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Viral effects on bacterial respiration, production and growth efficiency: Consistent trends in the Southern Ocean and the Mediterranean Sea

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Abstract

To investigate the potential effects of viruses on bacterial respiration (BR), production (BP) and growth efficiency (BGE), experiments were performed using natural microbial communities from the coastal Mediterranean Sea, from a typical high-nutrient low-chlorophyll (HNLC) region in the Southern Ocean and from a naturally iron (Fe)-fertilized algal bloom above the Kerguelen Plateau (Southern Ocean). Seawater was sequentially filtered and concentrated to produce a bacterial concentrate, a viral concentrate and a virus-free ultrafiltrate. The combination of all three fractions served as treatments with active viruses. Heating or microwaving was used to inactivate viruses for the control treatments. Despite the differences in the initial trophic state and community composition of the study sites, consistent trends were found. In the presence of active viruses, BR was stimulated (up to 113%), whereas BP and BGE were reduced (up to 51%). Our results suggest that viruses enhance the role of bacteria as oxidizers of organic matter, hence as producers of CO_2, and remineralizers of CO_2, N, P and Fe. In the context of Fe-fertilization, this has important implications for the final fate of organic carbon in marine systems.
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1. Introduction

It is well established that a significant fraction (often half or more) of the total carbon flux in marine food webs passes through bacterioplankton (Azam et al., 1983; Cole et al., 1988; Fuhrman, 1992). Heterotrophic bacteria perform two major functions in the carbon cycle: they are the only organisms producing a significant amount of new biomass (bacterial secondary production, BP) from dissolved organic carbon (DOC), and transform DOC into inorganic carbon (bacterial respiration, BR) (Williams, 1981; Cho and Azam, 1988; Pomeroy et al., 1991). A great deal has been learned on bacterial production and its regulation during the past several decades (Cole et al., 1988; Ducklow and Carlson, 1992; Kirchman, 1993; Kirchman et al., 1995; Carlson and Ducklow, 1996) but far less is known on the control of BR and bacterial growth efficiency (BGE), the latter exhibiting a very wide range of variation ($<5\%$ to more than 60%; del Giorgio and Cole, 1998).

This gap limits our understanding of the role and relative importance of bacteria in the carbon cycling of aquatic ecosystems. An important step has been achieved with the recent incorporation of marine viruses in studies of microbial food web dynamics (Fuhrman, 1999; Wommack and Colwell, 2000; Weinbauer, 2004). Growing evidence suggests that viruses, with abundances typically an order of magnitude higher than those of bacteria, are important

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players in microbial food webs, not only as agents of bacterial mortality but also as mediators of carbon and nutrient cycling. In an attempt to examine the potential impact of viruses on planktonic communities, Fuhrman (1999) modeled the carbon flux of a hypothetical steady-state food web where bacterial loss due to viral lysis was equivalent to the loss due to grazing by protists. In this model, viral lysis led to an increase in bacterial production and respiration, and to a decrease in carbon export to protozoan grazers. The few experiments that have addressed this issue suggest that viral activity promotes the recycling of carbon and nutrients by bacteria in the water column through the production of DOC from lysis of cells (Middelboe and Lyck, 2002; Middelboe et al., 1996; Gobler et al., 1997; Noble and Fuhrman, 1999). Middelboe et al. (1996) reported that lysis products reduce the growth efficiency of non-infected bacteria and increase their metabolic activity and growth rate. By reducing the carbon transfer to higher trophic levels (Thingstad et al., 1993; Fuhrman, 1999) viruses can influence the biogeochemical cycles by increasing the residence time of carbon and mineral nutrients in the euphotic zone and hence affect the functioning of all marine ecosystems.

Eukaryotic algae are responsible for the majority of the flux of organic matter to higher trophic levels and the ocean interior (Falkowski et al., 2004). In about one-third of the world ocean, in the so-called high-nutrient low-chlorophyll (HNLC) regions, phytoplankton growth is Fe-limited (Martin et al., 1991). Bacterioplankton, which generally dominates these waters, is thought to be the key player of the "microbial ferrous wheel" (Kirchman, 1996), since it contains more than twice iron per biomass than eukaryotic phytoplankton, thereby storing about 50% of the biogenic iron in that system (Tortell et al., 1996). Viral activity, in turn, has a potential impact on nutrient regeneration. In most of the cases, nutrients released as a result of viral lysis are thought to be organically complexed, which may facilitate their use by marine plankton (Rue and Bruland, 1997; Poorvin et al., 2004). Viral lysis can release more than 10% of ambient Fe concentrations (Gobler et al., 1997) and thus potentially relieve its limitation in depleted environments.

The role of heterotrophic prokaryotes and viral activity, however, has been largely ignored in the studies addressing iron control on phytoplankton growth (Boyd et al., 2007). The nine *in situ* mesoscale Fe-enrichment experiments performed so far in the HNLC regions have confirmed the role of Fe in regulating primary productivity but resulted in only small carbon export and depth sequestration (de Baar et al., 2005; Street and Paytan, 2005). To date, none of the studies have examined BR and viral activity during the final stages of the Fe induced algal bloom, the fate of the fixed carbon remains obscure.

Through the regulation of the metabolic activity of bacterioplankton, viral lysis drives the fate of organic carbon by influencing the rate of carbon production and export. Few quantitative data have been published on the effect of viruses on bacterial activity and many questions remain unanswered. For example, it is not known whether the influence of viruses on bacterioplankton is the same across systems. In the present study, we investigated the impact of viruses on bacterial metabolic activities in three contrasting environments: a typical nutrient-rich HNLC region in the Southern Ocean, a naturally Fe-fertilized area in the Kerguelen plateau and the coastal Mediterranean Sea.

2. Material and methods

2.1. Study sites and experimental approach

To assess the effects of viral enrichment on bacterial metabolism (BR, BP and BGE), experiments were carried out in the Mediterranean Sea in April and May 2002 (MED1 and MED2, respectively) and in the Southern Ocean in February 2005, including a typical HNLC region and the late stage of a naturally Fe-fertilized bloom above the Kerguelen Plateau (bloom). The Southern Ocean experiments were performed on board the R/V *Marion Dufresne* in the frame of the project KEOPS (KErguelen: comparative study of the Ocean and Plateau Surface waters). For experiments MED1 and MED2, surface-water samples (ca. 100 L), were collected in acid-rinsed 20-L polypropylene carboys from a coastal station in the Bay of Villefranche (43°41′00″N, 07°19′00″E; bottom depth: 95 m), transported to the laboratory and immediately pre-filtered through a 142-mm diameter polycarbonate membrane (Osmonics Inc., 0.8 μm pore size) to remove protists and larger cells. In the Southern Ocean, 1-μm pre-filtered seawater was obtained by pumping from 60 m depth at the HNLC station KEOPS C11 (51°38′53″S, 77°59′47″E, bottom depth: 3300 m) and at the bloom station KEOPS A3 (50°38′34″S, 72°05′17″E, bottom depth: 476 m). Data on environmental conditions at the time of sampling are summarized in Table 1. Prokaryotes were concentrated using a tangential flow system equipped with a 0.2-μm pore-size polycarbonate cartridge (Durapore and Pellicon, Millipore in the Mediterranean and Southern Ocean, respectively). To obtain virus-free seawater, 0.2-μm pore-size filtered seawater was passed through 0.1 N NaOH-washed polyethersulfone spiral cartridges (Millipore Prep/Scale TFF, 100 kDa cutoff) using a tangential flow ultrafiltration system. The retentate of this filtration process is the viral concentrate (between 100 kDa and 0.2 μm; viral size fraction). Prokaryotic concentrates were diluted with virus-free seawater to reach abundances 20–65% of the *in situ* concentrations and amended with viral concentrate to reach viral abundances ca. 0.7–2.4 times that in the field (V_1). All material used was acid-washed, rinsed with MilliQ and sample water prior to use.

For the inactivated virus control, a subsample from the viral concentrate was microwaved (850 W) in an ice bath at 20 s intervals for 2–3 min (Proctor and Fuhrman, 1992) for experiments in the Mediterranean (V_m) or heated at 80 °C

Table 1
Environmental conditions

Site/date	Environmental conditions								Experimental conditions		
	Temperature (°C)	Salinity	NO_3 (μmol L^{-1})	NO_2 (μmol L^{-1})	PO_4 (μmol L^{-1})	Chl *a* (μg L^{-1})	Bacteria (10^6 mL^{-1})	Virus (10^7 mL^{-1})	Treatment	Bacteria (10^6 mL^{-1})	Virus (10^7 mL^{-1})
MED											
April 2002	14.2	38.0	0.20	0.07	0.02	0.32	1.29	0.63	V_0	0.59	0.02
									V_m	0.83	1.43
									V_1	0.84	1.52
May 2002	15.8	37.9	1.45	bdl	0.04	0.40	1.43	0.67	V_0	0.72	0.04
									V_m	0.74	0.77
									V_1	0.81	1.08
S. Ocean											
HNLC	1.7	33.8	28.1	0.32	2.18	0.27	0.34	0.39	V_h	*	0.75
									V_1	0.10	0.70
Bloom	3.6	33.9	22.6	0.28	1.76	1.90	0.42	1.25	V_h	0.08	0.93
									V_1	0.09	0.83

In situ concentration of inorganic nutrients, chlorophyll *a* (Chl *a*), prokaryotes and viruses. bdl, below detection limit. The treatments used were: V_0 (no viral addition), V_m, V_h (inactivated viruses) and V_1 (active virus enrichment). *Data not shown, since bacterial abundance was ca. 2 times higher than in all other time points and treatments of this experiments and therefore probably an outlier.

for 20 min for experiments in the Southern Ocean (V_h). MED1 and MED2 received a supplementary control without viral addition (V_0). Note that in V_0, some viruses are present, since not all viruses are removed during the concentration of bacteria. Replicate 60-mL BOD bottles were filled and incubated in the dark at *in situ* temperature to measure respiration rates, bacterial and viral abundances, bacterial production as well as changes in total organic carbon.

2.2. Viral and bacterial abundances

Samples for viruses and cells were fixed with glutaraldehyde (0.5% final concentration), kept for 15–30 min at 4 °C, transferred to liquid nitrogen and finally stored at −80 °C pending enumeration. For MED1 and MED2, viral and bacterial abundances were determined by epifluorescence microscopy (EM) as described in Noble and Fuhrman (1998) after staining with SYBR Gold (Molecular Probes) according to Chen et al. (2001). For the HNLC and bloom experiments, viral particles and prokaryotes were stained with SYBR Green I (Molecular Probes) and quantified using a FACScalibur (Becton and Dickinson) flow-cytometer (FC) after dilution with TE buffer (10 mM Tris, 1 mM EDTA, pH 8). For viruses the optimized protocol of Brussaard (2004) was followed. Previous inter-calibration exercises using MED samples showed that differences in particle counts between EM and FC were lower than 10%.

2.3. Bacterial production, respiration and growth efficiency

The incorporation of ^{3}H leucine into protein (Smith and Azam, 1992) was used to estimate heterotrophic bacterial production. In MED1, triplicate samples and a trichloroacetic acid (TCA)-killed control received 12 nM of hot leucine and either 0, 40 or 80 nM of cold leucine (12, 52 and 92 nM final concentrations) in order to check for saturation conditions, while for MED2 a final concentration of 25 nM was used. In the Southern Ocean experiments, leucine (hot:cold = 1:9) was added at a final concentration of 40 nM to triplicate samples and the control. BP was estimated from leucine incorporation using a conversion factor of 1.5 kg C mol^{-1} for the Mediterranean samples and 3.1 kg C mol^{-1} for the Southern Ocean samples (Kirchman, 1993; Lemée et al., 2002), assuming a carbon content per cell of 20 fg C $cell^{-1}$ (Lee and Fuhrman, 1987). BR was estimated from oxygen uptake (Knap et al., 1996). At each time-point of the incubations (time 0 and after 24, 48 and 72 h), three to five 60-mL BOD replicate bottles were fixed with Winkler reagents for each treatment. Dissolved oxygen concentration was determined by the Winkler titration technique based on a potentiometric and photometric endpoint detection, in the Mediterranean and Southern Ocean, respectively. The respiration rate and its standard error were determined by regressing O_2 against time during the intervals of incubation. Bacterial growth efficiency was calculated as BGE = BP/(BP + BR) assuming a respiratory quotient of 1. Since BR and BP were measured at different time scales (over at least 24- and 1-h incubation, respectively), BGE was calculated using the average BP during the incubation (between time 0 and 24, 48 or 72 h). Bacterial carbon demand was calculated as BCD = BP + BR. The imbalance in incubation time is a potential problem for evaluating BGE and BCD data. However, the main focus of this work is not to obtain absolute values but to assess the effect of viruses.

2.4. Total organic carbon

Duplicate 10-mL samples were collected from each treatment in pre-combusted (450 °C for 24 h) glass ampoules (Wheaton), acidified with 12 μL of 85% phosphoric acid (Merck Chemicals), immediately flame-sealed and stored in the dark at room temperature pending analysis. TOC concentrations were determined as described by Lemée et al. (2002).

2.5. Ancillary parameters

The concentration of chlorophyll *a* was measured fluorometrically (Yentsch and Menzel, 1963). Nitrate (NO_3), orthosilicic acid ($SiOH_4$) and orthophosphate (PO_4) were determined as described by Tréguer and Le Corre (1975) using an Alliance-Instrument EV2 Autoanalyzer in the Mediterranean Sea and a Technicon autoanalyzer in the Southern Ocean following standard methods (Lorenzen, 1966; Strickland and Parsons, 1972).

2.6. Statistics

When assumptions of normality and homogeneity of variances were met, one-way analyses of variances were performed to determine statistical significance of bacterial metabolic parameters between treatments. Otherwise, a non-parametric Kruskal–Wallis test was used. Significant differences between the enrichment treatment and the control were assessed using two-tailed *t*-tests. Statistical significance was assumed when $p < 0.05$. Statistical testing was done with *SigmaStat* (Jandel Scientific/SPSS) or *StatView 4.5* (Abacus Inc., 1995) software. Data are reported as mean ± standard deviation (S.D.). The standard deviation of average BP, changes in TOC and percent stimulation of BR, BP, BGE and BCD were calculated by propagating the errors (Gans, 1992) assuming that the covariance terms are negligible as all bacterial processes were measured independently.

3. Results and discussion

3.1. Characterization of study sites

Physical, chemical and biological data of the study sites are summarized in Table 1. In the Mediterranean Sea, concentrations of nutrients and Chl *a* were comparatively low, whereas bacterial abundance was high. In the Southern Ocean, nutrient concentrations were high and Chl *a* concentrations were low at the HNLC site (KEOPS C11) and high at the bloom station (KEOPS A3). Bacterial abundance was much lower than in the Mediterranean Sea. Considering all investigated study sites, viral abundance was lowest in the HNLC station and highest at the bloom site. The virus to bacteria ratio (VBR) was about 5 for the Mediterranean, 10 for the HNLC site, which is similar to the open ocean VBR of the Southern Ocean (Marchant et al., 2000) and 30 for the bloom, comparable to values described from the coastal region of the Antarctic peninsula (Guixa-Boixereu et al., 2002). VBRs increased with nutrient load and productivity in agreement with the literature (Wommack and Colwell, 2000).

3.2. Temporal variations of bacterial and viral abundance

Bacterial abundance increased in all treatments of the MED1 and MED2 experiments (Fig. 1), and there was no significant difference between the treatments. In both experiments, the viral abundance in the V_0 represented 5% of *in situ* abundances demonstrating that the viral abundance was successfully reduced in this control. In the enrichment treatments, viral abundance was 20–37 times higher than in V_0.

In MED1, the viral abundance of the virus-enriched treatment decreased during the first 24 h and subsequently exhibited no significant changes. No significant difference was observed between V_1 and V_m, although the viral abundance was slightly higher in the V_1 (Fig. 1). In contrast, in MED2, viral abundance in the V_m and V_1 treatments increased significantly after 24 h (Fig. 1) and was significantly higher in V_1 than in the two controls.

In the Southern Ocean, the different trophic situations strongly influenced the dynamics of bacterial and viral abundance during the experiments. At the HNLC site, viral abundance increased linearly in the active virus treatment and remained roughly constant in the control. In contrast, in the active virus treatment, bacterial abundance decreased at the end of the experiment, whereas it remained constant in the control (Fig. 2). In the bloom experiment, viral abundance increased dramatically at the end of the incubation and was more than two-fold higher than in the control. Bacterial abundance showed a similar trend in both treatments but the decrease of bacterial abundance at the end of the experiment was more pronounced in the active virus treatment. However, this should be considered with caution, since viral and bacterial counts could not be replicated in the Southern Ocean.

It is worth mentioning that viral abundance increased in some controls. This might be due to incomplete inactivation. However, while this could have occurred in the microwave treatment, it seems unlikely in the heat treatment. The increase also could be attributed to the release of new viruses from bacteria already infected at the start of the experiment or from new infection, since viruses are also introduced in incubations along with bacterial concentrates.

In experiment MED2, viral production estimated from the increase in viral abundance between 24 and 48 h was significantly higher in the V_1 than in the V_m treatment. Viral production (VP) rate in the active virus enrichment was 7.9×10^6 particles $mL^{-1} d^{-1}$, which is 66 and 1.3 times higher, respectively, than in the V_0 and V_m controls. Considering a burst size of 23 phages $cell^{-1}$ as determined for the Bay of Villefranche (Weinbauer et al., 2002), the

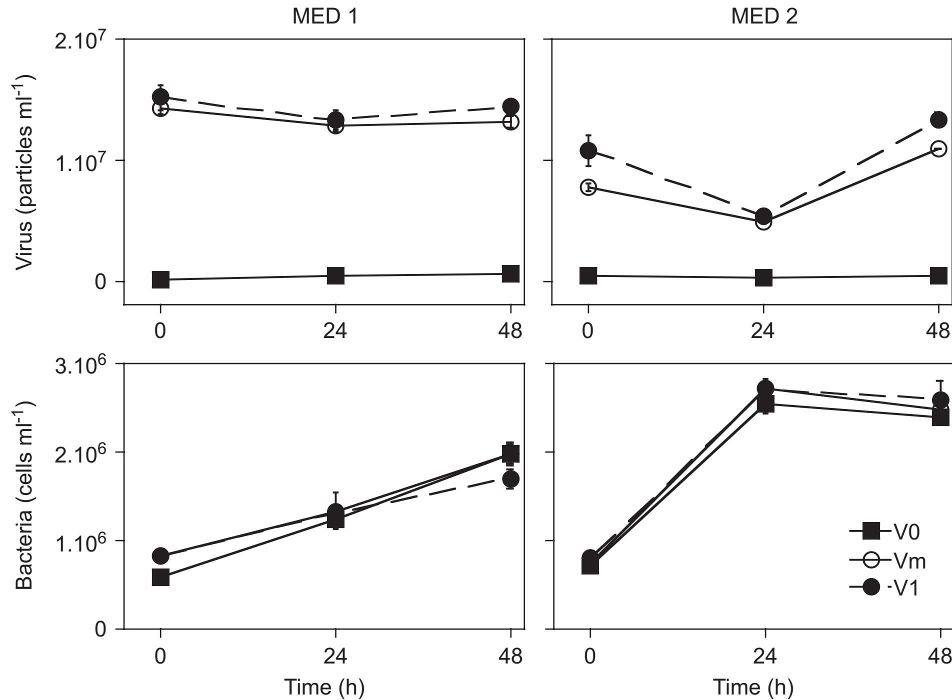

Fig. 1. Time course of viral and bacterial abundance in the virus-enriched (V_1) and control treatments (V_0 and V_m) in experiments MED1 and MED2. Values are given as means and the error bars are standard deviations of triplicate incubations. When no error bars are shown, the standard deviation was smaller than the width of the symbol.

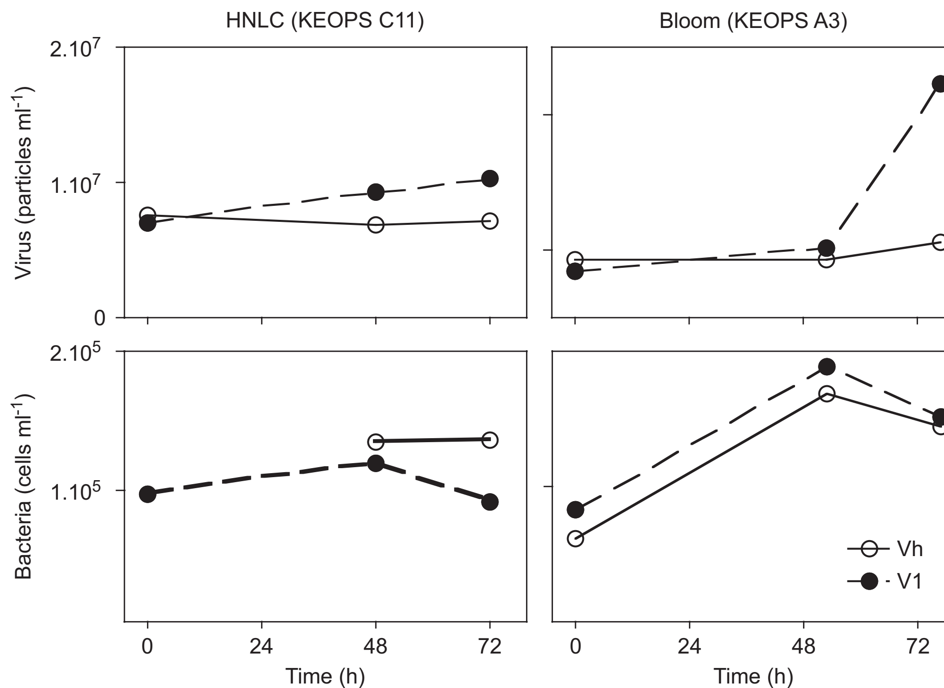

Fig. 2. Time course of viral and bacterial abundance in the virus-enriched (V_1) and control treatment (V_h) in experiments from the HNLC site and the algal bloom in the Southern Ocean. Values derive from single BOD bottle incubations.

estimated rate of lysis by viruses in the V_1 treatment was about 3.5×10^5 bacteria $mL^{-1} d^{-1}$, corresponding to a loss rate of BP of approximately 20%. This result is consistent with the generally assumed range from 10% to 30% losses of bacterial production due to viral lysis reported in the literature (Wommack and Colwell, 2000).

In the Southern Ocean at the HNLC site, VP was $1 \times 10^6 mL^{-1} d^{-1}$ corresponding to an estimated viral lysis

rate of 2.1×10^4 bacteria $mL^{-1}\,d^{-1}$ using a burst size of 50 phages $cell^{-1}$ as determined during the KEOPS cruise (Weinbauer, unpublished data). The resulting average loss rate of BP was 8%. Experiments from the natural Fe-fertilized bloom, revealed VP rates of $4.4\times10^6\,mL^{-1}\,d^{-1}$ in the virus-enriched treatment, which was more than 3 times higher than in the controls. The estimated viral lysis rate was 8.7×10^4 bacteria $mL^{-1}\,d^{-1}$ in the virus-enriched treatments corresponding to a loss rate of BP of 108% for last day BP, when viral abundance strongly increased. This suggests that viruses were entirely responsible for bacterial mortality in the late algal bloom, which is corroborated by feeble grazing rates of heterotrophic protozoa (Christaki et al., 2008). Interestingly, bacterial abundance decreased just slightly at this time-point. A similar decrease in bacterial abundance in the virus-free control may be a consequence of substrate limitation since there is no release of DOC in this treatment, whereas the high viral production in the virus treatment should be accompanied by the production of substrate for the non-infected bulk of the bacterial population thereby buffering the difference between virus treatment and control.

In some experiments, the viral abundance was only slightly higher in V_1 than in the control treatments. One of the reasons could be that the observed viral production of ca. $10^6\,mL^{-1}\,d^{-1}$ occurred at a high background of viruses of $10^7\,mL^{-1}$, making it difficult to detect the differences. An experiment performed in freshwater also did not detect changes in viral abundance between V_1 and V_h, although the frequency of infected cells in the virus treatment was more than twice higher than in the control (Weinbauer et al., 2007). Viruses also can cause cell lysis without production of viral progeny (Weinbauer, 2004). For example, "lysis-from-without", i.e. destruction of a cell by several simultaneously attaching phages, results in the release of viral lysis products, which can affect BP and BR without viral proliferation. Another example would be autolysis of cells after infection. Also, it is possible that we have missed a peak in virus abundance. Such mechanisms could also have resulted in an effect on metabolic activity and explain the lack of strong viral production in some experiments.

However, in the present study, the strongest viral effect on BP was detected in the bloom experiment, in which the increase in viral abundance was most pronounced although this was not reflected in a strong decrease in bacterial abundance. In experiments performed during a cruise in the Algerian Basin of the western Mediterranean Sea (Bonilla-Findji et al., unpublished data), we found that viral presence caused a decrease of net BP, while there was an increase in the total BA via the regulation of the proportion of the bacterial population with larger, high nucleic acid (HNA) content that are typically the highly active members of the community (Gasol et al., 1999; Vaqué et al., 2001; Lebaron et al., 2002). Thus, virus treatments might show low bacterial production because they are dominated by the less productive and smaller low nucleic acid (LNA) bacterial subpopulation. Consequently, a lower biomass accumulation was likely distributed in more but smaller cells. This could also explain why we did not find differences in bacterial abundance between treatments in all experiments, although bacterial production was always reduced in the active virus treatment (see Section 3.5).

3.3. Evaluation of controls for assessing viral effects on metabolic activity

In experiment MED1 after 24 h, BR was highest in the V_0 treatment and lowest in the V_m treatment, whereas BP was highest in the V_m control and similar in the two other treatments (Table 2). In experiment MED2, BR was higher and BP was lower in V_1 than in the two controls. However, the difference between V_0 and V_1 was larger than that

Table 2
Changes in bacterial metabolic parameters in experiments from the Mediterranean

Parameter	Time (h)	MED1			MED2		
		V_0	V_m	V_1	V_0	V_m	V_1
BR (μmol C $L^{-1}\,h^{-1}$) ± S.E.	24	0.087 ± 0.037	0.059 ± 0.009	0.072 ± 0.006	0.145 ± 0.016	0.164 ± 0.008	0.198 ± 0.012
	48	0.072 ± 0.015	0.105 ± 0.010	0.068 ± 0.005	0.119 ± 0.008	0.133 ± 0.009	0.165 ± 0.008
BP (μmol C $L^{-1}\,h^{-1}$) ± S.D.	24	0.017 ± 0.001	0.031 ± 0.004	0.019 ± 0.000	0.050 ± 0.004	0.046 ± 0.001	0.037 ± 0.001
	48	0.024 ± 0.002	0.039 ± 0.009	0.024 ± 0.003	0.046 ± 0.004	0.043 ± 0.001	0.038 ± 0.003
BGE (%) ± S.D.	24	16 ± 7	34 ± 8	21 ± 2	26 ± 4	22 ± 1	16 ± 1
	48	25 ± 6	27 ± 9	26 ± 4	28 ± 4	24 ± 2	19 ± 2
BCD (μmol C $L^{-1}\,h^{-1}$) ± S.D.	24	0.104 ± 0.046	0.090 ± 0.018	0.092 ± 0.008	0.195 ± 0.026	0.211 ± 0.011	0.235 ± 0.015
	48	0.096 ± 0.021	0.144 ± 0.037	0.092 ± 0.012	0.165 ± 0.020	0.176 ± 0.012	0.203 ± 0.020
Δ TOC (μmol C $L^{-1}\,h^{-1}$) ± S.D.	48	−0.05 ± 0.002	−0.10 ± 0.002	−0.43 ± 0.012	−0.18 ± 0.007	0.05 ± 0.001	−0.12 ± 0.003

BR, bacterial respiration calculated from regression vs time, $n = 9$–15; BP, average bacterial production over experimental time, $n = 6$; BGE, bacterial growth efficiency; BCD, bacterial carbon demand; ΔTOC, change of total organic carbon; S.D., standard deviation; S.E., standard error. The treatments used were: V_0 (no viral addition), V_m (inactivated viruses) and V_1 (active virus enrichment).

between V_m and V_1. The response of BR and BP to viral addition was only consistent between experiments when V_m was used as a control (Table 2).

Microwaved or heat-inactivated viruses have rarely been used in experimental enrichment studies investigating the effect of viruses on bacterial production and growth efficiency (Table 3). However, viruses inactivated by microwaving or heating are probably a better control than the absence of viruses because DOM in the viral size fraction is added back with the viral amendment. It has been shown that some bacterial phylotypes grow in the presence of the viral size fraction of DOM (whether the sample was inactivated or not) but not in the absence of this size fraction (Winter et al., 2004a). The possibility that microwaving modified the DOM composition of the virus concentrate cannot be ruled out. However, an analysis of the fluorescence spectra of colored DOM of a microwaved viral concentrate showed no difference compared to an untreated viral concentrate (data not shown). These lines of evidence suggest that V_m is a more reliable control than V_0. Consequently, viral effects are only discussed using V_m (and V_h) as a control in the following section.

3.4. Effect of incubation time on bacterial respiration

Two preliminary experiments in the Mediterranean focused on the virus-mediated effects on BR and their change at different time scales (data not shown). BR presented two different trends depending on the duration of the incubation. It was stimulated by the presence of viruses in a first phase and repressed in a second phase. This was also found in MED1 (stimulation, compared to V_m, after 24 h and repression after 48 h), whereas BR was stimulated after 24 and 48 h in MED2 (Table 2). The transition in the bacterial response might be a consequence of the changes in bacterial community composition and dissolved organic matter (DOM) cycling due to confinement. Indeed, it has been shown that confinement with and without the use of viral concentrates can trigger changes of the composition and the activity of bacterial communities and therefore impair the assessment of their activity (Massana et al., 2001; Gattuso et al., 2002; Winter et al., 2004b). Variable effects of viral enrichment on BR (from 78% reduction to 92% stimulation) were recently observed in 24-h incubations of natural bacterioplankton commu-

Table 3
Summary of published studies and approaches used to investigate the effects of viruses on the activity of bacterioplankton

Experimental design	Control(s)	Incubation time (h)	Parameter	Effect (% change)	Date	Source
Dilution culture. *Vibrio* sp. strain O6N-21, its virus O6N-21P, 5% seawater, natural bacterioplankton	*V* autoclaved	72	BGE	−66	nda	Middelboe et al. (1996)
		72	BR	+55	nda	
		72	BP	0	nda	
		96	DOC utilization	+72	nda	
Natural communities. <0.6 μm fraction, 2-fold HMWC	V_0 and *V* autoclaved	70	Net BP	+59 to +350	February 1995	Noble and Fuhrman (1999)
Natural communities. Use of BC<0.2 μm and <0.02 μm fractions	*V*− (reduction of viruses)	8	BGE	−43	nda	Middelboe and Lyck (2002)
		8	BR	0	nda	
		28	BR	+	nda	
		28	Net BP	−62	nda	
Natural communities. <0.6 μm fraction, 2-fold HMWC	*V*+ (viral enrichment/ natural abundance)	24	BR	−78	June 1999	Eissler and Quinones (2003)
		24	BR	+92	September 1999	
Natural communities. Use of BC, VC and <100 kDa fraction	V_0, V_m (1.2–2.4× enrichment)	24	BP	−38	April 2002	This study*
		24	BR	+21		
		24	BCD	−1		
		24	BGE	−39		
		24	BP	−20	May 2002	
		24	BR	+20		
		24	BCD	+11		
		24	BGE	−28		
	V_h (1.9× enrichment)	72	−32	−32	February 2005	
		72	−51	−51		
		72	+25	+25		

*Data from the present study are expressed relative to microwave inactivated viruses (V_m) as a control. BGE, bacterial growth efficiency; BR, bacterial respiration; BP, bacterial production; BCD, bacterial carbon demand; HMWC, high molecular weight concentrate (30 kDa cutoff); VC and BC, viral and bacterial concentrates; nda, no dates available; +, positive stimulation; −, negative stimulation (i.e. repression); 0, no effect. V_0, bacterial concentrate added to virus-free water (see Section 2).

nities in coastal waters of Chile (Eissler and Quinones, 2003). Therefore, the discussion of BP and BR focuses on data obtained during the first 24 h of incubation in the Mediterranean. This duration allowed detection of changes in oxygen concentration and viral effects on bacterial metabolic activity, while minimizing confinement effects. In the Southern Ocean, respiration rates only could be measured after 72-h incubation, probably due to low water temperatures, and these data are used in the following discussion.

3.5. *Effect of viruses on metabolic activity*

In experiment MED1, BR was slightly higher in V_1 than in the V_m control after 24 h, corresponding to a stimulation by 21%. Conversely, during a longer incubation BR was significantly ($p<0.05$) higher in V_m than in V_1. In experiment MED2, BR was ca. twice the rate measured in MED1. BR was significantly higher in the active virus-enriched treatment than in the control at 24 and 48 h, thus suggesting a stimulatory effect of viruses independent from the duration of the incubation. After 24 h, BR was stimulated by 20% relative to V_m, which is similar to the stimulation observed in the first experiment (Fig. 3). In the experiments performed in the Southern Ocean, BR was stimulated significantly after 72 h by 25% and 113% at the HNLC site and within the bloom, respectively. This suggests a stimulatory effect of viruses independent from the duration of the incubation length and trophic situation (Table 4). However, the stimulation of BR through viruses was considerably higher in the late algal bloom than in the HNLC waters along with higher viral production and bacterial mortality (see above).

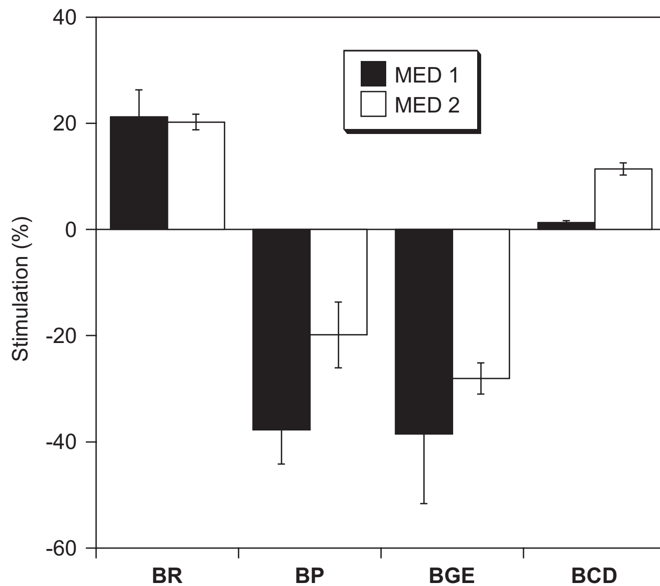

Fig. 3. Summary of virus-mediated effects on bacterial metabolism in the Mediterranean. Data are expressed as percent stimulation and the error bars are standard deviations after 24 h of the virus-enriched treatment (V_1) relative to the inactivated viruses control (V_m). BR, bacterial respiration; BP, bacterial production; BGE, bacterial growth efficiency; BCD, bacterial carbon demand.

BP was always significantly higher in V_m or V_h than in V_1. In the Mediterranean, BP in presence of active viruses was reduced by 38% and 20% relative to V_m, respectively, in MED1 and MED2 (Fig. 3). Similarly, in the Southern Ocean (Fig. 4), active viruses caused a 51% and 46% reduction of BP at the HNLC site and during the bloom, respectively. The BGE ranged from 16% to 34% at the Mediterranean sites and from 35% to 70% in the Southern Ocean. BGE showed the same significant trend as BP in all experiments: it was reduced by 39% and 28% in MED1 and MED2 after 24 h of incubation and by 32% and 48% at the HNLC and bloom after 72 h of incubation. BCD was stimulated in the presence of viruses by 1% in MED1 and by 11% in MED2, and stimulated by 4% in the bloom, whereas it was reduced by 28% at the HNLC site. However, the stimulation in the bloom was not statistically significant (Fig. 4).

TOC consumption was calculated for 48 h since some samples taken at 24 h seemed to be contaminated as indicated by higher values compared to t_0. In experiment MED1, 0.43 μmol C L^{-1} h^{-1} of TOC were consumed after 48 h in the presence of active viruses, whereas the consumption rate was 4 times lower in the V_m treatment, averaging only 0.10 μmol C L^{-1} h^{-1} (Table 2). In experiment MED2, the highest TOC consumption after 48 h was also observed in V_1 with 0.12 μmol C L^{-1} h^{-1}, while ΔTOC was close to zero in the inactivated control. Differences of

Table 4
Changes in bacterial metabolic parameters in experiments from the Southern Ocean

Parameter	Time (h)	HNLC (KEOPS C11)		Bloom (KEOPS A3)	
		V_h	V_1	V_h	V_1
BR (μmol C L^{-1} h^{-1}) ± S.E.	72	0.010 ± 0.003	0.012 ± 0.003	0.003 ± 0.001	0.007 ± 0.002
BP (μmol C L^{-1} h^{-1}) ± S.D.	72	0.022 ± 0.006	0.011 ± 0.006	0.007 ± 0.001	0.004 ± 0.0005
BGE (%)	72	70 ± 34	47 ± 37	68 ± 37	35 ± 14
BCD (μmol C L^{-1} h^{-1}) ± S.D.	72	0.031 ± 0.012	0.023 ± 0.013	0.010 ± 0.005	0.010 ± 0.004

BR, bacterial respiration calculated from regression vs. time, $n = 9$–10; BP, average bacterial production over experimental time, $n = 6$; BGE, bacterial growth efficiency; BCD, bacterial carbon demand; S.D., standard deviation; S.E., standard error. The treatments used were: V_0 (no viral addition), V_h (inactivated viruses) and V_1 (active virus enrichment).

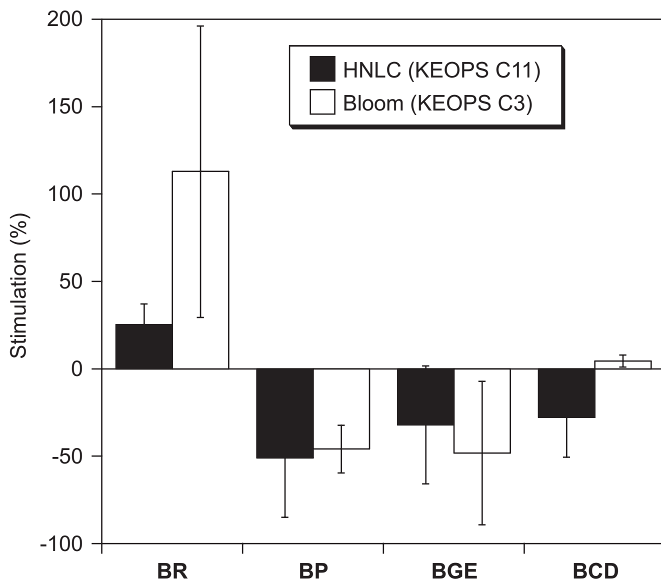

Fig. 4. Summary of virus-mediated effects on bacterial metabolism in the Southern Ocean. Data are expressed as percent stimulation after 72 h of the virus-enriched treatment (V_1) relative to the inactivated viruses control (V_h). BR, bacterial respiration; BP, bacterial production; BGE, bacterial growth efficiency; BCD, bacterial carbon demand.

less than 2.5 μmol C L^{-1} h^{-1} between measurements were not considered significant as it corresponds to the error of the measurement as defined by the instrument parameters. Rates of TOC consumption and BR theoretically should be the same. However, this was not the case in the MED1 and MED2 experiments, in which the V_1 incubations exhibited ΔTOC up to 6 times higher than BR. Several causes could explain this discrepancy, such as contamination or attachment to the incubation bottle walls of living and non-living organic matter. It is difficult to evaluate which of these factors is responsible for the higher ΔTOC values and it is probable that it is due to a combination of attachment and contamination.

Despite the different study sites and different initial conditions and viral and bacterial production rates throughout experiments, consistent trends concerning the effect of viral infection on bacterial activity could be observed. When differences across systems were assessed, BR values were significantly higher in the presence of active viruses than in the virus-inactivated controls, whereas BP was significantly lower (paired *t*-test). Differences in the magnitude and timing of the bacterial response to viral enrichment between the experiments might be due to differences in the trophic conditions, DOM availability, community composition or lysis rates at the respective study site. Other studies also have reported a virus-related increase in BR and reduction of BGE (Middelboe and Lyck, 2002; Middelboe et al., 1996). A model assuming that half of the bacterial mortality is due to viral lysis predicted that BR is stimulated by 33% compared to a virus-free system (Fuhrman, 1999), which is close to values found in the present study. We found a repression of BP in the presence of active viruses, which is in contrast to the observation of Noble and Fuhrman (1999) but supports the data previously reported by Middelboe and Lyck (2002).

One explanation could be that BP is only stimulated by viruses in the presence of grazing, when viral lysis diverts a significant fraction of carbon, which would otherwise be transferred to higher trophic levels, into lysis products. The reduced BGE (28–48% reduction relative to the controls) and increased respiration in the presence of active viruses might be due to recycling of organic matter by viral lysis as suggested by Middelboe and Lyck (2002). The hypothesis that viral infection directly increases BR but represses BP cannot be ruled out, although viral infection, at least during the initial part of the latent period, does not affect bacterial activity estimated by thymidine incorporation in bacterial isolates (Middelboe, 2000).

Our data from the Mediterranean suggest that about 20% of the bacterial production was removed by viral lysis after 24 h of incubation. At the HNLC site in the Southern Ocean, viruses removed a small fraction (8%) of bacterial production. In contrast, in the naturally Fe-fertilized bloom above the Kerguelen Plateau, the estimates indicate that viruses were responsible for the majority of bacterial mortality. These data are derived from 72-h incubations and therefore should be considered with caution. Nevertheless, mortality data based on a virus reduction approach (Weinbauer et al., 2002), which was assessed during the cruise but not presented in the present work, suggest the same trend. In addition, the other major mortality factor for bacterioplankton, protistan bacterivory, was lower during the bloom than in the HNLC sites (Christaki et al., 2008).

3.6. Biogeochemical implications

The higher respiration of bacterioplankton in the presence of viruses suggests that they enhance the role of bacteria as oxidizers of organic matter, hence as CO_2 producers, and remineralizers of N, P or Fe (Middelboe and Lyck, 2002; Middelboe et al., 1996). This is of major importance where some nutrients are scarce, such as Fe in HNLC regions of the Southern Ocean or phosphorus in the Mediterranean Sea. It is still unclear whether, under certain trophic or substrate conditions, enhanced bacterial activity increases competition with phytoplankton for inorganic nutrients or whether it stimulates phytoplankton production via remineralization. Through their lytic action viruses induce a short-circuit in the microbial loop (“viral shunt”), sequestering carbon into dissolved and smallest particulate forms, which are available for other bacteria (Wilhelm and Suttle, 1999). Our data suggest that DOM remineralization increases as indicated by stimulated respiration and reduced growth efficiency assuming that the N- and P-rich bacterial cell content is recycled. This could lead to the retention of nutrients in the euphotic zone (Fuhrman, 1999; Fuhrman and Suttle, 1993) and to reduced carbon

transport to the deeper ocean. In the context of the KEOPS program, we sampled the final stages of a naturally Fe-fertilized algal bloom (Blain et al., 2007). Viral production was considerably higher in the late algal bloom compared to the HNLC waters outside the naturally Fe-fertilized sites. The stimulation of viral production, viral-mediated increase in BR and of bacterial mortality by fertilization recycles organic matter in the microbial loop and the viral shunt, making less material available for export to the deep sea. This mechanism also could help explain why mesoscale *in situ* Fe-fertilization experiments have failed to show massive carbon export fluxes (Street and Paytan, 2005).

4. Conclusion

Our study from the oligotrophic coastal Mediterranean Sea, HNLC site in the Southern Ocean and a natural Fe-fertilized bloom is one of the few having investigated the viral impact on bacterial metabolism and adds to the growing experimental evidence that viruses affect bacterial metabolism and ecosystem function. Our data suggest that viral lysis plays a similar role for bacterioplankton activity and nutrient cycling in strongly contrasting environments and support the merging view that viruses are catalysts of nutrient cycling (Suttle, 2005). This result has important biogeochemical implications with respect to the carbon export into the deep sea.

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