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Fatty acid dynamics during viral infection of *Phaeocystis globosa*

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ABSTRACT: Previous studies have shown that viral infection can affect the lipid distribution of phytoplankton, specifically the fatty acid (FA) distribution, and has been hypothesized to affect the nutritional value of phytoplankton for higher trophic levels. Here, we report the bulk FA distribution as well as the FA distribution of individual intact polar lipid (IPL) classes of the alga *Phaeocystis globosa* infected with the lytic virus PgV-07T. Analysis of the virus PgV-07T itself showed that it contained shorter, more saturated bulk and IPL-bound FAs than the host. Viral infection did not affect the bulk or IPL-bound FA distribution after 24 h post-infection when cell lysis was initiated, but after 48 h the bulk FAs remaining in the particulate phase of the infected cultures contained 22% less polyunsaturated FAs (PUFAs) compared to the control cultures. This change in the bulk FAs was mainly due to the generation of PUFAs that occurred in the control cultures, suggesting that infection prevented *P. globosa* PUFA accumulation. Two of the 7 IPL classes, the monogalactosyldiacylglycerols and the sulfoquinovosyldiacylglycerols, showed about a 10% reduction in the percentage of PUFAs upon viral infection. In contrast, the digalactosyldiacylglycerols exhibited a 15% increase in PUFAs. This difference between the IPL-PUFAs and the bulk FAs is possibly due to a contribution to the bulk FA pool of e.g. triacylglycerols. Overall, these results suggest that grazing on infected cells and filter feeder uptake of post-lysis cell debris could lead to a transfer of relatively lower amounts of PUFAs to higher trophic levels.

KEY WORDS: *Phaeocystis globosa* · Virus · Fatty acids · Intact polar lipids

INTRODUCTION

Marine viruses affect phytoplankton population dynamics through the reduction in biomass and effects on interspecies competition and succession within a mixed phytoplankton community (Brussaard 2004b, Suttle 2007). They also play an important role in the production of dissolved organic matter, an essential step in the microbial loop (Wilhelm & Suttle 1999, Brussaard et al. 2005a). Grazing is often considered the main phytoplankton loss factor, although viral lysis can be equally important (see Evans et al. 2003, Baudoux et al. 2006). Knowledge on the relative importance of grazing and viral infection is critical to understanding ecological interactions and biogeochemical cycling in a natural system. However, these loss factors are not mutually exclusive, as it is known that virally infected microalgal cells can be grazed upon (Ruardij et al. 2005, Brussaard et al. 2007, Evans & Wilson 2008). Phytoplankton can synthesize polyunsaturated fatty acids (PUFAs), which cannot be produced by most of their grazers; hence, the phytoplanktonic PUFAs are essential dietary factors for organisms such as zooplankton (Fraser et al. 1989, Klein Breteler et al. 2005, Bell & Tocher 2009). Evans et al. (2009) noted that 151 h post-infection (p.i.), the fatty acid (FA) composition of the coccolithophore *Emiliania huxleyi* CCMP1516 showed a

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decrease in PUFAs relative to monounsaturated FAs (MUFAs) and saturated FAs (SFAs) during viral infection by the lytic virus EhV-86, and they hypothesized that viruses can affect the nutritional value of phytoplankton for higher trophic levels. Fulton et al. (2014) found that the changes in FAs observed by Evans et al. (2009) could be explained by specific changes in the distribution of polar glycerolipids and glycosphingolipids.

It is not known whether changes in PUFA content due to viral infection seen in *E. huxleyi* occur in other microalgae. In this study, we therefore examined the effect of viral infection on the FA distribution of the related phytoplankton species *Phaeocystis globosa*. Both haptophytes play a key role in the marine ecosystem and biogeochemical cycling, especially in the formation of blooms (see reviews by Paasche 2001 and Schoemann et al. 2005). A previous study examined the intact polar lipid (IPL) composition of virally infected *P. globosa* and its lytic virus PgV-07T; a selective acquisition of the IPLs from specific cell compartments such as the host's cytoplasm was suggested (Maat et al. 2014). In this study, we examined in detail the effect of viral infection on the bulk (i.e. those released by hydrolysis of the lipid extract) FA distribution on the host and the virus in order to gain insight into the effect of viral infection on the nature of the compounds which are transferred through ecological food webs and the microbial loop. We also compared the infection-induced changes in the bulk-FA distribution with those of the IPL-bound FAs.

MATERIALS AND METHODS

Culturing and sampling

The culturing and infection experiments have been previously described by Maat et al. (2014). Briefly, for the viral infection experiment, 4 replicate 2 l cultures of axenic, exponentially growing *Phaeocystis globosa* culture strain G (Culture collection Royal Netherlands Institute for Sea Research (NIOZ); 8.6×10^7 cells l^{-1}) were grown in a 1:1 (v/v) mixture of f/2 medium (Guillard 1975) and enriched artificial seawater (Harrison et al. 1980) modified after Cottrell & Suttle (1991) at 15°C over a light:dark cycle of 16:8 h with irradiance at 90 µmol quanta m^{-2} s^{-1}. Two cultures were inoculated with fresh 0.2 µm filtered (Minisart High-Flow syringe filter; Sartorius A.G.) axenic PgV-07T (PgV) lysate (culture collection NIOZ), at a virus:host ratio of 55:1 to guarantee a 1-step virus growth cycle. The 2 replicate non-infected control cultures were inoculated with the same volume of medium. Samples for algal and viral enumeration, IPL and FA analysis (150 ml) were taken at regular intervals until the cultures were completely lysed. Algal and viral abundances were determined by flow cytometry according to Marie et al. (1999) and Brussaard (2004a), respectively. IPL samples were filtered through 47 mm Whatman GF/F filters (Maidstone), folded in aluminum foil and flash frozen in liquid nitrogen and stored at −80°C until analysis. A very small percentage of PgV is usually retained on the GF/F filter, but this is negligible, especially considering the amount of viral lysate (10 l) needed for detection of viral lipids/FAs. For the IPL and FA analysis of PgV, 10 l of PgV lysate was produced in a separate batch under the same culture conditions. In short, viruses were concentrated by tangential flow filtration and further purified on an Optiprep™ density gradient (Maat et al. 2014). The purified viruses were filtered on a 0.02 µm Anodisc filter and stored at −80°C until analysis.

Intact polar lipid extraction and analysis

The filters containing the infected *Phaeocystis globosa* cells and the Anodisc filters containing the viral isolate were extracted using a modified Bligh and Dyer (BD) extraction, and analysis was carried out by HPLC electrospray ionization MS (HPLC-ESI-MS^n), using methods modified from Sturt et al. (2004), on an Agilent 1200 series LC equipped with a thermostated autoinjector, coupled to a Thermo LTQ XL linear ion trap with Ion Max source with electrospray ionization (ESI) probe (Thermo Scientific). Structural identification of the IPLs was carried out by comparison with fragmentation patterns of authentic standards as described in Brandsma et al. (2012). The chain length and number of double bonds of the IPL-bound fatty acids (FA) were determined by either the fragment ions or neutral losses diagnostic for FAs obtained in the MS^2 spectra (Brügger et al. 1997, Brandsma et al. 2012). The nomenclature used (e.g. $C_{32:5}$) describes the total number of carbon atoms and double bonds of the 2 FA moieties. Polyunsaturated IPLs were distinguished as those with a total of 3 or more double bonds in the combined FA moieties, while monounsaturated were those IPLs which contained 2 FA moieties with a total of 1 to 2 double bonds. We quantified the relative distribution of the FA, or combination of FAs, in each IPL class by integrating the base peak area of the individual ions and assuming similar ionization efficiencies within each IPL class.

Bulk fatty acid analysis

For FA analysis, aliquots of the BD extracts (with addition of a known amount of a C_{19} FA internal standard) were hydrolyzed by refluxing with 1 N KOH in MeOH solution for 1 h. After neutralization with a 2 N HCl/MeOH (1/1, v/v) solution, the FAs in the extracts were methylated with diazomethane in diethyl ether which was removed under a stream of N_2. Before analysis the extracts were treated with pyridine (10 µl) and BSTFA (10 µl) to derivatize alcohol groups and then brought to the final volume (40 µl) with ethyl acetate. FA methyl ester (FAME) identification and quantification was carried out using gas chromatography–mass spectrometry (Thermo Finnigan TRACE GC–MS). FAMEs were separated using a CP-SIL 5CB capillary column (length 25 m × internal diameter 0.32 mm, coating 0.12 µm) with the following oven conditions: initial temperature 70°C, increasing to 130°C by 20°C min^{-1}, then increasing to 320°C by 4°C min^{-1}. MS operating parameters were: electron multiplier 1663V; source temperature 250°C; full scan *m/z* 50–800; scan time 0.33 s. MS data were acquired and processed using the Thermo Finnigan Xcalibur software. FAMEs were identified based on literature data and library mass spectra. Double bond positions were determined, where possible, using dimethyldisulfide (DMDS) derivatization of the FAMEs. For this, extracts were derivatized in hexane (100 µl) with DMDS (Merck ≥99%; 100 µl) and I_2/ether (60 mg ml^{-1}; 20 µl) and heated overnight at 40°C. Hexane (400 µl) was then added with $Na_2S_2O_3$ (5% aqueous solution; 200 µl) to deactivate the iodine. The hexane layer was removed and the aqueous phase washed with hexane (×2). The hexane layers were combined and analyzed by GC–MS as described above. During our analysis we detected an unusual $C_{22:1}$ fatty acid, not reported in any other haptophyte algae. However, we also identified a $C_{22:1}$ fatty acid amide, a known contaminant (Grosjean & Logan 2007), and since long chain amides can be converted to carboxylic acids during hydrolysis we cannot rule out the possibility that the $C_{22:1}$ fatty acid is a contaminant rather than a natural product. Because of this uncertainty we did not include it in our analysis.

Where appropriate IPL and bulk FA results for infected and control treatments are presented as a mean of 2 replicate cultures with a standard deviation.

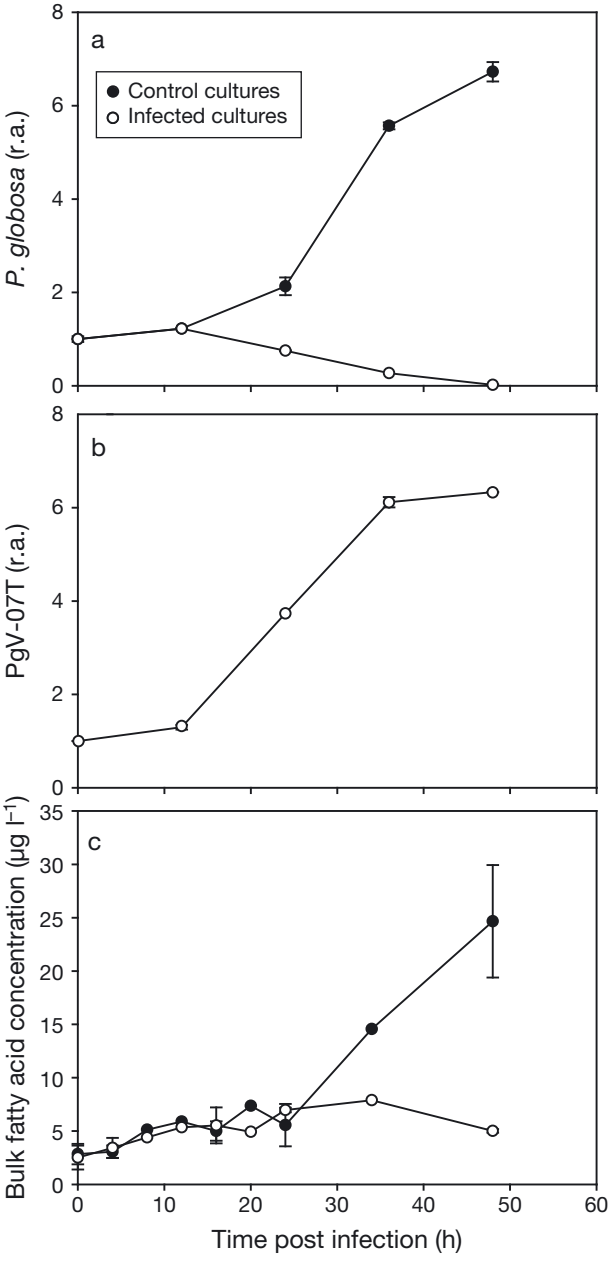

Fig. 1. Abundances (normalized to Time 0) of (a) algal host *Phaeocystis globosa*, (b) virus PgV-07T and (c) the concentration of fatty acids in culture (µg l^{-1}). Closed circles represent the non-infected cultures, open circles the virally infected cultures. Data for (a) and (b) reproduced from Maat et al. (2014). Error bars represent SD; r.a.: relative abundance

RESULTS

Algal, viral and bulk fatty acid dynamics

As reported by Maat et al. (2014), viral infection led to a decline of *Phaeocystis globosa* cell abundance within 24 h p.i. (Fig. 1a). The one-step viral growth curve showed a release of newly produced viruses within 12 h p.i. (Fig. 1b). Burst size was estimated

from the production of PgV and the loss of host cells, resulting in a burst size of 288 ± 1 PgV cell^{-1} (Maat et al. 2014). The total concentration of bulk FAs at the start of the experiment (average ± SD of all 0 h p.i. cultures) was 2.7 ± 0.9 µg l^{-1} (31 ± 2.8 fg cell^{-1}; Fig. 1c). FA concentration initially increased for both the control and the infected cultures at a rate of approximately 0.15 µg l^{-1} h^{-1} until 24 h p.i., after which it increased at a greater rate of 0.8 µg l^{-1} h^{-1} for the control cultures, concomitant with the increase in cell numbers (Fig. 1a), to reach a maximum of 24.7 ± 5.3 µg l^{-1} (43 fg cell^{-1}) at 48 h p.i. (Fig. 1c). In contrast, the bulk FA concentration of the infected cultures remained more or less constant at 5.0 ± 0.2 µg l^{-1} at 48 h p.i. The very low level of detectable cells, despite the continued presence of extractable FAs, indicates that a high proportion of the bulk FAs detected at 48 h p.i. were present in post-lysis cell debris.

Composition of bulk fatty acids

The initial FA composition of *P. globosa* (at 0 h p.i., average of control and infected cultures) comprised chain lengths between C_{14} and C_{22} and with 0 to 6 double bonds (Fig. 2; Table S1 in the Supplement at www.int-res.com/articles/suppl/a074p085_supp.pdf). During the first 24 h of growth p.i., the FA distribution in the control cultures exhibited little change, but between 24 and 48 h, several changes were observed. Overall, between 0 and 48 h p.i., there was an apparent increase in the percentage of polyunsaturated FAs (PUFAs) in the control cultures (from 51 to 65%, although this was not significant; *t*-test, n = 4; p = 0.08) (Table S1; Figs. 3 & 4a). Comparison of the FA distribution in the infected cultures with the control cultures revealed little difference at 24 h. Between 24 and 48 h p.i. there was a change in the FA distribution in the infected cultures, but not as great

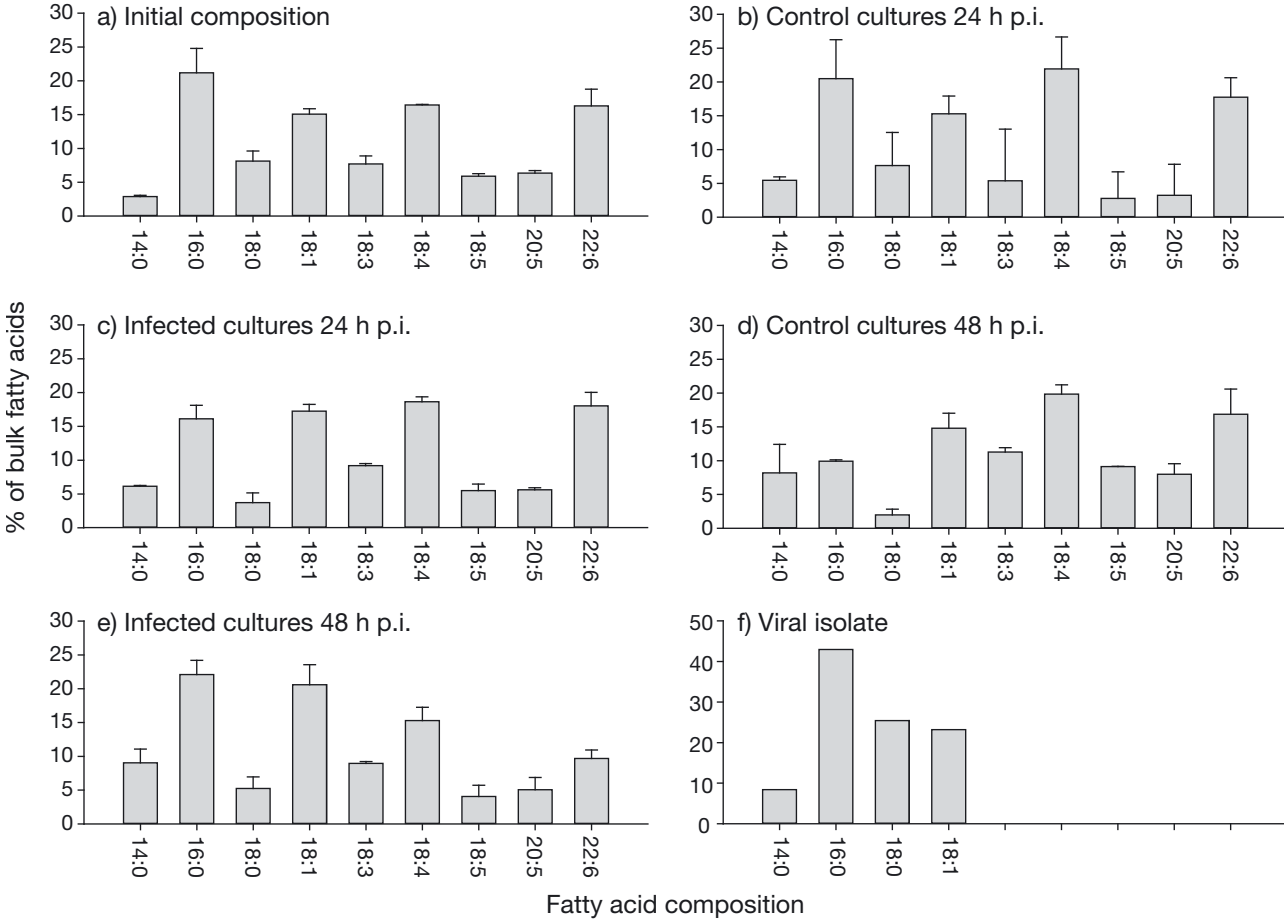

Fig. 2. The distribution of individual bulk FAs (% of Σ bulk FA) in (a) the initial composition (average of infected and control *Phaeocystis globosa* cultures at 0 h), (b) the control cultures at 24 h post-infection (p.i.), (c) the infected cultures at 24 h p.i., (d) the control cultures at 48 h p.i.,(e) the infected cultures at 48 h p.i. and (f) the viral isolate. Error bars represent SD between 2 replicate cultures

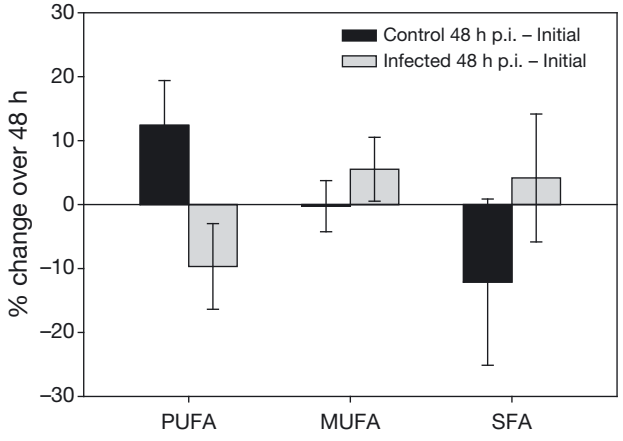

Fig. 3. Percentage change of the bulk PUFAs, MUFAs and SFAs over 48 h for the control and infected cultures. Error bars represent the summed 95% CI

as had been seen in the control cultures over the same period. After 48 h p.i. the percentage of PUFAs in the infected cultures was significantly lower compared to the control cultures (43 vs. 65%; *t*-test, n = 4, p = 0.02) (Table S1; Figs. 3 & 4a). The FA composition of the viral isolate was quite different from that of the host, i.e. it did not contain the $C_{18:3}$–$C_{18:5}$ FAs, the C_{20} or $C_{22:6}$ FAs and was dominated by the $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ FAs. Thus, it contained predominantly SFA (77%), less MUFA (23%) and no PUFAs (Table S1; Fig. 4a). In comparison with the initial composition of FAs in the host, the virus contained 45% more SFA.

Composition of IPL FAs

The intact polar lipids (IPLs) detected in both the host and virus included monogalactosyldiacylglycerols (MGDGs), digalactosyldiacylglycerols (DGDGs), dimethylphosphatidylethanolamines (DMPEs), phosphatidylglycerols (PGs), phosphatidylcholines (PCs) and diacylglyceryl hydroxymethyltrimethyl-β-alanines (DGTAs) and diacylglyceryl carboxyhydroxymethylcholines (DGCCs). Sulfoquinovosyldiacylglycerols (SQDGs) and phosphatidylethanolamines (PEs) were detected only in the host and not in the viral isolate. In this study, the PEs and DMPEs were rarely detected and were not examined further. Maat et al. (2014) previously reported the range of IPL-FAs; here, we quantified the relative contribution of these FAs in each IPL class and examined how this changed with growth and infection.

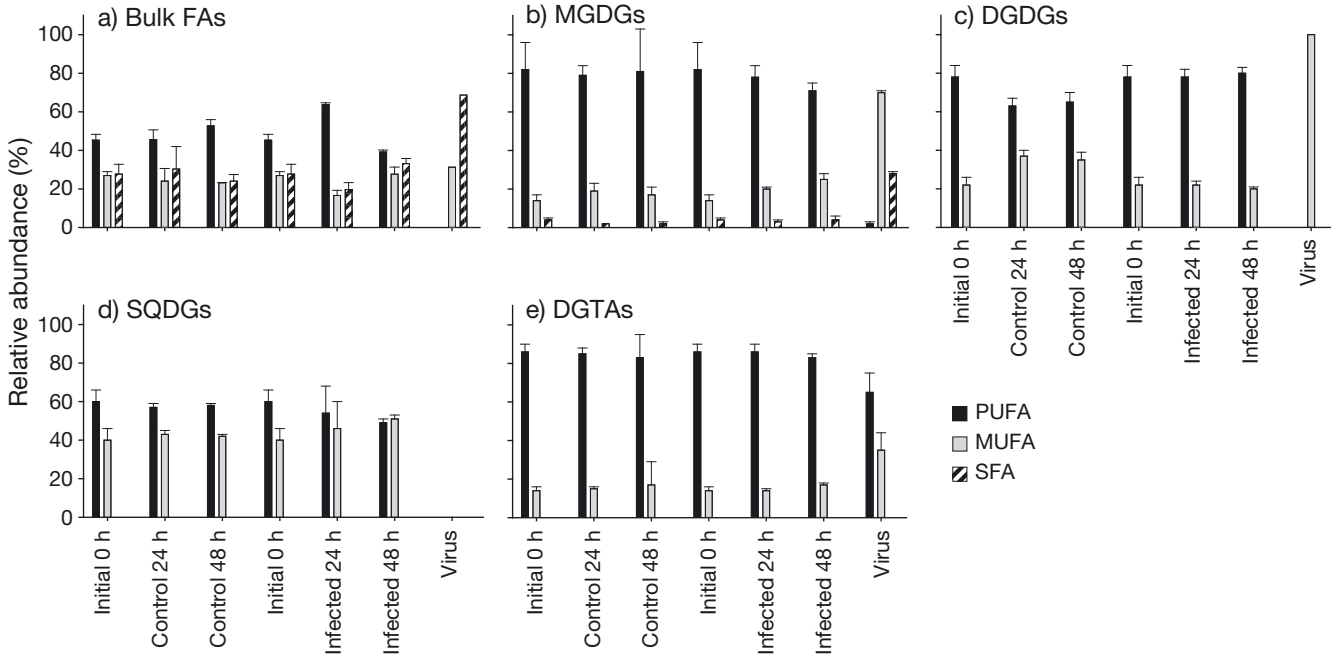

Fig. 4. The composition of IPL PUFAs, MUFAs and SFAs (% of Σ PUFA+MUFA+SFA) in the initial composition (average of infected and control *Phaeocystis globosa* cultures at 0 h), in the control and infected cultures at 24 h post-infection (p.i.) and 48 h p.i. and in the viral isolate for (a) bulk fatty acids, (b) the MGDGS, (c) the DGDGs, (d) the SQDGs and (e) the DGTAs. PCs/PGs/DGCCs not show as PUFA = 100% for all. No SFAs detected in (c–e). PUFA = polyunsaturated fatty acid, MUFA = monounsaturated fatty acid, SFA = saturated fatty acid. See text ('Composition of IPL FAs') for other abbreviations. Error bars represent SD between 2 replicate cultures

Glycolipids

In *P. globosa*, the sum of carbon number and double bond equivalents of the 2 FA moieties ranged from $C_{28:0}$ to $C_{36:10}$ for the MGDGs and from $C_{32:1}$ to $C_{36:10}$ for the DGDGs (Table S2), while only 2 SQDGs ($C_{32:1}$ and $C_{36:7}$) were detected (Table S3). The distribution of PUFA:MUFA:SFA (Fig. 4b) for the MGDGs remained essentially unchanged during growth in the control cultures, while the PUFAs in the DGDGs had significantly declined over 24 h from 78 to 63 % (*t*-test, n = 4, p = 0.04); thereafter, they remained stable at ca. 65 % until 48 h p.i. For both the MGDGs and DGDGs there were differences in FA composition between the control and infected cultures at 48 h p.i., but this was only significant for the DGDGs: the infected cultures contained a higher proportion of DGDG PUFAs than the controls (80 vs. 65 %; *t*-test, n = 4, $p < 0.05$). In contrast to the control cultures, the DGDG PUFA percentage in the infected cultures was more similar to the initial FA composition (Fig. 4c). The SQDG FA distribution did not change with growth (Table S3; Fig. 4d), while in the infected cultures the decrease in the percentage of the $C_{36:7}$ SQDG PUFA (58 to 49 %) with a concomitant increase in the $C_{32:1}$ SQDG MUFA (42 to 51 %) was not significant (*t*-test, n = 4, p = 0.05). Fulton et al. (2014) noted a similar shift in SQDG-FAs from primarily $C_{18:3}$ and $C_{18:4}$ to $C_{14:0}$ and $C_{16:0}$ FAs during infection of *Emiliania huxleyi*.

The MGDG FAs and DGDG FAs in the viral isolate generally had shorter chain lengths and fewer double bonds than those of the host (Table S2). The viral isolate contained $C_{28:0}$ to $C_{34:3}$ MGDGs, only one detectable DGDG ($C_{32:1}$) and no detectable SQDGs. The PUFA:MUFA:SFA distribution for the MGDGs and DGDGs in the viral isolate were quite distinct from the host, i.e. 2:70:28 and 0:100:0, respectively (Table S2; Fig. 4b,c).

Phospholipids

The PCs present in *P. globosa* were all PUFAs (Fig. 4e) and fell in the range of $C_{32:4}$ to $C_{44:12}$ (Table S4). During growth, changes were relatively small. Comparison of the infected cultures with the control cultures showed no significant differences at 48 h p.i. The FA distribution of the 3 PGs detected in *P. globosa* also comprised only PUFAs, $C_{36:7}$, $C_{38:6}$ and $C_{40:6}$, and remained relatively constant during growth (Table S4).

The viral isolate contained similar PC FAs to *P. globosa*, i.e. $C_{32:4}$ to $C_{44:12}$ but with additional $C_{34:7}$, $C_{36:8\text{-}10}$, $C_{38:4}$, $C_{38:10}$, $C_{42:12,}$ $C_{42:13}$ and $C_{44:11}$ (Table S4). The viral isolate also contained the $C_{38:6}$ and $C_{40:6}$ PGs, but $C_{36:7}$ could not be detected (Table S4). For both the PCs and PGs, the viral isolate, like *P. globosa,* was entirely composed of PUFAs (Fig. 4e).

Betaine lipids

The DGTA FA distribution in *P. globosa* ranged between $C_{34:1}$ and $C_{36:5}$ (Table S5). During growth of the non-infected algal cultures there was no significant change in the distribution (Fig. 4f), except for a decrease in $C_{36:5}$ DGTA (from 42 to 32 %; *t*-test, n = 4, p = 0.02; Table S5). For this class of betaine, the infected cultures exhibited the same trend as the controls, decreasing in $C_{36:5}$ DGTA from 42 to 36 %; however, this was a non-significant change (*t*-test, n = 4, p = 0.05; Table S5). The DGCCs were all PUFAs (Fig. 4e) and ranged between $C_{32:5}$ and $C_{44:12,}$ with $C_{34:5}$ being dominant (Table S5). There was some change in their distribution with growth over 48 h, i.e. $C_{34:5}$ decreased from 61 to 51 % (*t*-test, n = 4, p = 0.03) in the control cultures, while $C_{38:6}$ increased from 2 to 12 % (*t*-test, n = 4, p = 0.01). Conversely, after 48 h, the infected cultures contained 20 % higher $C_{34:5}$ DGCC relative to the control cultures (*t*-test, n = 4, p = 0.005) while several of the longer, more unsaturated PUFAs ($C_{36:6}$, $C_{38:6}$, $C_{44:12}$) were present in lower relative percentages (*t*-test, n = 4, p = 0.04, p = 0.01 and p = 0.03, respectively).

The $C_{36:2}$ and $C_{36:3}$ DGTAs, which were in low abundance (≤3 %) in the host, could not be detected in the virus (Table S5). However, due to the higher percentages of $C_{34:1}$ and $C_{34:2}$ DGTAs (27 and 8 %) than were seen in the host, the percentage of DGTA MUFA was higher at 35 % compared to the host (14 to 17 %) (Fig. 4). The DGCC distribution in the viral isolate was similar to that of the infected *P. globosa* cultures from 48 h, although the percentage of $C_{40:11}$ DGCC was significantly higher (19 vs. 9 %; *t*-test, n = 4, p = 0.04, Table S5).

DISCUSSION

Changes in the fatty acid composition of *Phaeocystis globosa*

During PgV proliferation and initial lysis of the host (24 h p.i.), both the control and infected *Phaeocystis globosa* cultures exhibited little difference in the bulk FA composition from their initial composition at the start of the experiment (0 h p.i.). Also by 48 h p.i., the

percentage of PUFA in the bulk FA in the infected cultures did not significantly decrease. However, at 48 h p.i. the control cultures contained 65% PUFA. This significant difference of 22% between the control and infected *P. globosa* cultures at 48 h p.i. was due mainly to a substantial increase in the percentage of PUFA in the control cultures over the 48 h experiment. By the end of the infection cycle, the cells were not limited in nutrients and neither were they entering stationary phase. We speculate that the increase in PUFAs was a process that occurred during population growth, possibly induced by cell density. To our knowledge, this has not been reported before, and further research is needed to clarify the underling mechanisms. In contrast, this increase in PUFA content was clearly halted during viral infection.

A decline in PUFAs was also observed by Evans et al. (2009) for infected *Emiliania huxleyi* (strain CCMP 1516 infected with EhV-86), from 70 to 44%. Fulton et al. (2014) suggested that the observations of Evans et al. (2009) may be explained by specific changes in the polar glycerolipids and glycosphingolipids of *E. huxleyi* during infection. The difference in FA dynamics between *P. globosa* and *E. huxleyi* could originate from the differences between both virus–host systems. Not only do the hosts have very different characteristics (*E. huxleyi* best known for its coccolith-bearing cells [Tyrrell & Merico 2004] and *P. globosa* for its formation of multicellular colonies [Schoemann et al. 2005]), but also the infection characteristics of the viruses involved seem to differ substantially. For example, how EhV-86 exits the host cell is different from PgV-07T (i.e. budding off vs. single burst event); in the infection pathway of EhV-86, viral glycosphingolipids are involved (Vardi et al. 2009), while this is not the case for PgV-07T (Maat et al. 2014). However, the differences in lytic cycle of PgV-07T in our study and EhV-86 in the study by Evans et al. (2009) could also explain the observed differences in the FA dynamics. The latent period (50 vs. 8–12 h), time until cell lysis (75 vs. 24 h) and duration of the experiment (151 vs. 48 h) for EhV-86 in the study by Evans et al. (2009) were all substantially longer than for PgV-07T in our study. This means that during infection of *E. huxleyi*, there was more time for potential changes in the FA profiles to take place. However, most phytoplankton–virus systems studied in the laboratory to date are known to lyse faster and have shorter latent periods than observed by Evans et al. (2009), i.e. within 24 to 72 h (Jacquet & Bratbak 2003, Brussaard 2004b, Lawrence et al. 2006, Fulton et al. 2014), including studies of *E. huxleyi* strain CCMP 1516 infected with EhV-86 (Evans et al. 2007, Rose et al. 2014). This suggests that the degree of PUFA impoverisation seen by Evans et al. (2009) may not always be the case for *E. huxleyi*. Furthermore, in the study of Evans et al. (2009) the infected cultures did not reach full lysis as occurred in this study. Instead, their final time point at 151 h p.i. appears to contain a cell density approximately equivalent to 36 h p.i. in the experiment from this study. Hence, for most virus–phytoplankton systems, changes in FA composition may not happen to the extent observed by Evans et al. (2009) but rather be more comparable to our results where no large changes in FA compositions were observed during infection.

The only significant change in the IPL-bound FAs observed in our study was exhibited by the DGDG-PUFAs, which at 24 h p.i. were already present in a higher percentage in the infected cultures than in the control cultures (80 vs. 65%; *t*-test, n = 4, p = 0.04). The fact that the bulk FAs exhibited a 22% difference in PUFA between the infected and the control cultures from 48 h p.i., while the IPL FAs did not, may be due to the bulk FA fraction containing additional inputs from non-IPL sources such as the triacylglycerols (TAGs), which can form an important fraction of the total FAs in algae (e.g. *Phaeocystis* sp., Al-Hasan et al. 1990). TAGs are utilized as storage lipids in algae and under stress conditions the production of TAGs in many algal species increases (Guschina & Harwood 2009). The production of TAG has also been shown to increase during senescence in leaves of the higher plant *Arabidopsis* sp. (Kaup et al. 2002), while a later study by Espinoza et al. (2007) showed that this plant shows similar transcription profiles during viral infection and senescence, including the transcription of genes involved in lipid metabolism. Hence, it could be expected that in the infected cultures these FA-containing lipids, which generally have fewer double bonds than FAs bound in polar lipids (Harwood 2004), would increase in relative concentration during the experiment. This would explain the 10% decrease in the relative percentage of PUFAs in the infected cultures over time. Similarly, the 12% increase in PUFAs in the control cultures over the same period may be explained by a decrease in the proportion of FAs that are associated with TAGs.

To investigate whether the membrane lipids of the total number of viruses produced could make up a high proportion of the host cell's biomass and hence could account for the changes seen in the FA composition of infected cells (as postulated by Evans et al. 2009), we calculated the potential contribution of viral FAs to the total FAs for *P. globosa.* For *P. globosa* with an average cell diameter of 5 µm (this study), we

estimated that the plasma membrane would span 7.9 $\times 10^7$ nm^2. The plasma membrane of a cell represents approx. 2% of the total membrane content (Alberts et al. 2002), so the total *P. globosa* membrane surface area would be 3.9×10^9 nm^2. The radius of the *P. globosa* virus PgV-07T is 75 nm (Baudoux & Brussaard 2005); hence, its envelope membrane would be approximately 7.1×10^4 nm^2. The burst size of *P. globosa* was 288 viruses cell^{-1} (this study), so the virus membrane lipids would be 0.5% of the host membrane lipids of a *P. globosa* cell. A similarly low percent (1.5%) was calculated for the *E. huxleyi* strain used by Evans et al. (2009): *E. huxleyi* CCMP 1516 cell diameter of 5.2 µm (Steinke et al. 1998), radius of the *E. huxleyi* virus 86 (EhV-86) of 90 nm (Mackinder et al. 2009), burst size of *E. huxleyi* 620 cell^{-1} (Castberg et al. 2002). As the virus membrane lipids make only a very small percentage of the total of membrane lipids in both an infected *E. huxleyi* cell and an infected *P. globosa* cell (<2%) they are unlikely to have significant effects on the bulk FA composition.

In the case of *P. globosa*, the distribution of bulk FA as well as IPLs was substantially different between the infected host and viral isolate, i.e. PgV contained elevated percentages of bulk FAs $C_{18:0}$ and $C_{16:0}$ compared with *P. globosa,* which results in distinctly different distributions of PUFA:MUFA:SFA in the viral isolate. Furthermore, PgV has been shown to contain a distinct IPL class distribution compared to the host (Maat et al. 2014). The PCs represented a more substantial part of the virus IPLs distribution than in the host, while the MGDG and DGDG contribution to the sum of IPLs was lower in the virus relative to the host (both infected and control) and the SQDGs were not detected at all. This confirms that the contribution of viral biomass to the infected host cells cannot be causing the decrease in the percentage of PUFAs seen in the infected cultures relative to the control cultures.

Comparison between virus and host lipid membrane

In previous work, we showed that PgV-07T acquired PCs from the host in the highest proportion, with lower proportions of PGs and the betaines, only trace amounts of MGDGs and DGDGs, and no SQDGs (Maat et al. 2014). The majority of MGDGs, DGDGs and SQDGs are associated with the chloroplast in algae and higher plants (Guschina & Harwood 2009, Sato & Wada 2010), which suggests that the chloroplast and its associated membranes were not the source of the recruited IPLs. Instead, it was hypothesized that PgV-07T selectively recruits its lipids from membranes in the host cytoplasm. It is this selective recruitment which probably resulted in the PUFAs, present in the bulk FAs of *P. globosa*, to be absent in the bulk FAs of PgV-07T (Fig. 2). Previous studies have also found that viral particles contain lower percentages of PUFAs relative to their hosts: mammal kidney cells infected with the rubella virus (Voiland & Bardeletti 1980) and the moth *Galleria mellonella* infected with a range of different invertebrate iridescent viruses (Williams & Thompson 1995). This too could be caused by the selective recruitment of lipids from specific cellular compartments. Indeed, studies have shown different FA distributions in different subcellular components of cells from higher plants (Devor & Mudd 1971, Schwertner & Biale 1973, Mackender & Leech 1974, Nozawa et al. 1974). Unfortunately, little is known about the FA composition of algal chloroplasts relative to the other cellular membranes (Harwood 2004).

In this study, the virus was found to contain PC species with FA combinations that are not found in uninfected cells (i.e. $C_{34:7}$, $C_{36:8\text{-}10}$, $C_{38:4}$, $C_{38:10}$, $C_{42:11\text{-}12}$, $C_{44:11}$). While the viral PCs could have been produced de novo during infection, it could also be that they were present in the host, but below the level of detection (Maat et al. 2014). In contrast, the DGCC FA composition of the virus was similar to that of the infected cells at 48 h p.i. Further experiments are needed to understand the reasons for these differences in distributions for the different IPLs.

Ecological significance

Phytoplankton are the main primary producers in the marine environment and form the base of most pelagic food chains. They are an important source of PUFAs for auxotrophic suspension feeders such as copepods and bivalves (Fraser et al. 1989, Taylor & Savage 2006, Pleissner et al. 2012). Changes in phytoplankton FA composition due to viral infection could thus affect the nutritional value of phytoplankton for higher trophic levels, as suggested by Evans et al. (2009). Furthermore, FA transfer to higher trophic levels may also occur after viral lysis of the phytoplankter bloom, as viral lysis derived cell debris may aggregate into transparent exopolymer particles (TEP) (Shibata et al. 1997, Brussaard et al. 2005a,b, Vardi et al. 2012). Several marine suspension feeders have been found to consume TEP, including copepods and bivalves (Passow & Alldredge 1999, Ling & Alldredge 2003, Kach & Ward 2008). A virally in-

duced decrease in PUFAs, as we have observed here for *P. globosa,* could thus also indirectly affect the PUFA intake of higher trophic levels.

CONCLUSIONS

Viral infection of *Phaeocystis globosa* prohibited the accumulation of PUFAs in bulk fatty acids (FAs). In contrast, the distribution of IPL bound-FAs changed little over the course of the experiment. This difference in the response of the bulk FAs and of the IPL FAs to viral infection suggests that the bulk FAs are affected by other FA-containing compartments, such as the triacylglycerol (TAG) storage lipids. The FA distribution of the PgV-07T virus itself was particularly different from the host, i.e. it contained shorter, more saturated and lower amounts of PUFAs, possibly due to the selective recruitment from the host of IPLs with low amounts of PUFAs. From an ecological perspective, some virally infected *P. globosa* cells may be grazed upon in a bloom environment, thus transferring FAs with a lower PUFA content than those found in non-infected cells to higher trophic levels. Post-lysis, PUFA-impoverished FAs may be transferred to higher trophic levels via filter feeder uptake of TEP.

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