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The response of the virus community to the SEEDS II mesoscale iron fertilization

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ABSTRACT

Although the important role of viruses in marine biogeochemical cycles has been established in recent years, virus activity (including changes in this activity) has been largely ignored during mesoscale iron (Fe)-fertilization experiments relative to other processes. This is of particular interest as viruses have been shown to be critical to the transformation of Fe from the particulate (i.e., biological) to the dissolved pools. The goal of the present study was to evaluate changes in the virus-mediated lysis of heterotrophic bacterial cells following a shift in ecosystem trophic status brought about by a mesoscale Fe addition in the subarctic Pacific Ocean. Virus production rates, estimated by a reduction and reoccurrence assay, were coupled with transmission electron microscopy estimates of burst size and direct counts of virus and bacterial abundance. Fe fertilization of the upper mixed layer resulted in significant yet weak increases in virus production rates during the 12 days of observation immediately after fertilization, although the burst size (viruses produced per lytic event) and the percentage of visibly infected cells remained constant. The results imply that increases in virus production rates were most likely tied to a decreased lytic cycle length or the stimulation of lysogenized cells following the stimulation of primary and secondary productivity by the addition of Fe. The results also indicate that virus-induced cell-lysis regenerated an estimated nearly 200 pmol L^{-1} Fe daily, providing a significant return of Fe back to the water column, which may be critical in the maintenance of this added Fe as resident.

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1. Introduction

The limitation of primary productivity by iron (Fe) availability in high-nutrient, low-chlorophyll (HNLC) regions, including the subarctic Pacific, equatorial Pacific, and the Southern Ocean, has been well studied since the development of trace-metal-clean sampling techniques (Bruland et al., 1979). Fe-addition experiments in the equatorial Pacific Ocean (Coale et al., 1996; Martin et al., 1994) the Southern Ocean (Boyd et al., 2000) and the subarctic Pacific Ocean (Boyd et al., 2004; Tsuda et al., 2003) have been used to test Martin's hypothesis (Martin et al., 1990) that Fe availability limits rates of phytoplankton productivity in HNLC waters.

Two Fe-fertilization experiments in the subarctic Pacific Ocean [the subarctic Pacific iron experiment for ecosystem dynamics study, "SEEDS" (Tsuda et al., 2003) and the subarctic ecosystem response to iron-enrichment study "SERIES"(Boyd, 2004)] have demonstrated that the availability of elemental Fe limits primary productivity in this region. Both studies reported significant increases in chlorophyll *a*, coupled with a rise in diatom abundance upon the addition of Fe. The SEEDS experiment resulted in a nearly 20-fold increase in chlorophyll *a* (Tsuda et al., 2003). While it is known that this bloom consisted primarily of the centric diatom, Chaetoceros debilis, there have been limited reports concerning other members of the microbial food web (Suzuki et al., 2005), and nothing is reported concerning the abundance or activity of viruses following the addition of Fe. In fact, during the six published Fe-fertilization experiments, the activity of the virus community has been left unstudied.

During a 2003 study ("FeCycle"), which produced an unperturbed geochemical budget for the pelagic Southern Ocean (Boyd et al., 2005), increased virus production rates were observed during a coincidental natural increase in both dissolved Fe and chlorophyll *a* concentrations (Strzepek et al., 2005). These

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results raised questions as to the potential response of viruses to changes in ecosystem productivity. Increases in bacterial abundance associated with changes in system productivity should result in increased rates of contact between viruses and their hosts (Murray and Jackson, 1992) and subsequently a marked increase in viruses specific for that host. Known as the "kill the winner" hypothesis (Thingstad and Lignell, 1997), this idea has been invoked in discussions of how viruses may be involved in the termination of algal blooms (Bratbak et al., 1993; Gastrich et al., 2004) and may in part answer G.E. Hutchinson's Paradox of the Plankton (Brussaard et al., 2008; Hutchinson, 1961; Wilhelm and Matteson, 2008). This hypothesized virus control of cell abundance suggests that prokaryotic abundance should stay low relative to increases in the rate of bacterial carbon production during fertilizations. Observations made during the original mesoscale Fe-fertilization experiment, IronEx (carried out in the eastern equatorial Pacific Ocean), 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) and (at least in terms of bacterial abundance) the original SEEDS survey (Suzuki et al., 2005).

In July and August 2004, a second subarctic Pacific Ocean iron experiment for ecosystem dynamics study (SEEDS II) took place at the same location of SEEDS I (48.5°N, 165°E). As a component of this program, changes in the abundance and activity of the virus community following the mesoscale Fe fertilization were monitored to test the hypothesis that virus activity would offset increases in bacterial abundance due to enhanced bacterial growth rates (be it from alleviation of Fe- or DOM-limitation). The virus reduction and reoccurrence method for estimating virus production rates (Wilhelm et al., 2002) were combined with TEM observations of burst size and visibly infection of cells (Binder, 1999; Proctor et al., 1993) during this study to monitor changes in virus activity. These observations have been used to determine if changes in ecosystem trophic status may affect short-term virus activity, as well as to determine influences on local geochemical cycles, heterotrophic bacterial abundance and the partitioning of nutrients between dissolved and particulate pools.

2. Materials and methods

2.1. Study site and sampling

Samples from the subarctic Pacific Ocean were collected between 16 July and 5 August 2004 in the same area as SEEDS I (Tsuda et al., 2003) while onboard the RV *Hakuho Maru* using acid-washed Niskin-X bottles and titanium wire. This cruise was the first of third to examine the evolution of the fertilized patch (and the only cruise our group participated on). Chemical and biological parameters were surveyed prior to the release of two infusions of Fe on as previously described (Tsuda et al., 2003, 2007; see also other articles in this volume). To track the water mass to which the Fe was added, the upper mixed layer of the water column was labeled with the inert tracer sulfur hexafluoride (SF_6) (Law et al., 1998), which was combined with iron sulfate dissolved in seawater, and the region was monitored for 12 days in areas denoted within and outside the fertilized region (the Fe "patch").

2.2. Chlorophyll a, bacterial and viral abundance, and bacterial production

Phytoplankton biomass was inferred from estimates of chlorophyll *a* concentrations. Samples (50 mL) for comparison to virus activity were collected onto 0.2-μm nominal pore-size polycarbonate filters (47-mm diameter; Millipore) and stored in the dark at −20 °C until processing. Chlorophyll *a* was extracted from samples with 90% acetone (24 h, 4 °C in the dark) prior to quantification using a Turner Designs TD700 fluorometer using the non-acidification approach (Welschmeyer, 1994).

Samples for the estimation of the abundance of virus and bacteria abundance were collected from stations both inside and outside of the fertilized patch and immediately preserved in glutaraldehyde (final concentration, 2.5%) to inhibit degradation. Samples (2 mL) for bacterial abundance measurements were collected onto 25-mm diameter, 0.2-μm nominal pore-size black polycarbonate filters (Poretics) and stained with Acridine Orange (Hobbie et al., 1977). To determine virus abundance, samples were diluted [80 μL sample plus 720 μL ultra-filtered seawater (<30 KDa)], collected on 25-mm diameter, 0.02-μm nominal pore-size Anodisc (Whatman) filters and stained with SYBR Green I (Noble and Fuhrman, 1998) immediately at sea (Wen et al., 2004), mounted on glass slides and stored frozen (−20 °C) until analysis. The abundance of virus-like particles was estimated by epifluorescence microscopy. Both bacterial and virus abundance were estimated with an epifluorescence microscope (Leica DMRXA) using a standard "wide blue" filter set ($Ex_\lambda = 450–490$ nm; $Em_\lambda = 510$ nm; suppression $filter_\lambda = 510$ nm). In each case, 200 particles or 20 fields of view were enumerated. Bacterial production rates at sea were estimated using the ^{3}H-leucine incorporation microcentrifuge method and conversion rates previously described (Kirchman, 2001).

2.3. Burst size and the percentage of visibly infected cells

Whole water (40 mL) was preserved with TEM grade glutaraldehyde (as above) and stored in the dark at 4 °C. These samples were subsequently collected onto carbon-coated collodion films (Electron Microscopy Sciences) atop 400-mesh electron microscope grids by centrifugation. Grids were rinsed with sterile water and stained with 0.75% uranyl formate. The percentage of visibly infected bacterial cells and burst size were determined by use of transmission electron microscopy [(TEM) Hitachi H-800; accelerating voltage, 100 KeV] (Weinbauer and Suttle, 1996). For each sample, three grids were prepared and at least 1000 bacteria cells were examined per grid. Burst size was defined as the average number of virus particles in all visibly infected cells (VIC). This is likely the minimum burst size, as more viral particles may accumulate within an infected cell before it lyses.

2.4. Viral production rates and virus-mediated bacterial mortality

Viral production rates and inferred virus-mortality were estimated using the dilution approach (Wilhelm et al., 2002) and TEM data (Binder, 1999; Proctor et al., 1993). For the dilution assay the planktonic community from 450 mL of seawater was gently collected over a 0.2-μm nominal pore-size polycarbonate filter (47-mm dia., Millipore) to reduce the abundance of free virus particles from the sample. Cells in the sample were continuously resuspended with a transfer pipette, while the initial volume was held constant with the addition of virus free, ultra-filtered (<30 kDa) seawater. Three subsamples (150 mL) were transferred to individual 500-mL polycarbonate bottles and incubated at in situ temperatures in the dark. A 4-mL subsample was collected from each bottle every 2.5 h. Each experiment was limited to 10 h to reduce the potential for infection of cells by newly produced viruses. Samples for virus enumeration were prepared and processed as described above. Mean virus production rates (viruses L^{-1} d^{-1}) were calculated from the reoccurrence of viruses

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in each replicate over time (e.g., Fig. 1). Virus-induced mortality rates from the dilution experiments (VIM_d) were inferred by dividing the virus production rates by the empirically determined burst size for each station, giving the abundance of bacterial cells lysed L^{-1} d^{-1} (Poorvin et al., 2004).

In laboratory studies, virus particles are only visible by TEM during the final ~10% of the lytic cycle (Valentine and Chapman, 1966). To this end, a range for the percentage of infected cells (FIC) is calculated from VIC obtained by TEM (Proctor et al., 1993) and these estimates used to calculate virus-mediated mortality of bacteria (the percentage of the total bacterial mortality that is attributable to viruses, VMB) according to the following equations (Binder, 1999):

$$FIC = 7.1VIC - 22.5VIC^2 \quad (1)$$

$$VMB = (FIC + 0.6FIC^2)(1 - 1.2FIC)^{-1} \quad (2)$$

Estimates of the rates of virus-induced bacterial lysis were subsequently calculated from the virus dilution assay as were the rates of nutrient regeneration (Poorvin et al., 2004; Wilhelm et al., 2002) following the virus-mediated lysis of bacterial cells using assumed bacterial cell quotas of 1.1 ag Fe $cell^{-1}$ (Tortell et al., 1996). Similarly, regeneration rates were calculated from the dilution approach using cellular quotas of 0.5 fg phosphorus $cell^{-1}$ (Heldal et al., 1996), 5.6 fg nitrogen $cell^{-1}$ (Lee and Fuhrman, 1987), and 23.3 fg carbon $cell^{-1}$ (Simon and Azam, 1989).

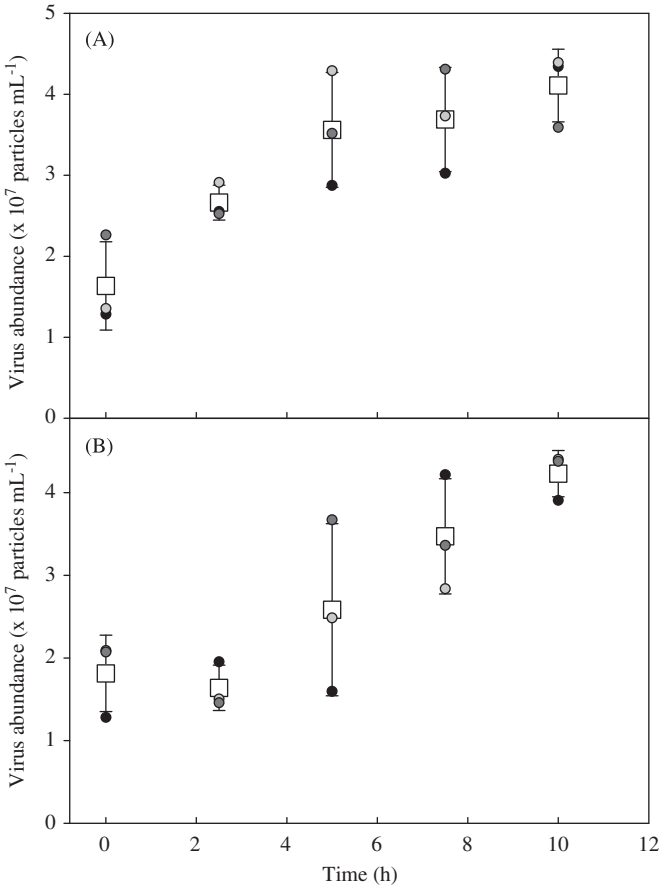


Fig. 1. The reoccurrence rate of viruses in dilution assay carried out on day 5 from inside (A) and outside (B) the fertilized patch. Virus abundances are given for individual replicates (small circles) as well as for the mean (square icons ± S.D.) of samples collected at the same time.

2.5. Tests for statistical significance

All statistical tests for differences between "in" and "out" stations (i.e., before or after Fe addition) were completed with independent *t*-tests. Statistical tests for differences between the two methods for measuring viral production were conducted by using paired sample *t*-tests using 95% confidence levels. The correlation between viral activity and chlorophyll *a* concentration were completed using Pearson correlation. All statistical analyses were conducted using SPSS 12.0 software and *p* values are reported when $p > 0.001$.

3. Results

3.1. Dissolved Fe and chlorophyll a

Inside the patch, dissolved iron (dFe) concentrations increased from 18 pmol L^{-1} before fertilization to ~300 pmol L^{-1} by day 4, and increased again after the second addition to 660 pmol L^{-1} by day 8. This dFe concentration in the patch was significantly different (higher) than measured concentrations prior to fertilization ($p = 0.033$). Dissolved Fe concentrations within the patch subsequently decreased until the end of the experiment. The dissolved Fe outside of the patch averaged 89.6 pmol L^{-1} and remained significantly lower ($p = 0.021$) than that inside the fertilized area (Fig. 2A).

Concurrent to the increase in dFe concentrations, chlorophyll *a* concentrations increased significantly ($p < 0.001$) from 0.36 μg L^{-1} before Fe addition to as high as 2.25 μg L^{-1} after fertilization. As was observed for iron, chlorophyll *a* remained significantly lower ($p = 0.001$) outside the patch than inside (Fig. 2B).

3.2. Bacterial and viral abundance

The highest bacterial abundance observed during our study occurred during the pre-fertilization survey, prior to the addition of Fe in the study area [mean (±S.D.) = 1.55 (±0.3) × 10^9 cells L^{-1}, $n = 3$]. These observations combined with water chemistry suggest this was potentially due to instability of the water mass (Tsuda, unpublished data). Following Fe fertilization, bacterial abundance significantly decreased ($p < 0.001$) and remained lower than the initial abundance [mean (±S.D.) = 5.07 (±1.8) × 10^8 cells L^{-1}, $n = 3$]. Although initial abundance estimates were high, the bacterial abundances found for the remainder of the study were not significantly different ($p = 0.59$) from those outside of the patch prior to day 12. There was, however, a significant increase ($p < 0.001$) in bacterial abundance observed on day 12 inside the Fe patch (Table 1).

Virus abundance was also high [mean = 5.3 (±0.03) × 10^{10} particles L^{-1}] prior to fertilization. After the Fe addition, a small though insignificant ($p = 0.60$) decrease was seen, but the abundance increased significantly ($p = 0.003$) by the end of the study period. As was observed for bacterial abundance, viral abundance inside the patch was not substantially different ($p = 0.701$) from estimates from outside (Table 1).

3.3. Viral and bacterial production measurements

Although viral production was not significantly correlated with the increase in chlorophyll *a* ($r = 0.173$; not shown), it significantly increased at the end of the study ($p = 0.001$). No significant increase in the percentage of visibly infected bacteria within the patch (Fig. 3a). At the beginning of the experiment, ca. 0.7–0.8% of the cells carried a visible virus infection. This estimate peaked on

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day 5 (~1.2% of cell) although it was not significantly different than the initial or final (~0.9%) estimates from within the patch. These results suggest that during the first 12 days after fertilization there was not a significant increase in the frequency of infections (or at least those with lytic cycles of <12 days).

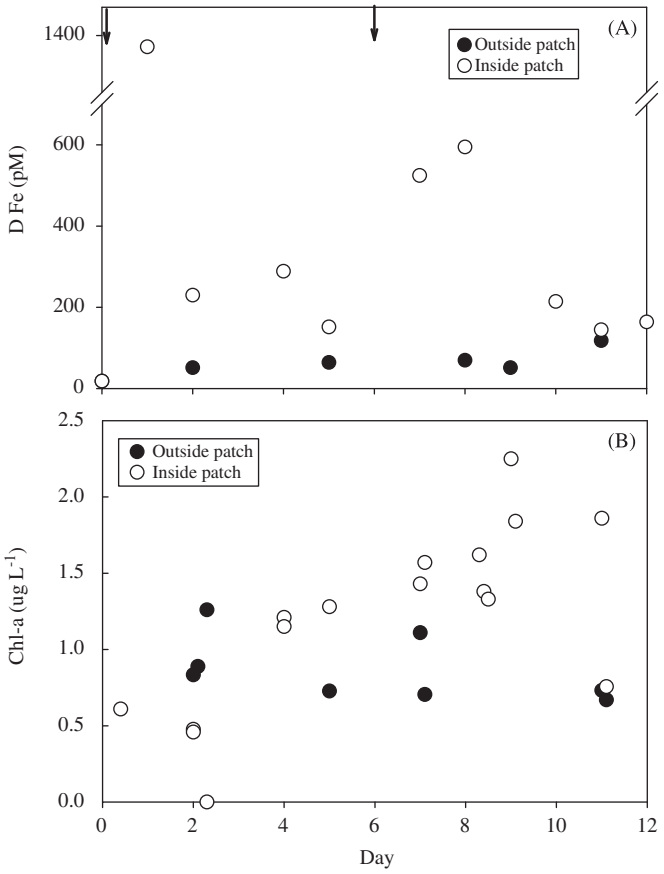


Fig. 2. Dissolved Fe and chlorophyll *a* inside and outside of the Fe patch (5 m) during SEEDS II. Samples for chlorophyll *a* were collected from the same samples where virus-production rates were estimated. Significant increases can be seen in both dFe (A; $p = 0.033$) and chl-*a* (B; $p < 0.001$) inside the patch following the addition of iron (arrows), but not outside.

Using the dilution method, VIM_d increased from 2.58 (± 0.61) $\times 10^9$ prior to Fe addition to 9.00 (± 2.5) $\times 10^9$ cells lysed $L^{-1}\,d^{-1}$ by the end of the study. Measurements taken inside the patch (after day 5) were significantly higher than those taken outside ($n = 4$, $p = 0.023$).

Bacterial production increased after the Fe fertilizations through the first 12 days, and correlated ($r = 0.647$, $p = 0.02$) with the increased concentrations of chlorophyll *a* in the water column (Fig. 4A). As part of these observations, the hypothesis that bacterial production and virus production would be correlated was also tested; indeed, although virus production remained constant at bacterial production rates up to ~3.0×10^8 cells $L^{-1}\,d^{-1}$, increases beyond this rate corresponded with increases in virus production rate (Fig. 4B). If we remove the outlining data point (from immediately after the fertilization as noted on the graph) the result is a positive correlation between bacterial production and virus production rates ($r = 0.780$, $p = 0.08$).

3.4. VMB, burst size and nutrient remobilization

Virus-mediated mortality of bacterial cells (VMB) estimated using TEM did not vary inside or outside of the Fe patch ($p = 0.921$; Table 1) and did not significantly change upon the addition of Fe ($p = 0.251$). The percentage of the heterotrophic population whose mortality was attributable to virus-mediated lysis was 7.32% (± 1.33) inside the patch and 7.16% (± 1.53) outside. Burst size followed similar trends, where values reflected no significant change after the addition of Fe ($p = 0.644$) and showed little difference inside or out of the patch ($p = 0.504$) during the study [mean = 12.9 (± 2.1), $n = 168$ inside; 12.1 (± 0.8), $n = 81$ outside].

The nutrient remobilization estimates found using the dilution assay are consistent with values given in previous studies (Poorvin et al., 2004; Wilhelm and Suttle, 1999). In the waters outside of the patch, viral lysis of bacterial cells was responsible for the daily remobilization of 191.8 pmol L^{-1} Fe, 9.73 μmol L^{-1} C, 2.00 μmol L^{-1} N, and 80.09 nmol P L^{-1} (Table 1). Within the patch virus

Table 1
Parameters of virus infection, virus and bacterial abundance ($n = 3$, ±SD) and estimated nutrient remobilization ($n = 3$, ± propagated SD from virus production estimates) during SEEDS II inside and outside the Fe-amended patch.

Sampling	Burst size	*n*	VMB (%)	Depth (m)	Bacteria ($\times 10^9$ L^{-1})	Viruses ($\times 10^{10}$ L^{-1})	Nutrient remobilization by virus activity ($L^{-1}\,d^{-1}$)			
							Fe (pmol)	C (μmol)	N (μmol)	P (nmol)
Inside the patch										
PS	12.0	31	8.3	5	1.55 (±0.29)	1.89 (±0.06)	125.7 (±2.9)	5.7 (±0.1)	1.2 (±0.0)	47.3 (±1.1)
PS	15.2	17	7.3	10	1.63 (±0.25)	7.12 (±0.72)	103.2 (±24.1)	5.2 (±1.2)	1.1 (±0.3)	42.9 (±10.0)
Day 02a	12.4	23	6.4	5	0.54 (±0.06)	2.16 (±0.39)	87.6 (±53.5)	2.5 (±1.5)	0.5 (±0.3)	20.7 (±12.6)
Day 02b	10.7	13	5.5	10	0.50 (±0.11)	7.68 (±1.23)	194. 8 (±61.2)	11.5 (±3.6)	2.4 (±0.7)	95.5 (±30.0)
Day 04	10.3	23	6.6	5	0.44 (±0.18)	2.07 (±0.36)	141.5 (±40.4)	8.0 (±2.3)	1.6 (±0.5)	66.3 (±18.9)
Day 05	14	11	10.1	5	0.49 (±0.06)	2.05 (±0.30)	80.7 (±18.1)	4.5 (±1.0)	0.9 (±0.2)	37.6 (±8.5)
Day 08a	12.1	31	8.9	5	0.40 (±0.08)	2.93 (±0.32)	179.1 (±66.9)	8.6 (±3.2)	1.8 (±0.7)	71.2 (±26.6)
Day 08b	12.5	28	7.7	10	0.45 (±0.09)	5.33 (±1.13)	105.5 (±58.0)	4.3 (±2.4)	0.9 (±0.5)	36.1 (±19.8)
Day 10	16.6	22	6.1	5	0.51 (±0.05)	2.95 (±0.34)	61.5 (±38.8)	2.0 (±1.3)	0.4 (±0.3)	16.7 (±10.5)
Day 12	15.6	17	7.1	5	0.97 (±0.14)*	6.35 (±2.21)	175.8 (±42.3)	7.9 (±1.9)	1.6 (±0.4)	66.6 (±16.0)
Outside the patch										
Day 2	11.5	20	5.7	5	0.60 (±0.10)	2.96 (±0.75)	152.5 (±20.9)	5.4 (±0.7)	1.1 (±0.2)	44.2 (±6.1)
Day 5	12.8	22	6.7	5	0.41 (±0.03)	2.26 (±0.27)	98.7 (±11.7)	2.3 (±0.3)	0.5 (±0.1)	19.1 (±2.3)
Day 11a	12.9	25	7.0	5	0.62 (±0.00)	4.55 (±0.74)	191.8 (±34.8)	9.7 (±1.8)	2.0 (±0.4)	80.9 (±14.7)
Day 11b	11.4	14	9.3	10	0.70 (0.07)	4.49 (±1.08)	339.8 (±108.7)	20.1 (±6.4)	4.2 (±1.3)	167.4 (±53.6)

Estimates for Fe and nutrient remobilization were determined from the estimates of cells lysed per day. VMB = virus-mediated bacterial mortality estimated from TEM observations. *n* = number of replicates examined by TEM for each experiment. *The bacterial abundance on day 12 was statistically higher ($p < 0.05$) than the abundance on previous days, post-fertilization. PS = pre-fertilization survey. Nutrient remobilization for individual nutrients was calculated as (virus production/burst size) × (cell quota).

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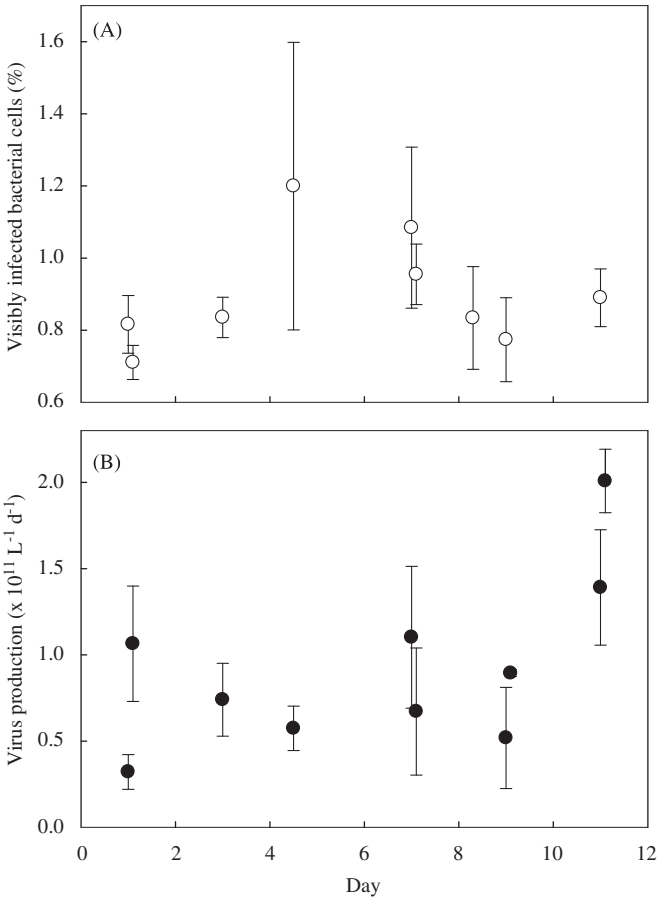


Fig. 3. Percentage of visibly infected cells estimated by TEM (A) and estimates of virus production from the virus dilution and reoccurrence assay (B) inside the patch during SEEDS II after Fe addition. Results are mean values for three replicates (±SD).

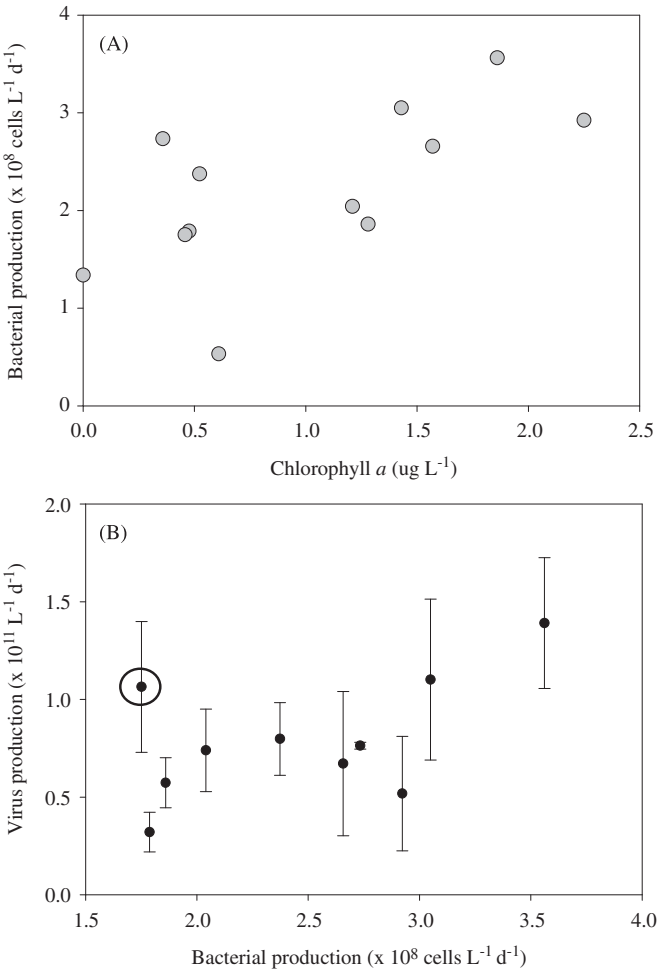


Fig. 4. Bacterial production within the patch relative to chlorophyll *a* (A) and dilution estimated virus production rates (B). Circle denotes point immediate after fertilization where a spike in virus production occurred. The correlation between virus and bacterial production rates without this point ($r = 0.78$, $p = 0.08$) is significant (when $p < 0.10$). Results for the virus production assays are means of independent triplicates (±SD).

activity released 194.8 pmol Fe L^{-1}, 11.47 μmol C L^{-1}, 2.36 μmol N L^{-1}, and 95.45 nmol P L^{-1} on day 2, just after the addition of Fe (Table 1).

4. Discussion

There are several important observations reported within these results. Although the addition of Fe did stimulate primary production, the fertilization resulted in a smaller increase in phytoplankton biomass than was observed in the previous study of this area or than was predicted at the onset of this work (Tsuda et al., 2007). As such, any conclusions or new hypotheses that arise from this data must be considered within these constraints. Within the framework of this study, some parameters associated with virus infection (i.e., burst size, the percentage of cells that were visibly infected) remained static, while the abundance of virus particles and the rate of virus production increased within the Fe infused patch at the end of our observations. These data and their implications for the ecology of marine prokaryotes and system geochemistry are discussed below.

The goal of this study was to examine how a shift in system trophic status (i.e., an increase in productivity) would influence virus–host interactions, and determine whether the virus community may act to constrain any increase in the abundance of bacteria resulting from Fe fertilization. The results support the hypothesis in that there was an observed increase in virus activity (production and abundance) after system productivity had been stimulated by the mesoscale addition of the limiting nutrient of photosynthetic carbon fixation (in this case, Fe). Unfortunately for this study, however, the time lag between the fertilization and the response led to these events only being documented as we prepared to leave the area.

Of interest is the significant increase in both bacterial and virus abundance that occurred by day 12. It was hypothesized at the onset of this study that virus production and abundance would increase after the addition of Fe. This was validated by the increase in virus production rates seen inside the patch relative to those outside of the fertilized patch at the end of our observations. While it is possible that these increases could be caused by the natural trends, it is most likely that the addition of Fe caused the rise in microbial activity observed in this study. Chlorophyll *a* concentrations increased after the addition of Fe (Fig. 2b), and the decay of phytoplankton would have released a substantial supply of dissolved organic matter (DOM) leading to an increase in bacterial abundance (Kirchman et al., 2000). Subsequent to this, the enhanced contact rates between viruses and the bacterial hosts would lead to increased infection rates, thus serving as a feedback on community bacterial production [the essence of the "kill the winner" hypothesis (Thingstad and Lignell, 1997)]. Therefore, while the addition of Fe most likely did not directly lead to an increase in bacterial or viral abundance, the indirect

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result of enhanced primary production was increases in both populations.

One important observation within this study was the delay in this response. It was anticipated that this series of events would have occurred within a few days of the Fe release based on observations from the FeCycle study (Strzepek et al., 2005). As such, although changes were detected during our field observations, additional sampling after day 12 would have been useful for constructing a clearer picture. In previous observations of a natural shift in available Fe (Strzepek et al., 2005) it was concluded that the accumulation of phytoplankton and virus abundance were more tightly linked. In the case of the current study, differences in community structure as well as other pressures (e.g., grazing) may have in some way also influenced the ontogeny of the bloom and the influence of viruses.

A second important observation in this study are the high rates of virus production, requiring that up to 100% (or more) of the in situ microbial biomass to be lysed. Similar high estimates of bacterial lysis due to virus activity have been seen in other systems (Wilhelm et al., 2002; Winter et al., 2004), reinforcing the point that aquatic microbial communities are not in steady state (Hutchinson, 1961). While there is little doubt that the imbalance may be from inaccuracies in the estimates of bacterial lysis by viruses (although within sample replication is generally quite high), the production of viruses through the lysis of phytoplankton (Brussaard, 2004; Kimmance et al., 2007) and the reliability of bacterial production estimates (Robarts and Zohary, 1993) could also complicate the results.

It is thus apparent from this and other studies (Weinbauer, 2004) that viruses impart significant mortality on marine microbial communities, and can play an important role in the remobilization of elements from the particulate (biological) to dissolved pool (Wilhelm and Suttle, 1999). Using established cellular quotas, estimations of the rate that nutrients were remobilized from the bacterial pool to the detrital (dissolved plus particulate) pool have been made. Fe remobilization estimates from outside of the patch and pre-survey stations [98.7–339.8 pmol L^{-1} d^{-1}, mean = 168.6 pmol L^{-1} d^{-1}] collected during this study are somewhat higher than those collected during previous studies (Poorvin et al., 2004), where virus-mediated Fe turnover was estimated to be 19.2–75.7 pmol L^{-1} d^{-1} [mean = 53.7 pmol L^{-1} d^{-1}] in samples collected from the Peruvian coast of the Pacific Ocean). In part, these high estimates may be due to the contrasts between the recycling-driven (North Pacific) and upwelling-driven (Peruvian coast of the eastern South Pacific) communities which persist in these two study areas. They may also be a function of the estimates themselves (e.g., see section on burst size, below). However, given the limited number of observations of virus activity in truly offshore marine systems (Rowe et al., 2008) these observations remain important. Further, it remains somewhat tenuous to compare these to rates in coastal environments where variations in light penetration, host (bacterial) abundance or community growth rates can be speculated to generate differences.

Fe-regeneration estimates within the patch were not different from those observed outside of the patch (61.5–194.8, mean = 128.3 pmol L^{-1} d^{-1}). While this was somewhat surprising, it may in part be due to the time lag associated with the shift in trophic status. Boyd et al. (2004) noted that bacterial production rates in a Fe-amended patch in a similar region did not double until days 21–25, well beyond the extent of the observations in the current study. As such, assuming that virus activity was tied to bacterial production (as per above), the Fe-regeneration rates may have been higher after the RV *Hakuho Maru* left the study site. Given that virus-regenerated Fe is highly bioavailable to marine plankton (Mioni et al., 2005; Poorvin et al., 2004), this would have facilitated continued production, albeit through the use of Fe-organics from lysates (and grazing byproducts) and not from the inorganic input from the initial fertilization. In previous fertilization efforts, increases (400%) in Fe-complexing ligands have been reported to occur within 2 days of Fe addition and were suggested to be an active response (e.g., siderophores) by the plankton community (Rue and Bruland, 1997). However, by their definition (Neilands, 1995) and based on observations within lab studies (Wilhelm, 1995; Wilhelm et al., 1998; Wilhelm and Trick, 1994) siderophores are only produced during deficits in Fe concentration. It is more likely in the case of Fe additions that Fe-binding organics are remobilized by grazers (Tsuda et al., 2007) or viruses (this study) during cell lysis.

An important factor in the interpretation of virus production data is burst size (the estimated abundance of virus particles produced per lytic event). As mentioned earlier, burst size estimates were low in this study [mean = 12.9 ($\pm$2.1) inside; 12.1 ($\pm$0.8) outside] relative to previously published estimates (Prada et al., 2006; Weinbauer, 2004) and did not change during the period of Fe fertilization. Virus-induced bacterial mortality derived from low burst sizes yields higher estimates of the abundance of cells lysed each day. For example in the Peruvian upwelling Fe-regeneration estimates (Poorvin et al., 2004), burst sizes were not empirically determined but were estimated to be 25 viruses per lytic event: changing this to the ~13, as was seen in the current study, would have nearly doubled the estimated Fe-regeneration rate and resulted in estimates of Fe remobilization similar to our current observations.

A major shortcoming in marine virus ecology is the lack of a non-invasive approach to measure the length of the lytic cycle (i.e., the time from infection to lysis). In the current study, it has been demonstrated that parameters associated with infection (burst size, percentage of visibly infected cells) do not change after stimulation of system trophic production. However, as it is probable that the length of the lytic cycle within an infected cell is tied to the host's energetics (and hence growth rate), the stimulation of bacterial production associated with enhanced primary producer biomass may have caused the increased virus production. Virus production rate is a function of the length of lytic cycle, burst size and virus-mediated mortality of bacteria, given by the following equation, where V_{prod} is viral production, T_{LC} is the length of the lytic cycle, VIC is the percentage of virus-infected cells, and BS is burst size.

$$V_{prod} \approx (\mathrm{VIC} \times \mathrm{BS}) T_{LC}^{-1} \quad (3)$$

Given this relationship and that there was not a significant change in either the BS or %VIC, the increase in V_{prod} after Fe addition may be due to a shortened lytic cycle associated with the increased bacterial growth rates.

There remain other possible explanations for these observations that include (1) the induction of lysogens by the addition of Fe, and (2) that changes in microbial community structure may have lead to the spike in virus production. The induction of lysogens in this case may explain the increased production of viruses in our samples. However, one would expect that, if lysogens were induced, we would have noted a loss in bacterial production and/or abundance. It may also be that this is offset by the increased growth rate of other cell types. As such, insight into the diversity of bacteria within the community and how this changed over time would be needed. Several studies have examined the impact of changes in Fe concentrations on bacterial diversity (Church et al., 2000; Eldridge et al., 2007; Hutchins et al., 2001) and have returned mixed opinions on Fe's affect on community structure. The addition of this information

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would have increased our ability to interpret our observations, and represents a data set that should be collected in future studies.

5. Conclusions

There remains a gap in our understanding of the intricacies of the microbial community's function in HNLC areas and the world oceans in general. While this study confirms that viral activity is responsible for the regeneration of substantial amounts of nutrients, the biological availability of these nutrients in situ, and how viruses influence planktonic community structure remain unclear. Historically researchers have focused on non-viral mechanisms of mortality (e.g., Tsuda et al., 2007) and ignored the viral component, which we show here to be important in nutrient regeneration. Of great importance is the fact that these nutrients are primarily organically complexed, and will therefore have altered availabilities to the nearby microbial consortium (Brussaard et al., 2008; Poorvin et al., 2004; Wilhelm and Matteson, 2008). These results highlight the potential importance of viral activity in HNLC regions in the recycling of nutrients, albeit that interpretation based on different methodologies requires caution. In the future, more complete examinations of microbial activity, and that of the viral community in particular with regards to nutrient regeneration, in the context of shifts in ecosystem trophic status should no doubt be considered prudent components of any study.

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