual ligands affects the availability of Fe. Macrellis et al. (2001) have demonstrated that the marine Fe-ligand pool is a complicated mixture of many different organic complexes. Unfortunately, the exact bioavailability (and source) of these compounds have yet to be determined. In freshwaters, several groups have used genetically engineered strains of cyanobacteria (Porta et al. 2003) and heterotrophic bacteria (Mioni et al. 2003) that report (via bioluminescence) on the in situ Fe physiology of the cells to gain insight into how these cells perceive bioavailable Fe in a background of naturally occurring ligands; unfortunately, similar strains have yet to be used in marine systems. As such, other than the use of model Fe-binding ligands (e.g., Hutchins et al. 1999b), estimates of variation in the bioavailability among Fe-ligand complexes in marine systems remain a mystery. The results of the current study highlight the fact that, although model ligands such as siderophores may provide useful insights into high-affinity transport systems used by prokaryotes (e.g., Butler 1998), these systems must interact and compete with a complex combination of different Fe-bound complexes.

Estimating Fe turnover by viruses in the eastern subtropical Pacific—To determine whether the estimated virus-mediated release rates of Fe were enough to satisfy phytoplankton demands, we measured ⁵⁵Fe-uptake rates at two stations in the Pacific Ocean. It should be noted that, as was mentioned in Results, the estimated release rates that we compare with the uptake rates are potentially confounded (both positively and negatively): Wilhelm et al. (1998) found that burst sizes of only 10–20 were common in oligotrophic waters of the Gulf of Mexico, with near-shore infected cells producing burst sizes of 30–60. As such, our estimates of virus-mediated Fe regeneration may be under- or overestimates by as much as 2-fold. A further caveat to this is that we assumed all the viral production to be the result of bacteriophage lysis of heterotrophic bacteria; although it has been shown that both cyanobacteria and eukaryotic phytoplankton can be infected by viruses, it is believed that the majority of viruses in marine systems infect heterotrophic bacteria (Fuhrman 1999; Wilhelm and Suttle 1999). Finally, we used the conservative estimate of 25% partitioning into

the dissolved phase for lysates. We hypothesize that, in field samples, this value is most likely higher, because lower cell densities in the field no doubt lead to less aggregation than in higher density lab cultures.

In total, the results imply that viruses could supply a major fraction of the daily algal demand for recycled Fe. Although our laboratory experiments show that heterotrophic bacteria are better able to take up Fe from lysis products than are algal cultures, in HNLC upwelling regimes heterotrophic bacteria have been shown to be limited by carbon, not Fe (Kirchman et al. 2000). Incubation studies in the South American eastern boundary current upwelling regime have demonstrated that the augmentation of ambient dissolved Fe concentrations by 0.1–2.5 nmol L⁻¹ can increase in phytoplankton biomass by up to 250% within 48–72 h (Hutchins et al. 2002; Eldridge et al. 2004). Given the sensitive nature of these natural populations to dissolved Fe concentrations, it becomes obvious that the Fe released by viral lysis may greatly affect the Fe bioavailability in this system. As well, given this work and previous studies using model ligands (Hutchins et al. 1999b; Eldridge et al. 2004), it appears that a key to understanding this cycle depends on the differentiation between the Fe(II) and Fe(III) pools as well as the mechanism by which Fe is recycled.

The chemical speciation of Fe in viral lysis products is not known, but Fe released from cells is most probably in a variety of different Fe(II) and Fe(III) organic complexes. Uncomplexed intracellular Fe is virtually nonexistent because of its toxic nature, and Fe(III) in cells is generally concentrated in storage components (e.g., bacterioferritins), which would presumably not be found in cells from Fe-limited environments. In contrast, regenerated Fe released as a by-product of grazing is a mixture of both organic and (primarily) inorganic forms: the inorganic Fe being generated as Fe-organic complexes pass through the acidic digestive system of the grazer (Fok et al. 1982), followed by the rapid oxidation of Fe(II) to Fe(III) in the oxic marine environment. In combination with photolysis in surface waters (Barbeau et al. 2001), these processes provide for distinct pools of the two valences of Fe that marine plankton can draw from.

To better visualize this, we have generated a schematic for the biological cycling of Fe in marine systems (Fig. 5) based on the assimilation efficiencies of Fe-ligand complexes described here as well as in Hutchins et al. (1999b). As was discussed above, the current dogma suggests that “new” Fe entering marine surface waters (as inorganic dust or from vertical advection) must be complexed to metal-free ligands to keep it in solution and make it bioavailable. The presence of siderophores in a system allows for siderophore-producing members of the community (which are mostly prokaryotes) (Wilhelm and Trick 1994; Butler 1998) to acquire this Fe. The Fe is then recycled back to either the inorganic pool (via grazing) or to the pool of organically complexed compounds (primarily via viral lysis). The organic Fe from the lysis products then becomes available to marine eukaryotes (potentially through surface reductases activity; Maldonado and Price 2001). The present study demonstrates that the release of Fe by viral activity may be a major pathway in this cycle.

Support for these ideas comes from previous mesoscale

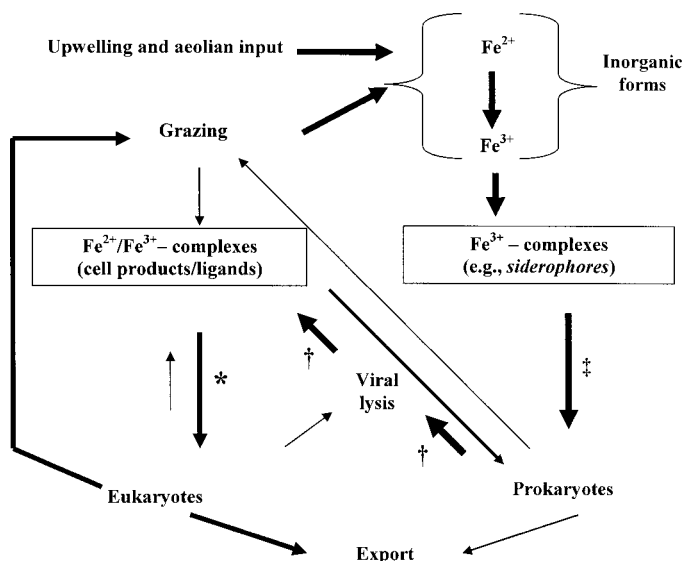


Fig. 5. Flow diagram of the major pathways for the steady-state biological cycling of iron in marine systems. Arrow weight reflects the hypothetical importance of pathways in HNLC environments. Results demonstrated that the phytoplankton Fe demand (*, 19.5–30.6 pmol L⁻¹ d⁻¹) can be completely offset by dissolved, organically complexed Fe generated from the virus-mediated lysis of bacterioplankton (†, 19.2–75.7 pmol L⁻¹ d⁻¹). The bacterial Fe uptake (‡, 160–181 pmol L⁻¹ d⁻¹) is sufficient to satiate this and is itself supported by regenerated Fe from grazing activity, virus-mediated lysis, and new Fe (aeolian and upwelling) inputs.

Fe addition experiments, which have demonstrated that the addition of Fe results in an increase in Fe binding organic ligands (Croot et al. 2001). Previous researchers have suggested that ligand production may be an active response to added Fe by the native microbial population. Although siderophore production may account for at least some of these compounds (Butler 1998), it is important to remember that siderophore production is a response to growth-limiting levels of Fe (Wilhelm and Trick 1994). In laboratory studies, siderophore production by marine bacteria and cyanobacteria is an exclusive response to decreases in available Fe (Wilhelm and Trick 1994, 1995; Butler 1998). So, although siderophores may play a significant role under normal conditions in Fe-limited environments, when Fe is added (as in mesoscale addition experiments), siderophore production should be repressed. We propose that the increased production of Fe-binding ligands is largely the result of microbial mortality. As pelagic assemblages respond to Fe inputs from fertilization, the increase in cell density results in a direct increase in microzooplankton grazing as well as viral infection. The latter point is because the frequency of virus-host contact is directly proportional to both the densities of the host cell and of the virus (Wilhelm et al. 1998). It is therefore evident that viral lysis rates will increase with Fe-induced biomass increases, and suggests that Fe-ligand concentrations could increase in marine systems after Fe fertilization because more cellular debris from viral activity will be produced.

This hypothesized viral control of the cell abundance suggests that prokaryotic abundance should stay low relative to

increases in carbon production during fertilizations: observations made during the IronEx fertilization experiments demonstrated only a ~1.7-fold increase in bacterial abundance, relative to a ~4-fold increase in bacterial carbon production (Cochlan 2001), with similar observations arising from bottle incubations in the Californian upwelling (Hutchins et al. 1998). Although this is usually attributed to a grazer-mediated control of bacterial biomass, it is evident that viruses should be major players in this process. The role of viral activity in the release of cellular Fe-ligand complexes is further supported by the fact that significant Fe(II) was detected during the SOIREE experiments, presumably bound to Fe(II) ligands (Croot et al. 2001). This Fe(II) could possibly have been released as a result of cell mortality.

Resolving the complex web of factors that influence marine Fe cycles is critical to our understanding of the biological role that marine plankton play in global carbon budgets and to an eventual resolution of the “ocean Fe fertilization” debate. Over the past two decades, we have gained an appreciation for the importance of the microbial components in marine systems and their influence on biogeochemical cycles: the present article highlights this role and demonstrates that even in their death microbes exert this considerable influence. Viral lysis produces a suite of Fe-binding compounds of varying bioavailability to marine plankton. Although there is little doubt that other “top-down” mechanisms and processes (e.g., heterotrophic consumption of Fe-ligand complexes leading to Fe release) are involved, our results demonstrate that viral activity is of vital importance and along with grazing regenerates supplies of organically complexed Fe that are critical to biological communities growing in Fe-limited HNLC conditions.

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