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Viral lysis of *Micromonas pusilla*: impacts on dissolved organic matter production and composition

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Abstract The viral mediated transformation of phytoplankton organic carbon to dissolved forms (''viral shunt'') has been suggested as a major source of dissolved organic carbon (DOC) in marine systems. Despite the potential implications of viral activity on the global carbon fluxes, studies investigating changes in the DOC composition from viral lysis is still lacking. *Micromonas pusilla* is an ecologically relevant picoeukaryotic phytoplankter, widely distributed in both coastal and oceanic marine waters. Viruses have been found to play a key role in regulating the population dynamics of this species. In this study we used axenic cultures of exponentially growing *M. pusilla* to determine the impact of viral lysis on the DOC concentration and composition, as estimated from lysate-derived production of transparent exopolymer particles (TEP) and two fractions of fluorescent dissolved organic matter (DOM): aromatic amino acids (excitation/emission; 280/320 nm; *F*(280/320)) and marine humic-like fluorescent DOM (320/410 nm; *F*(320/410)). DOC concentration increased 4.5 times faster and reached 2.6 times higher end concentration in the viral infected compared with the non-infected cultures. The production of *F*(280/320) and *F*(320/410) were 4.1 and 2.8 times higher in the infected cultures, and the elevated ratio between *F*(280/320) and *F*(320/410) in lysates suggested a higher contribution of labile (protein) components in viral produced DOM than in algal exudates. The TEP production was 1.8 times faster and reached a 1.5 times higher level in the viral infected *M. pusilla* culture compared with the non- infected cultures. The measured increase in both DOC and TEP concentrations suggests that viral lysis has multiple and opposite implications for the production and export processes in the pelagic ocean: (1) by releasing host biomass as DOC it decreases the organic matter sedimentation and promotes respiration and nutrient retention in the photic zone, whereas (2) the observed enhanced TEP production could stimulate particle aggregation and thus carbon export out of the photic zone.

Keywords Dissolved organic matter · Fluorescent DOM · *Micromonas pusilla* · Transparent exopolymer particles · Virus

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Introduction

The concentration of dissolved organic carbon (DOC) in seawater exceeds by one to two orders of magnitude that of particulate organic carbon (POC) (Hedges 2002), making the DOC pool the most important source of carbon for prokaryote growth in marine pelagic systems. In open oceans and coastal waters, not dominated by large terrestrial inputs, DOC originates largely from zooplankton sloppy feeding, extracellular release (percent extracellular release or PER), heterotrophic bacterial release, and viral cell lysis of host organisms (Nagata 2000). DOC can operationally be divided into 3 major categories depending on its accessibility to microbial degradation: a labile pool (<1 % of DOC) which is degraded within minutes to days, a semi-labile (~50 % of DOC) fraction utilized within months to years and a refractory part (~50 % of DOC) degraded over years to thousands of years (Kirchman 2004; Lønborg and Álvarez-Salgado 2012). DOC derived from viral-induced cell lysis of microbes has been shown to affect the transfer of organic matter through the microbial food web (Brussaard et al. 1995, 1996, 2005b; Bratbak et al. 1994; Middelboe et al. 1996, 2003; Gobler et al. 1997), however, still little is known of how and to what extent viral-derived lysates from phytoplankton affect the composition, lability and cycling of DOM in the ocean.

A major factor determining the underwater light environment in the ocean is chromophoric dissolved organic matter (CDOM), which is estimated to constitute 20–70 % of DOM in the ocean (Blough and Vecchio 2002). The CDOM pool absorbs light strongly in the UV and blue area of the light spectrum and a sub fraction of this pool can reemit this energy as fluorescence (termed: FDOM) at longer wavelengths. Two main types of FDOM have been identified: the aromatic amino acids, measured at excitation/emission wavelengths of 280/320 nm (F(280/320)) and marine humic–like detected at excitation/emission wavelengths of 320/410 nm (F(320/410)) (Coble et al. 1990). F(280/320) has been suggested as an indicator of labile DOM, while F(320/410) is believed to reflect more refractory DOM, partly of planktonic origin (Coble et al. 1990; Lønborg et al. 2010). Heterotrophic microbes, phytoplankton and zooplankton have been shown to produce FDOM during mineralization and growth (Rochelle-Newall and Fisher 2002; Lønborg et al. 2009; Romera-Castillo et al. 2010), while the role of viruses in the production of FDOM is currently not understood.

Phytoplankton derived organic matter can either be directed to higher trophic levels by grazing (classical food web), vertically exported from the euphotic zone by sinking (biological pump), or transferred to DOM via meso- and microzooplankton sloppy feeding, PER and viral cell lysis (microbial food web) (Brussaard et al. 1995, 2008; Weinbauer et al. 2010). These processes influence the cycling of energy and biogeochemically relevant elements differently, directly affecting the production/respiration ratio of the ocean and the efficiency of the biological pump (Brussaard et al. 2008). Typically 10–20 % of the photosynthetically fixed carbon is released by phytoplankton as PER, but this fraction is highly variable and can range between 1 and 70 % (Myklestad 2000). Reports show increased releases of DOM by active PER in times of nutrient depletion when the synthesis and exudation is often enhanced (Myklestad 2000). These compounds comprise a broad spectrum of biopolymers such as transparent exopolymer polysaccharides (TEP), whereby DOM can be converted into POM which subsequently can be vertically exported from the photic zone (Passow 2002). Viral induced mortality of phytoplankton influences the DOM pool in a different way than PER (Brussaard 2004a; Suttle 2007). Upon production of progeny viruses the phytoplankton host cell bursts releasing the new viruses as well as the host's cellular compounds. In contrast to PER, viral cell lysis therefore results in the release of all cell compounds (e.g. amino acids, carbohydrates, DNA), which are likely labile and relatively easy accessible for bacterial degradation (Brussaard et al. 2005b; Haaber and Middelboe 2009).

Viral cell lysis promptly affects the standing stock of labile DOC by destroying host cells and releasing the cell content as dissolved components, forcing the food web towards a more regenerative nature (Suttle 2007; Brussaard et al. 2008). Theoretical estimates suggest a high flow of ~10^9 tonnes of organic carbon per day being converted from host cell biomass into DOM through "the viral shunt" (Wilhelm and Suttle 1999). The very few studies that actually investigated the viral production of DOC imply that viral lysis influence the bulk (Gobler et al. 1997; Bratbak et al. 1998), amino acid and carbohydrate (Weinbauer and Peduzzi 1995; Middelboe and Jørgensen 2006) and

trace metals concentrations (Gobler et al. 1997), but a quantitative understanding of viral induced DOC production is still lacking. Viruses have also been shown to change the phytoplankton hosts cytological, physiological and biochemical pathways (Brussaard 2004a; Pagarete et al. 2009). These changes are thought to occur due to the use of enzymes and structural compounds (e.g. amino acids, nucleotides) for producing viral progeny, to synthesize viral intracellular signalling molecules, and to metabolize cellular compounds by virus controlled metabolic pathways (Brussaard 2004a; Pagarete et al. 2009). These changes have previously been shown to influence the phytoplankton cellular composition by affecting host fatty acid and pigment composition (Llewellyn et al. 2007; Evans et al. 2009), chlorophyll fluorescence (Balch et al. 2007), host DNA content (Brussaard et al. 1999), intracellular enzyme activity (Brussaard et al. 2001), disrupt the intracellular organelles (Levy et al. 1994), and increase the cellular levels of dimethylsulfoniopropionate (DMSP) and dimethylsulphide (DMS) (Evans et al. 2007). These findings suggest that viral infection changes the cellular composition of the infected phytoplankton and that viral lysis could play a vital role in shaping the released DOM. Still, a major gap in our understanding concerns factual information of how viral activity affects the DOM pool.

In this study we used axenic cultures of the marine eukaryotic alga *Micromonas pusilla* as a model organism to make a first step towards understanding how viral lysis impacts (1) the production of DOC and FDOM, (2) the optical signature of DOM and (3) the production of transparent exopolymer particles (TEP).

Materials and methods

Experimental design

In this study we compared DOM produced by photosynthetic extracellular release (PER) and viral lysis. In order to maximise the PER and obtain the upper limit of non-viral DOM release we let the non-infected cultures deplete the nutrients and reach stationary phase (Myklestad 2000).

The axenic algal-host-virus model system used in this study was the *M. pusilla* (Prasinophyceae) strain LAC38 and the dsDNA (200 kb genome size) virus MpV-08T (MpV) which belongs to the Phycodnaviridae, both were obtained from the culture collection at the NIOZ—Royal Netherlands Institute for Sea Research. The algae were cultured in a modified (1:1) mixture of f/2 medium (Guillard 1975) and enriched artificial seawater (ESAW) (Cottrell and Suttle 1995), containing 10 times lower vitamin and buffer amounts in order to reduce the organic carbon content of the medium and furthermore were nutrients added in lower amounts (194 μmol l^{-1} of KNO_3 and 11 μmol l^{-1} of KH_2PO). The *M. pusilla* culture was acclimated to these growth conditions by growing them in 4 replicate 5 l Erlenmeyer flasks and keeping them in the exponential growth phase over 5 generations using the same growth media and conditions (light and temperature) as used during the experiment. Following were an aliquot of this culture transferred to 64 different 1 l experimental bottles (500 ml headspace) containing 550 ml fresh autoclaved medium. The algae were cultured at 15 °C under a light:dark (L:D) cycle of 16:8 with a light intensity of 100 μmol photons m^{-2} s^{-1}. The viral lysate was produced by repeated lysis of *M. pusilla* cultures growing in 5 l Erlenmeyer flasks using the same experimental growth media and conditions; 5 ml of this viral-lysate was added to the experimental cultures (550 ml). To check that the algal cultures were kept axenic during the experiments, aliquots of 1 ml of each culture were fixed with 1 % paraformaldehyde + 0.05 % glutaraldehyde (final concentration), stained with 4,6diamidino-2-phenylindole (10 mg ml^{-1}, final concentration), and examined for the presence of bacteria using an Olympus BX61 epifluorescence microscope under blue and UV wavelength excitation at each sampling point.

During the experiment 4 replicate bottles were analysed for each sub-sampling at time −48, 0, 12, 24, 48, 72, 120 and 144 h after viral addition for measurements of algal and viral abundance, pulse-amplitude modulated fluorescence (PAM), and the concentration of dissolved organic carbon (DOC), dissolved organic matter fluorescence (FDOM), dissolved inorganic nitrogen (DIN; NH_4^+, NO_2^-/NO_3^-), dissolved inorganic phosphate (DIP; HPO_4^{2-}), total organic carbon (TOC) and transparent exopolymer particles (TEP). An additional four bottles were used to only determine *M. pusilla* abundance at time point −24 h, in order to follow the development of the growth curve. All glassware used in this study was first acid washed in 10 % HCl for 24 h, and then washed 3

times with ultraclean (Milli-Q) water and culture media before used.

Samples for the dissolved phase were filtered through 47 mm diameter 0.2 μm filters (Pall, Supor Membrane Disc). The DIN and DIP samples were collected into 50 ml acid washed (10 % HCl for 24 h) polyethylene bottles, while the DOC and TOC samples were collected in pre-combusted (450 °C, 12 h) glass ampoules and preserved with 50 μl 25 % H_2PO_4 per 10 ml sample.

Sample measurement

Algal abundance was determined using fresh samples diluted up to 10-fold in 0.2 μm (Minisart; Sartorius) filtered sterile culture medium and monitored using a Coulter Epics XL-MCL benchtop flow cytometer (Beckman Coulter Inc., Miami, FL, USA) equipped with a laser with an excitation wavelength of 488 nm (15 mW) and emission bands for the chlorophyll *a* autofluorescence (>630 nm) and phycoerythrin fluorescence (575 ± 20 nm).

Viral abundance samples (1 ml) were fixed with 25 % glutaraldehyde (0.5 % final concentration, EM grade; Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 4 °C, flash frozen in liquid nitrogen and stored at −80 °C until analysis. The viral abundance was determined using the method described by Brussaard (2004b). Thawed samples were diluted 100 to 1,000-fold in autoclaved 0.2 μm (Minisart; Sartorius) filtered TE buffer (10:1 Tris–EDTA, pH 8.0) and stained with the nucleic acid-specific dye SYBR Green I (Invitrogen-Molecular Probes) for 10 min at 80 °C. Prior to analysis samples were cooled at room temperature in the dark and analysed using a FACSCalibur flow cytometer. The trigger was set on the green fluorescence, the flow rate was 20 μl min^{-1} and the samples were analysed for 1 min. Virus counts were corrected for the blank consisting of 0.2 μm filtered TE-buffer and SYBR-Green I and analysed in the same way as the samples.

A PAM fluorometer (Pulse Amplitude Modulated–CONTROL Universal Control Unit, WATER-mode, Walz, Germany) was used to determine F_0 (chlorophyll *a* autofluorescence), F_m (maximum chlorophyll *a* fluorescence) and F_v/F_m (photochemical quantum efficiency, where $F_v = F_m - F_0$) after dark-acclimation of the algal cells for 5 min (Geider et al. 1993).

Inorganic nutrients (NH_4^+, NO_2^-/NO_3^- and HPO_4^{2-}) were determined by standard segmented flow analysis (TRAACS autoanalyzer) as described in Hansen and Koroleff (1999). TOC and DOC were measured using a Shimadzu TOC analyser (Pt-catalyst). Three to five replicate injections of 150 μl were performed per sample. Concentrations were determined by subtracting a Milli-Q blank and dividing by the slope of a daily standard curve of potassium hydrogen phthalate and glycine. Particulate organic carbon (POC) concentrations were calculated as the difference between TOC and DOC with the corresponding standard deviations (SD) calculated as $SD^2_{POC} = SD^2_{TOC} + SD^2_{DOC}$.

The FDOM fluorescence was measured in four replicates on a Shimadzu fluorescence spectrophotometer (Hitachi 2500). Measurements were performed at a constant temperature of 20 °C in a 1 cm quartz fluorescence cell. Milli-Q water was used as a reference, and the intensity of the Raman peak was checked daily. Excitation/emission (Ex/Em) measurements were performed for aromatic amino acids (average Ex/Em, 280/350 nm; termed *F*(280/320)) and marine humic-like substances (average Ex/Em 320/410 nm; termed *F*(320/410)). These DOM fluorescence peaks are consistent with those found by Coble (1996). Fluorescence measurements were expressed in quinine sulphate units (QSU), i.e. in μg eq QS l^{-1}, by calibrating at Ex/Em: 350/450 nm against a quinine sulphate dihydrate (QS) standard dissolved in 0.05 M sulphuric acid (H_2SO_4).

Transparent exopolymer particles (TEP) was measured colorimetrically in 4 replicates by filtration onto 47 mm 0.4 μm polycarbonate filters (Whatmann) and subsequent staining for <2 s with 1 ml of a 0.02 % aqueous Alcian Blue solution, rinsed with MQ water and frozen until analysed (within 2 weeks). When analysed the filters were soaked in 80 % H_2SO_4 for 2 h and the Alcian Blue bound to particles was determined by measuring the adsorption at 787 nm (adsorption max for alcian blue) and calibrating using Gum Xanthan (Passow and Alldredge 1995). TEP concentrations were expressed as μg Gum Xanthan equivalents per liter (μg Xequiv l^{-1}).

Statistical analysis

In this paper *t* tests were used to assess whether statistical significant difference were found between

the viral infected and non-infected cultures (Sokal and Rohlf 1995). Regression model II analyses as described in Sokal and Rohlf (1995) were used to calculate the DOC production rates. Prior to the regressions analysis, normality was checked, the confidence level was set at 95 % with all statistical analyses conducted in Statistica 6.0.

Results

Cell and viral abundance

The abundance of *M. pusilla* cells increased exponentially in both cultures until limited by either virus infection or nutrient availability (Fig. 1a). In the non-infected cultures the growth stopped after 48 h due to nutrient limitation (data not shown), as reflected in the declining F_v/F_m (Fig. 1a, b). The cell abundance in the infected cultures decreased rapidly 48 h after virus addition resulting in a progressive decline in F_v/F_m and increased viral abundance with a complete lysis after 120 h (Fig. 1a–c).

Organic matter dynamics

POC concentrations increased rapidly in both cultures reaching 1.7 times higher maximum concentrations in the non-infected cultures (Fig. 2a). In the non-infected cultures POC increased until 48 h remaining almost constant thereafter until the end of the experiment. In the viral infected cultures the POC started to decrease sharply 12 h after viral addition, reaching non-detectable levels at the end of the experiment (Fig. 2b). Calculating the DOC production rate, using time versus concentration for the period between virus addition and maximum concentration, showed that the DOC concentration increased 4.5-fold faster in the viral infected (22.2 ± 3.2 µmol C l^{-1} h^{-1}) compared with the non-infected cultures (5.0 ± 0.4 µmol C l^{-1} h^{-1}). The DOC end concentration was furthermore 2.6-fold higher (2.1 ± 0.1 mmol C l^{-1} vs. 0.7 ± 2 mmol C l^{-1}) in the viral infected cultures (Fig. 2b), corresponding to a DOC production of 0.11 ± 0.02 pmol C per lysed *M. pusilla* cell.

The viral produced DOM was characterized using the optical properties of aromatic amino acid (*F*(280/320)) and marine humic-like (*F*(320/410)) substances. The production of *F*(280/320) and *F*(320/410) were

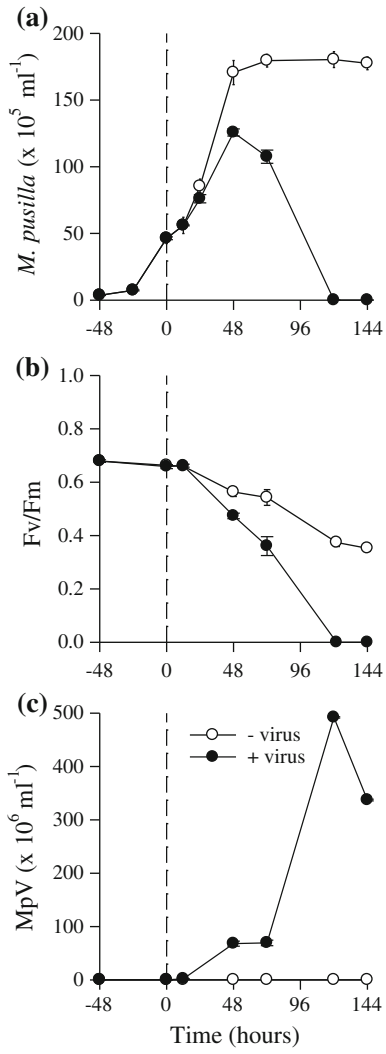

Fig. 1 Time course of **a** *Micromonas pusilla* cells, **b** the photochemical quantum efficiency (F_v/F_m) and abundance of **c** *M. pusilla* viruses (MpV), in the non-infected (−Virus) and viral cultures (+Virus). The *dashed line* indicates the time when viruses were added and *error bars* represent standard deviations of the mean ($n = 4$)

4.1 and 2.8 times larger, respectively, in the viral infected compared with the non-infected cultures (Fig. 3a, b). The ratio between *F*(280/320) and *F*(320/410) showed the relation between labile and refractory DOM components, suggesting that virus generated DOM has a higher contribution of labile (aromatic amino acids) compared with PER (Fig. 3c).

As most viruses pass the 0.2 µm filter used, viruses contributed to the DOC amounts measured. The potential influence of this contribution was tested by

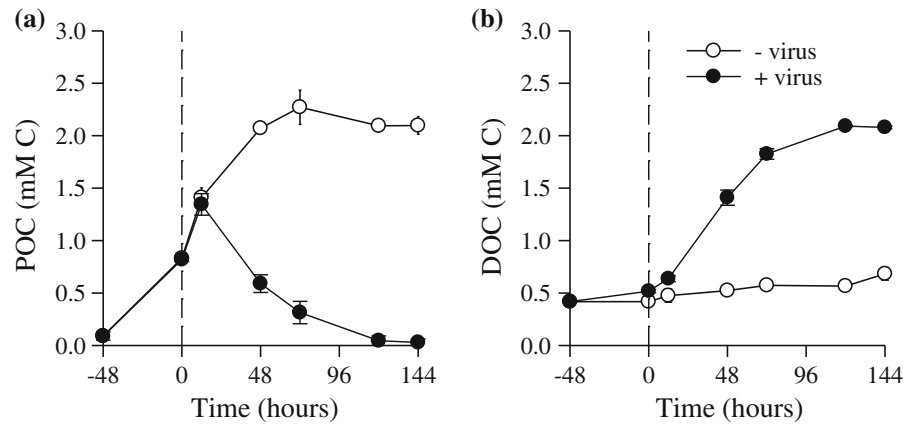

Fig. 2 Time evolution of **a** particulate (POC) and **b** dissolved organic carbon (DOC) in non-infected (−Virus) and viral infected (+Virus) cultures. The *dashed line* indicates the time when viruses were added and *error bars* represent standard deviations of the mean ($n = 4$)

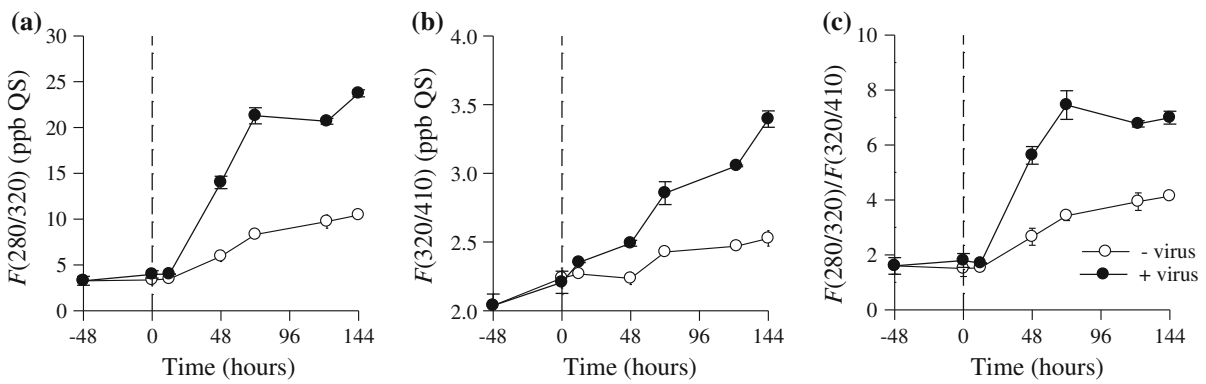

Fig. 3 Time course of **a** aromatic amino acid-like (F(280/320)), **b** marine humic-like fluorescence (F(320/410)) and **c** the ratio between F(280/320) and F(320/410) in non-infected (−Virus) and viral infected (+Virus) cultures. The *dashed lines* indicates the time when viruses were added and *error bars* represent standard deviations of the mean ($n = 4$)

ultra-centrifugating parts of the 0.2 µm filtrate to remove the viruses (50,000×g for 90 min) and subsequent measurements of DOC, FDOM and nutrients. The results showed no significant impact of the virus removal (paired t test, $p = 0.10$–0.16, $n = 8$), suggesting that viruses did not contribute significantly to the DOM measured in this study.

The TEP concentrations increased in both cultures, but the production was 1.8 times faster and reached a 1.5 times higher level in the viral infected cultures (Fig. 4).

Discussion

This study demonstrates that viral lysis results in a sudden and large release of DOC with a high contribution of labile (aromatic amino acids) components and stimulates the production of TEP, showing how viral lysis of eukaryotic phytoplankton can influences the DOM production and composition and could stimulate particle formation and organic carbon flux out of the photic zone due to enhanced TEP production.

The complex composition, variable supply rate and changing bioavailability of DOC impacts global element cycles, climate regulation and bacterial diversity in the ocean (Hansell et al. 2009; Teira et al. 2009; Lønborg and Álvarez-Salgado 2012). The question of what controls DOM production in pelagic systems is one of the most complex issues in marine biogeochemistry. Viral activity in the oceans results in $\sim 10^{29}$ infections per day, and upon production the host cell bursts and releases newly produced viruses

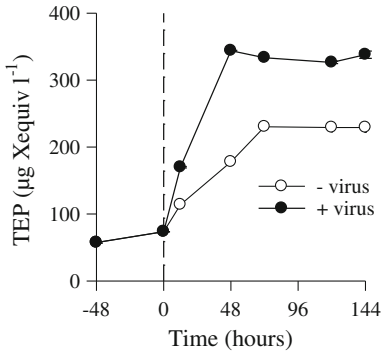

Fig. 4 Concentration of polysaccharides containing transparent exopolymer particles (TEP) in non-infected (−Virus) and viral cultures (+Virus). The *dashed line* indicates the time when viruses were added and *error bars* represent standard deviations of the mean ($n = 4$)

into the surrounding water thereby converting the cell content into DOM (Suttle 2005). This viral mediated transformation of organic carbon and nutrients from organisms to DOM (termed the "viral shunt"; Suttle 2005) has been suggested to be a major source of labile DOM, based on the known high impact of viruses on bacterial mortality (e.g. Suttle 2005) and the efficient bacterial turnover of viral lysates (Middelboe et al. 1996, 2003; Brussaard et al. 2005b). However, experimental evidence for the impact of phytoplankton viral lysis on marine DOC production and composition is limited (Bratbak et al. 1998; Haaber and Middelboe 2009).

In this study we show that viral lysis results in a 4.5 times faster and 2.6 times larger increase in DOC concentrations compared with photosynthetic extracellular release (PER) (Fig. 2b). This increase in DOC following infection corresponded to 98 % of the net decrease in POC suggesting a highly efficient transformation of cells into DOC by viral lysis. In the non-infected control cultures, 12 % of the POC in algal biomass was lost as PER during the incubation, which is in line with the previous studies (Myklestad 2000). In addition to DOC, viral lysis also results in the production of organic nutrients (e.g. amino acids) and trace metals, which in natural systems could sustain bacteria or phytoplankton growth, thus influencing carbon, nutrient and trace metal cycling in the pelagic environment (Middelboe et al. 1996; Gobler et al. 1997; Poorvin et al. 2004; Brussaard et al. 2005a). The viral induced production of DOC has in previous studies been estimated using isotope techniques, measuring changes in specific DOM pools (e.g. carbohydrates) and/or changes in host cell abundances (e.g. Bratbak et al. 1992; Weinbauer and Peduzzi 1995; Gobler et al. 1997; Noble and Fuhrman 1999; Middelboe et al. 2003). Comparing our direct measured DOC release, with values obtained using published carbon contents for *M. pusilla* (0.8–1.2 pg C cell^{-1}; Montagnes et al. 1994; Romera-Castillo et al. 2010), and the cell numbers observed in our study, we find a difference of 60–736 µmol C l^{-1}, i.e. the measured DOC release was 1.2–1.9 times higher than the expected release based on literature derived values. The values obtained from our direct measurements are therefore close to the results obtained using literature values, suggesting that these conversion factors can be used to provide estimates of the viral impact on DOC production. In natural environments *M. pusilla* often occurs at densities of up to 2×10^7 cells l^{-1} (Not et al. 2004). At such concentrations, viral lysis of the *M. pussilla* population would according to our values lead to the release of ~2.5 µmol C l^{-1}. Although this may seem negligible compared with the bulk DOC pool (often ~100 µmol C l^{-1}), we propose that an estimated input of 2.5 µmol C l^{-1} may constitute more than a doubling of the labile DOC pool (often <2 µmol C l^{-1}), considering that viral lysates contain highly labile compounds such as amino acids, carbohydrates and DNA, which provide high quality substrate for bacterial growth.

Fluorescent CDOM (FDOM) can be divided into two main DOM fluorophores: aromatic amino acid (*F*(280/320)) and humic-like (*F*(320/410)) compounds (Coble et al. 1990). The *F*(280/320) fluorescence is associated with the aromatic amino acids (tyrosine, tryptophan and phenylalanine) and has been suggested as an indicator of total hydrolyzable amino acids (THAA) (Yamashita and Tanoue 2003). The THAA pool is generally bioavailable and can contribute to the bacterial carbon and nitrogen demand in marine systems (Coffin 1989). Previous studies have found that *F*(280/320) is available for bacterial utilization (Cammack et al. 2004; Lønborg et al. 2010), however, different subcomponents of the *F*(280/320) pool may have different bioavailability, as some fractions are consumed more readily than others (Lønborg et al. 2010). The fluorescence of *F*(320/410) has been identified as a by-product of microbial respiration and a good proxy for refractory

DOM (Nieto-Cid et al. 2006; Lønborg et al. 2010). In this study we measured for the first time the viral production of FDOM and show that viral lysates and PER have different optical signatures indicating that viral released DOM has a relatively high content of amino acids (Fig. 3a–c) as previously suggested (Middelboe and Jørgensen 2006). The results thus show that viral lysates and PER provide significantly different contributions to the DOM pool in terms of substrate composition and quality, and the data support suggestions that lysates constitute a high quality contribution to the DOM pool (Brussaard et al. 2008). Other studies have shown that as much as ~75 % of viral lysates derived from marine prokaryotes are bioavailable for non-infected co-occurring bacteria (Middelboe et al. 2003), and our results support that viral lysis represents an important source of labile DOM in the marine environment, which could support pelagic bacterial production. This viral release of labile DOM would in natural systems not only affect the bacterial carbon cycling but also change the bacterial community composition as suggested previously (Brussaard et al. 2005b; Sheik 2012).

The enhanced production of $F(320/410)$ in the presence of viruses relative to the control cultures furthermore shows that viral lysates could be an important source of refractory DOM in the ocean (Jiao et al. 2010; Weinbauer et al. 2011). Previous studies have shown that bacteria, algae and zooplankton can produce FDOM (Steinberg et al. 2004; Lønborg et al. 2009; Romera-Castillo et al. 2010). Here we show that also viral lysis can contribute significantly to the production of FDOM thus emphasizing that viruses should be considered in future studies of CDOM dynamics in marine system.

The TEP particles (>0.4 μm) represent an intermediate stage at the border between DOC and POC (Verdugo et al. 2004). Because of its viscous nature, TEP plays a central role in the formation of aggregates during phytoplankton blooms, which thereby potentially could influence the formation of "marine snow" and sedimentation of organic matter (Fowler and Knauer 1986; Passow et al. 1994; Grossart and Simon 1998; Brussaard et al. 2005a). TEP has been shown to be formed from polysaccharides produced by both algal and bacterial cultures and during viral lysis of *Phaeocystis globosa* (Grossart et al. 1998; Stoderegger and Herndl 1999; Passow 2002; Brussaard et al. 2005a; Mari et al. 2005). In this study we demonstrate that the viral infection of the picoplankton species *M. pusilla* enhances the TEP production. The measured increase in both DOC and TEP concentrations, proposing that viral lysis could impact the biological pump in opposite directions. Our data thus provide experimental evidence supporting the suggestion by Brussaard et al. (2008), that viral lysis could (1) decrease the efficiency of the biological pump through the release of cellular host organic matter and nutrients into to the DOM pool and thereby activate the microbial food web, and (2) may stimulate aggregate formation and organic carbon flux out of the photic zone due to TEP production upon viral lysis. As 99 % of the *M. pusilla* biomass in our experiment was converted into DOC, it suggests that viral lysis is mainly decreasing the efficiency of the biological pump. But in order to understand these processes in more detail we need to verify these results for other species and under different environmental conditions.

Conclusions

Our results show that viral lysis (1) impacts the microbial food web by enhancing the production of both labile and refractory DOC and CDOM, (2) changes the optical signature of DOM and (3) could influence the particle aggregation by an enhanced TEP production. These effects of viral activity have multiple and opposite implications for the production and export processes in the pelagic ocean and it is therefore essential to increase our knowledge of the relative importance and contribution of these processes for obtaining a better understanding of the controls of oceanic biogeochemical cycles.

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