

Viral control of bacterial growth efficiency in marine pelagic environments

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Abstract

We tested the hypothesis that viruses can control bacterial growth efficiency (BGE) in marine pelagic environments. In the Bay of Villefranche, Northwestern Mediterranean, three experiments were conducted on different months to determine bacterial and viral variables in seawater cultures. In December, phosphorus (P) addition enhanced bacterial growth 6–9-fold with concomitant increase in viral production (4–15-fold), but little enhancement of bacterial respiration (BR). In other months, P enrichment increased BR 2–6-fold and viral production 2–5-fold, but did not increase in bacterial abundance (Aug, Feb) or growth (Feb). BGE depended on the fraction of bacterial production destroyed by viruses (shunting efficiency, ν ; i.e., when ν was low, nutrient enrichment enhanced BGE, whereas when ν was high, nutrient enrichment mainly led to low BGE). Viral production and bacterial production and respiration in the Western North Pacific and other data from the literature showed that BGE was negatively correlated with shunting efficiency. Predictions from a carbon flow model were consistent with the above results showing that decreased BGE over a broad range of values (from 0.7 to 0.001) could be largely explained by viral-induced conversion of bacterial biomass to dissolved organic carbon. Viruses exert the major influence on patterns in carbon fluxes mediated by bacteria in marine pelagic environments.

Bacteriophages (viruses) are widespread and abundant in marine environments, where they are major agents of bacterial mortality (Weinbauer 2004; Breitbart et al. 2008). Because bacteria dominate the oceanic biomass and consume a substantial fraction (20% to 60%) of the organic carbon from primary production in marine ecosystems (Azam and Malfatti 2007), viral-induced bacterial mortality, associated with transformations of materials, can affect marine food webs and biogeochemical cycles (Weinbauer 2004; Breitbart et al. 2008). However, there is still little information on the controls of viral production and the regulation of carbon and nutrient cycling mediated by bacteria–virus interactions in marine environments.

Because the production of viruses depends on host metabolism, the factors that limit bacterial growth could also limit viral production. In support of this notion, previous studies have suggested that nutrient addition enhanced viral production with concomitant increase in bacterial growth (Tuomi et al. 1995; Williamson and Paul 2004; Motegi and Nagata 2007). However, there are studies reporting that nutrient enrichment led to increased viral production with no apparent enhancement of bacterial growth. For example, Motegi and Nagata (2007) reported that addition of nitrogen resulted in increased production of viruses (up to 3-fold), but not of bacteria, at a station in the subtropical South Pacific. In order to explain this uncoupling in the responses of bacteria and viruses to nutrient addition, the authors suggested that the increase in bacterial growth was masked by enhanced mortality caused

by viruses. High viral-induced mortality relative to bacterial growth should result in effective conversion of biomass (biomass yield due to nutrient enrichment) to dissolved organic matter (DOM), which is at least partly available for bacterial re-consumption (Fuhrman 1999; Wilhelm and Suttle 1999). The resultant, cyclic flow of carbon, called 'viral shunt' (Wilhelm and Suttle 1999), is predicted to reduce the efficiency of conversion of DOM to bacterial biomass (Fuhrman 1999; Miki et al. 2008), with strong enhancement of bacterial respiration relative to production. Thus, an overall consequence of nutrient enrichment, accompanied by high viral-induced mortality, is high viral production and low bacterial growth (i.e., uncoupled response), with altered metabolic balance of the system as a whole toward catabolism. However, testing this hypothesis has been hampered by the lack of data relating the magnitude of the viral shunt to anabolic (production) and catabolic (respiration) parameters of marine bacterial communities.

It was reported that viral production is often limited by phosphorus (P) in aquatic environments (Tuomi et al. 1995; Williamson and Paul 2004). This has been explained by stoichiometric constraints posed by the P-rich nature of viruses (i.e., the C:N:P ratio of viruses has been estimated to be ~10:4.5:1 [Hewson et al. 2003]). In order to approach systematically the patterns and controls of carbon flows mediated by bacteria and viruses, we examined bacterial production, respiration, and viral production in cultures prepared with surface seawater collected in a P-limited environment.

In the Mediterranean Sea, microbial communities are generally P-limited or stressed, as indicated by high

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Table 1. Experimental set up of the seawater culture experiments, which were conducted on the basis of a 2×2 factorial design: active viruses and P are factors and addition and nonaddition are levels. Marks indicate addition (○) or nonaddition (–) of viral-free seawater, bacterial concentrate, viral concentrate, inactivated viral concentrate, or P (NaH_2PO_4 solution added at a final conc. $1 \mu\text{mol L}^{-1}$) to each treatment.

Treatments	Viral-free seawater	Bacterial concentrate	Viral concentrate	Inactivated viral concentrate	P
Control	○	○	–	○	–
V	○	○	○	–	–
P	○	○	–	○	○
P+V	○	○	○	–	○

phosphate turnover, especially in summer (Tanaka et al. 2004), and from results of bioassay experiments showing enhancement of bacterial activity in response to P addition (Thingstad et al. 1998). A dramatic, trophic propagation triggered by P enrichment has been reported in the eastern Mediterranean by Thingstad et al. (2005), who found that P-induced enhancement of bacterial production resulted in increased zooplankton egg production. However, no previous study has examined the responses of viruses to P addition in the Mediterranean.

In the present study, we conducted P addition experiments to surface-water samples from the Bay of Villefranche (Northwestern Mediterranean) to determine variations in viral and bacterial parameters over seasons and among treatments, covering a range of situations with variable extent of P limitation and viral-induced mortality. In addition, we collected data on viral production and bacterial production and respiration in surface waters of the Western North Pacific in order to examine relationships between bacterial growth efficiency and the extent of viral-induced destruction of bacteria. We also developed a carbon flow model to analyze the above data.

Methods

Seawater culture experiments in the Bay of Villefranche—We conducted experiments in August 2005, December 2005, and February 2006 using seawater samples collected at a station (Point B, $43^\circ 41.00' \text{N}$, $7^\circ 19.00' \text{E}$) in the Bay of Villefranche, Northwestern Mediterranean Sea. Water samples were collected at a depth of 10 m using a 20-liter Niskin bottle, transferred to 20-liter polycarbonate tanks, and brought back to the laboratory. Ninety liters of sample water were filtered through $0.8\text{-}\mu\text{m}$ pore-size filters (Isopore ATTP, diameter 142 mm; Millipore) by applying positive pressure (<67 cm Hg) using a filtration system consisting of a stainless-steel filter holder (YY3014236, Millipore), a positive pressure tank (XX6700P20, Millipore), and an air pressure pump. Particles in the $<0.8\text{-}\mu\text{m}$ size fraction were concentrated (final volume, 500–780 mL) using either a Durapore cartridge ($0.22\text{-}\mu\text{m}$ pore-size, CVDI01TPE; Millipore; Aug) or a Pellicon filter-cassette ($0.22\text{-}\mu\text{m}$ pore-size, PTGVPPC05; Millipore; Dec and Feb) to be used as ‘bacterial concentrate.’ The water that passed

through the $0.22\text{-}\mu\text{m}$ pore-size filters was filtered through a 100-kDa cut-off polyethersulfone membrane cartridge (Prep/scale-TFF, CDUF002TH; Millipore) to obtain a ‘viral concentrate’ (final volume, 320–480 mL) and ‘viral-free seawater’. Half of the viral concentrate was heated three times nearly to boiling by microwave and chilled on ice for 10 min to be used as ‘inactivated viral concentrate.’

Triplicate bottles (2-liter polycarbonate; Nalgene) were prepared for each of four treatments including a nonaddition control (control, a mixture of bacterial concentrate, inactivated viral concentrate and viral-free seawater), viral addition treatment (V, a mixture of bacterial concentrate, viral concentrate and viral-free seawater), P addition treatment (P, a mixture of bacterial concentrate, inactivated viral concentrate, viral-free seawater and phosphate), and P plus virus addition treatment (P + V, a mixture of bacterial concentrate, viral concentrate, viral-free seawater and phosphate; Table 1). The proportions of the added bacterial concentrate, viral concentrate (or inactivated viral concentrate) and viral-free seawater were adjusted to obtain final concentrations of bacteria and viruses in each treatment that were to be equal to the in situ concentrations of bacteria and viruses at the time of sampling in the Bay of Villefranche; later analyses was conducted to examine actual abundances (*see* Results). For the P and P + V treatments, NaH_2PO_4 was added at a final concentration of $1 \mu\text{mol L}^{-1}$. The bottles were incubated for 48 h at in situ temperature in the dark. Containers and plastic wares used for the sampling and preparations of the culture were rinsed before use with 1.2 N HCl followed by vigorous rinsing with Milli-Q water. Cartridge filters were cleaned with 0.1 N NaOH. During sample collection and handling, gloves were worn, and care was taken to minimize contamination.

We determined the bacterial production rate in December and February (but not in Aug because of logistic constraints) from the rate of leucine (Leu) incorporation (Kirchman 2001). Triplicate subsamples and 1 trichloroacetic acid (TCA)-killed control received 2 nmol L^{-1} [methyl- ^3H] Leu ($6.14 \text{ TBq mmol}^{-1}$, Amersham, TRK636) and 38 nmol L^{-1} cold Leu (Sigma, L8000) and were incubated for 30 min at in situ temperature in the dark. After incubation, samples were spiked with 100% TCA (final conc. 5%), filtered through $0.2\text{-}\mu\text{m}$ nitrocellulose filters (GS, 25-mm diameter; Millipore), and extracted with cold 5% TCA and 80% ethanol. Filters were placed into glass vials, dried, dissolved with ethyl acetate, and mixed with scintillation cocktail (7 mL, Ultima Gold; Packard Instruments) for measurement of radioactivity using a Perkin Elmer Tri-Carb 2900 TR scintillation counter. The coefficient of variations (CVs) of triplicate measurements were on average 20% (range = 2–62%). Values of disintegration per minute for the killed control were typically $<5\%$ of those for samples.

Bacterial respiration rates were estimated from the decrease of dissolved oxygen concentration during 48-h incubations of water samples dispensed into biochemical oxygen demand (BOD) bottles (60-mL capacity). Dissolved oxygen concentration was determined by Winkler titration using an automated titrator with a potentiometric end-

point detector (97-78-00; Thermo electron corporation; Knap et al. 1996).

Viral production rates were determined by the reduction method (Wilhelm et al. 2002) as modified by Winter et al. (2004) and Weinbauer et al. (2007). Bacteria from 250-mL incubated water samples were concentrated using a 0.2- μm pore-size polyethersulfone membrane cartridge (Vivaflow 50; Vivascience). The filtrate was passed through a 100 kDa cut-off membrane to produce virus-free seawater. One to 4 mL of the bacterial concentrate were added to 50 mL of viral-free seawater resulting in bacterial concentration of $\sim 20\%$ that in original sample water. The virus-reduced culture was incubated in a 50-mL conical tube (BD Falcon) at in situ temperature in the dark. Subsamples for counting viruses (*see below*) were withdrawn at 3-h intervals until the end of the 15-h incubation period. Viral production was calculated according to Winter et al. (2004) by summing the changes in viral abundance during the incremental phase(s) of viruses divided by corresponding time (i.e., the end of total increment time point *minus* the starting point of increment); the phase was defined for each treatment on the basis of graphical inspection of changes in average viral abundance over the incubation period.

Subsamples for counting bacteria and viruses were put in 2-mL cryovials, fixed with glutaraldehyde (final conc. 0.5%), stored for 30 min at 4°C, frozen in liquid nitrogen, and stored in a -80°C freezer. Before the analysis, samples were defrosted, diluted (only for viruses) with 0.2- μm filtered TE buffer (pH 8; viral contamination was negligible), stained with SYBR Green I (1:25,000 dilution of commercial stock; Molecular probes) for 10 min at room temperature (for bacteria) or at 80°C (for viruses), and analyzed with a flow cytometer (FACSCalibur; Becton Dickinson) equipped with a 488-nm Argon laser according to Brussaard (2004). Subsamples fixed with glutaraldehyde (final conc. 2%) were also served for counting protists by epifluorescence microscopy (Sherr et al. 1993).

Viral production and bacterial production and respiration in the Western North Pacific—We examined variations in viral production and bacterial production and respiration at three stations in the subarctic (Sta. 12: 44°00'N, 154°59'W), transitional (Sta. 16: 34°59'N, 154°59'W) and subtropical (Sta. 20: 19°58'N, 155°00'W) Western North Pacific during the R/V *Hakuho* KH08-2 cruise conducted between August and September 2008. Water samples were collected using clean Niskin X bottles (12 liters) at depths of 10 m, 50 m, 100 m, and 200 m at Sta. 12 and 16, and at depths of 10 m and 100 m at Sta. 20. Sample waters prefiltered by gravity through 0.6- μm pore-size filters (Nuclepore, diam. 47 mm; Whatman) were used for the determination of viral and bacterial parameters. Viral production was determined by the thymidine method (Noble and Steward 2001) with modifications of Motegi and Nagata (2007). Briefly, sample water contained in 14-mL sterile polypropylene tubes (BD Falcon) were inoculated with ³H-thymidine (2.74 TBq mmol⁻¹, GE Healthcare, TRK686, final conc. 10 nmol L⁻¹) and incubated for 24 h at in situ temperature in darkness. Viral nucleic acids were extracted by enzymatic digestion (Motegi and Nagata

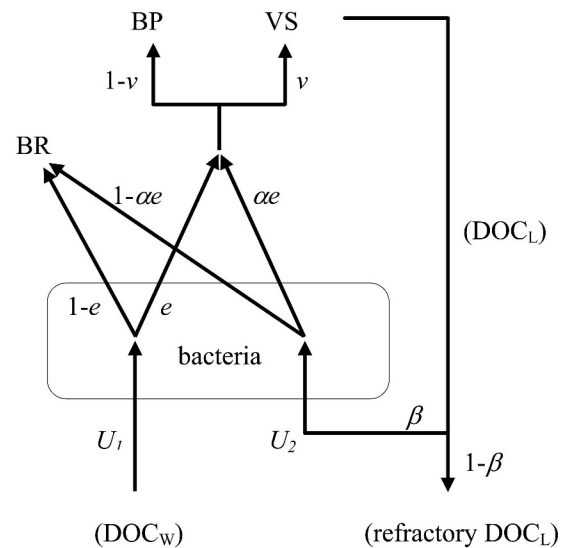


Fig. 1. Carbon flow model of the bacteria–virus system. See Table 2 for notation of variables.

2007) to be used for determination of radioactivity (Wallac 1400 scintillation counter with corrections for quenching). Viral production was estimated using the conversion factor of 6×10^{20} viruses per mole thymidine (Noble and Steward 2001). One TCA-killed control and triplicate samples were prepared. CVs for triplicate measurements were 0.6–30.8%. Bacterial production was determined from incorporation of ³H-Leu (5.92 TBq mmol⁻¹, GE Healthcare, TRK510, final conc. 20 nmol L⁻¹) as described above, except that centrifugation was used for the extraction of bacterial proteins (Kirchman 2001). CVs for triplicate measurements were 3.4–37.6%. Bacterial respiration was determined from oxygen consumption (48-h incubation) as described above, except that 300-mL capacity BOD bottles were used for the incubation and the Metromh Trino 793 (Metrohm AG) titrator was used for the high precision Winkler titration.

Model—In order to examine the effect of viral shunt on bacterial growth efficiency (BGE), we constructed a carbon flow model of the bacteria–viruses system (Fig. 1). This model describes the carbon fluxes as mediated by three mechanisms: bacterial respiration (BR), bacterial production (BP), and production of dissolved organic carbon (DOC) due to viral-induced bacterial mortality (VS). We assumed that bacteria are fueled by two sources of carbon (i.e., DOC_w, which was originally in the seawater, and DOC_L, which is supplied by the viral shunt [production of lysates and viral particles]); the uptake rates of DOC_w and DOC_L are denoted U_1 and U_2 , respectively. Of the total DOC_w consumed by bacteria, a fraction (e) is converted to bacterial biomass and the remainder ($1 - e$) is respired. The efficiencies of DOC_L conversion to bacterial biomass and BR are denoted αe and $(1 - \alpha e)$, respectively, where α accounts for different quality of DOC. A fraction of the newly produced bacterial biomass contributes to the production of DOC through viral-induced bacterial mortality, which can be regarded as the viral shunt (VS).

Table 2. Summary of notations for the variables and parameters used in the text, including the carbon flow model depicted in Fig. 1.

Abbreviation and symbol	Definition
ΔN_b	Changes in bacterial abundance, abundance at the end of incubation for 48 h <i>minus</i> abundance at the beginning of incubation, cells L ⁻¹
sBP	Cell-specific production of bacteria at the end of incubation for 48 h, fmol C cell ⁻¹ L ⁻¹
sBR	Cell-specific respiration of bacteria at the end of incubation for 48 h, fmol C cell ⁻¹ L ⁻¹
BR	Bacterial respiration, $\mu\text{mol C L}^{-1} \text{d}^{-1}$
BP	Bacterial production, $\mu\text{mol C L}^{-1} \text{d}^{-1}$
VS	Viral-induced bacterial mortality, VS=Viral production/Burst size×Bacterial cellular carbon content, $\mu\text{mol C L}^{-1} \text{d}^{-1}$
v	Shunting efficiency, $v=VS/(BP+VS)$, dimensionless
BGE	Bacterial growth efficiency, $BGE=BP/(BP+BR)$, dimensionless
DOC _w	DOC originally in the seawater
DOC _L	DOC supplied by the viral shunt
e	Fraction of DOC _w converted to bacterial biomass, dimensionless
α	Fraction of DOC _L converted to bacterial biomass divided by e , dimensionless
β	Fraction of DOC _L readily consumed by bacteria, dimensionless
U_1	Uptake rate of DOC _w , $\mu\text{mol C L}^{-1} \text{d}^{-1}$
U_2	Uptake rate of DOC _L , $\mu\text{mol C L}^{-1} \text{d}^{-1}$

Parameter v , the shunting efficiency, is proposed here as a new indicator of the fraction of bacterial production transformed into DOC by viruses as follows:

$$\text{Shunting efficiency } (v) = \frac{VS}{(BP + VS)} \quad (1)$$

In the model, fraction β of DOC_L is readily consumed by bacteria, whereas the remainder $(1 - \beta)$ is refractory. At steady state, conservation for the carbon fluxes (including BR, BP, and VS) is represented by the following three equations: $BR = U_1 \times (1 - e) + U_2 \times (1 - \alpha \times e)$, $BP = [U_1 \times e + U_2 \times (\alpha \times e)] \times (1 - v)$, and $U_2 = \beta \times VS = \beta \times v \times (e \times U_1 + \alpha \times e \times U_2)$. Although some fraction of the DOC released during viral lysis of bacteria can be refractory (Nagata 2000), this DOC production would be small relative to the total flux of carbon especially over a short time scale (<a few days). Hence, we assumed that $\beta = 1$. After algebraic calculations and rearrangement, BGE was related to e , v , and α with the following equation:

$$BGE = \frac{BP}{BP + BR} = \frac{e(1-v)}{1-\alpha ev} \quad (2)$$

This definition of BGE is consistent with the general definition in the literature. However, the BGE considered in this study reflects not only bacterial physiology but also overall features of carbon flows mediated by bacteria and viruses. In our model, e (fraction of DOC_w converted to bacterial biomass) is the value of the y-intercept, and α (fraction of DOC_L converted to bacterial biomass and divided by e) affects the bending of the curve. The above model does not fully embed the complexity in the lysogenic mode of viral infection (Miki et al. 2008), although the carbon flow mediated by the induction of prophage is implicitly incorporated (*see* Discussion). Variables and parameters used in the model are summarized in Table 2.

The data we had obtained from the seawater culture experiments, those we had determined in the Western North Pacific, and those we derived from the literature (*see*

below) were used to estimate BR (O₂ consumption rate was converted to C flux by assuming that the respiratory quotient = 1; del Giorgio and Cole 1998), BP (leucine incorporation was converted to C flux by assuming a conversion factor of 125 mol C per mol leucine; Kirchman 2001), and VS. We defined VS as follows:

$$VS = \frac{\text{viral production}}{\text{burst size}} \times \text{bacterial cellular carbon content}$$

where burst size and bacterial cellular carbon content were assumed to be 24 (Parada et al. 2006) and 1.0 fmol C per cell (Fukuda et al. 1998), respectively. Although the estimates of VS could have errors due to variations in burst size and bacterial cellular carbon content between months, among treatments and among regions, VS provides a first-order estimate of the flux of bacterial cellular carbon that is destroyed by viruses and becomes DOC (including colloids and viral particles). This DOC is potentially available for consumption by bacteria (Fuhrman 1999; Wilhelm and Suttle 1999). The estimated carbon fluxes mediated by bacteria and viruses (BP, BR, and VS) were used to calculate the shunting efficiency v (Eq. 1) and BGE (Eq. 2).

Results

Characteristics of the in situ seawater and seawater cultures used for the experiments conducted in the Bay of Villefranche—Water samples were collected in the upper layer (10 m) during periods of stratification (Aug, water temperature 24.2°C), partial mixing (Dec, 16.5°C) and overturn (Feb, 12.6°C; Table 3). Regardless of the season, the water used for the experiments was depleted in inorganic P (<0.01 $\mu\text{mol L}^{-1}$). Concentrations of nitrate and chlorophyll *a* (Chl *a*) were low in August (<0.01 $\mu\text{mol L}^{-1}$ and 0.12 $\mu\text{g L}^{-1}$, respectively) relative to the values in December (0.84 $\mu\text{mol L}^{-1}$ and 0.32 $\mu\text{g L}^{-1}$) and February (0.14 $\mu\text{mol L}^{-1}$ and 0.42 $\mu\text{g L}^{-1}$). Bacterial and viral abundances ranged from

Table 3. Physical, chemical, and microbiological characteristics of the water used for the seawater culture experiments. Mean values and standard deviations (\pm SD) were calculated for bacterial and viral abundance ($n = 3$).

Experiment (date)	Temp. ($^{\circ}$ C)	Salinity	PO ₄ (μ mol L ⁻¹)	NO ₃ (μ mol L ⁻¹)	Chl <i>a</i> (μ g L ⁻¹)	Bacterial abundance ($\times 10^8$ cells L ⁻¹)	Viral abundance ($\times 10^9$ viruses L ⁻¹)
Aug (18 Aug 2005)	24.2	38.5	<0.01	<0.01	0.12	8.2 \pm 0.6	12.5 \pm 0.4
Dec (07 Dec 2005)	16.5	38.2	<0.01	0.84	0.32	8.0 \pm 0.6	9.2 \pm 0.1
Feb (27 Feb 2006)	12.6	38.2	<0.01	0.14	0.42	6.9 \pm 0.1	4.8 \pm 0.3

6.9 to 8.2 $\times 10^8$ cells L⁻¹ and 4.8 to 12.5 $\times 10^9$ viruses L⁻¹, respectively.

In the seawater cultures, bacterial abundances were lower (by 30–60%) than the in situ abundance because of incomplete recoveries. Viral abundance in the V-treatment accounted for 30% to 170% of the in situ abundance. However, virus to bacteria abundance ratios at time zero were 12 to 27 in the V-treatment, being close to the ratios commonly observed in natural waters (Parada et al. 2006). Viral abundance in the ‘viral-free seawater’ represented 1% of the in situ abundance. We did not detect protist in 0.8-

μ m-filtered samples used for the preparation of seawater cultures.

Responses of bacteria and viruses to P addition in seawater cultures—Bacterial and viral variables determined at the end of incubation (after 48 h) were compared among treatments. These variables included: changes in bacterial abundance (Δ Nb, cells L⁻¹; i.e., abundance at the end of incubation for 48 h minus abundance at the beginning of incubation), cell-specific production of bacteria (sBP, fmol C cell⁻¹ d⁻¹; except for Aug experiment), cell-specific

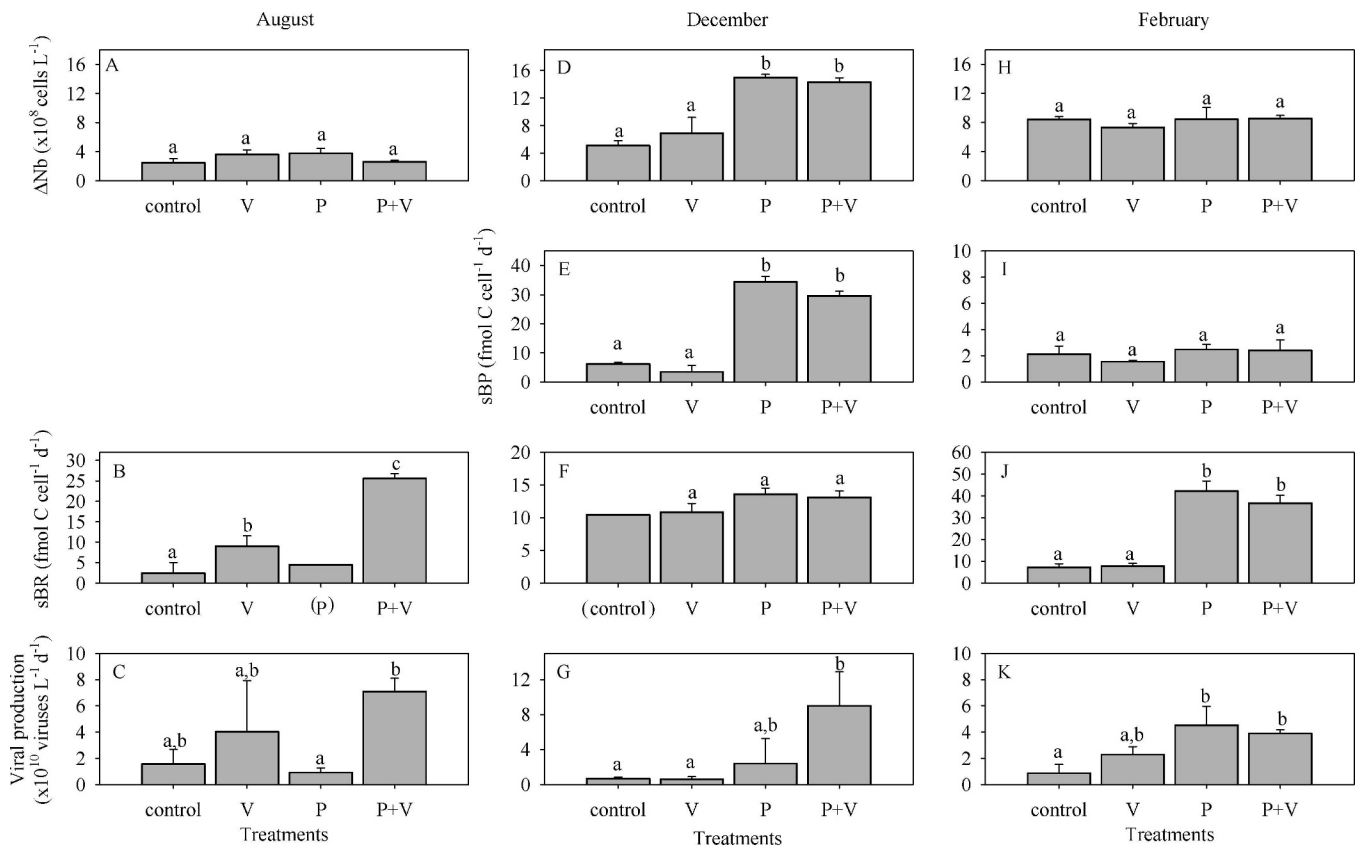


Fig. 2. Responses to P and/or virus additions of (A, D, H) change in bacterial abundance (Δ Nb), (E, I) cell-specific production rate of bacteria (sBP), (B, F, J) cell-specific respiration rate of bacteria (sBR) and (C, G, K) viral production. Data from (A–C) August, (D–G) December, and (H–K) February experiments. Error bars are standard deviations for triplicate bottles ($n = 3$), except for sBR in the P treatment in August and the control in December for which there is only one datum (a single bottle). Multiple comparisons (ANOVA with Bonferroni corrections) among treatments: different letters identify the treatments for which the mean values differed significantly ($p < 0.05$).

Table 4. Bacterial and viral parameters at the end of incubations. Values are means and standard deviations ($n = 3$). N.D. means no data available.

Month	Treatments	BP ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	BR ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	VS ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	BGE BP/(BP+BR)	ν VS/(BP+VS)
Aug	control	N.D.	1.4 \pm 1.7	3.2 \pm 2.3	N.D.	N.D.
	V	N.D.	5.9 \pm 1.3	8.4 \pm 8.1	N.D.	N.D.
	P	N.D.	3.4*	1.9 \pm 0.7	N.D.	N.D.
	P+V	N.D.	14.6 \pm 0.8	14.8 \pm 2.1	N.D.	N.D.
Dec	control	4.5 \pm 0.2	8.1*	1.4 \pm 0.4	0.36*	0.23 \pm 0.05
	V	3.1 \pm 1.9	9.7 \pm 1.2	1.2 \pm 0.6	0.23 \pm 0.11	0.33 \pm 0.25
	P	59.1 \pm 1.7	23.4 \pm 1.1	5.0 \pm 6.0	0.72 \pm 0.00	0.07 \pm 0.08
	P+V	48.9 \pm 0.8	21.7 \pm 2.2	18.7 \pm 8.1	0.69 \pm 0.03	0.27 \pm 0.09
Feb	control	2.7 \pm 0.8	9.3 \pm 2.3	1.8 \pm 1.4	0.22 \pm 0.02	0.38 \pm 0.25
	V	1.8 \pm 0.1	8.9 \pm 1.2	4.8 \pm 1.2	0.17 \pm 0.02	0.72 \pm 0.05
	P	3.1 \pm 0.3	52.9 \pm 0.8	9.4 \pm 3.0	0.06 \pm 0.01	0.74 \pm 0.06
	P+V	3.1 \pm 1.1	46.6 \pm 3.5	8.1 \pm 0.6	0.06 \pm 0.02	0.73 \pm 0.06

* Single measurement.

respiration of bacteria (sBR, $\text{fmol C cell}^{-1} \text{d}^{-1}$), and viral production (viruses $\text{L}^{-1} \text{d}^{-1}$; see Table 2 for the summary of abbreviations). Viral to bacterial abundance ratio (VBR) was also determined at the end of incubation. VBR varied in the range of 4–27, displaying no clear patterns among treatments and months.

In August (Fig. 2A–C), ΔNb ($2.5 \pm 0.6 \times 10^8$ to $3.8 \pm 0.7 \times 10^8$ cells L^{-1}) did not differ significantly ($p > 0.1$) among treatments, whereas the sBR value in the P + V treatment (26.0 ± 1.2 $\text{fmol C cell}^{-1} \text{d}^{-1}$) and the V treatment (9.0 ± 2.6 $\text{fmol C cell}^{-1} \text{d}^{-1}$) were significantly (ANOVA with a Bonferroni correction, $p < 0.001$) higher (4- to 11-fold) than those in the control (2.4 ± 2.7 $\text{fmol C cell}^{-1} \text{d}^{-1}$). sBR in the P treatment (determined from a single bottle) was 4.5 $\text{fmol C cell}^{-1} \text{d}^{-1}$. The high sBR value in the P + V treatment corresponded to the highest viral production ($7.1 \pm 1.0 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$) in that treatment. Viral production did not differ significantly ($p > 0.05$) among treatments, except that the value in the P + V treatment was significantly ($p < 0.05$) higher (8-fold) than that in the P treatment.

In December (Fig. 2D–G), ΔNb and sBP in the P ($15.0 \pm 0.4 \times 10^8$ cells L^{-1} and 34.3 ± 1.8 $\text{fmol C cell}^{-1} \text{d}^{-1}$, respectively) and the P + V treatments ($14.3 \pm 0.6 \times 10^8$ cells L^{-1} and 29.6 ± 1.6 $\text{fmol C cell}^{-1} \text{d}^{-1}$) were significantly ($p < 0.001$) higher (3- to 6-fold) than those in the nonaddition control ($5.1 \pm 0.7 \times 10^8$ cells L^{-1} and 6.1 ± 0.6 $\text{fmol C cell}^{-1} \text{d}^{-1}$) and the V treatment ($6.9 \pm 2.3 \times 10^8$ cells L^{-1} and 3.4 ± 2.3 $\text{fmol C cell}^{-1} \text{d}^{-1}$). In contrast, sBR did not differ significantly ($p > 0.1$) among treatments (overall average \pm stand deviation [SD] = 12.0 ± 1.6 $\text{fmol C cell}^{-1} \text{d}^{-1}$). Viral production in the P + V treatment ($9.0 \pm 3.9 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$) was significantly ($p < 0.05$) higher (13- to 15-fold) than that in the control ($0.7 \pm 0.2 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$) and the V treatment ($0.6 \pm 0.3 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$), although the difference in viral production between the P ($2.4 \pm 2.9 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$) and the P + V treatments was not significant ($p = 0.061$).

In February (Fig. 2H–K), ΔNb and sBP did not differ significantly ($p > 0.1$) among treatments, with an overall

average (\pm SD) of $8.2 \pm 0.6 \times 10^8$ cells L^{-1} and 2.1 ± 0.4 $\text{fmol C cell}^{-1} \text{d}^{-1}$, respectively. However, there was a significant increase in bacterial respiration in response to the P addition; sBR values in the P (42.2 ± 4.6 $\text{fmol C cell}^{-1} \text{d}^{-1}$) and the P + V (36.6 ± 3.6 $\text{fmol C cell}^{-1} \text{d}^{-1}$) treatments were significantly ($p < 0.001$) higher (5- to 6-fold) than those in the control (7.3 ± 1.7 $\text{fmol C cell}^{-1} \text{d}^{-1}$) and the V treatment (7.8 ± 1.4 $\text{fmol C cell}^{-1} \text{d}^{-1}$). High sBR in the P and P + V treatments corresponded to high viral production in these treatments; the rates of viral production in the P ($4.5 \pm 1.5 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$) and P + V treatments ($3.9 \pm 0.3 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$) were significantly ($p < 0.05$) higher (4- to 5-fold) than those in the control ($0.9 \pm 0.7 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$), although viral production in the V treatment ($2.3 \pm 0.6 \times 10^{10}$ viruses $\text{L}^{-1} \text{d}^{-1}$) was not significantly different ($p > 0.05$) from the corresponding values in other treatments.

Viral shunt and bacterial growth efficiency in seawater culture experiments—We calculated BGE and ν for the data obtained from seawater culture experiments (Table 4). Shunting efficiency was generally low in December (range = 0.07–0.33), with the lowest value in the P treatment, relative to the corresponding values in February (range = 0.38–0.74). In reverse to the ν trend, BGE was high in December (range = 0.23–0.72) and low in February (0.06–0.22). The highest BGE value (0.72) was obtained in P enriched cultures in December, whereas the lowest value (0.06) was obtained in P enriched cultures in February.

The scatter diagram of BGE as a function of ν for the whole data set obtained in December and February shows that these two parameters were significantly negatively correlated ($\text{BGE} = -0.74 \times \nu + 0.65$; $r^2 = 0.64$; $p < 0.001$, $n = 22$; Fig. 3A). This strong correlation cannot be fully explained by variations in BP, which was used for the calculation of both BGE and ν , because the partial correlation BGE and ν with control of BP was significant ($r = 0.73$; $p < 0.001$). Excluding the data obtained for P- and P + V treatments in December, which form a cluster of plots with low ν and high BGE (Fig. 3A), we still found a

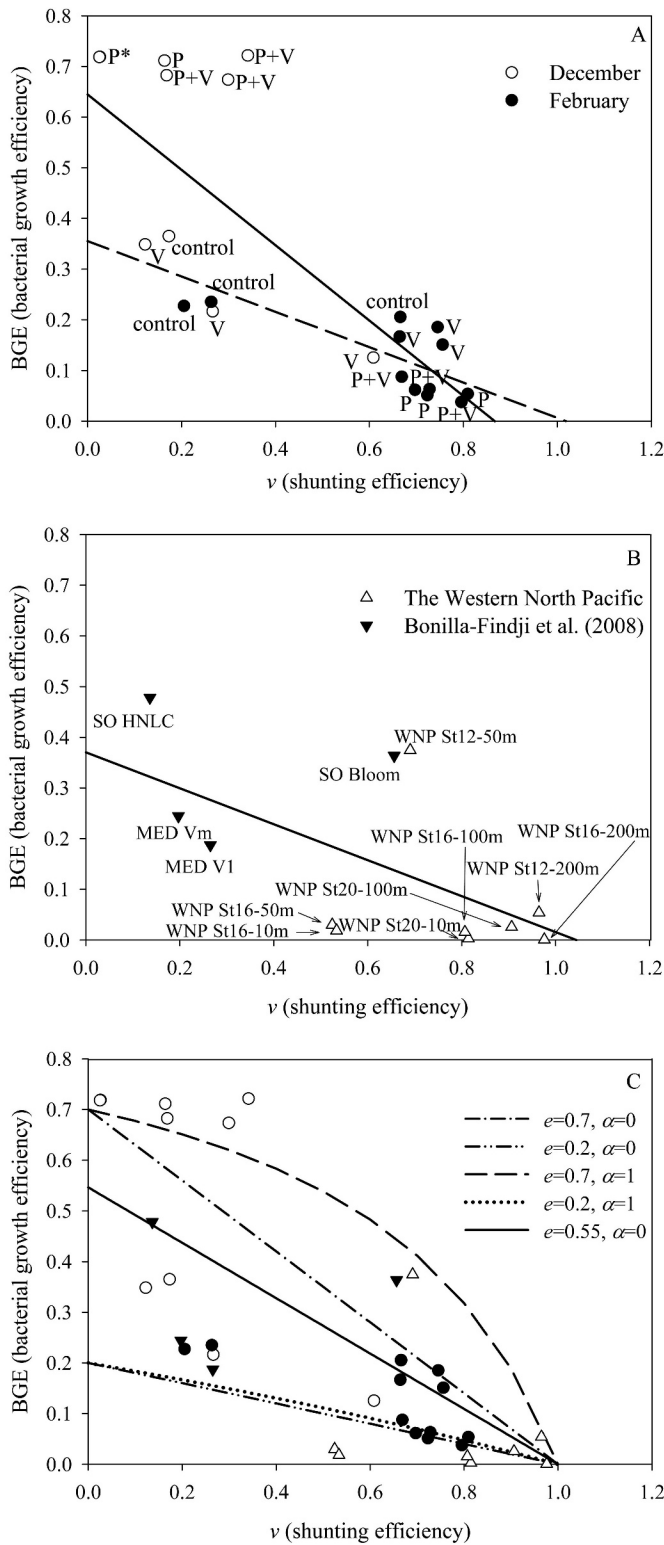


Fig. 3. (A) Relationship between shunting efficiency (v) and bacterial growth efficiency (BGE) for the data from the seawater culture experiments conducted in the Bay of Villefranche in December and February. Each point represents a datum from a single bottle. P* indicates that two values for the December P treatment overlapped. Solid line is the linear regression for the whole experimental data set: $BGE = -0.74 \times v + 0.65$ ($r^2 = 0.64$,

significant, negative correlation between BGE and v : $BGE = -0.35 \times v + 0.36$ ($r^2 = 0.73$; $p < 0.001$, $n = 16$).

Bacterial growth efficiency and shunting efficiency in the Western North Pacific and other oceanic regions—In order to examine the relationship between BGE and v in additional marine environments, we determined viral and bacterial variables in the Western North Pacific. Oceanographic parameters for each sampling station at the time of sampling are summarized in Table 5. Temperature ranged from 1.5 to 29.5°C, and salinity from 32.5 to 34.9. Chl *a* displayed large variations over latitude and depth (range = 0.01–0.62 $\mu\text{g L}^{-1}$), reflecting transitions in physical conditions across the subarctic and subtropical gyres of the North Pacific basin. For these open-ocean samples, viral production ranged from 0.93 ± 0.23 to $2.99 \pm 0.45 \times 10^9$ viruses $\text{L}^{-1} \text{d}^{-1}$, with the highest value at 100-m depth in the subtropics (Sta. 20). Bacterial production and respiration ranged from 0.001 ± 0.000 to $0.098 \pm 0.007 \mu\text{mol C L}^{-1} \text{d}^{-1}$ and 0.04 ± 0.05 to $3.31 \pm 0.21 \mu\text{mol C L}^{-1} \text{d}^{-1}$, respectively (Table 6). Corresponding BGE and v ranged from 0.001 to 0.37 and 0.38 to 0.98, respectively.

We also used the data of BP, BR, and viral production from the literature (Bonilla-Findji et al. 2008) to obtain estimates of BGE and v (Table 6). In the Bay of Villefranche, the estimated values for BGE and v ranged from 0.19 to 0.24 and 0.20 to 0.27, respectively, and in the Southern Ocean, from 0.36 to 0.48 and 0.14 to 0.66, respectively. The overall plot of data from the Pacific Ocean and the literature, which represent a wide variety of marine regions with a broad range of oceanographic conditions, showed that BGE was significantly and negatively correlated with v ($BGE = -0.36 \times v + 0.37$; $r^2 = 0.36$; $p < 0.05$, $n = 12$; Fig. 3B). The slope and y-intercept were close to those of the linear regression equation for the seawater culture experiments with the exclusion of the data for the P- and P + V treatments in December (Fig. 3A).

Comparison of data and model prediction—We examined if the relationships between BGE and v described above were consistent with predictions from our carbon flow model (Fig. 1). The relationships between BGE and v with

$p < 0.001$, $n = 22$). Dashed line is the regression for a subset of data that excluded the cluster of points with high BGE and low v (i.e., Dec P- and P + V treatments): $BGE = -0.35 \times v + 0.36$ ($r^2 = 0.73$, $p < 0.001$, $n = 16$). (B) Relationship between BGE and v for our data from the Western North Pacific (WNP) and from Table 6 (Med and SO refer to the Mediterranean and the Southern Ocean, respectively). Linear regression line for the whole data set: $BGE = -0.36 \times v + 0.37$ ($r^2 = 0.36$, $p < 0.05$, $n = 12$). (C) Model prediction of the relationship between BGE and v . Prediction lines based on four combinations of e and α illustrate the sensitivity of predictions to these two parameters (see Fig. 1 and Table 2 for explanations of e and α). Regression line (solid): the parameters of the best-fit solution of the nonlinear regression for the whole data set from experiments (Fig. 3A) and field observations (Fig. 3B) are $e = 0.55$ and $\alpha = 0.0$ (adjusted $r^2 = 0.54$; see text for explanations).

Table 5. Characteristics of the sampling stations in the Western North Pacific. Data are from the cruise report of the KH08-2 cruise (Ocean Research Institute, The University of Tokyo).

Location (station)	Depth (m)	Temp. (°C)	Salinity	Chl <i>a</i> ($\mu\text{g L}^{-1}$)
44°00'N, 154°59'W (Sta. 12)	10	17.0	32.5	0.52
	50	3.0	33.1	0.38
	100	1.5	33.2	0.04
	200	3.3	33.8	0.01
34°59'N, 154°59'W (Sta. 16)	10	28.4	34.0	0.12
	50	24.2	34.5	0.62
	100	20.5	34.7	0.11
19°58'N, 155°00'W (Sta. 20)	10	17.8	34.8	0.01
	10	29.5	34.4	0.03
	100	24.3	34.9	0.19

different sets of parameter values for e (0.2 to 0.7) and α (1 to 0) are illustrated in Fig. 3C, showing that the general trend is consistent with the data obtained in our P-addition experiments (Fig. 3A) and those for the Western North Pacific, the Bay of Villefranche, and the Southern Ocean (Fig. 3B). In the three panels, BGE decreases with increasing v . A nonlinear regression analysis using the dynamic fit algorithm of SigmaPlot (V10.00, Systat Software), with the constraints $e > 0$ and $\alpha \geq 0$, yielded an overall best-fit solution of $e = 0.55$ and $\alpha = 0.0$ (adjusted $r^2 = 0.54$) for our entire data set (Fig. 3C).

Discussion

The goal of our study was to test the hypothesis that viruses can affect BGE in marine pelagic waters. Specifi-

cally, we examined if BGE decreases with increasing magnitude of viral-induced bacterial mortality in bottle-contained seawater cultures with and without amendment of P. Our data from the Northwestern Mediterranean in February were consistent with this hypothesis, because they showed that P-induced enhancement of viral production resulted in reduction of BGE. Our data from the Western North Pacific and those we extracted from the literature displayed large variability, which may be partly due to differences in methodologies used for the determination of bacterial and viral variables; nonetheless, these data showed a trend of reduced BGE with increasing v . Taken together, these data are consistent with the prediction from our carbon flow model that viral-induced conversion of bacterial biomass to DOC, followed by consumption of DOC by bacteria (i.e., viral shunt; Fuhrman 1999; Wilhelm and Suttle 1999), is a major regulator of BGE in marine pelagic waters.

Considerations on treatment effects—Treatments of water samples by prefiltration and concentration of bacteria and viruses using tangential filtration could result in disturbances in organic matter and nutrient conditions (e.g., via the release of carbon and nutrients from fragile cells broken during the filtration) and alteration of bacteria–virus communities, which in turn may affect bacterial activity. In our culture experiments, these treatment effects might have resulted in the enhancement of BP and BR. Indeed, the maximum levels in our P-enriched cultures of BP ($59.1 \pm 1.7 \mu\text{mol C L}^{-1} \text{d}^{-1}$ in the P treatment in Dec) and BR ($52.9 \pm 0.8 \mu\text{mol C L}^{-1} \text{d}^{-1}$ in the P treatment in Feb; Table 4) were high relative to the range of BP (0.004 to $0.43 \mu\text{mol C L}^{-1} \text{d}^{-1}$) and BR (0.03 to $3.06 \mu\text{mol O}_2 \text{ L}^{-1} \text{d}^{-1}$) previously determined in the Bay

Table 6. Bacterial and viral variables from our study in the Western North Pacific (means and SD; $n = 3$). Data for the Northwestern Mediterranean and the Southern Ocean are from Bonilla-Findji et al. (2008).

Region	Location (Station)	Depth or treatment (m)	BP ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	BR ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	Viral production ($\times 10^9$ viruses $\text{L}^{-1} \text{d}^{-1}$)	VS ($\mu\text{mol C L}^{-1} \text{d}^{-1}$)	BGE (BP+BR)	v (VS/(BP+VS))
Western North Pacific	44°00'N, 154°59'W (Sta. 12)	10	0.098 ± 0.007	—*	1.45 ± 0.37	0.06	—*	0.38
		50	0.023 ± 0.002	0.04 ± 0.05	1.21 ± 0.01	0.05	0.37	0.69
		100	0.003 ± 0.000	—*	1.14 ± 0.02	0.05	—*	0.94
		200	0.002 ± 0.000	$0.040 \dagger$	1.30 ± 0.31	0.05	0.05	0.96
	34°59'N, 154°59'W (Sta. 16)	10	0.035 ± 0.013	1.14 ± 0.18	0.93 ± 0.23	0.04	0.03	0.52
		50	0.039 ± 0.008	2.09 ± 0.53	1.08 ± 0.09	0.04	0.02	0.53
		100	0.009 ± 0.000	3.31 ± 0.21	0.96 ± 0.11	0.04	0.003	0.81
	19°58'N, 155°00'W (Sta. 20)	100	0.001 ± 0.000	2.01 ± 0.27	1.15 ± 0.07	0.05	0.001	0.98
		10	$0.012 \dagger$	0.74 ± 0.77	1.16 ± 0.36	0.05	0.02	0.81
		100	0.013 ± 0.001	0.49 ± 0.3	2.99 ± 0.45	0.12	0.03	0.91
Northwestern Mediterranean‡	—	V1	0.91	3.96	7.90	0.33	0.19	0.27
	—	Vm	1.03	3.19	6.08	0.25	0.24	0.20
Southern Ocean‡	HNLC	V1	0.26	0.29	1.00	0.04	0.48	0.14
	Bloom	V1	0.10	0.17	4.40	0.18	0.36	0.66

* No significant decrease in dissolved oxygen concentration was detected during the incubation.

† Single measurement.

‡ Seawater samples filtered through 0.8- μm or 1.0- μm pore-size filters were used to prepare two types of seawater cultures, with addition of viruses (V1) or heat-inactivated viruses (Vm). Bacterial production (^3H -leucine method), bacterial respiration (consumption rate of dissolved oxygen), and viral production (changes in abundance) were determined. In the Southern Ocean, the samples were collected in a high-nutrient low-chlorophyll region (HNLC) or within a patch of algal bloom (Bloom).

of Villefranche (Bonilla-Findji 2005), although they were close to BP and BR in more productive environments (Lopez-Urrutia and Moran 2007). However, the range of BGE we observed (0.06 to 0.72) is within the range of values previously reported for bacterial communities in the Bay of Villefranche (0.005 to 0.64; Bonilla-Findji 2005) and aquatic systems in general (0.01 to 0.7; del Giorgio and Cole 1998). In short, although our BP and BR values in P-enriched cultures were close to the upper, albeit not unusual, range for these two variables, the key parameter that we addressed (i.e., BGE) was within the observed range of variation in seawater.

Responses of the bacteria–virus system to P addition in the Bay of Villefranche—We found two types of response of the bacteria–virus system to P addition. One was a coupled response of bacterial growth and observed viral production (i.e., P addition resulted in enhancing ΔN_b and sBP with concomitant increase of viral production, although there was no significant enhancement of sBR [Dec]). The other type was an uncoupled response of bacterial growth and viral production, (i.e., P enrichment enhanced sBR and viral production, but not ΔN_b [Aug and Feb] and sBP [Feb]). For all the experiments, viral production did not differ significantly between the control and V treatment, suggesting that productivity of host, rather than encounter probability between viruses and host, limited viral production in seawater cultures with no P addition.

Interestingly, viral production was high in the P-treatment in February, being equivalent to the level in the P + V treatment. The same responses to P enrichment of viral production in both the bottles with and without addition of ‘free’ viruses (V + P and P treatments, respectively) suggest that the viral production enhancement might be due to the shift from lysogenic to lytic mode of infection (prophage induction) in response to the alleviation of P limitation. Consistent with this notion, previous research has reported that lysogeny is common in marine environments especially in oligotrophic waters (Williamson et al. 2002) including the Mediterranean (Weinbauer et al. 2003). In addition, studies have suggested that nutrient addition to natural communities can lead to prophage induction (Williamson et al. 2002; Motegi and Nagata 2007). Thus, lysogeny may play a major role in controls of BGE and oceanic carbon cycles. It is important for future studies to clarify variations in the extent of lysogeny and mechanisms by which prophage induction is regulated in marine environments.

Viral shunting efficiency, BGE, and trophic transfer—Despite the apparent complexity in the patterns of responses of sBP and sBR to virus- and P-additions among different months, the overall results from our seawater culture experiments indicated that BGE decreased with increasing v . Even if the plots of the BGE as a function of v were discontinuous, with a cluster of data corresponding to the P- and P + V treatments of December, the negative correlation between the two variables held even after excluding these data. In addition, our results from seawater culture experiments agreed well with those derived from

observational data collected in various marine environments that covered a broad range of BP rates (0.001–1.03 $\mu\text{mol C L}^{-1} \text{d}^{-1}$). On the basis of these considerations, we conclude that BGE generally decreases with increasing v as predicted from our carbon flow model.

The nonlinear regression analysis of our whole data set yielded a best-fit solution of $\alpha = 0.0$, indicating that BGE was linearly related to v (i.e., $\text{BGE} = e(1 - v)$). This means that DOC_L was completely respired by bacteria, implying that DOC_L chemically differed from DOC_w . This is consistent with the observation of Middelboe et al. (1996) who found that the lysate of *Vibrio* sp. produced during viral infection was mostly respired with no concomitant increase of bacterial biomass, which they interpreted as reflecting the polymeric and less labile nature of the lysate. However, our conclusion concerning DOC_L is very preliminary given the wide scatter of the data points around the regression line (Fig. 3C). Also, we know that predictions based on our model have limitations because of our steady-state assumptions.

Previous studies have suggested that over a broad range of marine environments, BGE tends to increase with decreasing temperature (Rivkin and Legendre 2001) or with increasing level of organic resource (Lopez-Urrutia and Moran 2007). In our seawater culture experiments, these factors did not explain the observed variations in BGE, because values tended to be lower in February (12.6°C) than in December (16.5°C), and did not show a significant correlation ($p = 0.2$) with BP + BR (a proxy of the level of resource supply).

Thingstad et al. (2005) found that the addition of P to surface water in the Eastern Mediterranean resulted in enhancement of bacterial production, which led to increased egg production by mesozooplankton. In contrast, Obernosterer et al. (2003) had found that the addition of P to surface waters of the Sargasso Sea resulted in increasing BR with no concomitant increase in bacterial biomass (implying low efficiency of trophic transfers). These apparently contradictory results concerning the response of microbial systems to P addition can be reconciled by a general model that follows from the results of the present study. In this model, when v is low, nutrient enrichment results in enhancing BGE and, thus, the production of higher trophic levels via the enhancement of carbon fluxes through the $\text{DOC} \rightarrow \text{bacteria} \rightarrow \text{protist}$ pathway, whereas when v is high, nutrient enrichment mainly leads to low BGE (i.e., enhanced BR). The applicability of our new conceptual model to broader regions of the ocean needs to be tested under diverse environmental settings.

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