Environmental Microbiology (2007) **9**(2), 287–297 doi:10.1111/j.1462-2920.2006.01137.x

Key role of selective viral-induced mortality in determining marine bacterial community composition

T. Bouvier[1]* and P. A. del Giorgio[2]
[1]*CNRS-UMR5119, Université de Montpellier II, case 093, Place Eugène Bataillon, 34095 Montpellier cedex 5, France.*
[2]*Université du Québec à Montréal, Dépt des Sciences Biologiques, CP 8888, Succ. Centre Ville, Montréal (Québec), H3C 3P8 Canada.*

Summary

Viral infection is thought to play an important role in shaping bacterial community composition and diversity in aquatic ecosystems, but the strength of this interaction and the mechanisms underlying this regulation are still not well understood. The consensus is that viruses may impact the dominant bacterial strains, but there is little information as to how viruses may affect the less abundant taxa, which often comprise the bulk of the total bacterial diversity. The potential effect of viruses on the phylogenetic composition of marine bacterioplankton was assessed by incubating marine bacteria collected along a North Pacific coastal-open ocean transect in seawater that was greatly depleted of ambient viruses. The ambient communities were dominated by typical marine groups, including *alphaproteobacteria* and the *Bacteroidetes*. Incubation of these communities in virus-depleted ambient water yielded an unexpected and dramatic increase in the relative abundance of *bacterial* groups that are generally undetectable in the *in situ* assemblages, such as *betaproteobacteria* and *Actinobacteria*. Our results suggest that host susceptibility is not necessarily only proportional to its density but to other characteristics of the host, that rare marine bacterial groups may be more susceptible to viral-induced mortality, and that these rare groups may actually be the winners of competition for resources. These observations are not inconsistent with the 'phage kills the winner' hypothesis but represent an extreme and yet undocumented case of this paradigm, where the potential winners apparently never actually develop beyond a very low abundance threshold *in situ*. We further suggest that this mode of regulation may influence not just the distribution of single strains but of entire phylogenetic groups.

Received 17 February, 2006; accepted 8 August, 2006. *For correspondence. E-mail tbouvier@univ-montp2.fr; Tel. (+33) 467144188; Fax (+33) 467143719.

Introduction

Aquatic prokaryotic communities harbour a significant fraction of the global genetic diversity in the biosphere, and yet the factors that determine microbial community composition, even the distribution of the major phylogenetic groups of prokaryotes, are still not well understood. Microbial composition and diversity are influenced both by environmental factors, such as the nature of the organic substrates, nutrients and water chemistry, and by biological factors, including competition and predation (Šimek *et al*., 1999; Cottrell and Kirchman, 2000; Bouvier and del Giorgio, 2002). In this regard, there is increasing speculation that viral infection may be one of the key factors in the regulation of the structure and composition of prokaryotic communities in aquatic ecosystems (Hewson *et al*., 2003; Schwalbach *et al*., 2004; Weinbauer and Rassoulzadegan, 2004; Winter *et al*., 2004; 2005), but empirical evidence is still equivocal and neither the intensity nor the outcome of this regulation is well understood.

If phages are present that can infect a certain strain, collision rates between phage and bacteria will increase in proportion to the abundance of the strain, so that the probability of infection increases proportionately. Lytic phage infection thus represents a density-dependent form of regulation that has been hypothesized to play a major role in the control of the most competitive and numerically dominant bacteria, preventing them from displacing competing taxa, in what has been termed the 'phage kills the winner' hypothesis (Fuhrman and Suttle, 1993; Thingstad and Lignell, 1997). This density-dependent effect may be modulated by the development of resistance to co-occurring phages, which may allow the persistence of dominant taxa even at high cell densities (Waterbury and Valois, 1993). Lysogenic bacteria, on the other hand, acquire immunity to further infection from other homologous virus, but this resistance is partial and does not extend to all different types of lytic viruses that can potentially infect the strain (Riemann and Middelboe, 2002). The current consensus is that phage infection will preferentially impact the population dynamics of strains that are

more abundant, and that their effect on bacterial community composition will be to enhance bacterial diversity by allowing the coexistence of strains through the control of the more competitive taxa.

The impact of viruses on bacterioplankton composition and diversity has been investigated using modelling (Murray and Jackson, 1992; Thingstad, 2000; Wommack and Colwell, 2000), or experimentally, generally by enriching natural samples with *in situ* viral concentrates and following the response of the bacterial community over time using polymerase chain reaction-based techniques (Fuhrman and Schwalbach, 2003; Hewson *et al*., 2003; Schwalbach *et al*., 2004; Weinbauer and Rassoulzadegan, 2004). These approaches have confirmed that viruses may indeed impact the dominant bacterial strains, but have provided little information as to how viruses may affect the less abundant taxa, which often comprise the bulk of the total genetic pool. In addition, it is unclear whether viruses impact bacterial strains independently from each other (Sano *et al*., 2004), or if larger phylogenetic groups may be collectively more affected than others by viral infection and thus more vulnerable to viral regulation.

In this study, we have explored the potential effects of viral infection on bacterial community composition at broad phylogenetic levels using fluorescence *in situ* hybridization (FISH), by manipulating the viral communities to which bacterioplankton from distinct Northeast Pacific water masses were exposed. Our main objective was to physically uncouple bacteria from viruses, i.e. to remove the selective killing mechanisms, to determine if the phylogenetic groups that developed and become dominant in these virus-depleted incubations resembled those that developed in the presence of the ambient viral assemblage.

Results

In situ *characteristics of the shelf, slope and open ocean sites*

The study was carried out as part of a cruise that span coastal waters off the coast of Oregon (USA), to open water sites at the edge of the northern Pacific Gyre. The experiments were carried out in three stations along the transect, characterized by distinct water masses. The coastal shelf station was dominated by colder (8.2°C) and high-salinity (32.2) upwelling waters, the slope station had influence of the warmer (10.9°C), low-salinity (30.4) Columbia River plume whereas the open ocean station had a strong influence of gyre waters (salinity 32.3) (for more details, see the study by Longnecker *et al*., 2005). The stations had similar total bacterial abundances (from 1.5×10^6 cells ml^{-1} to 1.3×10^6 cells ml^{-1}). Chl *a* concentration was higher in the shelf station (2.1 μg l^{-1}) than in the slope and open sea stations (0.2 μg l^{-1} and 2.1 μg l^{-1}), and the microplankton composition was drastically different between stations: The shelf samples were overwhelmingly dominated by small diatoms, the slope stations had the highest cyanobacterial densities and the open ocean station had a mixed community dominated by pico-eukaryots and cyanobacteria (data not shown). Bacterioplankton community composition varied greatly along the transect, and within the *bacteria*, there was increasing dominance by the *alphaproteobacteria* towards the open ocean site and a more diverse assemblage towards the coastal sites, with significant contribution of groups such as the *gammaproteobacteria* (Fig. 1A). The proportion of the members of the *Bacteroidetes* also increased seaward and were the dominant bacteria in the slope region (43%). Other major bacterial groups, such as the *betaproteobacteria*, the *Planctomycetales*, the *Actinobacteria* and the SAR324 lineage were characteristically at low densities or undetectable along the transect. The patterns in prokaryotic composition that we found along this transect are in good agreement with parallel studies from the same region that report phylotypes of all the major groups [*Bacteroidetes*, *Proteobacteria* and *Actinobacteria* (Longnecker *et al*., 2005)], and from other similar oceanic environments (Fuhrman and Ouverney, 1998; Nold and Zwart, 1998; Rappé *et al*., 2000).

Effect of the presence and absence of viruses on bacterial phylogenetic structure

The incubation of resident bacterial assemblages in virus-depleted ambient water resulted in an unexpected and dramatic increase in the relative abundance of bacterial phylogenetic groups that were numerically very rare in the ambient waters, and in the disappearance of groups that dominated the community *in situ* (Fig. 1). The two experiments carried out using the shelf and slope samples (St. 15 and 35) yielded very similar results. After 70 h of incubation in virus-depleted water, both assemblages were dominated by *betaproteobacteria* (46% and 55% of total cell counts respectively); these groups were typically undetectable *in situ* (< 1%; Fig. 1A and C). Groups that were dominant in the ambient assemblage, such as the *alphaproteobacteria*, *Bacteroidetes* and *gammaproteobacteria* were much less abundant in the virus-depleted incubation. The third experiment, carried out in the open ocean station (St. 85) also resulted in a dramatic shift in composition, although this response differed somewhat from that of the other two incubations. The community that developed in the virus-depleted treatment from this offshore station was dominated by the *gammaproteobacteria* (45%) and the *Actinobacteria* (34%), also with a

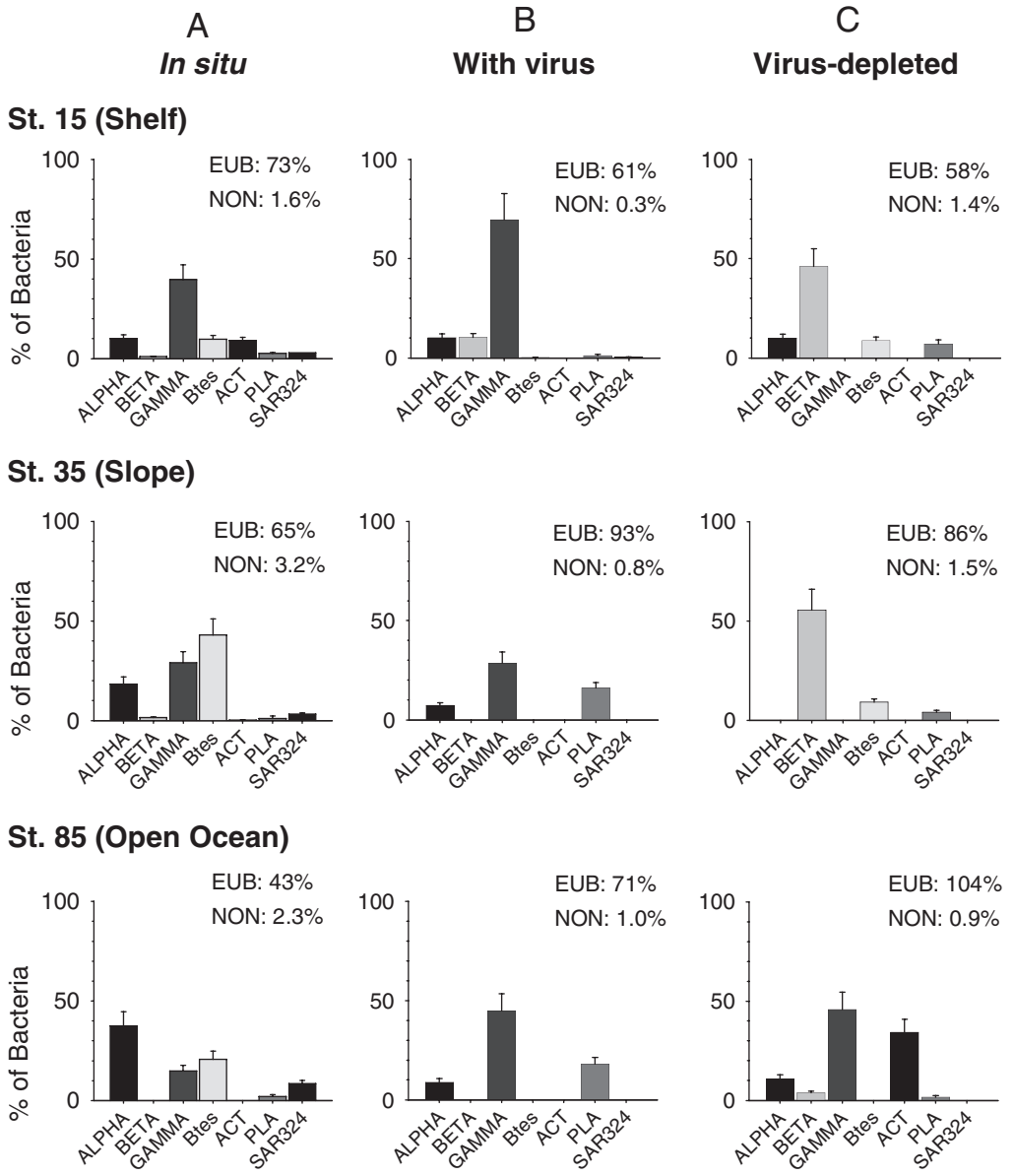

Fig. 1. A. *In situ* bacterioplankton community composition in shelf (station 15), slope (station 35) and open ocean (station 85) regions.
B and C. Bacterioplankton phylogenetic composition after 70 h of incubation in (B) the presence of autochthonous viruses at ambient densities and in (C) virus-depleted water (< 5% of ambient density). Data are expressed as per cent of total *bacteria* counts. The number (as per cent) that appears in each panel represents the proportion of cells detected with the *bacteria* probes relative to total number of cells in that sample. Bars represent an average error determined for a subset of replicated samples (see *Experimental procedures* section). *Alphaproteobacteria* (Alpha), *betaproteobacteria* (Beta), *gammaproteobacteria* (Gamma), *Bacteroidetes* (Btes), *Actinobacteria* (ACT), *Planctomycetales* (PLA), SAR324 lineage (SAR324).

substantial increase in the proportion of *betaproteobacteria* (4%), the latter two groups also being undetectable in the ambient waters. The *alphaproteobacteria* and the *Bacteroidetes* that dominated the assemblage *in situ* decreased to low levels in the virus-depleted incubation. These changes in the relative abundance of the different groups far exceed the average counting error of FISH samples. Bacterial composition in the presence of virus also changed relative to the *in situ* assemblages, with appearance or disappearance of particular phylogenetic groups. For example, at the slope and open ocean stations, the *Planctomycetales* greatly developed in the incubations with virus and became more abundant than they were *in situ*. In contrast, *Bacteroidetes*, which were abundant *in situ*, greatly declined in incubations in the presence of virus.

Table 1. Average bacterial specific production (10^{-19} mol leucine cell^{-1} h^{-1}) over 70 h-incubations of bacterioplankton in the presence of ambient viral assemblage and in the absence of virus at the shelf, slope and open ocean sites.

	With virus	Virus-depleted	CV
Shelf	13.1 (1.2)	17.3 (3.5)	13
Slope	4.4 (0.5)	5.3 (0.5)	9
Open ocean	10.7 (0.9)	12.6 (1.1)	8
Total average	9.4 (3.6)	11.7 (4.9)	10

CV, coefficient of variation between both incubations.

Patterns in bacterial growth during incubations

The incubations were carried out in a semicontinuous mode, with dilution rates selected to match the turnover times of bacterial communities that were previously measured in these same sites. These dilutions were thus aimed at maintaining bacterial growth as close as possible to *in situ* rates. The average specific leucine incorporation rates during the experimental incubations, which we take as an index of bacterial growth rate, varied from 4×10^{-19} to 13×10^{-19} mol cell^{-1} h^{-1} (Table 1), and were lowest at the slope and highest in the coastal site. These regional differences in bacterial turnover rate are in agreement with previous studies in the region (Sherr *et al*., 2001) and we conclude that dilution was effective in maintaining realistic levels of bacterial growth in the semicontinuous incubations. Differences in phylogenetic composition at the end of incubations could conceivably be due to differences in the turnover rates of bacteria between treatments (incubations with and without viruses). Within each oceanic region, there was less than 13% variability in the specific production rate between the virus and virus-depleted treatments, and no significant ($P < 0.05$) difference was observed between the two (Table 1). The absence of significant differences in growth rates among treatments suggests that there were no major effects of the sample manipulation on specific bacterial production, as we discuss in sections later, and thus that the dramatic differences in composition observed cannot be attributed to major differences in bacterial growth rates between treatments. It is interesting to note, however, that specific rates were on average 1.2-fold higher in the virus-depleted treatment relative to the samples incubated with the ambient viral assemblage (Table 1). Although these differences between both treatments were not statistically significant, they occurred in all oceanic regions. As we point out below, our experimental controls show that sample manipulation and filtration did not lead to enhanced bacterial growth during incubations, and thus our results suggest that bacteria developing in virus-depleted incubations may have had higher intrinsic growth rates.

Growth of individual bacterial groups during incubations

Although the overall bacterial specific production and growth rates at the community level were relatively similar between treatments, the treatments affected very differently the growth patterns of the different bacterial groups that developed within the communities, based on the observed changes in abundance during incubations (Table 2). For example, *gammaproteobacteria* had higher growth rates in all treatments with virus, but barely grew in virus-depleted treatments. Likewise, *alphaproteobacteria* grew faster (shelf and slope) in the samples incubated with virus and less in the samples incubated in virus-depleted treatment. The *Planctomycetales* grew generally faster in presence of virus and the SAR324 did not show any measurable growth in all treatments and sites. *Betaproteobacteria*, on the other hand, had higher growth rates in the virus-depleted treatments, compared with the growth samples that were incubated with virus. The *Actinobacteria* had a very low growth rate in all treatments and stations with the noticeable exception in virus-depleted treatment at the open ocean site. The *Bacteroidetes* did not show any trends in growth rates between treatments and stations and when measurable, they grew much more slowly than the other groups.

Table 2. Growth rate of specific bacterial groups (day^{-1}) in the presence (+virus) and absence (–virus) of the ambient viral assemblage at the shelf, slope and open ocean sites.

	Alpha	*Beta*	*Gamma*	*Bacteroidetes*	*ACT*	*PLA*	SAR324
Shelf							
+virus	0.35	0.01	0.92	NC[a]	NC[a]	NC[a]	NC[a]
–virus	NC[a]	0.97	NC[a]	0.36	NC[a]	NC[a]	NC[a]
Slope							
+virus	0.38	NC[a]	0.76	NC[a]	0.04	0.56	NC[a]
–virus	NC[a]	0.72	NC[a]	0.25	NC[a]	0.14	NC[a]
Open ocean							
+virus	0.21	NC[b]	1.39	NC[a]	NC[b]	1.17	NC[a]
–virus	0.18	0.05	1.79	NC[a]	1.17	0.02	NC[a]

NC, the growth rate could not be calculated because of either a decrease ([a]) or an absence of significant pattern ([b]) in cell abundance during the incubation.

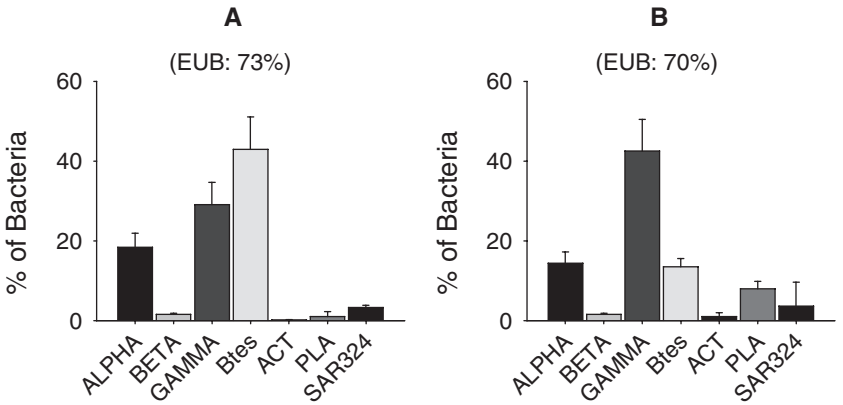

Fig. 2. The effect of confinement on bacterial composition: the *in situ*, unmanipulated bacterial assemblage at time zero (A) and after 70 h of incubation (B). Bars represent the per cent of each phylogenetic group determined by FISH relative to the total *bacteria* counts. The number (as per cent) within each plot represents the proportion cells detected with the *bacteria* probes relative to total number of cells. Example from station 35.

Altogether, these specific responses of the different groups contributing to bacterial community structure translated into dominance of *betaproteobacteria* in the virus-depleted treatments at the shelf and slope stations, and of *gammaproteobacteria* and the *Actinobacteria* at the open ocean station. These results further suggest that rare marine groups such as the *betaproteobacteria*, were capable of attaining growth rates that were similar or even exceeded those observed for more common marine groups.

Effect of sample manipulations on the bacterial community composition

These experiments involved extensive sample manipulation, and we carried out a series of experiments to control for potential experimental artefacts. Figures 2 and 3 show examples of these experiments designed to assess possible methodological artefacts of the experimental set-up that could have influenced the observed results. The controls carried out in other stations yielded very similar results. The simple confinement of untreated samples during 70 h resulted in changes in composition in all samples relative to the ambient communities (Fig. 2), with a general dominance of *gammaproteobacteria*. We also tested the potential effect of the tangential filtration on the growth rates of bacteria, due to release of organic and inorganic material present in the cartridge or to cell breakage. Results showed that both specific leucine incorporation rates and the final composition were similar in the AP15-filtered samples and in the reconstituted samples, and in all cases drastically different from that obtained in the virus-depleted treatments (Fig. 3). In addition, average specific leucine incorpora-

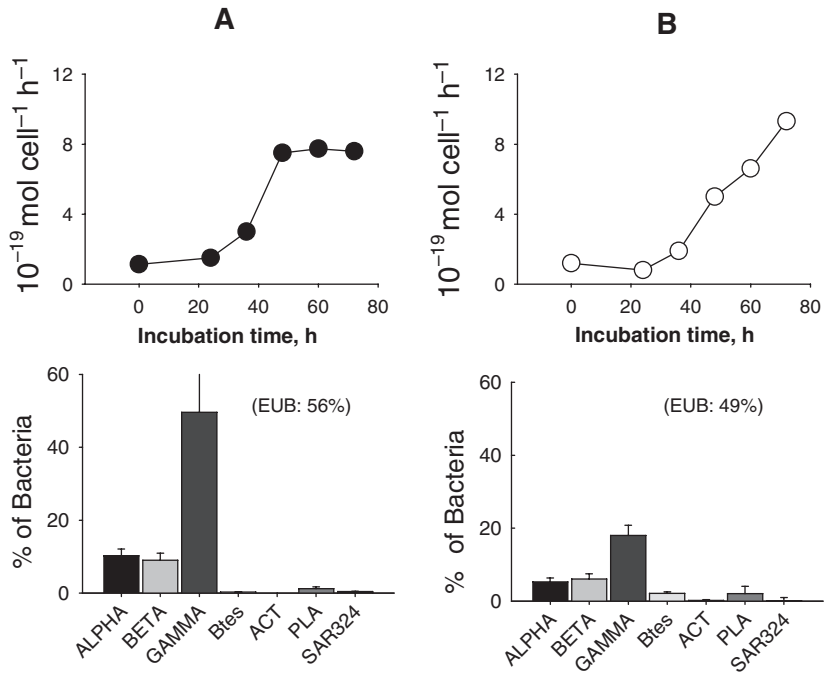

Fig. 3. The effect of sequential and tangential flow filtration: comparison of the treatment containing the unmanipulated bacterial and viral fractions obtained by filtration of bulk water through an AP15 filter (~1.0 μm pore-size) (A), with the reconstituted sample, obtained by recombining the viral and bacterial fractions that were previously isolated by sequential filtration (1.0 μm and 0.1 μm) and tangential filtration (B). Dot plots show leucine incorporation rates per cell during the incubation, bar charts show bacterial composition after 70 h of incubation and the number (as per cent) within each plot represents the proportion cells detected with the *bacteria* probes relative to total number of cells. Example from station 35.

tion rates were within 3% between treatments and these reconstituted controls, and were always lower in the latter (data not shown), suggesting that sample manipulation did not result in changes in carbon and nutrient availability that could have enhanced or altered bacterial growth.

Sample filtration aimed at separating bacteria from viruses also slightly modified the proportion of the major bacterial groups relative to the unfiltered samples but did not fundamentally alter the composition of the assemblages used at the start of the different experiments (data not shown). Therefore, differences in composition at the end of the incubations cannot be attributed to differences in the initial composition of the assemblages. In summary, although sample manipulation resulted in changes in composition of the bacterial assemblage, these shifts bore no resemblance at all to those obtained in the virus-depleted incubation (Fig. 1). Moreover, our controls suggest that sample manipulation and filtration did not lead to major differences in nutrient or carbon availability that could have influenced bacterial growth and turnover time among incubations.

Discussion

We have experimentally shown that the presence or absence of viruses rapidly led to drastically different patterns of bacterial community composition at the class and subclass level, with the development of rare bacterial groups in the absence of viruses. Methodological artefacts could account for at least some of these patterns, because it is conceivable that some of phylogenetic groups were responding not to the absence or presence of viruses but to changes in nutrient or dissolved organic carbon concentration or quality resulting from the experimental manipulation. In particular, the sequential sample filtration may result in cell lysis and dissolved organic carbon release, whereas tangential ultrafiltration removes not only viruses but also colloidal organic matter that is potentially bioreactive, and these effects could impact both the rates of growth and the dominant bacterial groups. Our controls confirmed that filtration and sample manipulation *per se* neither resulted in the development of these rare groups nor in drastically different rates of bacterioplankton growth, which would indicate major changes in substrate or nutrient availability. The incubation of the same bacterial communities in the presence of the ambient viral assemblages resulted in community shifts relative to the *in situ* assemblages and this type of phylogenetic shift during confinement has been noted before, often due to opportunistic groups such as the *gammaproteobacteria* (Fuchs *et al*., 2000). However, these changes bore no resemblance to those obtained in the virus-depleted incubations.

It is conceivable that some of the differences in composition could have been driven by differences in the turnover of the bacteria in the different treatments, but our data would suggest this was not the case. There was no significant difference in average growth rate among treatments and we did not observe any significant difference between treatments and reconstituted controls. Furthermore, growth rates in reconstituted controls were generally lower whereas we expected higher value due to possible enrichment. In addition, although for brevity we report here the results after 70 h, measurements taken at intermediate points along the time-course suggest that community composition in each treatment developed within the first 2 days of the incubation and remained relatively uniform thereafter. These lines of evidence suggest that although sample manipulation, filtration and confinement had an effect on bacterial community composition, none of these factors can explain the patterns in composition observed under virus-depleted conditions.

The dominance of rare marine prokaryotic phylogenetic groups in absence of viruses

The observation that in virus-depleted incubations, the bacterial phylogenetic groups that developed and become dominant did not resemble those that developed in the presence of the unmanipulated viral assemblage corroborates previous finding that prokaryotic diversity is influenced by viruses (Weinbauer and Rassoulzadegan, 2004). However, the development of *betaproteobacteria* and *Actinobacteria* in the virus-depleted incubations is remarkable, as none of these groups are naturally abundant in marine surface waters and are typically considered freshwater groups (Methé *et al*., 1998). It is generally thought that surface marine waters do not represent favourable environments for the survival and growth of these groups, and their presence in surface ocean waters and in the coastal areas has been attributed not to growth but to transport from continental areas (Rappé *et al*., 2000). Results from our incubations, however, show that *betaproteobacteria* and *Actinobacteria* not only remained viable in open ocean sites, but could also develop in ambient marine waters, even outcompeting other typical marine phylotypes in a virus-depleted environment. Although these groups were at the limit of detection of FISH in the ambient waters, particularly in the open ocean station, a parallel study of the same sites that used denaturing gradient gel electrophoresis of polymerase chain reaction-amplified 16S rRNA genes and sequencing confirmed the presence of these groups *in situ*, reporting several phylotypes belonging to the *Burkholderiales*, the *Hydrogenophilales* and the *Rhodocyclales* orders of the *betaproteobacteria*, and the marine Gram-positive cluster

of the *Actinobacteria* (Longnecker *et al*., 2005). These groups are thus present and detectable by DNA amplification but numerically rare *in situ*, as our FISH results show.

Our results thus suggest that these marine groups may be strongly impacted by lytic viral infection, despite their extremely low ambient abundances. For such a stringent regulation to exist, the rareness of the host must necessarily be compensated by other factors that enhance infection, such as higher number of viruses or a much greater bacterial susceptibility to viral infection.

The differences in susceptibility to lytic viral attack among major prokaryotic groups may result from a number of different processes. It has been established that bacteria may develop resistance to lytic viral infection (Lenski, 1988; Middelboe *et al*., 2001), but the extent and nature of this resistance in marine prokaryotes is still not well understood (Fuhrman, 1999). There is increasing evidence of intrinsic differences in cell metabolism among major bacterial groups (Fuchs *et al*., 2000; Gasol *et al*., 2002; Kirchman, 2002a; Cottrell and Kirchman, 2003; Yokokawa and Nagata, 2005), which could influence susceptibility to infection because cells with higher growth and metabolic rates are generally more susceptible to viral infection and may also tend to have higher virus yields (Lenski, 1988; Middelboe, 2000). Higher intrinsic cellular activity could thus support a higher viral abundance that in turn may increase collision rates with their rare host cells. Little is known concerning systematic differences in burst size among phylogenetic groups, and so this aspect will remain completely speculative.

We hypothesize here that one of the mechanisms that might explain the strong impact of viruses on the development of rare bacteria in ocean waters is that these bacteria may have higher intrinsic rates of growth than the numerically dominant marine bacteria. This scenario is supported by our own results that show the bacterial groups that dominated the virus-depleted incubations generally had higher growth rates than the groups that dominated the bacterial communities exposed to viruses (Table 2). This was further reflected at the community level, because the bacterial communities that developed in the virus-depleted treatments had higher average growth rates than those in the virus treatments (Table 1). As we discussed earlier, our controls show that these higher growth rates were not the result of experimental artefacts, because our reconstitution controls yielded rates that were lower and similar to those observed in the unfiltered water incubations. This hypothesis is also supported by recent studies that have reported in coastal marine environments the highest growth rates for the less abundant phylogenetic groups (Yokokawa *et al*., 2004; Yokokawa and Nagata, 2005).

Viral regulation of bacterial community composition

The changes in composition that we observed when viral pressure was relaxed corroborate recent suggestions that viruses may influence oceanic bacterial community composition (Hennes *et al*., 1995; Wommack and Colwell, 2000; Hewson *et al*., 2003; Schwalbach *et al*., 2004; Weinbauer and Rassoulzadegan, 2004; Winter *et al*., 2005), but further suggest that: (i) host susceptibility to infection is not necessarily only proportional to its density but also to other characteristics of the host; (ii) rare marine bacterial groups may be more susceptible to viral-induced mortality; and (iii) these rare groups may actually be the winners of competition for resources. These observations are not inconsistent with the 'phage kills the winner' hypothesis (Fuhrman and Suttle, 1993; Thingstad and Lignell, 1997) in the sense that once the selective killers were removed, we observed the emergence of a winner as predicted by the hypothesis. This, however, represents an extreme and yet undocumented case of this paradigm, where the potential winners apparently never actually develop beyond a very low abundance threshold *in situ*. Furthermore, our data do not necessarily support the idea that low host density represents a refuge against viral-induced mortality, or conversely, that numerical dominance necessarily increases the incidence of viral-induced mortality.

These results are in accordance with current views on selective processes that generate pattern in diversity in microbial communities, and further point to the fact that for bacteria, winning is not only based on competition for resources but also on avoiding mortality, including that generated by viral infection (Thingstad *et al*., 2005). It has been hypothesized that there are trade-offs associated to being effective in exploiting resources, and being able to avoid or resist predation or viral mortality, so that organisms that develop the most effective defence strategies may not be those that exploit resources most effectively, and vice versa (Lenski, 1988). It has been further hypothesized that the relative importance of these strategies will differ depending on the trophic status of the systems, with dominance of resource specialists at low resource concentration, and defence specialists at high resource availability (Bohannan and Lenski, 2000). In this case, the 'apparent' winner, in terms of competition for resources, is not necessarily the most abundant population. Our observations are consistent with such a theory and confirm that in marine microbial communities, both strategies coexist and that they are probably associated to significant trade-offs. These trade-offs seem to be at least in part related to differences in the growth rates that these different groups can potentially express under the ambient conditions. It is interesting to note that whatever the trophic status of sampled waters, rare groups appear to be more competi-

tive in terms of resource acquisition, and much more vulnerable to viral induced mortality. While this is coherent with predictions for more eutrophic areas (in our case the shelf) (Bohannan and Lenski, 2000), it is not what is expected for the most oligotrophic conditions. Indeed, at the open sea station, rare bacterial groups that appear to be highly vulnerable to viral infection can also express very high growth rates, suggesting that the numerically dominant groups may not be those that are most effective in exploiting resources but rather the ones most resistant to viral mortality, even in areas with low resource availability. These observations are also coherent with results obtained in cultures and chemostat where viruses seem to reproduce by infecting less abundant but sensitive cells, whereas the majority of the cells are resistant to viral infection (Lenski, 1988; Waterbury and Valois, 1993; Middelboe *et al*., 2001).

Interestingly, incubation in the presence of virus also leads to changes in community composition relative to the *in situ* communities, albeit with very different end results than those obtained in the absence of virus. Because of the experimental set-up, the incubations with virus had very low initial protist densities, so it is conceivable that the differences observed between the communities that developed in these incubations and those found in the ambient waters could be in part due to the relaxation of the predation pressure on these communities. Thus, although it was not our intention to explore the effect of predators on bacterial diversity, our results may provide indirect evidence of the effect of selective predation pressure on bacterial diversity. If this were the case, our results would further suggest that groups that are most vulnerable to viral infection are not those that are the most vulnerable to selective predation, a pattern that has been previously suggested (Šimek *et al*., 2001). The corollary of this scenario would be that the marine bacterial groups that are numerically dominant *in situ* may be those that have developed effective defence strategies against either selective predation or viral infection (or perhaps both), most likely at the expense of their performance *in situ*. This emphasizes the fact that the winner of the competition *in situ* may not be competitive when the selective mortality factor is removed, and therefore that selective mortality must play a role that is at least as important as resource availability, in shaping the structure of these marine communities.

Virus effect at broad phylogenetic level

This study based on FISH targeted major phylogenetic bacterial groups, and we have shown that the manipulation of viruses resulted in the emergence of some rare groups and the almost complete disappearance of other major groups that are common *in situ*. An effect at the broad phylogenetic level is certainly unexpected considering that bacteriophages are typically strain-specific. Viral/bacterial dynamics have been assumed to depend entirely on the abundance and characteristics of the individual strain, and thus proceed independently from all the other coexisting taxa. The fact that we have recorded an effect at the class or subclass level would suggest that the underlying mechanisms involved, for example, higher susceptibility to infection, are not strain-specific but rather that there may be more general eco-physiological features involved, which are shared by multiple strains within a broader phylogenetic group. There is increasing evidence that phylotypes within broad phylogenetic groupings may share functional and metabolic traits, such as patterns of substrate utilization or intrinsic levels of cellular activity (Cottrell and Kirchman, 2000; Yokokawa and Nagata, 2005), but to date there has been no evidence to link any of these common metabolic traits to aspects of community regulation. The possibility that viral regulation might operate not only at the individual strain level, as has always been assumed, but also at the scale of broad phylogenetic groupings, should prompt a fundamental change in the way we incorporate viruses into conceptual and mathematical models of microbial food webs.

Conclusions

Based on these experimental results, we propose a scenario of bacterial–viral interactions, where the dominant bacterial phylotypes of the surface oceans may have a lower susceptibility to lytic viral infection, e.g. due to the development of resistance or to low intrinsic metabolic activity. We further propose that bacterial groups that are usually undetectable or numerically unimportant may be characteristically more susceptible to lytic viral infection, because of either higher intrinsic activity, higher burst sizes that increase the virus to bacteria ratio, lower incidence of resistance or a combination of these processes. These rare groups appear to be more effective in resource exploitation than the bacterial groups that are numerically dominant in the ambient waters, thus suggesting that selective mortality must play a key role in shaping bacterial community structure in these ecosystems, even in the most oligotrophic waters. This density-independent form of the ‘phage kills the winner’ interaction would provide a relatively constant background regulation of bacterial composition, which would coexist and complement a density-dependent form of the ‘phage kills the winner’ process, which might provide periodic control of major episodic host oscillations associated to blooms and other events (Thingstad and Lignell, 1997). Viral infection has generally been regarded as a process that enhances total prokaryotic diversity by allowing less competitive taxa to coexist with the dominant competitors.

Our results, however, suggest that strong viral regulation within certain broad phylogenetic groups may in fact decrease the overall prokaryotic diversity of these marine communities, by maintaining these groups at such low abundances that their contribution to both the total DNA pool and to the overall functioning of the community would be minimal. Whether this scenario is borne out will be a matter of further research.

Experimental procedures

Sampling area

Water samples were collected in the Pacific Ocean along the Newport sampling line off the Oregon Coast, USA in June 2002. Samples were collected at three stations along the transect at 15 (St. 15, shelf), 35 (St. 35, slope) and 85 (St. 85, open ocean) nautical miles offshore with a conventional CTD rosette equipped with 10 l Niskin bottles taken within the top 5 m, and processed as described later.

Experimental manipulations

Two types of incubations were carried out: bacteria growing in virus-depleted ambient seawater, and bacteria growing in the ambient water in the presence of the autochthonous viral assemblage at *in situ* concentration. The virus-depleted incubation was prepared by filtering 1 l of seawater through a ~1.0 μm pore-size glass fibre filter (AP15 Millipore®), to remove nanoplankton and most picoplankton, and then by recovering the bacteria in the filtrate on a 0.1 μm pore-size polycarbonate filter. The bacteria retained on this 0.1 μm filter were rinsed four times with 50 ml of virus-depleted seawater and the final virus-depleted bacterial concentrate was diluted back to the original volume (1 l) with virus-depleted seawater and transferred to a chemostat bottle. The incubation of bacterioplankton with ambient viruses consisted of 1 l of AP15-filtered seawater, containing most ambient viruses and bacteria, and < 10% of the native picoautotroph and protist communities (P.A. del Giorgio, unpubl. data).

All incubations were semicontinuous, carried out in acid-washed 1 l bottles in the dark at ambient sea temperature for 70 h. Dilution rates were calculated to maintain cell growth at rates similar to those measured between 0.04 and 0.30 per day on the basis of previous re-growth experiments carried out in the same sites (Sherr *et al*., 2001), and were similar for all treatments from a given site. Dilutions were carried out every 4 h with virus-depleted seawater. These virus-depleted media that were used as growth media for the virus-depleted incubation and as dilution media for the other incubations were prepared onboard by tangential filtration with 300 kDa cut-off cartridge. Preliminary experiments showed that this system removes all bacteria and over 95% of viruses. The abundance of virus and bacteria was followed throughout the incubations as described in studies by Noble (2001) and Bouvier and del Giorgio (2002) respectively. Viral density remained below 10^6 ml^{-1}, i.e. < 5% of *in situ* concentration, in the virus-depleted treatment for the entire length of the incubations, although bacterial density increased on average by 2.6-fold, suggesting that the dilution was effective. In the ambient virus treatment, viral density remained at around 2×10^7 ml^{-1} throughout the incubation (data not shown). Bacterial growth rates during the incubations were obtained by dividing the rate of ^{3}H-leucine incorporation rate (Bouvier and del Giorgio, 2002) by bacterial abundance at each time point. Most incubations followed a very similar pattern in bacterial abundance and production (see later and Fig. 3) suggesting that the dilution rates used were effective in compensating for intrinsic differences in bacterial growth among treatments. The turnover time of the bacterial populations was thus of similar magnitude among treatments and regions, and this facilitates the comparison of community structure at the same fixed time point of 70 h. Because of the complexity of the experimental protocol and time and space constraints on board, it was not possible to replicate the experiments, although all analyses were carried out in duplicate or triplicate.

Experimental controls

The experimental protocol used in this research involved extensive manipulation and disruption of the samples, and it is certain that the protocol itself induced shifts in bacterial composition. In the three oceanic regions investigated, we carried out a series of controls, to ensure that changes observed in the treatments were not due to an experimental bias. In particular, we assessed the possible alteration of the phylogenetic bacterial composition due to: (i) simple enclosure effects; (ii) the filtration through polycarbonate or AP15 membranes, used to separate bacteria from other plankton and viruses and to generate the initial bacterial inoculum; and (iii) the tangential filtration, used to generate virus-depleted ambient water and also to generate bacterial-free viral concentrates used in the cross-experiments. The effect of the enclosure (i) was tested by following bacterial composition in incubations of the unmanipulated, *in situ* bacterial assemblage. The effect of sample prefiltration (ii) was assessed by comparing the initial composition of bacteria in all treatments with the *in situ* composition of bacteria in the ambient waters. This controlled for the direct effect of the filtration through AP15 filters and manipulation of the bacterial assemblages onto 0.1 μm filters, e.g. the retention of bacteria onto membrane. We further tested the effect of the tangential filtration (iii), potentially through the release of organic matter or nutrients present in the cartridge or due to cell breakage, on both the composition and growth rates. After separating the bacterioplankton and virioplankton as described earlier by sequential filtration followed by tangential filtration, we reconstituted the sample by recombining the virus-depleted water with the viral and bacterial fractions. This reconstituted sample was incubated in parallel to the same water that had only been filtered through AP15 but that had not undergone the subsequent filtration steps.

Phylogenetic diversity

Fluorescent *in situ* hybridization reactions were carried out essentially as described previously using oligonucleotides probes conjugated with CY3 and designed to target specifically the domain *bacteria* (mixture of EUB338 I, II and II)

(Daims *et al*., 1999) and seven of its main phylogenetic groups (the *alpha*-, *beta*-, *gammaproteobacteria*, the *Bacteroidetes*, the *Planctomycetales*, the *Actinobacteria* and the SAR324 lineage, Roller *et al*., 1994; Wright *et al*., 1997; Bouvier and del Giorgio, 2002). After 30 min of fixation at 4°C with formalin (2%, final concentration), 5–10 ml (depending on the sampling site) was filtered onto a 25 mm diameter, 0.2 μm white, polycarbonate filter under a vacuum of < 5 mmHg. The filters were air-dried and stored in a Petri dish between two aluminium foil pieces at −20°C until hybridization. Each filter was cut into equal sections. The filter sections were placed on a 5 μl droplet of hybridization solution containing 2 ng of probe per microlitre on a parafilm-covered slide. Slides were incubated at 46°C overnight in an equilibrated chamber. The filter sections were transferred to a 1.5 ml microfuge tube containing 0.5 ml of prewarmed (48°C) wash solution and incubated at 48°C for 15 min (Bouvier and del Giorgio, 2002). The filter pieces were dried on Whatman filter paper, placed on slides, covered with 10 μl of Citifluor solution (Citifluor, London, UK) and a cover slip. Orange CY3-labelled cells were counted within 1 day of hybridization using an Olympus-BX61 epifluorescence microscope under green light excitation; counts were corrected for non-specific binding by subtracting the counts for the control probe NON338. The fraction of autofluorescent cells and cells non-specifically stained with the control probe (NON338) was low in all samples, averaging less than 2% of total-counts cells. *Bacteria* accounted from 21% to 104% (mean 69%) of the total cells (detected by flow cytometry), which is in agreement with previous observations (Bouvier and del Giorgio, 2003). Here we report the abundance of each phylogenetic group as a percentage of the total number of *bacteria* cells detected by the EUB338 I, II, III probe mix. The error associated with replicate FISH counts ranged from 12% to 27% (mean 19%), based on a subset of 24 samples from stations 15, 35 and 85 for which we conducted independent replicate FISH preparations (3). The error associated with total bacterial cells count by flow cytometry ranged from 1% to 7% (mean 4%). The error on the per cent of bacteria numbers is then 23%, i.e. the sum of the errors for counting the bacterial group and for total bacteria, according to standard propagation of error equations. This error was applied to all subsequent FISH samples.

Bacterial growth rate at the community and phylogenetic group levels

Rates of bacterial production were estimated from the incorporation of ^{3}H-leucine following the centrifugation method of Smith and Azam (1992). Specific incorporation rates were calculated by dividing isotope incorporation rates by bacterial abundance at each time point measured. We use these specific incorporation rates as an index of bacterial growth rate, so we have avoided to further converting isotope incorporation rates and cell abundance into carbon units (Kirchman, 2002b). Growth rates of bacteria belonging to different phylogenetic groups were determined on the basis of the changes in abundance of individual phylogenetic groups during incubations, calculated as $\mu = \ln(A_T/A_0)/T$, where A_0 is the initial bacterial abundance, A_T the bacterial abundance at time t, T the unit time interval and μ the specific growth rate (Kirchman, 2002b). For phylogenetic groups that were undetectable at T_0 of the incubation and that developed during the incubation, the time frame considered for their growth rates calculation begun to the first time they were detectable.

Acknowledgements

We thank the captain and crew of the R/V *Wecoma* as well as K. Longnecker and E. and B. Sherr for their help during the research cruise, C. Bouvier for FISH analyses, F. Guillemette for viral counts, R. Condon for bacterial production, D. Bird, J. Apple, C. Duarte, P. Gasol, J. Fuhrman, I. Hewson, C. Legrand, R. de Wit and two anonymous reviewers for critical and constructive comments on the manuscript, and HSC for encouragements.

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