

Synechococcus growth in the ocean may depend on the lysis of heterotrophic bacteria

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In experiments designed primarily to investigate viral lysis, we found that the presence of viruses had a positive effect on the growth of *Synechococcus*. A Landry-Hassett-type stepwise dilution experiment conducted during a *Synechococcus* bloom in the Gulf of Mexico used both (i) 0.2- μ m filtered seawater in which the abundance of bacteria and grazers were reduced but the majority of viruses were retained, and (ii) ultrafiltered (30 000 MW cutoff) virus-free seawater in which the abundance of viruses, bacteria and grazers were reduced. High growth rates and frequency of dividing cells (FDCs) were recorded in 0.2- μ m filtered treatments while growth was inhibited in incubations with a high proportion of virus-free ultrafiltered water. In two subsequent experiments using Mediterranean Sea populations, a two-point dilution approach in which viral abundance was reduced by 80–90% yielded similar results, and showed that *Synechococcus* only grew well in the presence of viruses, bacteria and grazers. In four further Mediterranean experiments viruses removed via ultrafiltration were added back, either untreated, or inactivated by a heat treatment. Growth rates and FDCs were higher in the presence of untreated viruses than with viruses inactivated by heat, suggesting that it was not organic matter in the virus-size fraction but rather the presence of infectious viruses which sustained growth. While *Synechococcus* was also infected by viruses during these experiments, our data imply that growth of *Synechococcus* may depend upon viral lysis of heterotrophic bacteria. This finding is consistent with the view that nutrient cycling by viral lysis of heterotrophic bacteria may control phytoplankton growth and ecosystem scale carbon production.

KEYWORDS: viruses; growth control of cyanobacteria; heterotrophic bacterioplankton

INTRODUCTION

Unicellular cyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are significant primary producers in the ocean, contributing up to 50% of total primary production in some areas (Li, 1994; Liu *et al.*, 1998; André *et al.*, 1999). Consequently, defining the mechanisms controlling their growth rates and the fate of the carbon they produce is important for understanding nutrient and energy flow in the ocean. Grazing by heterotrophic nanoflagellates frequently appears inadequate to control the abundance of *Synechococcus* (e.g. Dolan and Simek, 1999). Another source of cyanobacterial mortality is lysis by viruses (Suttle, 2000). Viruses infecting *Synechococcus* (Suttle *et al.*, 1990a; Suttle and Chan, 1993) as well as those infecting *Prochlorococcus* (Sullivan *et al.*, 2003) are readily isolated from seawater. Cyanophages infecting *Synechococcus* are often abundant, with concentrations coupled to those of their putative hosts (Waterbury and Valois, 1993; Suttle and Chan, 1994; Wang and Chen, 2004). However, several studies have shown that viral lysis is usually only a moderate source of *Synechococcus* mortality *in situ* (Waterbury and Valois, 1993; Suttle and Chan, 1994; Garza and Suttle, 1998; Ortmann *et al.*, 2002; Baudoux *et al.*, 2007), in marked contrast to virus-mediated controls on heterotrophic bacteria.

Current food web models assume that phytoplankton growth in nutrient-limited systems is sustained primarily by nutrients recycled during grazing, which releases organic and inorganic nutrients (e.g. Buitenhuis *et al.*, 2010). Obviously, other sources of cell death such as viral lysis may have similar consequences for phytoplankton growth (Wilhelm and Suttle, 1999; Middelboe *et al.*, 2003; Weinbauer, 2004). With regard to heterotrophic bacteria, several studies have shown that bacterial respiration is stimulated in the presence of viruses (relative to their absence) suggesting that lysis products stimulate metabolism in the non-infected community (Middelboe *et al.*, 1996; Fuhrman, 1999; Middelboe and Lyck, 2002; Bonilla-Findji *et al.*, 2008). Viral lysis products originating from phytoplankton and bacteria are assimilated rapidly and fuel bacterial production (Gobler *et al.*, 1997; Noble and Fuhrman, 1999) implying a significant role for viruses in bacteria-driven processes. Potentially, the uptake of lysis products by heterotrophic bacteria could lead to enhanced rates of re-mineralization. Viral lysis could thereby indirectly enhance nutrient supply rates to phytoplankton. Notably, a more direct route is possible with regard to *Prochlorococcus* and *Synechococcus* as they can directly take up dissolved organic nitrogenous compounds (Zubkov *et al.*, 2003) and organic Fe (Wilhelm and Trick, 1994) so viral lysis products could directly stimulate growth.

Similarly, marine phytoplankton can assimilate Fe from Fe–organic complexes released by virus-mediated lysis (Poorvin *et al.*, 2004). Thus, viral lysis of heterotrophic bacteria potentially benefits *Synechococcus* by supplying nutrients while simultaneously removing their potential competitors (heterotrophic bacteria) for inorganic and organic nutrients.

Here, we report the results of experiments designed to study the interactions between *Synechococcus*, heterotrophic bacteria and viruses. The experiments allowed us to determine the effect of viruses on the growth of *Synechococcus* in the Gulf of Mexico and the NW Mediterranean Sea, areas where *Synechococcus* is abundant. In both environments, *Synechococcus* can contribute significantly (>50%) to primary production (Hagström *et al.*, 1988), and in the Gulf of Mexico can also form large monospecific blooms (Garza and Suttle, 1998). Initial experiments were performed to assess viral production and virus-induced mortality in *Synechococcus*. However, the results suggest that while potential agents of mortality, viruses may also sustain *Synechococcus* growth, probably via nutrient regeneration from lysis of co-occurring heterotrophic bacteria.

METHOD

Study sites

Seawater for the experiments was collected using Go-Flo bottles on 26 June 1995, during a *Synechococcus* bloom in the western Gulf of Mexico (shelf station E; 26°47'N, 96°38'W), and in the northern-western Mediterranean Sea in the Bay of Villefranche-sur-Mer (43°41'N, 7°19'E) on 23 September 2003, 4 June 2004, 12 March 2005 and 12 and 26 April 2005.

Gulf of Mexico experiment

The experiment was designed to estimate mortality of *Synechococcus* due to viruses as well as grazers. Seawater samples were diluted serially using a modified Landry–Hassett approach (Landry and Hassett, 1982) to reduce grazing (0.2- μ m filtered water) or grazing plus virus-mediated cell lysis (ultra-filtered water). Theoretically, viral mortality is estimated as the difference between expected higher growth in the ultra-filtered series, without viruses and growth recorded in the series with only grazers removed (e.g. Evans *et al.*, 2003; Baudoux *et al.*, 2006). Samples were serially filtered through a 1.2- μ m nominal pore-size glass-fibre filter (147-mm diameter; Gelman GC50) and a 0.2- μ m pore-size polycarbonate cartridge filter (Nuclepore) to remove

zooplankton, phytoplankton and most bacteria. Viruses from the 0.2- μm filtrate were removed using ultrafiltration (Amicon S10Y30 and S1Y30 cartridges; 30 000 MW cutoff) (Suttle *et al.*, 1991). Untreated seawater was diluted to 80, 60, 30 and 10% using either 0.2- μm filtrate or virus-free filtrate, sealed in rinsed polyethylene Whirl-pack bags and maintained at *in situ* temperatures in a flowing seawater bath at 37% ambient irradiance. Triplicate containers were sampled for cyanophage and *Synechococcus* abundances at \sim 6-h intervals. Experiments were begun at dawn and terminated 24 h later.

Mediterranean experiments

The basic design was a simplification of the dilution approach comparing two treatments: untreated and highly diluted. This simplification of the original dilution design is now used to assess grazing (e.g. Landry *et al.*, 2010). For the experiments, viruses were removed using a 100 000 MW cutoff polyethersulfone cartridge (PrepScale-TFF, Millipore). Virus-free ultrafiltrate from the PrepScale cartridge was used to reduce viral (and cell) abundance in untreated seawater by 90%. This treatment was compared with untreated seawater.

In four experiments performed in June 2004 and March–April 2005, we also examined the effects of heat-inactivation of material in the virus-size fraction to determine if living material was responsible for the consistent trends observed in the previous experiments (see results). Cells were separated from viruses by a 0.2- μm pore-size polycarbonate cartridge filter (Nuclepore) operated at low pressure (<100 mmHg) and viruses were then concentrated using a PrepScale cartridge. Half of this virus concentrate was treated for 30 min at 80°C to inactivate viruses, and the heat-treated and untreated viruses were added back to virus-free water. Cell concentrates obtained using the 0.2- μm cartridge were added back at \sim 20% of the *in situ* abundances in this reconstitution approach. Flow cytometric counting of bacteria and viruses in the concentrates (see below) was used to adjust the volumes of cell and virus concentrates added back to the ultrafiltrate. For all Mediterranean experiments, triplicate containers were incubated at *in situ* temperature in a flowing seawater bath with irradiation attenuated to \sim 40% of ambient.

Data from the Mediterranean Sea (Bay of Villefranche) indicate that the fraction of dividing cells (FDCs) of *Synechococcus* is highest after dawn and before midnight (Dolan and Simek, 1999; Bettarel *et al.*, 2002). The sampling interval for *Synechococcus* parameters (abundance and FDCs) in experiments performed in 2003 and 2004 was 4 h and confirmed the trend found

before in the same bay (data not shown); experiments lasted for 28 h in 2003 and 32 h in 2004 to obtain samples from the time period with the potentially highest FDC values. In the 2004 experiments, samples for enumeration of *Synechococcus* were collected in \sim 4 h intervals for 24 h. Samples for enumeration of prokaryotes and viruses were only taken at start-point and end-point.

Cyanophage abundance

For the Gulf of Mexico samples and the Mediterranean samples from 2003, the titre of infectious cyanophages was determined using a most-probable-number (MPN) assay with *Synechococcus* sp. DC2 as the host organism (Suttle and Chan, 1993). Titre was determined by adding 200 μL of exponentially growing cells to each well of a 96-well microtitre plate (Corning #25803). Samples to be titered were taken through a seven step, 10-fold dilution series using artificial seawater as the diluent (Cottrell and Suttle, 1991) and a 100 μL aliquot from each step of each dilution was added to each of the 16 wells of the microtitre plate. The number of wells in which lysis occurred was scored and the viral titre was determined using a BASIC program (Hurley and Roscoe, 1983). Data are presented as means and standard deviations of triplicate samples.

Synechococcus abundance and growth

Samples for estimating the abundance and FDCs of *Synechococcus* were filtered onto 0.2- μm pore-size black polycarbonate filters (Nuclepore). Abundance and FDCs was determined by counting a minimum of 200 autofluorescent cells in a minimum of 20 microscope fields using an epifluorescence microscope with green-light excitation (Suttle and Chan, 1993). In the Mediterranean 2005 experiments, *Synechococcus* was counted by flow cytometry (Jacquet *et al.*, 2006). The apparent growth rates of *Synechococcus* were calculated from the increase of \ln transformed cell numbers.

Bacterial and viral abundance; growth, turnover and decay

In the Gulf of Mexico experiments, heterotrophic bacteria were stained with diamidinophenylindole (DAPI) (Porter and Feig, 1980), filtered onto 0.2- μm pore-size polycarbonate filters and enumerated by epifluorescence microscopy. Bacteria and flagellates were also enumerated using DAPI in some of the samples from the Mediterranean experiments.

Estimates of viral abundance in the Gulf of Mexico employed epifluorescence microscopy of Yo-Pro stained samples filtered onto Anodisc (Whatman) 0.02- μm pore-size filters (Hennes and Suttle, 1995; Weinbauer and Suttle, 1997). For the Mediterranean samples, viruses and bacteria were stained using SYBRGreen I and counted by a FACSCalibur (BD Biosciences) flow cytometer as described previously (Gasol and del Giorgio, 2000; Brussaard, 2004).

Estimation of the frequency of visibly infected *Synechococcus* and burst size

Cells from 40 mL of the Gulf of Mexico seawater were collected quantitatively by centrifugation in a swinging-bucket rotor (SW 40; 20 000 g for 20 min) onto triplicate Formvar-coated, 400-mesh electron microscope grids, stained for 30 s with 1% uranyl acetate and thrice rinsed with distilled water using transmission electron microscopy (TEM). *Synechococcus* were distinguished from heterotrophic bacteria based on size and shape, and confirmed based on the relative abundance of *Synechococcus* and heterotrophic bacteria in parallel samples as determined using epifluorescence microscopy. Burst size was estimated by counting the number of virus particles in *Synechococcus* completely filled with viruses (maximum burst size *sensu* Weinbauer and Suttle, 1996). The percentage of visibly infected *Synechococcus* and burst size was determined for each of three electron-microscope grids by counting cells until a minimum of 10 visibly infected *Synechococcus* cells was enumerated on each grid (>3000 cells inspected with ~ 30 visibly infected). This analysis was only done in the Gulf of Mexico, where an exceptionally high abundance on *Synechococcus* (see results) allowed for this analysis.

Statistical analysis

Significant differences ($P < 0.05$) between treatments for specific time points were tested using Mann–Whitney U test statistics.

RESULTS

In situ data

The Gulf of Mexico site was characterized by a *Synechococcus* bloom with maximum abundances of $1.1 \pm 0.3 \times 10^5$ *Synechococcus* cells mL^{-1} and $6.3 \pm 2.7 \times 10^5$ infective cyanophages mL^{-1} (Table 1). Estimates by TEM indicated that $1.1 \pm 0.3\%$ of *Synechococcus* cells were visibly infected with an estimated burst size of 81 ± 17 viruses. At the Mediterranean site, *Synechococcus* and cyanophage abundances were 3.5×10^4 and 1.4×10^4 mL^{-1} , respectively, in September 2003, while *Synechococcus* abundance in June 2004 and in March–April 2005 ranged from 0.9 to 5.9×10^4 mL^{-1} (cyanophage data not available). For the Mediterranean samples, the abundance of *Synechococcus* was too low for TEM estimates of burst size and the percent of visibly infected cells.

Gulf of Mexico experiment

Regression analyses indicated that the abundances of *Synechococcus* were proportional to dilution with 0.2- μm filtrate and ultrafiltrate ($r^2 = 0.99$ and 0.98 , respectively; $P < 0.05$). The abundance of cyanophages also changed proportionally to dilution with ultrafiltrate ($r^2 = 0.97$, $P < 0.05$), and did not change significantly in 0.2- μm filtrate ($r^2 = 0.57$, $P > 0.21$). Viral and

Table 1: *In situ* characterization of study sites and abundances of viruses and cell types

Environment	T (°C)	Nitrate (μM)	Phosphate (μM)	Chl <i>a</i> ($\mu\text{g l}^{-1}$)	Bacteria (10^5 mL^{-1})	Viruses (10^6 mL^{-1})	<i>Synechococcus</i> (10^4 mL^{-1})	Cyanophages (10^4 mL^{-1})
Gulf of Mexico								
26 June 1995	28.4	ND	ND	0.17	6.2	18.4	11 ± 0.32	63 ± 2.7
Mediterranean Sea								
23 Sept 2003	22.8	0.27	0.03	0.15	6.6	10.9	3.5 ± 0.34	1.4 ± 0.31
4 June 2004	19.0	ND	ND	0.27	9.0	11.7	1.1	ND
15 March 2005	13.0	1.46	0.04	0.65	4.1	9.0	0.9	ND
12 April 2005	13.8	0.13	0.00	0.49	7.8	13.6	5.7	ND
26 April 2005	14.2	0.00	0.00	0.37	5.5	9.4	5.9	ND

ND, not determined. Data are given as means \pm SD of triplicate measurements where appropriate.

bacterial abundances were not determined in the experiment.

Regardless of treatment or dilution, cyanophage abundance remained constant or decreased during the experiments, while *Synechococcus* abundance increased over the first 20 h (data not shown). However, the growth rates of *Synechococcus* in untreated seawater ($0.65 \pm 0.03 \text{ day}^{-1}$; data not shown) and seawater diluted 90% with 0.2- μm filtrate ($0.69 \pm 0.06 \text{ day}^{-1}$; Fig. 1A) were similar. In contrast, the growth rate in seawater diluted 90% with virus-free ultrafiltrate was much lower ($0.06 \pm <0.01 \text{ day}^{-1}$; Fig. 1A). Evidence of the effect of removing viruses on *Synechococcus* growth rate is also seen in Fig. 1A + B; the FDCs at $t = 15 \text{ h}$

(corresponding to the early evening peak in FDCs) and growth rate at $t = 24 \text{ h}$ was much greater in treatments diluted 70 or 90% with seawater in which viruses were present than in treatments in which they were removed (Mann–Whitney U test, $P < 0.05$).

Mediterranean experiments

In September 2003 and June 2004, seawater samples were either diluted with ultrafiltrate to $\sim 10\%$ of their initial abundance to reduce contact rates between viruses and host cells or left untreated, and incubated for 28 or 32 h, respectively. In the diluted samples, the abundances of viruses, infectious cyanophages, bacteria and *Synechococcus* ranged from 12 to 16% of those in untreated seawater, indicating that the encounter rates between viruses and host were strongly reduced. Over the course of the experiments, in untreated seawater the viral abundances increased at a net turnover rate of $0.79 \pm 0.26 \text{ day}^{-1}$ (2003) and $0.13 \pm 0.04 \text{ day}^{-1}$ (2004), respectively, while bacterial abundance was either not significantly different or decreased slightly (Table II). In contrast, in the 90% dilution, viral abundance declined slightly in both experiments at a decay rate of 0.12 ± 0.07 and $0.47 \pm 0.03 \text{ day}^{-1}$, respectively, while bacterial abundance increased markedly at net growth rates of 0.63 ± 0.08 and $0.98 \pm 0.15 \text{ day}^{-1}$, respectively. In the 2003 dilution experiment, *Synechococcus* abundance increased in the untreated incubations (with viruses and grazers present) with net growth rate of 0.40, but decreased when the viruses and grazers were diluted by 90% with ultrafiltrate (Fig. 2). In the 2004 dilution experiment, growth rate was lower in the diluted treatment than in the untreated incubations, however, this difference was not significant (Fig. 3; Mann–Whitney U test, $P > 0.05$). In both experiments, the FDCs of *Synechococcus* were significantly higher in the untreated seawater than in the 90% dilution with virus-free ultrafiltrate (Mann–Whitney U test, $P < 0.05$). The abundance of infectious cyanophages in the diluted and untreated samples remained either constant or decreased (in the 2003 experiment) (data not shown); no cyanophage data are available for the 2004 experiment.

The decreases in *Synechococcus* growth rates in Mediterranean samples diluted with ultrafiltrate could be the result of chemical or biological activity, and if the latter, either heterotrophic flagellates or viruses could be responsible. To distinguish a biological from a chemical effect, cell concentrates and untreated and heat-treated viral concentrates were added back to ultrafiltered seawater for the June 2004 and the 2005 experiments. Bacterial and *Synechococcus* cells were diluted to

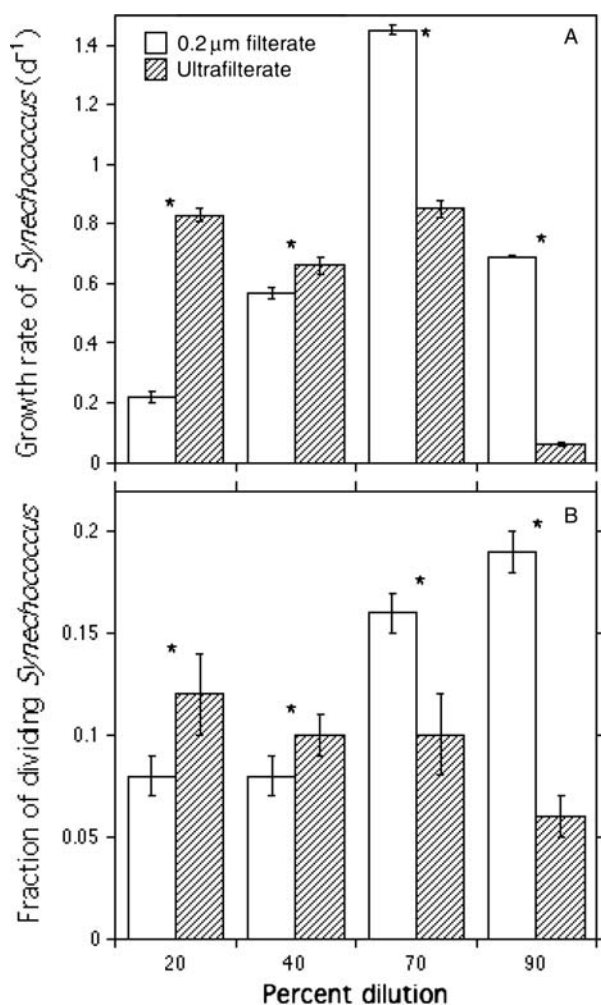


Fig. 1. *Synechococcus* parameters in a dilution experiment from the Gulf of Mexico. (A) Growth rate, (B) FDCs. Apparent growth rate and fraction of dividing *Synechococcus* cells were estimated in samples that were untreated or serially diluted with 0.2- μm filtrate (containing viruses) or virus-free ultrafiltered seawater. Means and error bars (\pm SD) are from triplicate incubations. *Significant difference between treatments ($P < 0.05$; Mann–Whitney U test); FDCs, fraction of dividing cells. FDCs: $T = 15 \text{ h}$; growth rate: $T = 24 \text{ h}$.

Table II: Bacterial growth rates and viral turnover and decay rate in the experiments from the Mediterranean Sea

Experiment	Treatment	Bacteria		Viruses	
		Growth rate (day ⁻¹)	Significance (P)	Turnover/decay rate (day ⁻¹)	Significance (P)
23 September 2003	Untreated seawater	0.10 ± 0.09	<0.05	0.79 ± 0.26	<0.05
	Virus + cell reduced	0.63 ± 0.08		-0.12 ± 0.07	
4 June 2004	Untreated seawater	-0.04 ± 0.03	<0.05	0.13 ± 0.04	<0.05
	Virus + cell reduced	0.98 ± 0.15		-0.47 ± 0.03	
4 June 2004	Active viruses	1.23 ± 0.06	NS	0.14 ± 0.02	<0.05
	Inactivated viruses	1.39 ± 0.32		-0.21 ± 0.06 ^a	
15 March 2005	Active viruses	0.20 ± 0.03	<0.05	0.71 ± 0.151	<0.05
	Inactivated viruses	0.65 ± 0.20		-0.42 ± 0.082 ^a	
12 April 2005	Active viruses	0.29 ± 0.11	NS	1.10 ± 0.282	<0.05
	Inactivated viruses	0.35 ± 0.30		-0.34 ± 0.332 ^a	
26 April 2005	Active viruses	0.19 ± 0.01	<0.05	0.69 ± 0.121	<0.05
	Inactivated viruses	0.43 ± 0.09		-0.32 ± 0.133 ^a	

Rates were calculated from abundances at T0 h and end of experiments (T24–32 h). Data are given as means ± SD of triplicate measurements. P-values are from Mann–Whitney U tests for treatments. NS, not significant. ^aDecay rates of inactivated viruses.

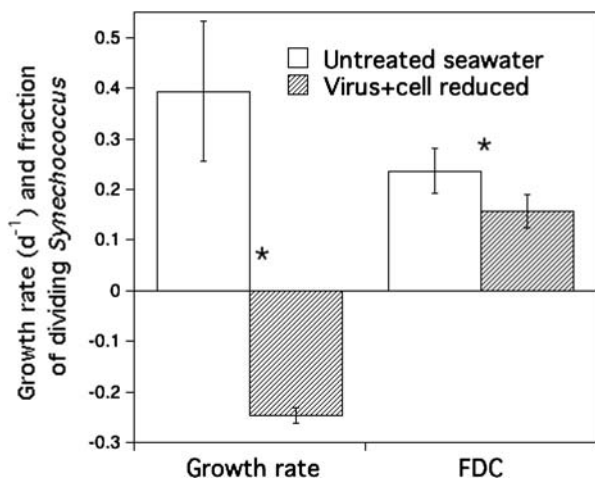


Fig. 2. *Synechococcus* parameters in a dilution experiment from September 2003 in the Mediterranean Sea. (A) Growth rate, (B) FDCs. Apparent growth rate and fraction of dividing *Synechococcus* was estimated in samples that were untreated or diluted 90% with ultrafiltrate (viruses, bacteria and grazers reduced). Means and error bars (±SD) are from triplicate incubations. *Significant difference between treatments ($P < 0.05$; Mann–Whitney U test); FDCs, fraction of dividing cells. FDCs: $T = 28$ h; growth rate: $T = 28$ h. A significant difference was also found for FDCs at $T = 8$ h.

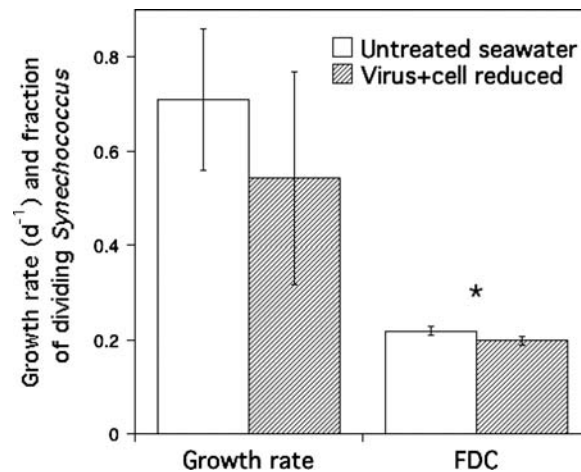


Fig. 3. *Synechococcus* parameters in a dilution experiment from June 2004 in the Mediterranean Sea. Apparent growth rate and fraction of dividing *Synechococcus* was estimated in samples that were untreated or diluted 90% with ultrafiltrate (viruses, bacteria and grazers reduced). Means and error bars (±SD) are from triplicate incubations. *Significant difference between treatments ($P < 0.05$; Mann–Whitney U test), FDCs, fraction of dividing cells. FDCs: $T = 32$ h; growth rate: $T = 32$ h. A significant difference was also found for FDCs at $T = 8$ h.

~20% of *in situ* abundances, while viral abundances in the virus-amended treatment averaged $118 \pm 26\%$ of *in situ* viral abundances indicating that the reconstitution with viruses was successful. Flagellates were not detected at the end of the experiments indicating that flagellate grazing was not significant. Viral growth in the active-virus treatment was detected in all experiments, whereas viral decay was observed in the inactivated virus treatments (Table II). Bacterial growth was detected in both treatments and was lower in the active

compared with in the inactivated virus treatment; however, this difference was only significant in two of the experiments (Mann–Whitney U test, $P < 0.05$).

In the 2004 experiment, the growth rate of *Synechococcus* was significantly higher (Mann–Whitney U test, $P < 0.05$) in the incubations containing untreated virus concentrates compared with the heat-inactivated virus concentrate (Fig. 4). Up to 15% of *Synechococcus* were dividing, a portion 1.7 times greater in the active than in the inactivated virus treatments (Mann–Whitney

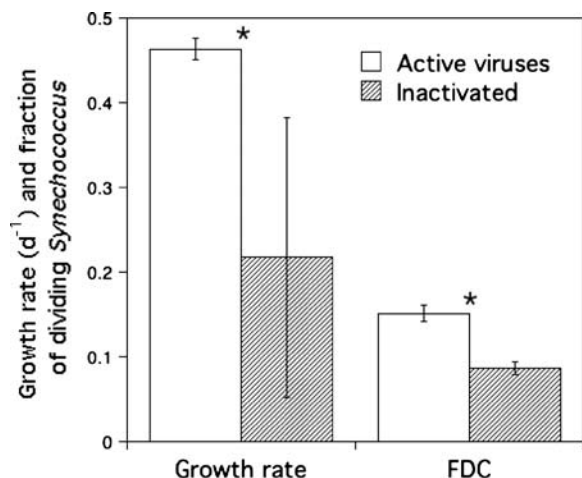


Fig. 4. *Synechococcus* parameters in a reconstitution experiment from June 2004. Apparent growth rate and fraction of dividing *Synechococcus* was estimated in samples to which either untreated (active) or a heat-treated (inactivated) viruses were added. Grazers were undetectable. Means and error bars (\pm SD) are from triplicate incubations. *Significant difference between treatments ($P < 0.05$; Mann–Whitney U test), FDCs, fraction of dividing cells. FDCs: $T = 32$ h; growth rate: $T = 32$ h.

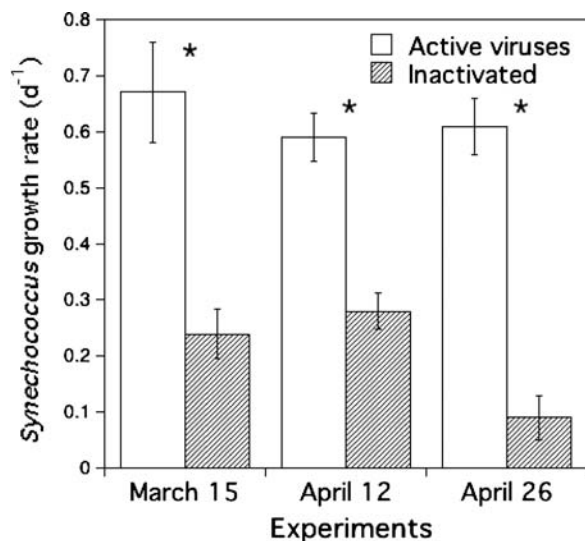


Fig. 5. Apparent growth rate of *Synechococcus* parameters in reconstitution experiments from March to April 2005. Apparent growth rate *Synechococcus* was estimated ($T = 24$ h) in samples to which either untreated (active) or a heat-treated (inactivated) viruses were added. Grazers were undetectable. Means and error bars (\pm SD) are from triplicate incubations. *Significant difference between treatments ($P < 0.05$; Mann–Whitney U test).

U test, $P < 0.05$). In all 2005 experiments, the growth rate of *Synechococcus* was significantly higher (Mann–Whitney U test, $P < 0.05$) in the active virus than in the virus inactivated treatment (Fig. 5; FDCs were not estimated in the 2005 experiments).

DISCUSSION

Data from several experiments were obtained in the Gulf of Mexico and the Mediterranean Sea. Although different approaches and analysis techniques were used, this body of work provides persuasive evidence that the presence of active viruses stimulates the growth of *Synechococcus*. The most likely explanation for these results is that viral lysis of heterotrophic bacteria releases nutrients required for the growth of *Synechococcus*. These findings are considered in more detail below.

Decreased *Synechococcus* growth when virus abundance is reduced

In the Gulf of Mexico (Fig. 1) and Mediterranean (Figs 2–5) experiments, growth rates of *Synechococcus* were reduced when contact rates between infectious viruses and host cells were decreased by dilution with virus-free seawater or seawater containing only heat-treated viruses. For heterotrophic bacteria, lower virus contact rates resulted in decreased lysis rates and increases in the abundances, thus less nutrients released as the result of bacterial cell lysis. In contrast, in samples that were untreated or reconstituted with infectious viruses, viral abundance increased relative to $t = 0$, bacterial abundance and growth was lower than in the virus-reduced (diluted or inactivated) treatment, and growth rates of *Synechococcus* were higher.

It is noteworthy that similar results, diminished growth of *Synechococcus* in virus-free water, have been reported previously. Baudoux *et al.* (Baudoux *et al.*, 2008) investigated virus versus grazing mortality of picoplankton in the North Sea using parallel dilution series (0.2- μ m and ultrafiltered seawater). In two out of the six experiments, a negative mortality of *Synechococcus* due to lysis was recorded indicating higher growth of *Synechococcus* in the presence of viruses compared with incubations in waters with greatly reduced viral abundances.

Viral stimulation of *Synechococcus* growth could be direct, indirect, or some combination of the two. Direct stimulation could occur via the uptake of lysis products. Viral action could indirectly stimulate *Synechococcus* growth by reducing the abundance of its competitors for inorganic nutrients, heterotrophic bacteria; or again indirectly through catalysing nutrient cycling by heterotrophic bacteria. With regard to direct stimulation, viral lysis of heterotrophic bacteria releases dissolved and particulate organic matter (Riemann and Middelboe, 2002; Middelboe and Jorgensen, 2006). We know that these products of viral lysis are taken up rapidly by heterotrophic bacteria (Noble and Fuhrman, 1999; Middelboe

et al., 2003). However, cyanobacteria can also take up dissolved organic material (Zubkov *et al.*, 2003; Poorvin *et al.*, 2004) so it would seem reasonable to assume that growth of *Synechococcus* can be directly stimulated by the uptake of lysis products. Another, non-exclusive, possibility is that as heterotrophic bacteria can compete with phytoplankton for inorganic nutrients (Currie and Kalff, 1984; Suttle *et al.*, 1990b; Thingstad *et al.*, 1993; Kirchman and Wheeler, 1998), less viral lysis of bacteria, resulting in increased bacterial growth, reduces nutrients available to *Synechococcus* (Thingstad and Lignell, 1997). The third possibility, also non-exclusive, is that viral lysis stimulates enzymatic activity and respiration of bacteria (Middelboe *et al.*, 1996; Middelboe and Lyck, 2002). Stimulation of bacterial respiration in the presence of viruses has been observed for the Mediterranean study site (Bonilla-Findji *et al.*, 2008; Motegi *et al.*, 2009). The uptake of lysis products with elemental ratios similar to host ratios should result in the production of inorganic nutrients such as N, P or Fe, since uptake and incorporation of organic matter requires respiratory carbon (Wilhelm and Suttle, 1999). Indeed, it has been argued that the catalytic recycling of nutrients is a major role of viruses (Suttle, 2005; Brussaard *et al.*, 2008b). Thus, heterotrophic bacterial remineralization of inorganic nutrients from the products of viral lysis may stimulate the growth of *Synechococcus*.

Organic matter in the virus size fraction can stimulate the growth of some bacteria (Winter *et al.*, 2004). Cyanobacteria are also able to exploit dissolved organic matter (Zubkov *et al.*, 2003; Poorvin *et al.*, 2004). Whether or not *Synechococcus* could also be directly stimulated by organic matter in the virus-size fraction can be examined by comparing *Synechococcus* growth in incubations with heat-treated and untreated viruses. The growth of *Synechococcus* was higher in the presence of active versus inactivated viruses (Table II). Thus, a heat-labile component stimulated the growth of *Synechococcus*, which is consistent with live viruses, rather than organic matter in the viral size fraction, enhancing the growth rate.

P limitation occurs in the Gulf of Mexico during blooms of *Synechococcus* (Wawrik *et al.*, 2004), and also in the Bay of Villefranche (the NW Mediterranean Sea) from late spring to early fall (Dolan *et al.*, 1995; Thingstad *et al.*, 1998), when *Synechococcus* also has a strong affinity for phosphate (Tanaka *et al.*, 2004). Thus, viral lysis may have stimulated phosphate recycling by heterotrophic bacteria, which in turn enhanced the growth of *Synechococcus*. However, the mechanisms suggested above are not mutually exclusive, and our experiments were not designed to discriminate among them.

Nutrient-limited growth of *Synechococcus*?

Data from dilution experiments using nutrient-enrichments and non-supplemented water support the view that *Synechococcus* growth may depend on locally recycled nutrients (e.g. Ayukai, 1996). Rough calculations can be made for the Mediterranean experiments to assess whether enough nitrogen and phosphorus is potentially released by lysis of heterotrophic bacteria to support the observed growth of *Synechococcus* in our experiments. To begin, we can estimate virus production from the viral stock at the start of an experiment and the net growth rate. The rate of bacterial lysis can be estimated by dividing viral production by a burst size of 30, which is slightly higher than the highest values reported from the Mediterranean study site (Weinbauer *et al.*, 2002). The concentration of nitrogen and phosphorus released was estimated by assuming 20 fg C cell⁻¹ (Lee and Fuhrman, 1987) and a C:N:P ratio of 45:9:1 for bacteria (Goldman *et al.*, 1987). The ‘virus-supported’ production of *Synechococcus* was calculated from the difference in the growth rate in whole seawater versus the virus + cell reduced treatment (or the active versus inactivated virus treatment) and the abundance of *Synechococcus* in the whole seawater and active virus treatment, respectively. To estimate nitrogen and phosphorus requirements, a range of 20.0–50.2 fg N and 0.47–3.34 fg P *Synechococcus* cell⁻¹ was used; these values were found for *Synechococcus* strains grown in P-replete and P-limited conditions (Bertilsson *et al.*, 2003). Using the highest N and P quota for *Synechococcus*, the nitrogen requirement can on average be met 1.2 times, whereas on average 2.0 times more phosphorus is produced than needed. Using the lowest quota, the nitrogen requirements are met on average 2.9 times and the phosphorus requirement 14.0 times (Table III). These estimates are conservative, since viral net production was not corrected for decay rates. Our rough calculations suggest that viral lysis of heterotrophic bacteria could have released enough nitrogen and particularly phosphorus to sustain the observed growth stimulation of *Synechococcus* in the presence of viruses.

Difficulty in estimating viral mortality in *Synechococcus*

The original rationale for these studies was to apply the dilution approach used to estimate grazing rates by microzooplankton (Landry and Hassett, 1982) to assess lysis rates of *Synechococcus* by cyanophages. The approach assumes that dilution with virus-free water results in increased growth rates of *Synechococcus* as viral contact

Table III: Estimation of nutrient concentrations released by viral lysis of bacteria compared to the nutrient concentrations needed by *Synechococcus* for the observed growth stimulation in the presence of viruses

Experiments	Viral production ($10^6 \text{ mL}^{-1} \text{ day}^{-1}$)	Bacterial lysis ($10^5 \text{ mL}^{-1} \text{ day}^{-1}$)	<i>Synechococcus</i> stimulation ($10^3 \text{ mL}^{-1} \text{ day}^{-1}$)	Ratio of nutrient concentrations released by lysis to nutrient concentrations needed by <i>Synechococcus</i>			
				High cell quota		Low cell quota	
				N	P	N	P
23 September 2003	9.9	3.3	22.5	0.9	1.6	2.3	11.1
4 June 2004	7.0	2.3	2.2	1.7	2.8	4.2	20.1
4 June 2004	0.9	0.3	0.4	2.4	4.1	6	28.8
15 March 2005	1.4	0.5	0.7	0.3	0.5	0.7	3.3
12 April 2005	2.7	0.9	3.5	1.4	2.3	3.4	16.5
26 April 2005	1.7	5.6	6.1	0.4	0.6	0.9	4.3
Average \pm SD				1.2 ± 0.8	2.0 ± 1.4	2.9 ± 2.0	14.0 ± 9.8

For details of calculation see text. Following cell quota were used for *Synechococcus*: 20.0 and 50.2 fg nitrogen cell⁻¹ and 0.47 and 3.34 fg phosphorus cell⁻¹ (Bertilsson *et al.*, 2003). N, Nitrogen; P, Phosphorus.

rates, and hence lysis rates decrease. However, in both the Gulf of Mexico and Mediterranean Sea, *Synechococcus* growth rates decreased when the abundance of infectious viruses was reduced by dilution or heating. Moreover, the addition of ultrafiltrate did not directly reduce growth rates. Similarly, in the Mediterranean experiments *Synechococcus* growth was stimulated by the addition of concentrated viruses to samples which were diluted by ultrafiltrate, consistent with ultrafiltrate not inhibiting the growth rate of *Synechococcus*.

Although dilution experiments have been used to estimate viral lysis rates of eukaryotic phytoplankton (Evans *et al.*, 2003; Baudoux *et al.*, 2006) our results indicate that it might not be suitable for estimating viral-mediated mortality of *Synechococcus*. This is because of the confounding effects of growth stimulation by viral lysis products released from heterotrophic bacteria. In fact, in every experiment we conducted, growth rates of *Synechococcus* were lower in treatments in which the concentration of viruses was reduced.

This may be the reason that significant viral lysis rates for *Synechococcus* and *Prochlorococcus* have not been always observed or were low compared with viral lysis of eukaryotic phytoplankton in other studies where the dilution approach has been employed (Baudoux *et al.*, 2007; Kimmance *et al.*, 2007; Baudoux *et al.*, 2008; Brussaard *et al.*, 2008a). Also, in two out of six experiments reported by Baudoux *et al.* (Baudoux *et al.*, 2008) *Synechococcus* showed negative mortality in the presence of viruses thus indicating problems with the dilution approach. It is possible that the application of the method may be environment specific; nonetheless, non-linear environmental effects on the length of the lytic cycle, burst size and adsorption rates suggest that the

specific host–virus system should be well defined before inferences are made on mortality rates *in situ*.

Conclusion

Contrary to both expectation and intuition, the presence of viruses and bacteria in seawater stimulated the growth of *Synechococcus*. Although these experiments do not definitively show the mechanism responsible for this enhancement, it seems likely that uptake of viral lysis products by other members of the bacterial community resulted in remineralization of inorganic nutrients that enhanced the growth of *Synechococcus*. In experimental studies, increasing the abundance of viruses has been shown to reduce growth rates of phytoplankton (Suttle *et al.*, 1990a; Suttle, 1992), but ultimately lead to higher phytoplankton yield (Peduzzi and Weinbauer, 1993). This suggests that primary production is in part dependent on virus-mediated nutrient cycling by bacterioplankton (Poorvin *et al.*, 2004; Weinbauer, 2004; Suttle, 2005). Here, we present evidence that the natural virus community not only stimulated the growth of *Synechococcus*, but was essential for maintaining productivity. This represents a previously not fully appreciated control mechanism of primary productivity in the ocean.

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