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Effect of turbulence and viruses on prokaryotic cell size, production and diversity

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ABSTRACT: A factorial design was used to assess the roles of turbulence and viral infection in prokaryotic production and diversity in a spring phytoplankton bloom experiment in the Bay of Villefranche, France. Several consistent trends were observed in 2 experiments: (1) turbulence stimulated prokaryotic production, (2) prokaryotic cell length increased in experimental turbulence and virus treatments, and (3) organic micro-aggregates with attached prokaryotes formed only in the turbulence treatments and seemed to be reduced in the presence of viruses. We conclude that turbulence likely influenced prokaryotes indirectly by affecting micro-aggregate formation and nutrient availability. Turbulence and viruses had only small influences on the number of bacterial and archaeal bands detected by 16S rRNA gene denaturing gradient gel electrophoresis. However, taking into account presence versus absence of specific bands and their intensities, we detected strong effects in the experiments. We not only detected a negative effect of viruses, but also found that some bands increased in intensity in the presence of active viruses, e.g. one of 3 phylotypes affiliated with the *Rhodobacteriaceae*. In both experiments, several consistent patterns were found: (1) a phylotype affiliated with *Roseobacter* was negatively affected (in terms of band intensity) by viruses and turbulence, (2) the relative band intensity of a *Rhodobacter* increased in the turbulence treatments, and (3) a phylotype related to *Oceanospirillum* was detected only in the turbulence treatment. We suggest that turbulence and viruses play a significant and previously neglected role in shaping prokaryotic diversity, aggregation and production.

KEY WORDS: Virus infection · Prokaryotic activity · Morphology · *Roseobacter* · *Oceanospirillum*

INTRODUCTION

Prokaryotic viruses play an important role in aquatic food web processes through top-down control of the microbial community, thereby influencing biogeochemical cycles in the sea (Wommack & Colwell 2000, Weinbauer 2004). Estimates indicate that virus infection can be as important as protistan grazing for total bacterial mortality (Fuhrman & Noble 1995). However, a broad range of lysis to grazing ratios has been reported from different depths and locations, suggesting that the contribution of lysis to overall bacterioplankton mortality will depend greatly on environmental conditions and host community structure (Wommack & Colwell 2000). The model by Wilhelm & Suttle (1999) suggests that through viral lysis, up to 26% of photosynthetically produced organic carbon is directed to the dissolved organic carbon (DOC) pool. Viruses can also influence bacterial diversity in various ways; however, comparatively few studies with natural communities have been performed (e.g. Schwalbach et al. 2004, Winter et al. 2004b).

The persistence and decay of free viruses are controlled by biotic factors, such as extracellular enzymes, and abiotic factors, such as solar radiation and adsorption onto particles (Suttle & Chen 1992, Noble & Fuhrman 1997). In addition, any process that brings host and virus together is important for the fate

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of the virus (Murray & Jackson 1992). Turbulence and attachment to aggregates are such potential processes. Viruses are transported by diffusion to their hosts. Naturally occurring turbulent or laminar flow may increase particle encounter between viruses and prokaryotes by up to 3.5% (Karp-Boss et al. 1996), depending directly on host particle diameter and inversely on the diffusion coefficient for viruses. However, in a modeling exercise, fluid motion had an insignificant influence on contact rates between viruses and bacteria (Murray & Jackson 1992). The calculated effects of fluid motion on viral contacts are based on the assumption that prokaryotes are distributed evenly. The assumption may be unrealistic as up to 90% of the total prokaryotic community can be attached to transparent exopolymeric particles (TEP) (Mari & Dam 2004) and may be exposed to increased virus contact rates, as implied from the direct dependence of flux increase on particle size (Karp-Boss et al. 1996). For example, for a particle of 20 µm, virus transport in turbulent flux should increase by about 90% compared to still water. In addition, turbulence may also indirectly increase total abundance of attached viruses, since turbulence triggers coagulation and TEP formation (Kiørboe et al. 1990, Beauvais et al. 2006). Consequently, there are good reasons to suppose that encounter probability between viruses and hosts will increase with turbulence and organic particle load.

Turbulence may not directly influence bacteria. Fluid shear rates in natural systems (<2.1 s^{-1}, $\varepsilon = 0.04$ cm^{-2} s^{-3}) do not increase leucine or glucose uptake by suspended bacteria (Logan & Kirchman 1991). However, turbulence sometimes stimulates prokaryotic production, and it is believed that this is mainly due to an effect of turbulence on trophic interactions between cells and grazers (Peters et al. 2002) and on aggregation or algae (Logan & Hunt 1987, Logan & Dettmer 1990, Bergstedt et al. 2004, Malits et al. 2004, Pinhassi et al. 2004). Since viruses influence particle formation (Proctor & Fuhrman 1991), they may also affect bacterial metabolism in an indirect way. However, studies on the effects of turbulence on prokaryotic communities have thus far been performed in the presence of viruses and the turbulence effects detected might have been influenced by virus-host interactions.

We performed experiments in order to (1) test the effect of turbulence on cell size, production and diversity of a natural prokaryotic community, and (2) assess how viruses are involved in these processes. Our study in the Bay of Villefranche, France (NW Mediterranean Sea) showed that prokaryotic production is stimulated by turbulence, probably by formation of organic aggregates, and that viruses have the potential to modify this aggregate formation.

MATERIALS AND METHODS

Sampling and experimental set-up. We collected 100 l water samples for the 2 experiments (performed March 22 and April 14, 2004, and denoted TV 1 and TV 2, respectively) with acid-rinsed buckets at 0.5 m water depth in the northwestern Mediterranean Sea at the entrance to the Bay of Villefranche, France (Point B, 43° 41′ N, 7° 19′ E), where physical, chemical and biological parameters are monitored weekly. Chlorophyll *a* and inorganic nutrient concentrations were measured by the sampling program Service d'Observation du Milieu Littoral (SOMLIT).

Prokaryotes were concentrated using 0.2 µm pore-size polycarbonate cartridges (Durapore, Millipore). To obtain viral concentrates and virus-free seawater, 0.2 µm pore-size filtered seawater was processed with a spiral polyethersulfon cartridge (Prep/Scale 100kDa, Millipore). Recoveries of prokaryotes and viruses by the concentration methods were about 20 and 40%, respectively. Prokaryotic concentrates were diluted to approximately the original volume (95 ± 10% with respect to *in situ* concentrations) with virus-free seawater and amended with viral concentrate at 74 and 36% (with respect to *in situ* concentrations) in TV 1 and TV 2, respectively. To inactivate viruses, the viral concentrate was heated in a water-bath at 80°C for 20 min. In TV 1, most flagellates were killed in the concentration step (data not shown), whereas in TV 2, heterotrophic flagellates and eukaryotic phytoplankton were removed by 0.8 µm prefiltration. A factorial experimental design was used with 4 treatments run in triplicate to test the effects of turbulence and viruses: (1) turbulent with active virus (T-active), (2) still (i.e. no turbulence) with active virus (S-active), (3) turbulent with inactivated virus (T-inactive), and (4) still with inactivated virus (S-inactive). Turbulence was created with vertically oscillating grids in 2 l cylindrical plexiglas containers with a set-up described in detail by Peters et al. (2002). The energy dissipation rate was $\varepsilon = 0.04$ cm^2 s^{-3}. Experiments lasted for 3 d and were conducted in darkness.

Samples for prokaryotic production (PP), abundance of prokaryotes (PA) and virus-like particles were taken daily from the containers. Samples for morphology and size of prokaryotes (image analysis) and denaturant gradient gel electrophoresis (DGGE) were taken at the start of the experiment (t_0) and at the end of the experiment (t_{end}). Subsamples (2 ml) for enumeration and image analysis were fixed with glutaraldehyde (0.5% final concentration), incubated for 15 min at 4°C, flash-frozen in liquid nitrogen and stored at –80°C until analysis. For DGGE, 1000 to 1500 ml water samples (about 10^8 prokaryotes) were filtered onto 0.2 µm pore size polycarbonate membranes (47 mm, Whatman) and stored at –80°C until further processing.

Enumeration of viruses and prokaryotes. Viruses and prokaryotes were stained with SYBR Green I (Molecular Probes) and quantified using a FACScalibur (Becton and Dickinson) flow-cytometer after dilution with TE buffer (10 mM Tris, 1 mM EDTA, pH = 8) (Gasol & del Giorgio 2000). For viruses, an optimized protocol by Brussaard (2004) was used. Three DNA content subpopulations were distinguished based on fluorescence of particles measured by flow cytometry (Brussaard 2004). Abundances were calculated by the measured flow rate.

Prokaryotic production. The incorporation of ^{3}H leucine into protein (Smith & Azam 1992) was used to estimate the production of heterotrophic prokaryotes. Radioactively labeled leucine solution (1 µM; Amersham) was mixed with non-radioactive leucine (hot: cold = 1:9) and added at a final concentration of 40 nM to triplicate 1 ml aliquots and a trichloroacetic acid (TCA)-killed control. Isotope saturation of 40 nM was determined from samples that received leucine at final concentrations of 10 nM, 20 nM, 40 nM and 80 nM. Samples were incubated in the dark at *in situ* temperature. After 60 to 90 min, the incubations were stopped with TCA (final concentration of 5%).

Viral production. Viral production (VP) was calculated as

$$VP = (V_2 - V_1) / (t_2 - t_1) \quad (1)$$

where V_1 and V_2 are viral abundances, and $t_1 - t_2$ the elapsed time. Dividing the number of phages produced by viral abundance at t_1 gives the viral turnover rate.

Prokaryotic cell volume and organic aggregates. To estimate prokaryotic cell size and to calculate mean cell volume, cells were stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma) (following the method of Porter & Feig 1980) at a final concentration of 0.25 µg ml^{-1}; 2 ml of stained sample were filtered onto 0.2 µm pore size black polycarbonate membranes (25 mm) and sized using a semi-automated image analysis system (Image-Pro Plus 4). Images of 200 to 400 cells per sample were recorded with a CCD color video camera (COHU 2252-1040). Cell dimensions (pixel area, perimeter) were estimated after edge detection with a second derivative filter. Cell volume was calculated assuming prokaryotes had a cylindrical shape, with 2 half spheres at their ends, using the equation

$$\text{Cell volume} = \pi/4\ w^2\ (l - w/3) \quad (2)$$

where w is the width and l is the length of the prokaryotic cell.

These preparations were also used to identify aggregated prokaryotes, and to estimate the size and number of attached prokaryotes. Furthermore, these filters were used to confirm that no growth of flagellates occurred during the incubations.

DNA extraction and manipulation. Nucleic acids from filters were extracted as described by Winter et al. (2001), with slight modifications. Briefly, after 4 freeze–thaw cycles in liquid nitrogen and in a water bath at 37°C, an enzyme treatment with lysozyme (1.25 mg ml^{-1} final concentration; Fluka BioChemika #62970) at 37°C for 30 min, and Proteinase K (100 µg ml^{-1} final concentration; Fluka BioChemica #82456) for 2 h at 55°C was performed. The phenol-chloroform extraction step of the original protocol was replaced by extraction of nucleic acids with 4.5 M NaCl and chloroform, followed by isopropanol precipitation, since this avoids a toxic chemical and yields same DGGE profiles (M. Agis unpubl. data). The pellets were then re-suspended in 60 µl of water. With 1 µl of template DNA, a fragment of the 16S rRNA gene was amplified in 50 µl reactions (1.5 mM $MgCl_2$, 0.25 µM of each primer and 2.5 U *Taq* polymerase; Sigma; #D 5930) together with a positive and a negative control using the primer pairs 341F-GC/907R (Schäfer & Muyzer 2001) and 344F-GC/917R (Casamayor et al. 2000) specific for *Bacteria* and *Archaea*, respectively (fragment lengths: 566 bp for *Bacteria* and 573 bp for *Archaea*). Conditions of the touchdown PCR were set as described by Schäfer & Muyzer (2001). PCR products were quantified by agarose gel electrophoresis with a molecular size standard in the gel (Bioline). We then seperated 100 ng of PCR products on a DGGE gel (Muyzer et al. 1993) made with a DGGE device (Ingeny phorU).

DGGE analysis. DGGE gels were imaged with the gel documentation system GelDoc EQ (Bio-Rad) after 15 min SYBR Gold staining (Molecular Probes; #S11494) by distributing a 10× SYBR Gold solution on the gel. DGGE banding pattern was analyzed with Quantity One Software (Bio-Rad). This software allows for quantification of band intensity by avoiding signal saturation. Similarity of band patterns was analyzed by a cluster analysis based on a Bray-Curtis similarity matrix.

Selected DGGE bands were excised from the DGGE gels, the DNA was eluted overnight in autoclaved Milli Q water at 4°C, checked for purity by re-running it on a gel, and sequenced (MWG-Biotech, Ebersberg). Trace files generated during DNA sequencing were edited using the freeware program 4Peaks 1.6 (A. Griekspoor and T. Groothuis, available at: www.mekentosj.com). About 500 bp of the 16S rRNA gene were used in a BLAST (http://blast.ncbi.nlm.nih.gov/BLAST.cgi) search. These sequence data were submitted to the GenBank database under accession numbers EF018060 through EF018063.

Ancillary parameters. Nitrate (NO_3^-), and orthophosphate (PO_4^{3-}) were determined as described in Tréguer & Le Corre (1975) using an Alliance-Instru-

ment EV2 autoanalyzer following standard methods (Lorenzen 1966, Strickland & Parsons 1972). Concentration of chl *a* was measured fluorometrically (Yentsch & Menzel 1963).

Statistics. All statistical analyses (analyses of variance, ANOVA; analysis of covariance, ANCOVA, with time as a covariate; unpaired *t*-test) were done with the StatView 4.5 (Abacus, 1995) software package. Differences among means were considered significant at $p < 0.05$.

RESULTS

In situ conditions

In situ concentrations of chl *a* on the 2 sampling dates ranged from 0.11 to 0.41 µg l^{-1}, whereas orthophosphate concentration was 0.1 µM and total nitrogen concentration was ca. 0.8 µM. *In situ* abundances of prokaryotes and viruses ranged from 8.5 to 9.4 × 10^5 ml^{-1} and from 1.1 to 1.4 × 10^7 ml^{-1}, respectively.

Abundances of prokaryotes and viruses

Prokaryotic abundances increased in all treatments in both experiments. However, time-course changes differed between the 2 experiments with respect to the treatment factors (turbulence and viruses) (Fig. 1). In experiment TV 1, prokaryotic abundance was significantly higher in the turbulence than in the still treatment (Table 1). In TV 2, prokaryotic abundance was

Table 1. Effects of turbulence and the presence of active viruses on prokaryotic abundance and production, with time as a covariate (factorial ANCOVA). PA: prokaryotic abundance; PP: prokaryotic production. +: stimulating effect; –: repressing effect. ns: not significant. Interactions between factors (turbulence and viruses) were only found for PA in TV 1

Exp	Treatment	PA	PP
TV 1	Turbulence	+, $p < 0.0001$	+, $p < 0.0001$
	Viruses	ns	–, $p < 0.01$
TV 2	Turbulence	ns	+, $p < 0.01$
	Viruses	+, $p < 0.0001$	+, $p < 0.0001$

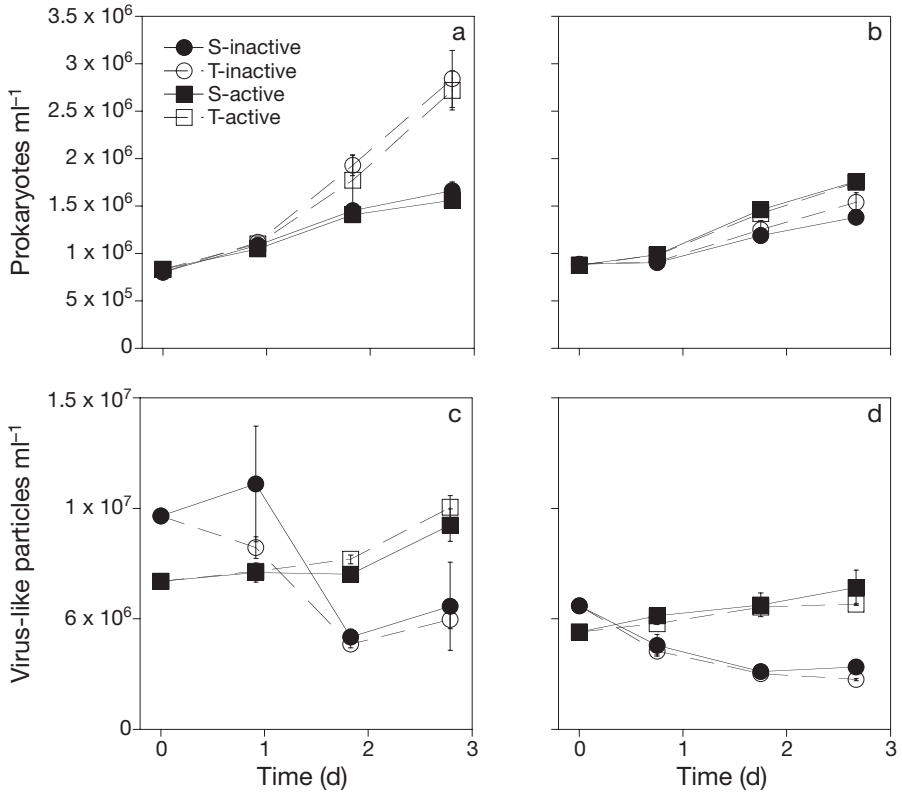


Fig. 1. Time course of prokaryotic and viral abundances in the turbulent (T) treatments and still controls (S) with the amendment of active and inactivated viruses in experiments (a,c) TV 1 and (b,d) TV 2. Values are means ± SD, n = 3 (when not visible, SD bars are within the size of symbols)

significantly higher in the presence than in the absence of active viruses. No other significant effects were found for prokaryotic abundance.

The heat-killing treatment to inactivate viruses resulted in slightly higher abundance of virus-like particles than in the active virus treatment at the start of the experiments (Fig. 1). However, viral abundance declined towards the end in both experiments in the inactive virus treatment, but increased in the active virus treatment. Turbulence did not affect the abundance of active viruses in TV 1, but in TV 2, viral abundance was slightly, but significantly, higher in the still treatment. Viral turnover rate in the active virus treatments ranged from 0.11 to 0.19 d^{-1} in both experiments, with no significant differences between experiments (unpaired *t*-test). Viral turnover in TV2 was significantly higher in the still than in the turbulent treatment. Viral net production rate was on average higher in TV 1 ($1.06 \pm 0.26 \times 10^6$ viruses ml^{-1} d^{-1}) than TV 2 ($0.65 \pm 0.23 \times 10^6$ viruses ml^{-1} d^{-1}), in which viral net production was lower in the turbulent treatments (unpaired *t*-test).

Morphology and size of prokaryotes; micro-aggregates

In the presence of turbulence and active viruses, prokaryotes were significantly elongated at the termination of both experiments (Table 2). In addition, micro-aggregate formation and attachment of bacteria to micro-aggregates occurred only in the turbulent treatments of both experiments. In TV 1, the presence of active viruses did not affect the number and size of micro-aggregates significantly (although the average number and size were lower in the presence of active viruses), while in TV 2, micro-aggregates were in general less abundant and did not develop at all in the virus treatment (Table 2). In general, the micro-aggregates were small, with an average size of 1.9 to 2.4 µm. In TV 1, about 40 to 50% of prokaryotes aggregated, while in TV 2, fewer prokaryotes (ca. 15%) were found on micro-aggregates.

Prokaryotic production

In the virus-inactivated and virus-active treatments of both experiments, leucine incorporation rates (as a measure of prokaryotic production) were significantly higher in the turbulent than in the still treatment (Fig. 2, Table 1). The addition of active viruses had a significant negative effect on prokaryotic production in experiment TV 1, but a significant promoting effect in TV 2. Both the repressing (TV 1) and stimulating viral effects (TV 2) were higher in the still than in the turbulence treatment, and this trend was significant for TV 2, but not for TV 1. Cell-specific prokaryotic production showed the same trends (data not shown).

Prokaryotic diversity

16S rRNA gene and DGGE based archaeal and bacterial fingerprints showed little variation (among experimental triplicate incubations) in band numbers and relative intensities. In addition to confinement effects (54 to 70% reduction of the number of archaeal bands and 57 to 59% of bacterial bands relative to t_0), we also found virus and turbulence treatment effects.

Archaea

DGGE profiles revealed a reduction in the number of archaeal bands after 3 d of incubation from 10 to 7 bands in TV 1 and from 13 to 7 bands in TV 2.

Table 2. Lengths and cell volumes of prokaryotes, and abundances and diameters of aggregates at t_0 and Day 3. Means ± ranges of length (*L*) and volume (*V*) of single prokaryotes are from duplicate incubations. Means ± ranges of abundances and diameters (*D*) of aggregates are from image analysis of at least 10 frames. For treatment labels see section 'Materials and methods'. Significant effects of virus (V) and turbulence (T) treatments (ANOVA) are given by probability values. ns: not significant ($p \geq 0.05$)

Expt	t_0	S-inactive	T-inactive	S-active	T-active	Treatment effects
TV 1						
cell *L* (µm)	0.60	0.74 ± 0.04	0.82 ± 0.02	0.80 ± 0.01	0.90 ± 0.01	T: $p < 0.05$, V: $p < 0.05$
cell *V* ($µm^3$)	0.049	0.074 ± 0.005	0.079 ± 0.006	0.074 ± 0.000	0.080 ± 0.000	ns
aggregates ml^{-1}	0	0	$8.14 \pm 5.5 \times 10^5$	0	$6.11 \pm 2.1 \times 10^5$	T: $p < 0.0001$, V: ns
aggregate *D* (µm)	0	0	2.27 ± 0.52	0	2.43 ± 0.60	T: $p < 0.0001$, V: ns
TV 2						
cell *L* (µm)	0.60	0.71 ± 0.00	0.79 ± 0.01	0.73 ± 0.01	0.83 ± 0.00	T: $p < 0.001$, V: $p < 0.05$
cell *V* ($µm^3$)	0.049	0.061 ± 0.001	0.065 ± 0.003	0.065 ± 0.001	0.074 ± 0.003	ns
aggregates ml^{-1}	0	0	$1.03 \pm 1.8 \times 10^5$	0	0	T: $p < 0.05$, V: $p < 0.05$
aggregate *D* (µm)	0	0	1.92 ± 0.53	0	0	T: $p < 0.05$, V: $p < 0.05$

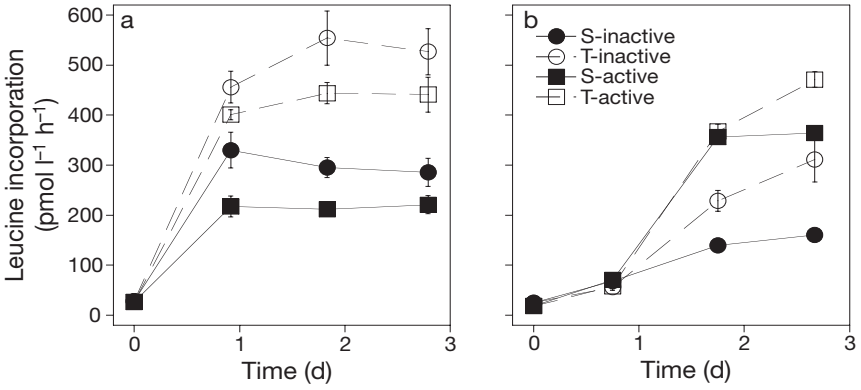


Fig. 2. Time course of leucine incorporation in experiments (a) TV 1 and (b) TV 2. Values are means ± SD, n = 3 (when not visible, bars are within the size of symbols). Symbol labels as in Fig. 1

Turbulence and virus treatments significantly influenced the number of archaeal bands in TV 1, but not in TV 2. Furthermore, some bands showed treatment effects on relative band intensities (Fig. 3). In the 2 experiments, between 2 and 5 bands (26 to 50% of total per experiment at t_{end}) were influenced by viruses, and between 1 and 3 bands (13 to 30%) were influenced by turbulence; in TV 1, 1 band was influenced by both viruses and turbulence. Between 2 to 3 bands (26 to 30%) were stimulated by the presence of viruses, and 2 bands (20%) were repressed. For turbulence, the values were 1 to 2 bands (13 to 20%) for stimulation and 1 band (10%) for repression.

Bacteria

Confinement resulted in a reduction from 27 to 16–17 bacterial bands in TV 1, and from 30 to 17–21 bands in TV 2. In TV 1, the number of bacterial bands was not affected by the virus or turbulence treatment, whereas in TV 2, turbulence increased the number of bands by 5%, and viral presence significantly decreased the number by 8%. Cluster analyses for both experiments showed that the community composition of bacteria differed among all treatments (Fig. 4). However, the clusters differed between the 2 experiments. In TV 1, the turbulence treatment with inactive viruses clustered separately from the other treatments, whereas in TV 2, we found 2 main clusters, one with active viruses, the other with inactivated viruses.

More specific treatment effects on band intensity are shown in Fig. 5. Virus and turbulence effects were found for both relatively dominant (>10% band intensity) and less dominant bands (≤5% band intensity). In the 2 experiments, between 8 and 9 bands (27 to 33%) were influenced by the virus treatment and 9 to 13 (30 to 48%) by the turbulence treatment; 4 to 7 of these

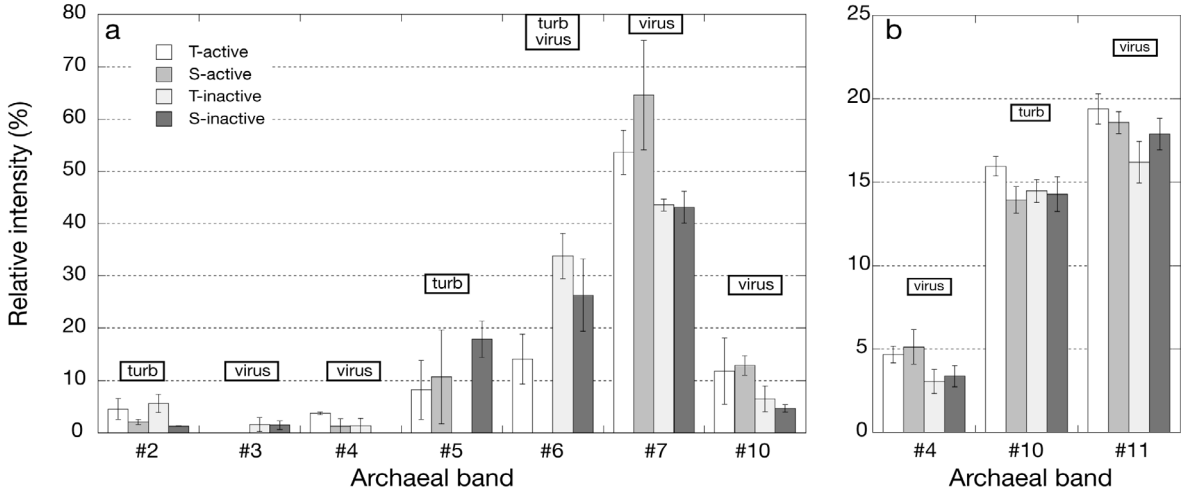


Fig. 3. Relative band intensities of archaeal bands from the final assemblage in (a) TV 1 and (b) TV 2. Only data for bands significantly influenced by viruses and/or turbulence are shown. Values are means ± SD, n = 3. The experimental factor(s) [virus and turbulence (turb)] are indicated above the columns. Note the different scales on *y*-axes. Treatments factors are: turbulent (T) and still controls (S), and amendment with active or inactivated viruses in a fully crossed design

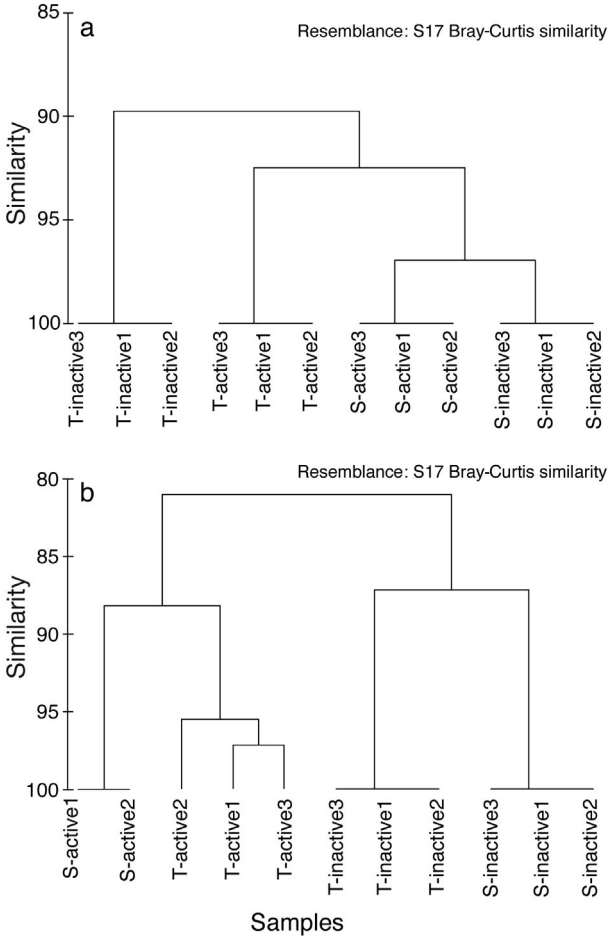


Fig. 4. Cluster analysis of bacterial community composition from individual DGGE samples of experiments (a) TV 1 and (b) TV 2. The dendrogram is constructed from a Bray-Curtis similarity matrix based on the absence or presence of bands. Band patterns from replicate samples for all treatments were similar (>95% similar in all cases). Treatments factors are: turbulent (T) and still controls (S), and amendment with active or inactivated viruses in a fully crossed design

bands (13 to 26%) were affected by both treatments (viruses and turbulence). Between 2 to 3 bands (7 to 11%) were stimulated by the presence of viruses, and 5 to 6 bands (17 to 22%) were repressed. For turbulence, the values were 4 to 5 (15 to 17%) for stimulation and 3 to 9 bands (10 to 33%) for repression. In addition, in TV 2, there was a statistically significant interaction between viruses and turbulence treatment for a relatively dominant (ca. 10%) band (#31; Fig. 5). In the presence of viruses, turbulence promoted this phylotype, but repressed it in the absence of viruses.

Sequences were obtained for 4 bands that were dominant in at least in one experiment. In the following section, these bands will be used to describe the variable influence of viruses and turbulence on the single phylotypes. A phylotype with a sequence identity of 99.8% to *Roseobacter* sp. isolates (*Roseobacter* 'a') from the Mediterranean (Fig. 6, Band a) was significantly less abundant (in terms of band intensity) in the turbulent than in the still treatment in TV 1, but significantly more abundant in the presence than in the absence of viruses in TV 2. Another phylotype with a high sequence similarity (98%) to *Roseobacter* clade-affiliated strains (*Roseobacter* 'b') (Fig. 6, Band b) showed a consistent pattern in both experiments. Relative band intensity was higher in the still than the turbulent treatments and decreased significantly under viral impact (Fig. 5). A phylotype affiliated with the *Rhodobacter* group of the *Alphaproteobacterial* subclass (Fig. 6, Band c) was significantly more abundant in the turbulent treatments in both experiments, whereas no virus effect was found. In both experiments, we detected a turbulence-specific band showing 97% identity of the partial 16S rRNA gene sequence to an *Oceanospirillum* sp. isolate from surface waters of the eastern Mediterranean (Fig. 6, Band d).

DISCUSSION

Critical evaluation of the experimental set-up and confinement effects

We applied a heat-killing treatment to inactivate viruses, since previous studies showed that some phylotypes seem to need organic matter in the virus size-fraction (Winter et al. 2004a). Abundance of viruses at time zero in both experiments was higher in the heat-killed treatments. Similar data were reported by Weinbauer et al. (2007). The heat-killing treatment might have disaggregated viral particles and broken some viral DNA genomes. Indeed, the higher abundance was just at the level of the low fluorescence viral subgroup, while other fluorescence viral subgroups were almost one order of magnitude more abundant in the untreated samples (data not shown). Furthermore, FCM analysis showed that the low fluorescence viruses were difficult to separate from the background noise in this treatment at t_0. Later in the experiment, viral abundance decreased in the inactive viruses treatment, but increased in the active viruses treatment, suggesting that we had successfully inactivated viruses by heat treatment.

The number of bacterial bands in the inoculum decreased in the experiments independently of the initial trophic conditions and bacterial assemblage. Massana et al. (2001) showed that confinement promotes a few opportunistic strains and thereby generates significant changes in the community composition that are accompanied by a decreased number of bands. Inter-

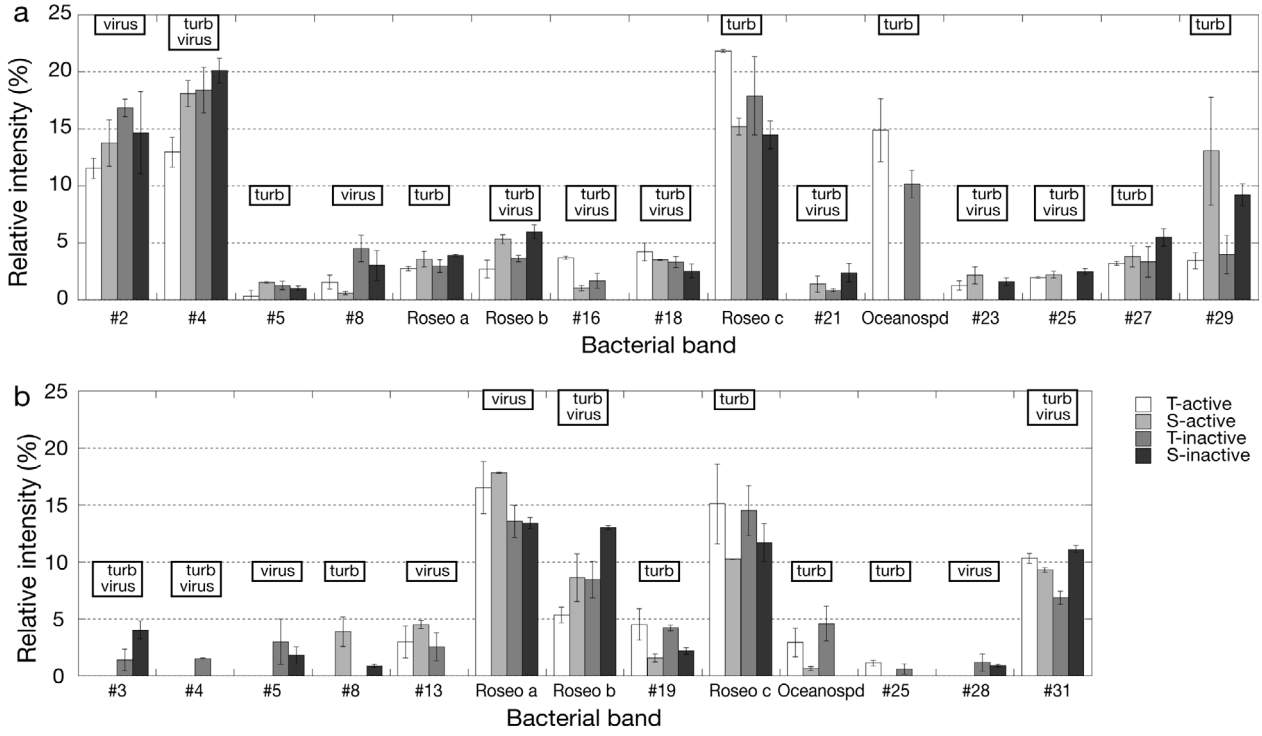
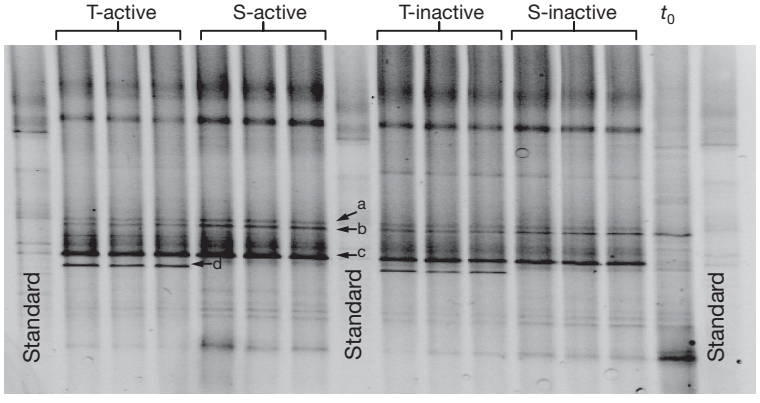


Fig. 5. Relative band intensities of all bacterial bands from the final assemblage in (a) TV 1 and (b) TV 2 that were influenced significantly by viruses and/or turbulence. Values are means ± SD, n = 3. The experimental factor(s) [virus and turbulence (turb)], are indicated above the columns. Treatment labels as in Fig. 3



Band	Accession no	Closest relative (accession no.)	Similarity	Alignment	Taxonomic description
a	EF018062	9IX/A01/152 (AY612764)	100	490/490	*Alphaproteobacteria*
		Nereida ignava (AJ748748)	99.8	489/490	*Rhodobacteraceae*
		Roseobacter sp. (AY136102)	99.8	455/456	*Rhodobacteraceae*
b	EF018061	DC11-80-2 (AY145625)	98.8	481/487	*Alphaproteobacteria*
		Roseobacter sp. (AF245632)	98.8	481/487	*Rhodobacteraceae*
c	EF018060	PI_4a9f (AY580451)	98.4	479/487	*Alphaproteobacteria*
		PI_4j11h (AY580443)	98.4	479/487	*Alphaproteobacteria*
		D058 (AF367385)	98	477/487	*Rhodobacteraceae*
d	EF018063	*Oceanospirillum* sp. (AY136116)	96.5	518/537	*Gammaproteobacteria*

Fig. 6. DGGE gel from TV 1 comparing initial (t_0) and final bacterial assemblages from incubations with inactivated and active viruses under turbulence (T-inactive and T-active, respectively) or still conditions (S-inactive and S-active, respectively). Bands excised from the gel and sequenced are indicated by arrows and were affiliated with (a–c) *Roseobacter* sp. and (d) *Oceanospririllum* sp. Similarity is given in %. Some of the closest BLAST alignments in the GenBank database are given in the table below the gel image

estingly, in the present study, the most dominant bands throughout all treatments were affiliated to the *Rhodobacteriaceae* of the *Alphaproteobacteria* group. In contrast, enrichment preferentially selected for the gamma-subclass of *Proteobacteria* in other experiments (e.g. Eilers et al. 2000). This may be due partially to the fact that the primers used select against some *Gammaproteobacteria*. However, the *Rhodobacter*-related phylotype identified became more dominant in terms of band intensity in the experiments compared to *in situ*, and the *Roseobacter* relatives increased in band intensity in some treatments. This indicates that at least for the community identified, experimental set-ups and confinement did not always select for r-strategists of the *Gammaproteobacteria* or that confinement was not important in our experiments, since experiments lasted for only 3 d.

Viral control of prokaryotes

Models and experimental data suggest that prokaryotic production should increase in the presence of viruses; however, other studies have also shown that viruses can reduce prokaryotic production (Fuhrman 1999, Noble & Fuhrman 1999, Middelboe & Lyck 2002, Zhang et al. 2007). This discrepancy could be partially due to the trophic level considered, i.e. food web (stimulation by viruses) or prokaryote-virus interactions (reduction of production by viruses) (Weinbauer et al. 2007). However, in our experiments, both trends were evident. Similar data are also available for other coastal environments (Zhang et al. 2007). In our study, differences in initial conditions might explain differences between experiments in the viral effect on prokaryotic production. In TV 1, the water for the experimental set-up was not prefiltered and tangential flow filtration should have killed flagellates and, consequently, enriched the samples with DOC. In TV 2, the DOC enrichment through viral lysis may have been relatively more important compared to TV 1, leading to a net-stimulation of prokaryotic production. Clearly, the conditions resulting in a net stimulation or repression of prokaryotic production by viruses are not well understood.

We found an effect of viruses on morphology. The length of prokaryotic cells increased in the presence of active viruses (Table 2) and a virus-mediated increase in volume was also shown for 2 non-turbulence experiments from the same environment (data not shown). Phages may force their hosts to increase cell size and thus the number of offspring (burst size), and this might also increase the average size of bacterioplankton (Parada et al. 2006). In addition, lysis products might stimulate the growth rates of infected and non-infected cells and thereby induce an increase in cell size.

The 'killing the winner' hypothesis predicts that phages influence host diversity by keeping in check phylotypes dominating the outcome of competition for nutrients, and this should sustain diversity by allowing less competitive bacteria to survive (Thingstad & Lignell 1997). Several authors have indeed found evidence for a viral control of the community structure of *Bacteria* and *Archaea* (Schwalbach et al. 2004, Winter et al. 2004b). However, few studies have reported an increase of number of bands in the presence of viruses (Weinbauer et al. 2007, Zhang et al. 2007). Considering single bands, we found 3 responses of *Bacteria* and *Archaea* to viral addition: (1) no effect, (2) increase in band intensity or appearance of bands and (3) decrease in band intensity or disappearance of bands. The simplest explanations for these responses are (1) resistance (or lack of specific phages), (2) stimulation by lysis products or reduction of competitors and (3) mortality due to viral lysis (Weinbauer et al. 2007). It is noteworthy that the effects of stimulation and reduction are considered here on a relative scale. For example, a stimulation of a phylotype (higher band intensity) by the virus treatment might indeed mean an increase in cell numbers (e.g. by lysis products or by removal of competitors); however, it is also possible that a reduction in cell number of a phylotype by viral lysis did not affect the absolute but only the relative abundance of another, the 'stimulated' phylotpye. While constant band patterns and intensities cannot be considered as proof for the lack of change in diversity, a change in the intensity of bands is very likely due to changes in the relative abundances of phylotypes, i.e. eveness of the community. This should be kept in mind in the following discussion.

Interestingly, we found in the presence of viruses a significant increase in band intensity for the most intense archaeal band. Since *Archaea* are rarely detected by CARD-FISH (catalyzed reporter deposition-fluorescence *in situ* hybridization) in the Bay of Villefranche (M. G. Weinbauer unpubl. data), their abundance was likely very low. In such a situation, control by lysis is unlikely, if infection were a density dependent process. Rather, *Archaea* benefited from lysis of competitors and their lysis products, assuming that band intensity reflected an increase in relative cell abundance. In the presence of viruses, we found increases in band intensity of 25 to 50% for a *Roseobacter* relative (band a) (TV 2). Since the band intensity of the phylotypes was high, it was probably a dominant bacterium in the experiments. This may have been due to viral lysis products or to removal of competitors. However, this also indicates that such phylotypes showed some resistance against infection, assuming

that infection is a density dependent process and that band intensity was related to cell abundance. This is compatible with an emerging idea that the dominant phylotypes detected *in situ* may be resistant to infection and grazing and that fast growing, but non-resistant phylotypes, are a greater source of viruses than previously assumed (Bouvier & del Giorgio 2007, Zhang et al. 2007). Nevertheless, a dominant *Roseobacter* band (band b) from the initial bacterial community of the bloom and post-bloom assemblages decreased in intensity in the presence of viruses. This may indicate that moderately or very abundant phylotypes also do not exhibit full resistance against infection. Along with findings of Bouvier & del Giorgio (2007), our data suggest that the influence of phages on prokaryotic diversity is more complex than predicted by the 'killing the winner' hypothesis. Instead, the data indicate moderate deviations from the 'killing the winner' hypothesis, which might be addressable in a refined model, rather than indicating fundamental problems with the original model.

Turbulence effects on prokaryotes

Despite theoretical assumptions (Karp-Boss et al. 1996) that fluid motion should not significantly increase nutrient flux to single prokaryotic cells, turbulence had a consistent positive effect on prokaryotic production in the present study. Recent freshwater (Bergstedt et al. 2004) and marine (Pinhassi et al. 2004) studies found similar results. However, trends are not always clear (Peters et al. 2002, Malits et al. 2004). A potential explanation is based on the concept of seawater as a gel-like polymeric matrix (Chin et al. 1998) consisting of structures such as cross-linked polymers, colloids, nano- and microgels. It has been shown that TEP are detectable after 0.2 µm filtration (Passow 2002), either due to spontaneous reformation from smaller TEP or by large TEP passing filters due to their flexible nature. These TEP could then be the target of turbulence and result in the micro-aggregates detected. Indeed, in our study, formation of micro-aggregates occurred in the turbulence treatment and this is consistent with previous results (Stoderegger & Herndl 1999, Malits et al. 2004). Thus, increased prokaryotic production in the turbulence treatments may have been caused by a better utilization of nutrients bound in these 'hot spots'. If this idea holds true, effects of turbulence on prokaryotic production should be linked to TEP and micro-aggregate concentration.

In the turbulence treatments, there was a shift to significantly larger single prokaryotes as well as a taxonomic shift in the prokaryotic community. The morphological shift might simply be a physiological consequence of unbalanced growth when protein synthesis is favored compared to cell division in response to variations in nutrient stoichiometry in the hot spots, thus resulting in larger cells (Malits et al. 2004).

In accordance with previous phytoplankton-free experiments (Pinhassi et al. 2004), we did not find clear effects of turbulence on the number of bands. However, when individual bands were investigated, a clear effect of turbulence on community composition was detected, with some bands disappearing or showing a lower intensity, and others appearing or becoming more intense (Figs. 3 & 5). In the turbulence treatment of both experiments, a bacterial phylotype appeared, which was affiliated with the *Gammaproteobacterial Oceanospirillum* clade. This phylotype was also likely active, as indicated by its presence on a 16S rRNA DGGE gel (data not shown). The few existing studies show that *Oceanospirillum* is less competitive in enrichment cultures than other members of the *Gammaproteobacterial* subclass group (Eilers et al. 2000, Pinhassi & Berman 2003); however, it is able to out-compete them under low-nutrient conditions (Pernthaler et al. 2001, Pinhassi & Berman 2003). Furthermore, Pernthaler et al. (2001) found by comparing the growth kinetics of *Pseudoalteromonas* sp. and *Oceanospririllum* sp. that the gradual addition of substrates to stationary-phase co-cultures is clearly disadvantageous for *Pseudoalteromonas* populations. Thus, *Oceanospririllum* populations seem to be favored when there is substrate patchiness, such as that induced by fluid motion. The high band intensity of a *Rhodobacter*-related phylotype in the turbulence treatment of both experiments might also be linked to turbulence-induced aggregate formation. This is supported by findings from the same environment that cells detected by a *Rhodobacter*-specific FISH (fluorescence *in situ* hybridization) probe had preferential growth in the presence of high molecular weight DOM (dissolved organic matter) (Weinbauer et al. 2006). Our data clearly show that turbulence is an important niche parameter for some phylotypes and that turbulence has to be included in concepts explaining the persistence of prokaryotic diversity in aquatic systems.

Viral control and aggregate formation

It has been argued that viruses might affect aggregate formation (e.g. by lysis products) and dissolution (e.g. by releasing enzymes during lysis) (Proctor & Fuhrman 1991); however, knowledge is sparse. Phage lysis products can coagulate to form larger colloids (Shibata et al. 1997), and adding viruses increases the size and stability of algal aggregates (Weinbauer 2004). We present evidence (TV 2) that viruses can also reduce or

delay prokaryotic aggregation and, thus, we extend the sparse knowledge on the influence of viruses on micro-aggregate formation by prokaryotes. This finding for prokaryotes is compatible with data from rolling tank experiments showing that in an initial phase, the phytoplankton bloom and algal floc formation were delayed by adding viruses (Weinbauer 2004). Among the potential reasons for such a delay are enzymes released during lysis (Proctor & Fuhrman 1991), the depolymerasing enzymatic activity of viral particles (Weinbauer 2004), or viral control of aggregate-forming prokaryotes. The formation of aggregates is a major biogeochemical process in the ocean, since it is linked to the transport of organic carbon into the interior of the ocean and by that to climate control. Thus, a delayed or reduced aggregate formation by viruses might influence the magnitude or time-scale of organic carbon sequestration.

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