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The organic ties of iron

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Chapter 7

Phytoplankton virus production negatively affected by iron limitation

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Abstract

Fe-limited monocultures of the ubiquitous algae *Micromonas pusilla* and *Phaeocystis globosa* were infected with their respective viruses (MpV and PgV) to ascertain the effect of Fe-limitation on phytoplankton host-virus dynamics. The effect of the viral shunt on Fe concentrations and bioavailability is starting to gain attention, since not only is Fe released through lysis, but also its solubility is increased by the simultaneous release of Fe-binding dissolved organic ligands. However, the effect of Fe-limitation on the process of viral lysis itself is poorly understood. In this study fine adjustment of a seawater-based culture medium including the use of ultra-clean trace metal conditions and protocols allowed for Fe-limited growth at nanomolar amounts as opposed to micromolar amounts typically employed in culturing. Viral lysates derived from Fe-limited and Fe-replete (for comparison) hosts were cross-inoculated in hosts of both Fe treatments, to judge the quality of the resulting lysate as well as the effect of Fe introduction after initial infection. For both phytoplankton host-virus systems, the virus burst size reduced strongly under Fe stress, i.e. on average $28 \pm 1\%$ of replete. Moreover, the MpV virus progeny showed highly reduced infectivity of $30 \pm 7\%$, whereas PgV infectivity was not affected. A small addition of Fe to Fe-limited cultures coming from the Fe-replete lysate counteracted the negative effect of Fe-limitation on phytoplankton virus production to some extent (but still half of replete), implying that the physiological history of the host at the moment of infection was an important underlying factor. These results indicate that Fe-limitation has the strong potential to reduce the loss of phytoplankton due to virus infection, thereby affecting the extent of Fe-cycling through the viral shunt. To what extent this affects the contribution of viral lysis-induced organic ligand release needs further study.

7.1. Introduction

Phytoplankton form the base of most marine pelagic food webs and are important in sequestering atmospheric carbon dioxide (CO₂) through photosynthesis. The production of phytoplankton is controlled by physicochemical variables (bottom-up) as well as by biological factors (top-down). Main bottom-up controls of phytoplankton are light and nutrient availability (Behrenfeld et al., 2006). The latter can be subdivided into major (nitrate, phosphate and silicate) and micro-nutrients (e.g. iron) (de Baar et al. 1990; Martin et al. 1990). Top-down factors, e.g. grazing and viral infection, influence the organic matter flux differently (Wilhelm and Suttle, 1999; Weitz and Wilhelm, 2012). While grazing transfers photosynthetically fixed carbon and organic nutrients up the food chain (Calbet and Landry, 2004), viral lysis results in the release of the hosts' cellular content into the surrounding water (Gobler et al., 1997; Wilhelm and Suttle, 1999). Thereupon, the flow of nutrients through the microbial food web is stimulated by bacterial recycling of the dissolved and dead particulate matter (Suttle 2005; Brussaard et al. 2005; Brussaard and Martínez 2008). Virally-induced mortality of various different natural phytoplankton groups was found to be at least an equally important loss factor as microzooplankton grazing (Baudoux et al., 2007; Mojica et al., 2016).

In order to understand and predict changes in phytoplankton community composition, it is important to elucidate how bottom-up and top-down factors interact and affect phytoplankton population dynamics. Several studies using phytoplankton host-virus culture systems showed that major nutrient availability influences viral production (Maat and Brussaard 2016 and see review by Mojica and Brussaard 2014). For example, phosphorus (P) limitation of the virally infected phytoplankton host results in a prolonged latent period, i.e. the time between infection and the initial release of progeny viruses from the host cell, for the infecting viruses (Maat et al., 2014). Moreover, P-stress resulted in reduced viral burst size, i.e. the number of newly formed viruses released per lysed host cell (Bratbak et al., 1998; Maat et al., 2014). These studies proposed shortage of phosphorus as a viral production substrate as well as possible host energy deficiency as reasons for the lower and delayed viral particle yield. There is, however, virtually nothing is known on the effect of micronutrient limitation on phytoplankton host-virus interactions.

Furthermore, as iron (Fe) solubility in seawater is low (Millero, 1998; Liu and Millero, 2002), marine phytoplankton depend on Fe-binding ligands to increase solubility and therefore bioavailability (Gledhill and van den Berg, 1994; Rue and Bruland, 1995). The redox state of Fe is an important factor in Fe bioavailability. The oxidized Fe(III) state is the more stable and thus prevalent

state in marine conditions, while the reduced Fe(II) state is the more bioavailable (Breitbarth et al., 2010; Shaked and Lis, 2012). Release of reactive oxygen species during phytoplankton growth has been shown to contribute to bioavailability of Fe by facilitating reduction of Fe(III) (Kustka et al., 2005; Garg et al., 2007). Furthermore, organic exudates have been connected to lowered Fe(II) oxidation rates experimentally (González et al., 2014). Part of the Fe-binding ligand pool is thought to be of marine biological origin. Strong Fe-binding organic ligands called siderophores are purposefully produced by bacteria (Butler, 2005; Mawji et al., 2011). Humic acids and polysaccharide excretions are other recognized Fe chelators with a biological origin (Laglera et al. 2011; Hassler et al. 2011). The highest Fe-binding ligand concentrations generally correlate with biological activity (Rue and Bruland, 1995; Gerringa et al., 2006; Ibisani et al., 2011). Viral lysis, releasing organic substances in seawater, may well be an important contributor to the ligand pool (Gobler et al. 1997; Poorvin et al. 2004; Poorvin et al. 2011). In these studies by Poorvin and others, it was found that bacterial and cyanobacterial lysates provided organically bound Fe in a form more bioavailable than supplied inorganic, ethylenediaminetetraacetic acid (EDTA) bound or desferrioxamine B (DFB) bound Fe. In comparison to the studied bacteria's self-produced siderophores, lysates were also found to contain more bioavailable Fe.

Fe-limitation negatively affects phytoplankton physiology and growth (Behrenfeld et al. 1996; de Baar et al. 1990; Martin et al. 1990; Timmermans, Gerringa, et al. 2001). Besides energetic consequences of Fe-limitation in terms of the cell's ability to harvest light energy (Geider and La Roche, 1994), Fe is also found to be an essential micronutrient for DNA replication (Netz et al., 2012; Zhang, 2014). As parasites viruses are dependent on the metabolism of their host for the production of their progeny. We hypothesize that viral production depends on the degree of Fe-stress of the host. Viral lysis in turn affects the production of Fe-binding organic ligands and thus the solubility of the limiting Fe. Thus far, in terms of impact on Fe cycling, studies focussed solely on the release of Fe or Fe-binding organic ligands upon viral lysis and not on the virus growth cycle (Gobler et al. 1997; Poorvin et al. 2004; Poorvin et al. 2011). Here we examine virus production characteristics under Fe-limitation for two key ecologically relevant phytoplankton hosts: the nanoeukaryotic bloom-forming Prymnesiophyte *Phaeocystis globosa* and the picoeukaryotic Prasinophyte *Micromonas pusilla*. *Phaeocystis* is a globally occurring, bloom-forming genus (Vaulot et al., 1994), with *P. globosa* ecologically relevant in temperate marine waters (Schoemann et al., 2005). Viruses have been found to drive *P. globosa* bloom decline (Brussaard, 2004a; Brussaard et al., 2005; Baudoux et al., 2006). *M. pusilla* is a common species that is distributed globally

(Not et al., 2004, 2005; Vaultot et al., 2008). It has been speculated that viral control of this species is continuous (Cottrell et al., 1995). As model species for diverse regions and ecological niches, these species were chosen in this study to offer a broad insight in the response to Fe-limitation of phytoplankton host-virus systems in world oceans subject to changing conditions. Limiting concentrations were of ecological relevance to represent a natural context.

7.2. Methods

7.2.1. Experimental design and sampling

Steady state exponentially growing phytoplankton cultures were subdivided per treatment (Fe-limited and Fe-replete) in 6 replicate 500 mL culture flasks. Two days later the viral infection experiment started 3 h into the light period. For each treatment, 2 replicate cultures received viruses produced on Fe-limited host culture (VL), 2 replicates received viruses produced on Fe-replete host culture (VR), and 2 replicates did not receive viruses and served as non-infected controls (C). Fe-limited lysates were added not only to the respective Fe-limited host cultures, but also to the Fe-replete host in order to test for the reduced infectivity we observed under Fe-limitation (see Results). For this reason and to still guarantee a one-step infection cycle we aimed to add 20-25 viruses per algal cell for *P. globosa*. Given lower yields for *M. pusilla*, we endeavoured to add at least 5-10 viruses per algal cell, while still maintaining a ~10% v/v addition. Similarly, Fe-replete lysate was also added to Fe-limited host cultures. This caused a Fe-spike of about 0.9 μM (10% v/v of Fe-replete medium containing lysate), which allows testing whether a spike of Fe influences virus proliferation.

At steady state, i.e. after at least 8 volume changes and consistent phytoplankton counts ($2.1 \pm 0.4 \times 10^6$ and $2.1 \pm 0.7 \times 10^6$ for *P. globosa* and *M. pusilla*, respectively), samples were collected for dissolved macronutrients (nitrogen and phosphorus) and Fe, as well as pigment composition. Nutrient samples (5 mL after washing of filter and tube) were 0.2 μm filtered (25 mm diameter Acrodisc, Pall) and frozen at -20°C until analysis. GF/C filtered (1.2 μm nominal pore size, 25 mm diameter, Whatman) algal pigment samples of 50 mL were frozen at -80°C until analysis. The number of infective phytoplankton viruses was determined using the most probable number (MPN) endpoint dilution assay according to Suttle (1993). In short, 10-fold dilution series were set up in 5 replicate tubes using a dilute Fe-replete phytoplankton culture at a density of $\sim 10^6$ cells mL^{-1} . A row of uninfected control tubes was added to each analysis. Cell lysis was regularly scored by eye and the final score after 14 days post-infection was used to calculate the number of infectious viruses. Dividing this number of infectious by the total number of PgV or MpV provided the %

infectious viruses. MPN data was analysed using the University of British Columbia Computer Science department's Assay Analyser software program (Passmore et al., 2000).

The moment viruses were added, cultures were maintained in-batch. We examined the effect of frequent handling by taking along a control subculture per Fe-limited treatment that was only gently mixed once a day. Samples for phytoplankton and virus abundance as well as photosynthetic capacity (F_v/F_m) were taken every 4 h until full lysis of the cultures (48 h for *P. globosa* and 96 h for *M. pusilla*). Phytoplankton abundance and F_v/F_m were determined directly upon sampling, while viral abundance samples were fixed with glutaraldehyde (EM-grade, 0.5% final concentration), flash frozen in liquid nitrogen and stored at -80°C (Brussaard et al., 2010).

7.2.2. Additional analyses

High Performance Liquid Chromatography (HPLC) pigment analysis was performed on steady state phytoplankton samples after Zapata et al. (2000). Concentrations of dissolved inorganic macronutrients nitrate and orthophosphate were verified as per chapter 2 and were non-limiting at all times ($> 128 \mu\text{M}$ nitrate and $> 8 \mu\text{M}$ phosphate). Verification of the dissolved Fe concentration was done using flow injection analysis after Klunder et al. (2011), also detailed in chapter 2. The detection limit of this method was 0.01 nM.

7.3. Results & Discussion

7.3.1. Steady state

Exponential growth rate (μ_{max}) for both Fe-limited and Fe-replete *P. globosa* and Fe-replete *M. pusilla* was $0.99 \pm 0.11 \text{ d}^{-1}$. *M. pusilla* showed, however, reduced growth under Fe-limitation ($0.63 \pm 0.07 \text{ d}^{-1}$). Initial difficulties encountered with consistent semi-continuous culturing of *M. pusilla* required us to increase the Fe concentration to 3 nM as compared to the 1 nM for *P. globosa*. Still, the lower steady state μ_{max} found for *M. pusilla* under Fe-limitation suggests a less efficient Fe-uptake or utilization of *M. pusilla* as compared to *P. globosa*. However, the photosynthetic capacity (F_v/F_m) of the Fe-limited *M. pusilla* remained high around 0.6. Thus the smaller-sized *M. pusilla* requires more Fe to grow, albeit at a lower μ_{max} , while it is capable of retaining F_v/F_m at a value similar to Fe-replete conditions. Reduced F_v/F_m and growth rate under Fe-limitation has been reported for a small diatom species (Timmermans et al., 2001a); however, both variables were reduced for the same species and not one or the other as found in present study. Our result do not support the earlier reports that small phytoplankton (diatoms and cyanobacteria) are growing better under Fe-limitation than larger phytoplankton (Price et al. 1994; Timmermans, Gerringa,

et al. 2001; Timmermans et al. 2004). Whether the differences are due to species-specific or phytoplankton group related responses is currently unclear.

The cellular Chlorophyll-a concentration at steady state was lower under Fe-limitation compared to the Fe-replete control cultures for both algal species (Tables 1 and 2). For *M. pusilla* the reduction was larger, i.e. Chlorophyll-a concentration was only 40% of Fe-replete concentration, compared to 63% for *P. globosa*. In the Fe-limited *P. globosa* cultures, cellular Chlorophyll-c concentration was reduced (to a similar extent; Table 1), whereas for *M. pusilla* the Chlorophyll-b concentration also decreased compared to Fe-replete (Table 2). Furthermore, Fe-limitation led to a different distribution of light-harvesting xanthophylls in *P. globosa* (Table 1). Cellular 19'-hexafucoxanthin concentration was found 5-fold higher in the Fe-limited cultures as compared to Fe-replete *P. globosa* (4.8×10^{-12} g cell⁻¹), while 19'-butanoyloxyfucoxanthin and fucoxanthin concentrations were 0 and 89% of Fe-replete, respectively. The photoprotective xanthophyll derivatives in Fe-limited *P. globosa* are increased relative to Chlorophyll-a, i.e. the diadinoxanthin concentration over Chlorophyll-a is 162% of Fe-replete (0.58 and 0.57×10^{-11} g x cell⁻¹, respectively) and the diatoxanthin concentration is 181% of Fe-replete (0.08 and 0.07×10^{-11} g x cell⁻¹, respectively). Fe-limited *M. pusilla* cultures (Table 2) had lower cellular Chlorophyll-b and light-harvesting xanthophyll concentrations. Relative to the Chlorophyll-a concentration, photoprotective xanthophyll derivatives antheraxanthin, zeaxanthin and lutein were present in higher amounts. Only violaxanthin remains lower both in absolute and relative terms.

Overall, the pigment analysis shows that for both phytoplankton species the photosystem shifted towards a more photoprotective character under Fe-limited conditions. The lower concentrations of chlorophyll and most light-harvesting xanthophylls furthermore indicated that the Fe-limited cells suffered a lower light-harvesting capacity. Fe is essential for all life and earlier studies showed that Fe-deficiency can lead to anemia in mammals, the dysfunction of Fe-dependent enzymes in yeast, the reduction of the amount of electron-transferring complexes and induction of chlorosis in plants, and a decrease in Fe-intensive light harvesting pigment synthesis and increased photoprotective pigments in phytoplankton (Geider et al., 1993; Mengel, 1994; van Leeuwe and Stefels, 2007; van de Poll et al., 2009; Zhang, 2014). In line with the fact that the Fe concentrations in the Fe-limited cultures were always below the limit of detection, these results confirm that both phytoplankton cultures were indeed Fe-limited, despite that *M. pusilla* showed healthy F_v/F_m . The more pronounced shift to photoprotective pigment production relative to light-harvesting Chlorophyll-a in *M. pusilla* compared to *P. globosa* is in agreement with the strong decline in steady state *M. pusilla* cell abundance with Fe-limitation while

F_v/F_m was unaffected. However, *M. pusilla* was unable to grow at 1 nM Fe while *P. globosa* grew well. Our results imply that *P. globosa* was more affected energetically by Fe-limitation, while *M. pusilla* suffered instead in overall cellular production.

Table 1 Steady state phytoplankton pigment composition in non-infected Fe-limited (first column) and Fe-replete (second column) *P. globosa* cultures. Concentrations of pigments are expressed in 10^{-11} g cell⁻¹. The third column shows the ratio of Fe-limited over Fe-replete cultures, serving to indicate relative shifts in pigment composition. Chlorophylls (1), light harvesting xanthophyll derivatives (2) and photoprotective xanthophyll derivatives (3) are grouped together.

Pigments	Treatment		ratio
	<i>limited</i>	<i>replete</i>	
Chlorophyll a ¹	1.31	2.08	0.63
Chlorophyll c2 ¹	0.31	0.37	0.86
Chlorophyll c3 ¹	0.23	0.48	0.47
19'-hexafucoanthin ²	0.48	0.09	5.16
19'-butanoyloxyfucoxanthin ²	0.00	0.20	0.00
Fucoxanthin ²	1.13	2.01	0.56
Diadinoxanthin ³	0.58	0.57	1.02
Diatoxanthin ³	0.08	0.07	1.07

Table 2 Steady state phytoplankton pigment composition in non-infected Fe-limited (first column) and Fe-replete (second column) *M. pusilla* cultures. Concentrations of pigments are expressed in 10^{-11} g cell⁻¹. The third column shows the ratio of Fe-limited over Fe-replete cultures, serving to indicate relative shifts in pigment composition. Chlorophylls (¹), light harvesting xanthophyll derivatives (²) and photoprotective xanthophyll derivatives (³) are grouped together.

Pigments	Treatment		Ratio
	<i>limited</i>	<i>replete</i>	
Chlorophyll a ¹	2.70	6.78	0.40
Chlorophyll b ¹	2.06	4.50	0.46
Neoxanthin ²	0.44	0.88	0.50
Prasinolanthin ²	0.71	1.52	0.47
Antheraxanthin ³	0.27	0.12	2.33
Zeaxanthin ³	0.17	0.10	1.75
Lutein ³	0.13	0.29	0.44
Violaxanthin ³	0.29	1.14	0.25

7.3.2. Viral infection characteristics

Infection of both phytoplankton species resulted in one-step infection cycles with full lysis of the cultures whereas the non-infected controls grew or maintained constant cell number (Figures 1A,B and 2A,B). Cell growth in the non-infected Fe-replete cultures reflects the synchronized cell division during the dark period (Brussaard et al., 1999). In the Fe-limited cultures growth of *P. globosa* halted, which was due to stress from the frequent sampling, since the Fe-limited subcultures that were sampled only once a day did show some growth (data not shown). The F_v/F_m of the uninfected Fe-replete phytoplankton cultures remained constant (Figures 1C,D and 2C,D), whereby the small variations observed in the Fe-replete *P. globosa* cultures relate to the light:dark cycle (Figure 1D). The Fe-limited non-infected control cultures showed a decline in F_v/F_m over time as a result of the Fe deprivation, but to a lesser extent than the infected cultures.

When infected with the corresponding Fe-treatment virus (i.e. Fe-limited host with VL and Fe-replete host with VR), Fe-limitation resulted for the infected *P. globosa* in slightly delayed (about 4h) and slower cell lysis than for the Fe-replete cultures (full lysis occurring about a day later; Figures 1A,B). F_v/F_m showed similar differences in temporal dynamics between the Fe-limited and Fe-replete infected cultures upon infection with these viruses (Figures 1C,D). At large, the infected *M. pusilla* cultures showed similar results (Figure 2). The

alterations in host physiological condition in the Fe-limited cultures resulted for both phytoplankton species in slower release of virus progeny and reduced virus yield when infected with VL as compared to Fe-replete cultures (Figures 1E,F and 2E,F). The latent period, e.g. the time period to extracellular release of newly produced viruses, was however unaffected by Fe-limitation of the host. Experiments with the same strain of *M. pusilla* did show prolonged latent periods when under phosphorus (P) and nitrogen (N) limitation (Maat et al., 2014; Maat and Brussaard, 2016). These were suggested to be due to reduced substrate and host energy availability resulting from impaired photophysiology under major nutrient limitation. In our study Chlorophyll-a concentration was comparably reduced but F_v/F_m of Fe-limited steady state *M. pusilla* was not impaired. Then again, F_v/F_m of *P.globosa* cells prior to infection was reduced compared to Fe-replete, still not prolonging the latent period. We cannot be sure of the exact underlying mechanism from the here presented data, but the proliferation of DNA viruses is directly dependent on Fe due to the essential role Fe plays in the catalytic centre of ribonucleotide reductase, produced early in infection to support dNTPs production needed for viral DNA synthesis (Romeo et al., 2001). As such, it can be speculated that not the time to produce a virus is affected but instead the number of viruses that can be produced before cell lysis occurs. The burst size of MpV and PgV indeed decreased strongly under Fe-limitation, i.e., to 24 and 29% of the burst size produced under Fe-replete conditions (20 and 196 viruses lysed host cell⁻¹; Table 3). In comparison to *P. globosa*, the reduced growth rate of Fe-limited steady state *M. pusilla* did not significantly affect the extent of reduction in virus burst size. Nonetheless, *M. pusilla* required a higher cellular Fe concentration. This implies that Fe-limited *P. globosa*, able to grow at the lower Fe concentration, displays a more efficient virus proliferation (despite the low F_v/F_m at the start of infection).

Noteworthy, the higher Fe concentration needed to allow for sustainable growth of *M. pusilla* under Fe-limitation did not prevent the loss of infectivity of the virus progeny. Infectivity of the Fe-limited MpV lysate was reduced to 30±7%, while in contrast PgV did not show decreased infectivity for Fe-limited hosts. Our results signify that a stronger Fe-stress experienced by the Fe-limited *M. pusilla* (expressed in reduced growth rate) is more likely responsible for the production of impaired virus progeny than a changed photosynthetic capacity (F_v/F_m 0.2 for *P. globosa* compared to 0.6 for *M. pusilla*). The fact that total virus abundance, as measured after staining with a nucleic acid dye, was higher than the infective abundance indicates that (i) virus particles and (ii) viral nucleic acids were produced. Still, impaired capsid proteins or host receptors may explain a loss of infectivity. Alternatively, processes known to be Fe-intensive are DNA replication and repair (Netz et al., 2012; Zhang, 2014). Failure of DNA

repair could indeed explain the result of reduced percentage infective viruses. Future research should not only focus on the causal aspects per se but also study what causes the dissimilarities in loss of infectivity between the algal viruses since different responses will directly regulate community composition. Furthermore, future studies should screen if and how other marine taxa and different viruses are affected by virus proliferation under Fe-limitation. For example, non-marine tailed bacteriophages have been found to contain iron ions in the tail proteins that can utilize the siderophore-bound iron receptors on the host cell membrane for attachment of the phage and subsequent infection of the bacterial host (Bartual et al., 2010). It is likely that similar interactions also exists for marine bacteriophages (Bonnain et al., 2016). Still open questions are whether such phages will obtain fewer iron ions in their tail under Fe-deprivation, and if this will negatively affect their infectivity. Furthermore, the recently proposed 'Ferrojan Horse Hypothesis' by Bonnain and colleagues (2016) posits that introduction of phage-attached Fe may aid the host. More study is needed to test this theory and its potential interference with siderophore-specific uptake mechanisms and effect on Fe cycling.

For all Fe-replete cultures (i.e. high Fe concentration and consequently high F_v/F_m of around 0.6) and the Fe-limited *M. pusilla* the lysis dynamics and decline in F_v/F_m for the VR- and VL-infected cultures were largely comparable (Figure 1D and 2D). However, the Fe-treatment history of the virus (VL or VR) did matter in combination with Fe-limited *P. globosa* cells, i.e. infection with VR did delay the decline in F_v/F_m with more than a day (Figure 1C). Infection with a VR lysate is analogous to a relief in Fe-limitation at the time of infection. When the Fe-limited *P. globosa* cultures were infected with a VR lysate, they were effectively spiked with an Fe increase of $\sim 10\%$ relative to Fe-replete conditions (0.9 μM). The 100- to 300-fold increase of Fe with the addition of an Fe-replete lysate (0.9 μM vs. 1-3 nM) takes the culture Fe concentration well out of limitation ranges which are generally considered to be in nano- to picomolar ranges (de Baar et al. 1990; Martin et al. 1990; Brand 1991). The concentration increase in our experiment is comparatively drastic to assure lifting of Fe-limitation to levels nearer normal replete cultures. This cross-inoculation provides insight in the potential effects of sudden introduction of Fe, e.g. with (seasonal) dust deposition or terrestrial runoff. The effect of this spike with Fe was apparently enough to prevent instant loss of photosynthetic capacity in the Fe-limited *P. globosa* cells. For both phytoplankton species, the improved physiological condition upon infection of Fe-limited host with VR virus resulted in enhanced viral production compared to Fe-limited infected with VL virus (by 1.6 and 2.3-fold for PgV and MpV, respectively; Table 3). The effect on burst size is thus stronger for the more Fe-sensitive *M. pusilla*, indicating that *M.*

pusilla is more capable of mobilizing the Fe added for viral production. Utilization of the limiting macronutrient P when added post infection was also found to stimulate virus production of *M. pusilla* (Maat et al., 2016). Although our results indicate that an infected host is capable of mobilizing the limiting Fe for viral production upon addition post infection, it did not lead to a complete recovery of virus production as compared to Fe-replete conditions (around 50% of the replete treatment; Table 2).

Table 3 Burst sizes (number of virus progeny per lysed host cell) of PgV-07T and MpV-08T infecting Fe-replete and Fe-limited *P. globosa* (Pg) and *M. pusilla* (Mp), respectively. Viruses were derived from a Fe-replete host (VR) or a Fe-limited host (VL). Burst sizes for Fe-limited cultures are also expressed as a percentage of the corresponding infections in Fe-replete cultures.

<i>Treatment</i>	<i>Burst size</i>	<i>% of replete</i>
Pg replete + VR	671 ± 62	
Pg replete + VL	679 ± 5	
Pg limited + VL	196 ± 35	29 ± 0.2
Pg limited + VR	308 ± 4	46 ± 2.3
Mp replete + VR	84 ± 14	
Mp replete + VL	83 ± 6	
Mp limited + VL	20 ± 0	24 ± 5.2
Mp limited + VR	46 ± 2	54 ± 0.5

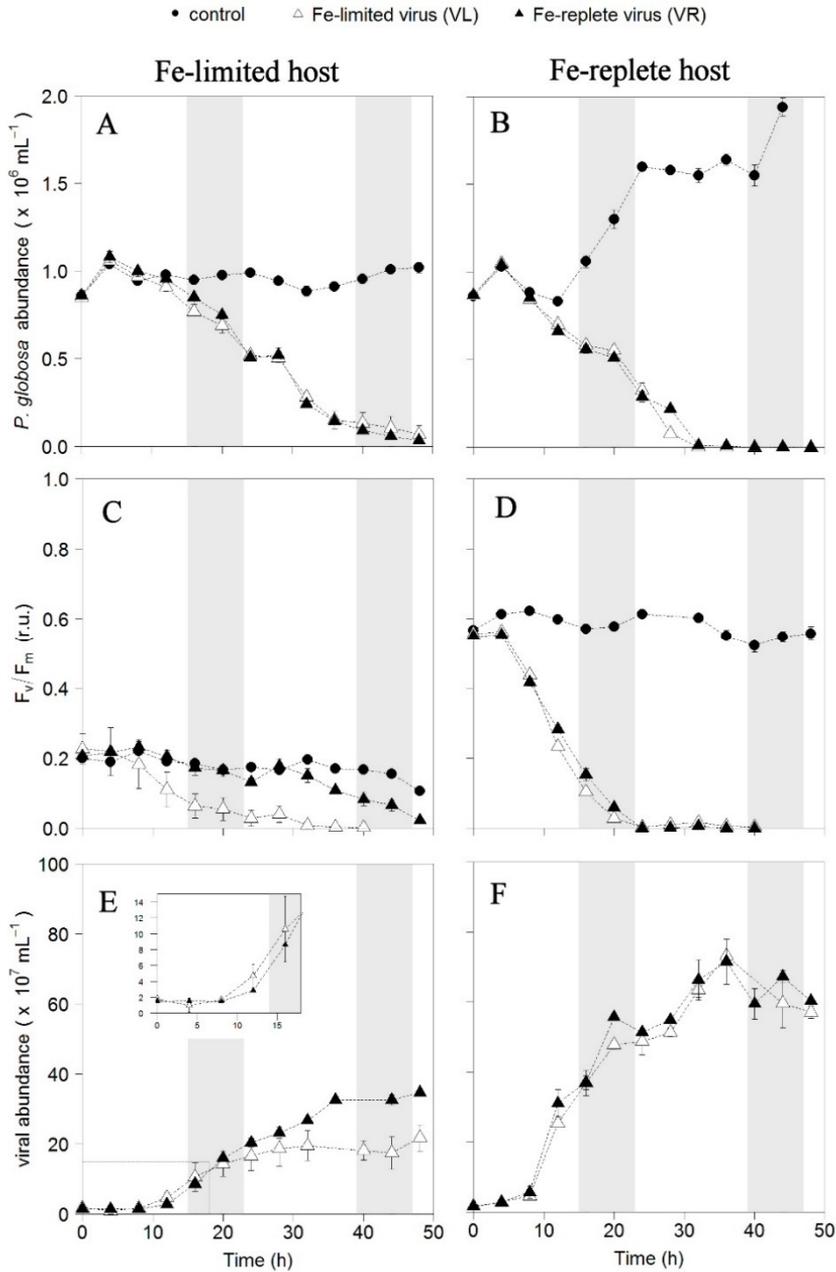


Figure 1 Temporal dynamics of *P. globosa* viral infections: host cell abundance over time (h) in Fe-limited (A) and Fe-replete cultures (B); photosynthetic capacity (F_v/F_m) over time (h) of Fe-limited (C) and Fe-replete cultures (D); viral abundance in Fe-limited (E) and Fe-replete (F) cultures. Circles represent non-infected controls, black triangles represent infections with Fe-replete host derived viruses (VR) and white triangles represent infections with Fe-limited host derived viruses (VL). Error bars indicate deviation of replicates (N=2) and fall within symbols when not visible. Shaded areas indicate dark periods. Inlay in (E) magnifies the first 20 hours post-infection.

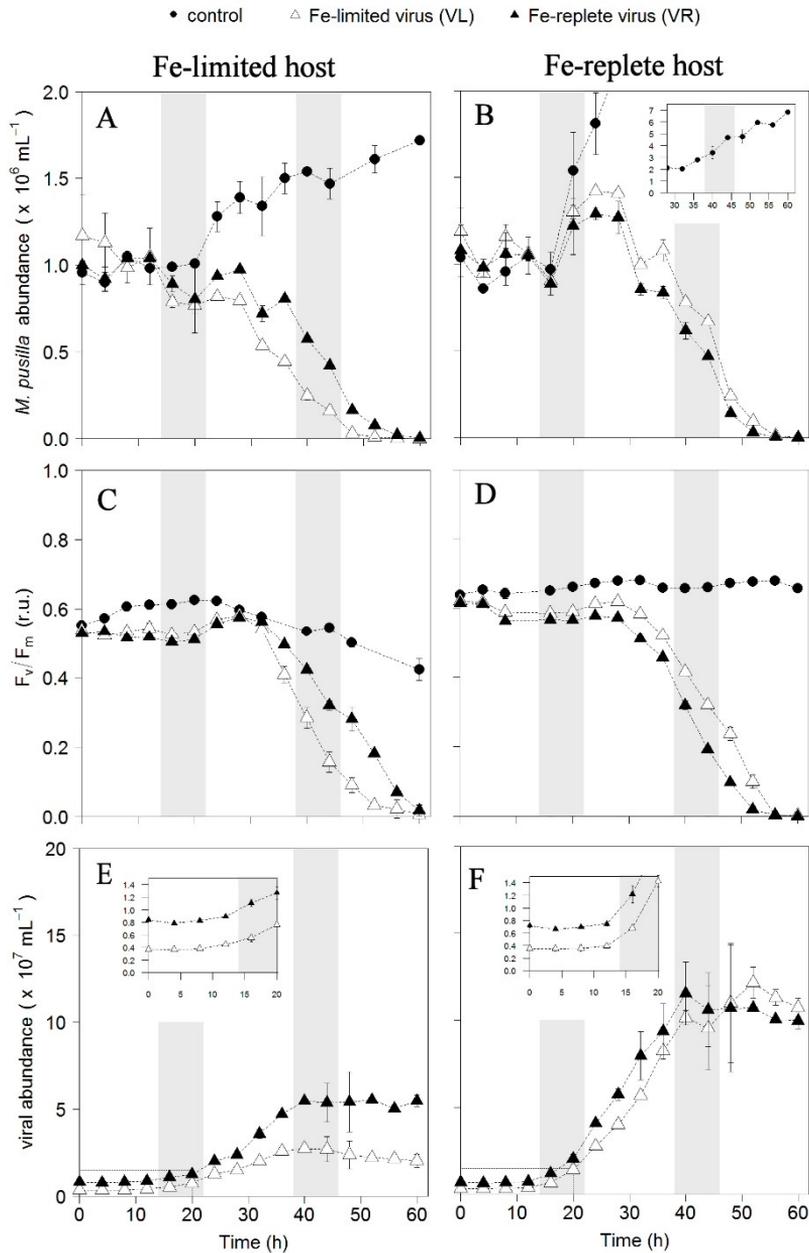


Figure 2 Temporal dynamics of *M. pusilla* viral infections: host cell abundance over time (h) in Fe-limited (A) and Fe-replete cultures (B); photosynthetic capacity (F_v/F_m) over time (h) of Fe-limited (C) and Fe-replete cultures (D); viral abundance in Fe-limited (E) and Fe-replete (F) cultures. Circles represent non-infected controls, black triangles represent infections with Fe-replete host derived viruses (VR) and white triangles represent infections with Fe-limited host derived viruses (VL). Error bars indicate deviation of replicates (N=2) and fall within symbols when not visible. Shaded areas indicate dark periods. Inlay in (B) shows off scale host control growth, inlays in (E and F) magnify the first 20 hours post-infection.

In conclusion, viral infection of both phytoplankton species is distinctly influenced by Fe-limitation. The differences in sensitivity of the host to Fe-limitation subsequently affected the progeny virus growth properties. *Phaeocystis* and *Micromonas* occur in Fe-limited and Fe-replete conditions alike (Not et al. 2004, 2005; Schoemann et al., 2005). Viral lysis has been shown to be an important mortality term for *P. globosa* under natural conditions, and also *M. pusilla* is readily infected (Cottrell et al., 1995; Brussaard, 2004a; Brussaard et al., 2005; Baudoux et al., 2006; Martínez Martínez et al., 2015). Virus burst sizes became strongly reduced (on average by 70%) under Fe-limiting relative to Fe-replete conditions. Although addition of Fe at the time of infection was utilized by the infected Fe-limited host to increase virus production, it was not to the level found under Fe-replete conditions. Thus Fe-limitation irrevocably interfered with viral productivity. Additionally, Fe-limited *M. pusilla* demonstrated an evident effect on the quality of the viruses produced as only 30% were infective. The lowered virus infectivity and/or virus yield impair viral control of the specific host species under Fe-limitation. However, at the same time, reduced viral lysis may affect the productivity of remaining non-infected cells and of other phytoplankton species, because viral lysis is considered a driver of Fe cycling by releasing ligand-bound dissolved Fe-species back into the dissolved organic matter pool (Gobler et al. 1997; Poorvin et al. 2004; Poorvin et al. 2011). What relative contributions different ligands have, and in how far their release is facilitated or impaired by viral lysis requires further study. Further experimental and *in-situ* study of phytoplankton host-virus dynamics under Fe-limitation is essential to elucidate the level of response specificity, but also the effects on the viral shunt in terms of nutrient cycling in general as well as Fe speciation.

