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The response of *Vibrio* and *Rhodobacter*-related populations of the NW Mediterranean Sea to additions of dissolved organic matter, phages, or dilution.

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running head: Bacterial life strategies

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Abstract

We investigated the growth response of the heterotrophic prokaryotic community focusing on *Vibrio* and *Rhodobacter*-related populations (SRF3), to variation in the availability of dissolved organic matter (DOM), population density dependant effects and prokaryotic virus (phage) infection in coastal and offshore waters of the NW Mediterranean Sea. We tested the response of the prokaryotic community to three different DOM fractions prepared by ultrafiltration. One of the DOM fractions contained phages (<0.2-m); a second was virus-free (<100 kDa); and a third contained only low-molecular-weight (<1 kDa). The proportion of *Vibrio* and SRF3 populations as determined by fluorescent hybridization (FISH) in the community ranged from <0.1 to 6.2% and from 3.2 to 6.3%, respectively. Based on changes in cell numbers growth rates ranged from 2.1 to 3.1 d^{-1} for *Vibrio* and from 0.8 to 1.2 d^{-1} for SRF3. Growth rates of *Vibrio* were similar or higher than that of the total prokaryotic community, whereas the ability of *Vibrio* to use high molecular weight (HMW) DOM and the responses to additions of phage-rich material were lower. Growth rates of SRF3 were lower than that of the community. Susceptibility to infection of SRF3 was sometimes lower than in the community, whereas the growth stimulation of HMW DOM was similar or lower. Reducing the cell concentrations of the prokaryotic community by dilution stimulated the overall growth of the community, including that of its constituent *Vibrio* and SRF3 populations, but the effect was smaller on the SRF3 and greater on *Vibrio* populations than for the total community. Comparisons with the community also revealed that life strategy traits of bacterial populations differed between coastal and offshore waters. Overall, our

data suggest that *Vibrio* is a *r*-strategist or opportunistic population in the NW Mediterranean Sea, whereas SRF3 is a *K*-strategist or equilibrium population.

Introduction

The data accumulated during the last 2-3 decades on marine heterotrophic bacterioplankton show that the *Bacteria* and *Archaea* are phylogenetically diverse and metabolically versatile groups, which drive biogeochemical cycles in the ocean (20). Nevertheless, the ecology of specific prokaryotic species in marine systems, especially heterotrophic forms, ***remains unknown*** ~~is obscure~~. This is due to a low isolation efficiency, which often results in a cultivation of only less than 1% of the cell numbers of the prokaryotic community (36). Taxonomic and functional antibodies have been developed to study bacterial populations in situ (39, 42) and in experiments with natural communities (45). While such approaches have produced valuable information on prokaryotic ecology, isolation is still required for developing antibodies.

16S rRNA gene sequencing and probe design have been used to circumvent the cultivation problem and identify taxonomic groups of prokaryotes in situ (1). Using fluorescent in situ hybridization (FISH) a large number of studies has investigated the geographical distribution of taxonomic groups such as alpha-, beta- and gamma-Proteobacteria and assessed their abundance in situ. Also, the relative contribution of *Bacteria* and *Archaea* to total prokaryotic abundance was assessed in various parts of the ocean using FISH (18, 29). The combination of microautoradiography with FISH permits now the allocation of functions to these groups (8, 21, 25).

Some studies have used probes targeted against narrow taxonomic groups. For example, it was shown that single clusters or groups can constitute up to half of the prokaryotic community in surface waters (10, 23, 34) and contribute significantly to

the carbon and sulfur cycle (46, 47). Bacterial groups such as *Roseobacter*, *Alteromonas* and SAR86 show differences in the uptake of bromodeoxyuridine, an analogue for thymidine (28) ***suggesting that their growth rates are different***, and bacterial populations such as *Pseudoalteromonas* and *Oceanospirillum* differ in their responses to substrate addition (27). In experiments manipulating grazing pressure in a reservoir, the removal of grazers resulted in a strong increase of a narrow group of beta-*Proteobacteria*, while filamentous growth forms of a *Flexibacter* lineage developed only in the presence of grazers (35). Such data increase our knowledge on the ecology of bacterial genera/species or narrow taxonomic groups.

Attempts have been made to ecologically differentiate bacterial populations by concepts such as opportunistic versus equilibrium populations (30, 45), oligotrophs versus copiotrophs (31), or the *r-K* selection continuum (4). Although these concepts are not fully compatible (33), they provide a useful framework for tackling the ecological characteristics of these populations. Life history concepts are often seen as evolutionary trade-offs in resource abundance versus resource scarcity (40, 41). Indeed, the significance of dissolved organic matter (DOM) and inorganic nutrients as limiting resources in the ocean is well established (20). There is increasing evidence that defense against grazing (14, 17) and antiphage defense systems (43) are important survival strategies of bacteria in aquatic systems. Nonetheless, a***n*** ~~thorough~~ assessment of life history traits has not been often performed for marine bacteria ***except for isolates*** (33).

In this study, we used a clone and a genus specific probe and FISH to detect bacterial populations within marine communities and size fractionation of DOM to

assess the effect of bioavailability of DOM, phage infection and dilution on the growth of these bacterial populations. ***Studies investigating simultaneously the effect of the potential control mechanisms DOM bioavailability, phage impact and dilution on narrow taxonomic groups as assessed by FISH have not been performed***
5 ***before in marine systems. In addition, we*** ~~We~~ used the deviation from the average growth rates of a prokaryotic community to evaluate life strategy traits of bacterial populations and their variability in coastal and offshore habitats.

Material and Methods

10 **Study sites and sampling**. 100L surface water samples were collected in the northwestern Mediterranean Sea in the Bay of Villefranche at the station Point B (43°41'N, 7°19'E) and at the oligotrophic French JGOFS station Dyfamed, 30 nautical miles off the coast of Nice (43°25'N; 7°51'E) using Niskin bottles or surface pumps. Offshore water is separated from coastal water by the Ligurian current. Coastal
15 samples were collected on July 17, 1998 and on June 8, 1999. Offshore samples were obtained on July 22, 1998 and on June 14, 1999.

Dissolved organic matter size fractionation and experimental approach. To test the effect of different size fractions of DOM on the growth of bacterial populations and the entire prokaryotic community, 100L seawater was prefiltered by
20 10-µm pore-size Nitex screening to remove larger zoo- and phytoplankton and passed through 0.2-µm pore-size polycarbonate filter cartridge (Nuclepore) to concentrate bacterioplankton (to ca. 700 ml) and obtain total DOM (TDOM) which contained most of the viral community (virus-rich TDOM). Subsequently, 40 l of prefiltered

water was passed through a 100 kDa cutoff spiral cartridge (Amicon S10Y100) using a tangential-flow ultrafiltration system (Amicon M12). The rationale behind this treatment was to reduce the abundance of viruses but maintain dissolved organic carbon (DOC) concentrations. DOC concentrations in the virus-size fraction obtained

5 from water collected at Point B did contribute significantly to total DOC concentrations, and viruses were not found in the permeate of the 100kDa cutoff cartridges (data not shown). Thus, this fraction of seawater was termed virus-free TDOM fraction. Thirty liters of the 0.2-µm pore-size filtered water was passed through a 1kDa cutoff spiral cartridge (Amicon S10Y1) to obtain the low molecular

10 weight (LMW) DOM fraction.

Aliquots of the prokaryotic concentrate were added to the three DOM fractions corresponding to 10% of the prokaryotic abundance in situ (without considering losses of bacteria during the concentration procedure), in order to 1) reduce contact rates between bacteria and flagellates, 2) reduce the average distance

15 between cells and thus competition between bacteria, and 3) avoid nutrient limitation. The virus-free TDOM fraction was also amended with prokaryotic concentrates corresponding to 100% of the initial concentration in order to study density-dependant effects on bacteria in the absence of viruses. Ten liter hard plastic containers were cleaned with hydrochloric acid and rinsed with MilliQ water and 2

20 times with 1L of the DOM fraction later used in the experiments. Duplicate containers were filled with 8 liter of treatments and incubated at ca. in situ temperatures ***in the dark***. Samples were removed periodically to perform prokaryotic and viral counts and to determine bacterial community composition. Growth rates of

the entire prokaryotic community and the two populations were calculated as the slope of ln(2)-transformed data of prokaryotic abundance versus time (typically within 24 h) assuming exponential growth. Duplicates were averaged and the standard error is the range of data from duplicate incubations.

5 **Enumeration of specific bacterial populations using oligonucleotide probes**. The oligonucleotide probes used in this study were developed from bacteria collected at the Dyfamed sampling site using dilution to extinction cultures to isolate strains or make clone libraries (13). The probe *G V* (5'-AGGCCACAACCTCCAAGTAG-3') is group-specific for the genus *Vibrio* and the

10 probe SRF3 (5'-CTCAAGACTACCAGTATTAG-3') is specific for a clone in the *Rhodobacter* lineage. For more details on probe development and specificity see Giuliano et al. (10, 13). Additional material is available at http:/bioinfo.unice.fr (section publications), which allows for verification of these probes.

Probes were Cy3 labeled at the 5' end (MWG Biotech AG) for use in

15 epifluorescence microscopy. Samples were preserved in formaldehyde (2% final concentration) over night at 4°C. Ten-ml and 50-ml subsamples were filtered onto white 0.2-µm pore-size polycarbonate filters (20 mm diameter, respectively) and stored frozen at -70 °C. Filters were cut into pieces and hybridization and washing conditions were used as described previously (10, 13). Hybridization was performed

20 in 2ml Eppendorf tubes in an Eppendorf thermomixer. Contrast staining of prokaryotic communities was done by using 4'6-diamidino-2-phenylindole (DAPI; final concentration, 1 µg ml^{-1}). Filters were mounted on glass slides.

Enumeration of prokaryotes and viruses. Samples were preserved in formaldehyde (1% final concentration), kept at 4°C and further processed within a few hours. Prokaryotes and viruses were collected on 0.02-µm pore-size Anodisc filters (Whatman), stained with SYBR Green I (10,000 x in DMSO; Molecular Probes, Chemical No. S-7567; concentration of staining solution, 2.5 x 10^{-3} of stock) and enumerated using epifluorescence microscopy as described by Noble and Fuhrman (24).

Prokaryotic production. Prokaryotic production was estimated by the [^{3}H] thymidine (Tdr) (83.0 Ci mmol^{-1}, Amersham) incorporation method (6, 12). Ten-ml replicates of samples were added in triplicate to 15-ml Falcon tubes and spiked with a Tdr at a final concentration of 10 nM. Two tubes containing formaldehyde (4% final concentration) and radioactive label served as control. After incubation for 1-2 hrs at in situ temperature, incorporation of label into bacteria was stopped with formaldehyde. Samples were filtered onto cellulose nitrate filters (Millipore GSWP, 0.22-μm pore-size), and unincorporated [^{3}H]Tdr was removed by two incubations (10 min) with 5% ice-cold trichloroacetic acid. The filters were dissolved with scintillation cocktail and the incorporated level was determined with a Packard Tricarb 8500 scintillation counter.

Statistical analyses. All data were log transformed for statistical analyses. Analysis of variance (ANOVA) and Fisher's Protected Least Significant Difference (PLSD) post-hoc tests (for pair-wise comparisons) were used to test, whether growth rates were significantly different between the DOM fractions, seasons and stations. A probability of < 0.05 was considered significant.

Results

Study sites. Summary physical and microbial characteristics of the study sites are shown in Table 1. Temperature varied from 21.3 to 24.5 °C at the sampling sites. Prokaryotic and viral abundances ranged from 1.0 - 1.2 x 10^9 L^{-1} and 10.0 to 22.6 x 10^9 L^{-1}, respectively, and were higher in 1999 than in 1998. Prokaryotic production was
5 higher at the coastal station Point B than at the offshore station Dyfamed.

Effect of DOM size fractions on microorganisms. *Growth of prokaryotic and viral communities.* ***Bacterial abundance at T0 was < 30% higher in virus-rich TDOM than in the two other treatments suggesting that the number of tiny bacteria passing the 0.2-µm pore-size filters was small.*** An example of time-course changes in
10 the concentrations of total viral and prokaryotic abundance in containers subjected to additions of different the DOM size fractions is shown in Fig. 1. Prokaryotes displayed exponential growth with additions of all DOM size fractions and typically entered stationary phase at the end of the experiment. Prokaryotic abundance was highest in the virus-free TDOM fraction and lowest in the LMW DOM fraction.
15 ANOVA tests statistics revealed that these differences were significant ($P < <0.0001$) for all DOM size fractions (post-hoc tests, $P < 0.05$). Viral abundances increased strongly after a lag phase of ca. 12h in the virus-rich TDOM fractions. In the other two fractions, viral abundances increased only slightly or remained constant.

Prokaryotic growth rates ranged from 0.06 to 0.15 d^{-1} (Fig. 2). Growth rates
20 were always lowest in treatments of LMW DOM addition and highest in the virus-free TDOM fractions. ANOVA test statistics showed that there was a significant difference between treatments ($P < 0.0005$) and post-hoc tests revealed a significant difference between all DOM size fractions ($P < 0.05$). No significant difference in

growth rate was found between coastal and offshore stations or between seasons. Assuming that the differences in growth rates between virus-reduced and virus-rich TDOM fractions are due to viral lysis, viruses removed between 5 and 36% of prokaryotic production (Table 2). HMW DOM stimulated prokaryotic growth by 39-156%.

Abundance and growth of bacterial populations. The abundance of SRF3 populations ranged from 3.5 to 5.8 % of the total bacterial counts in the experiments. Growth rates of SRF3 populations ranged from 0.03 to 0.05 d^{-1}. Abundance and growth rates were lowest in bottles to which LMW material was added and typically higher in virus-free than in the virus-rich TDOM fractions (Fig. 2). Growth rates of SRF3 differed significantly between DOM addition treatments ($P<0.05$). Post hoc tests showed that the LMW DOM fraction was significantly lower than the other two fractions ($P < 0.05$). Growth rates also differed significantly between seasons ($P < 0.01$) and between coastal and offshore waters ($P < 0.05$). Assuming that the differences in growth rates between virus-reduced and virus-rich TDOM fractions are due to viral lysis, viruses reduced growth of SRF3 by ~~removed between~~ 4 ~~and~~ - 9 % ~~of prokaryotic production~~. HMW stimulated growth by 13-57% (Table 2).

The relative abundance of *Vibrio* in the experiments ranged from close to the detection limit (< 1% or ca. 10^2 cells ml^{-1}) to 6.2 % of the total prokaryotic community. The abundance of the *Vibrio* population at the end of the experiments was always highest in the virus-free TDOM addition treatments (Fig. 3). Growth rate estimates of *Vibrio,* based on an increase of cell abundance with time, ranged from 0.09 to 0.13 d^{-1} (Fig. 2). *Vibrio* growth rate was highest in the virus-free TDOM fraction

(Fig. 2) and an ANOVA post-hoc test showed that the differences between virus-free TDOM and LMW DOM were significant ($P < 0.05$). Growth rate of *Vibrio* did not differ significantly between coastal and offshore stations. However, growth rate was significantly higher in 1998 ($P < 0.0005$) than in 1999. Assuming that the differences in growth rates between virus-reduced and virus-rich TDOM fractions are due to viral lysis, viruses ~~removed between~~ ***reduced growth of Vibrio by*** 2 ~~and~~ - 11% ~~of prokaryotic production~~. HMW stimulated growth by 6-15% (Table 2).

Dilution effects. The effects of cell numbers were assessed in the absence of viruses by adding prokaryotic concentrates at 10% and 100% of the initial volume to the virus-free TDOM fractions and estimating prokaryotic growth rate. In all experiments, the reduction of cell numbers in the virus-free TDOM fraction resulted in an increased growth rate of the populations and the community. This growth stimulation ranged from 3.6 to 7.5 fold for communities, from 1.7 to 2.1 fold for SRF3 and from 15 to 30 fold for *Vibrio* (Table 2).

Characterization of life history traits of bacterial populations by comparison with prokaryotic community parameters. The comparison of populations with communities was done in two ways. First, growth rates were compared directly. Compared to the community growth rate, rates of the SRF3 populations were always lower, whereas growth rates of *Vibrio* populations were often higher than the growth rates of the entire prokaryotic community (Fig. 2). In three of the four experiments, *Vibrio* populations grew faster than the prokaryotic community in the LMW DOM (1.5 - 2.2 fold) and virus-rich TDOM fractions (1.1 – 1.5 fold). This effect was stronger in 1998 than in 1999.

Second, life history traits such as the effect of bioavailability of DOM, susceptibility to infection and dilution on population growth, were compared to the effect on community growth (Table 2). The stimulatory effect of HMW DOM was stronger for communities than for populations except for SRF3 populations in
5 offshore waters. With the exception of the experiment performed with offshore waters in 1999, the negative effect of viruses on prokaryotic growth was stronger for the whole community than for SRF3 and *Vibrio*. With respect to the stimulatory effect of HMW DOM on the growth of populations and the susceptibility to phage infection, both populations showed at best small differences between coastal and offshore
10 waters. However, when compared to the community mean, the bacterial populations were more similar to the community in offshore than in coastal waters. Dilution effects were stronger for *Vibrio* than for the community but weaker for SRF3.

Discussion

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Effect of bioavailability of DOM and viral lysis on growth of prokaryotic communities. The size-reactivity continuum of DOM predicts that HWM DOM is on average more bioavailable than LMW DOM (2, 3). In our study, the growth rate of the prokaryotic community was significantly higher in virus-free TDOM (containing
20 both HMW and LMW) than in LMW DOM fractions. Moreover, thymidine incorporation at the start of the experiments and thus the cell specific prokaryotic production was also higher in virus-free TDOM than in LMW DOM fractions (data

not shown). Thus, our data support the concept of the size-reactivity continuum of DOM utilization.

We estimated the effect of viral lysis on bacteria by monitoring prokaryotic growth in the presence and absence of the natural viral community. Assuming that the differences in growth rates between virus-rich and virus-reduced TDOM treatments are due to viruses, viral lysis removed between 5 and 36% of prokaryotic production. Similar data have been reported before in the same environments using a transmission electron microscopy approach (7, 44).

Evaluation of probes. Since the sequences of the probes used in this study were published several years ago, a reevaluation seemed necessary. This analysis indicated that SRF3 is still a valid probe for this clone, and the *Vibrio* probe detects many strains of this genus as well as some strains of the genus *Photobacterium* (see methods). The validity of the *Vibrio* probe was also shown for *Vibrio* strains (10). The populations detected by this probe in our experiments were probably rather homogeneous, since sequences of *Vibrio* bands excised from 16S rRNA gene based single strand confirmation polymorphism gels analysis from the experiments performed in 1998 were identical (data not shown). However, we cannot exclude the possibility that more than one type of *Vibrio* was present in our experiments. Indeed, a high variability of *Vibrio slpendidus* types has been reported for the coastal North Atlantic (37, 38). For the SRF3 probe, it is difficult to assess the taxonomic level due to the lack of isolates, however, the phylogenetic analysis available as supplementary material (see methods) suggests a quite narrow specificity.

Effect of different DOM size-fractions, phage presence and dilution on bacterial populations. SRF3 and *Vibrio* populations grew better in the presence of HMW-DOM than on LMW DOM alone but SRF3 populations seemed to use HMW DOM better. Differences in HMW DOM use have also been shown for two freshwater populations using a DOM size fractionation approach (45), and phylogenetic groups differed in the uptake of LMW and HMW compounds (8). This indicates that the molecular weight of DOM is an important niche parameter for bacterial populations.

The population size of *Vibrio* was close to the detection limit in natural seawater, however, the relative contribution of *Vibrio* to total prokaryotic abundance increased in the dilution experiments. A similar phenomenon was observed in other studies (10, 26). A small population size in situ could be due to high mortality induced by flagellate grazers (5, 15). Viral impact on the growth rate of *Vibrio* was ***rather*** small ***(max. 11% reduction of growth)*** ~~compared to that in the prokaryotic community~~ and thus can probably not explain the low in situ abundances. This result was surprising, since vibriophages have frequently been isolated from marine systems (19) suggesting a potential for viral control of this genus. Production of extracellular slime *in situ* has been shown for *Vibrio* populations (10) and this could be a defense mechanism of these populations against phage infection.

A low competitive ability could be another reason for low *Vibrio* abundances, since *Vibrio* was more strongly affected by dilution than the entire prokaryotic community~~, and growth rates of *Vibrio* were low in undiluted incubations~~. Competition can be in terms of resource acquisition or direct antagonistic interaction.

Since marine *Vibrio* strains are quite resilient against inhibitory substances produced by other bacteria (22), the strong response to dilution was likely due to relieving nutrient limitation. As *Vibrio* did not use HMW DOM well ~~in our study even in dilutions~~ **compared to the community** (Table 2), availability of LMW DOM substances has likely limited the growth of *Vibrio*. The rapid response of *Vibrio* to glucose addition in seawater mesocosms (26) ***and mixed substrate additions in microcosms*** ~~thecould indicate that this LMW substances were monomers. Substrate limitation was also shown for *Vibrio* in the North Sea~~ (9) ***support these findings***. ~~Our data suggest that grazing and substrate availability are important factors controlling the abundance of *Vibrio* in situ~~. ***Growth rates of Vibrio in situ are not known, however, it has been suggested that it has the potential to respond rapidly to changes in growth conditions, i.e. due to high RNA content per cell (9). Overall, our data support previous reports showing that Vibrio has a strong growth potential (9, 11).***

To the best of our knowledge, nothing is known on defense mechanisms of SRF3 populations against grazing or on effects of cell concentration. Viral impact was small as well. The data indicate that effects of cell concentration were less important for SRF3 than for the entire community. In other studies, bacterial populations belonging to the *Rhodobacter* group decreased or vanished during incubation in mesosocosm experiments (32). However, the results are not directly comparable, since incubations lasted for 336 h and no dilutions or DOM size fractionation were used in the latter study. Since the growth rates of SRF3 were lower than those of the community in our study, it is possible that a longer incubation would have resulted in a reduced representation of SRF3 in our experiments. ***These data present the first study***

on ecological aspects of SRF3, an as-of-yet uncultured bacterial population, which is moderately abundant in marine waters.

Conclusion. One way to apply life history concepts is to directly compare two or more species in a system (30). In addition, comparison with community parameters, i.e. the deviation from the growth rate of the entire community facilitates a characterization of life strategy traits in this system (Table 2). ***Some of such*** ~~Such~~ traits are listed and compared in Table 3 for the *Vibrio* and SRF3 populations. Considering the low variability of abundance, low growth rates and weak dilution effects, SRF3 can be described as *K*-strategist or equilibrium population, whereas the variability of abundance, the high growth rates and the strong dilution effects affiliate pelagic *Vibrio* as *r*-strategist or opportunistic population in the ~~NW Mediterranean Sea~~ ***investigated environments***. The finding that mixed substrate and glucose addition stimulated growth of *Vibrio* and that this population might store carbon in vacuoles (26) suggests that *Vibrio* might survive by means of carbon storage, until a stochastic nutrient input allows for strong cell propagation. Such a feature is in accordance with *Vibrio* being *r*-strategists. ***Based on growth responses to nutrient additions, Vibrio has been termed an r-strategist before (9). Here we compile more information, which supports this affiliation (Table 3). Interestingly, these data suggest that Vibrio is an r-strategist in eutrophic (North Sea) and oligotrophic coastal (Bay of Villefranche) and oligotrophic offshore waters (station DYFAMED). Other life history concepts might be applicable as well. For example, the feast-or-famine concept (31) has been applied to Vibrio (9).***

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References

10 1. Amann R. I., W. Ludwig and K.-H. Schleifer (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microb Rev 59:143-169.

2. Amon R. M. W. and R. Benner (1996) Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. 41:41-51.

15 3. Amon R. M. W. and R. Benner (1994) Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. Nature 369:549-552.

4. Andrews J. H. and R. F. Harris (1986) r- and K-selection and microbial ecology. Adv. Microb. Ecol. 9:99-147.

5. Beardsley C., J. Pernthaler, W. Wosniok and R. Amann (2003) Are readily 20 culturable bacteria in coastal North Sea waters suppressed by selective grazing mortality? Appl Environ Microbiol 69:2624-30.

6. Bell R. T. (1986) Further verification of the isotope dilution approach for estimating the degree of participation of [^{3}H]thymidine in DNA synthesis in studies of aquatic bacterial production. Appl. Environ. Microbiol. 52:1212-1214.

25 7. Bettarel Y., J. Dolan, K. Hornak, R. Lemée, M. Masin, M.-L. Pedrotti, E. Rochelle-Newall, K. Simek and T. Sime-Ngando (2002) Strong, weak and missing links in a microbial community of the N.W. Mediterranean Sea. FEMS Microbiol Ecol 42:451-462.

8. Cottrell M. and D. Kirchman (2000) Natural assemblages of marine 30 proteobacteria and members of the *Cytophaga-Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. Appl Environ Microbiol 66:1692-1697.

9. Eilers H., J. Pernthaler and R. Amann (2000) Succession of pelagic marine bacteria during enrichment: a close look at cultivation-induced shifts. Appl Environ Microbiol 66:4634-40.

35 10. Eilers H., J. Pernthaler, F. O. Glockner and R. Amann (2000) Culturability and In situ abundance of pelagic bacteria from the North Sea. Appl Environ Microbiol 66:3044-51.

11. Flaten G., T. Castberg, T. Tanaka and F. Thingstad (2003) Interpretation of nutrient-enrichment bioassays by looking at sub-populations in a marine bacterial 40 community. Aquat Microb Ecol 33:11-18.

12. Fuhrman J. A. and F. Azam (1982) Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66:109-120.

13. Giuliano L., M. De Domenico, E. De Domenico, M. Höfle and M. Yakimov (1999) Identification of culturable oligotrophic bacteria within naturally occurring bacterioplankton communities of the Ligurian Sea by 16S rRNA sequencing and probing. Microb. Ecol. 37:77-85.
5 14. Hahn M. and M. Höfle (1999) Flagellate predation on a bacterial model community: Interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. Appl. Environ. Microbiol. 65:4863-4872.
15. Hahn M. W. and M. G. Höfle (1998) Grazing pressure by a bacterivorous flagellate reverses the relative abundance of *Comamonas acidovorans* PX54 and *Vibrio*
10 *sp*. CB5 in binary chemostat cultures. Appl. Environ. Microbiol. 64:1910-1918.
16. Holmquist L. and S. Kjelleberg (1993) Changes in viability, respiratory activity and morphology of the marine *Vibrio s*p. strain S14 during starvation of individual nutrients and subsequent recovery. FEMS Micobiol Ecol 12:215-224.
17. Jürgens K. and H. Güde (1994) The potential importance of grazing-resistant
15 bacteria in planktonic systems. Mar. Ecol. Prog. Ser. 112:169-188.
18. Karner M. B., E. F. DeLong and D. M. Karl (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409:507-10.
19. Kellogg C. A., J. B. Rose, S. C. Jiang, J. M. Thurmond and J. H. Paul (1995) Genetic diversity of related vibriophages isolated from marine environments around
20 Florida and Hawaii, USA. Mar. Ecol. Prog. Ser. 120:89-98.
20. Kirchman D. (ed.). 2000. Microbial Ecology of the Oceans. Wiley-Liss, Inc., New York.
21. Lee N., P. H. Nielsen, K. H. Andreasen, S. Juretschko, J. L. Nielsen, K. H. Schleifer and M. Wagner (1999) Combination of fluorescent in situ hybridization and
25 microautoradiography-a new tool for structure-function analyses in microbial ecology. Appl Environ Microbiol 65:1289-97.
22. Long R. A. and F. Azam (2001) Antagonistic Interactions among Marine Pelagic Bacteria. Appl Environ Microbiol 67:4975-83.
23. Morris R. M., M. S. Rappé, S. A. Connon, K. L. Vergin, W. A. Siebold, C. A.
30 Carlson and S. J. Giovannoni (2002) SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420:806-10.
24. Noble R. T. and J. A. Fuhrman (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat. Microb. Ecol. 14:113-118.
35 25. Ouverney C. C. and J. A. Fuhrman (1999) Combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types in situ. Appl Environ Microbiol 65:1746-1752.
26. Øvreås L., D. Bournde, R.-A. Sandaa, E. Casamayor, S. Benlloch, V. Goddard, G. Smerdon, M. Heldal and T. F. Thingstad (2003) Response of bacterial and viral
40 communities to nutrient manipulations in seawater mesocosms. Aquat Microb Ecol 31:109-121.
27. Pernthaler A., J. Pernthaler, H. Eilers and R. Amann (2001) Growth patterns of two marine isolates: adaptations to substrate patchiness? Appl Environ Microbiol 67:4077-83.
45 28. Pernthaler A., J. Pernthaler, M. Schattenhofer and R. Amann (2002) Identification of DNA-Synthesizing Bacterial Cells in Coastal North Sea Plankton. Appl Environ Microbiol 68:5728-36.
29. Pernthaler A., C. M. Preston, J. Pernthaler, E. F. DeLong and R. Amann (2002) Comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the
50 detection of pelagic marine bacteria and archaea. Appl Environ Microbiol 68:661-7.
30. Pianka E. T. (1988) Evolutionary Ecology, 4th ed. Harper & Row, New York.
31. Poindexter J. (1981) Oligotrophy. Feast and famine existence. Adv Microb Ecol 5:63-89.
32. Schäfer H., L. Bernard, C. Courties, P. Lebaron, P. Servais, R. Pukall, E.
55 Stackebrandt, M. Troussellier, T. Guindulain, J. Vives-Rego and G. Muyzer (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. FEMS Microbiol Ecol 34:243-253.

33. Schut F., R. A. Prins and J. C. Gottschal (1997) Oligotrophy and pelagic marine bacteria: facts and fiction. Aquat. Microb. Ecol. 12:177-202.
34. Selje N., M. Simon and T. Brinkhoff (2004) A newly discovered Roseobacter cluster in temperate and polar oceans. Nature 427:445-8.
35. Simek K., J. Pernthaler, M. Weinbauer, K. Hornák, J. Dolan, J. Nedoma, M. Masin and R. Amann (2001) Changes in bacterial community composition, dynamics and viral mortality rates associated with enhanced flagellate grazing in a meso-eutrophic reservoir. Appl. Environ. Microbiol. 67:1723-2733.
36. Staley J. T. and A. Konopka (1985) Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. Annu Rev Microbiol 39:321-46.
37. Thompson J. R., S. Pacocha, C. Pharino, V. Klepac-Ceraj, D. E. Hunt, J. Benoit, R. Sarma-Rupavtarm, D. L. Distel and M. F. Polz (2005) Genotypic diversity within a natural coastal bacterioplankton population. Science 307:1311-3.
38. Thompson J. R., M. A. Randa, L. A. Marcelino, A. Tomita-Mitchell, E. Lim and M. F. Polz (2004) Diversity and dynamics of a North Atlantic coastal Vibrio community. Appl Environ Microbiol 70:4103-10.
39. Tuomi P., T. Torsvik, M. Heldal and G. Bratbak (1997) Bacterial population dynamics in a meromictic lake. Appl. Environ. Microbiol. 63:2181-2188.
40. Velicer G. and R. Lenski (1999) Evolutionary trade-offs under conditions of resource abundance and scarcity: experiments with bacteria. Ecology 80:1168-1179.
41. Velicer G., T. Schmidt and R. Lenski (1999) Application of traditional and phylogenetically based comparative methods to test for a trade-off in bacterial growth rate at low versus high substrate concentration. Microb Ecol 38:191-200.
42. Ward B. B. and A. F. Carlucci (1985) Marine ammonia- and nitrite-oxidizing bacteria: seriological diversity determined by immunofluorescence in culture and in the environment. Appl. Environ. Microbiol. 50:194-201.
43. Weinbauer M. (2004) Ecology of prokaryotic viruses. FEMS Microbiol Rev 28:127-181.
44. Weinbauer M., I. Brettar and M. Höfle (2003) Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic waters. Limnol Oceanogr 48:1457-1465.
45. Weinbauer M. G. and M. G. Höfle (1998) Distribution and life strategies of two bacterial populations in a eutrophic lake. Appl Environ Microbiol 64:3776-3783.
46. Zubkov M. V., B. M. Fuchs, S. D. Archer, R. P. Kiene, R. Amann and P. H. Burkill (2001) Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulphoniopropionate in an algal bloom in the North Sea. Environ Microbiol 3:304-11.
47. Zubkov M. V., B. M. Fuchs, G. A. Tarran, P. H. Burkill and R. Amann (2002) Mesoscale distribution of dominant bacterioplankton groups in the northern North Sea in early summer. Aquat. Microb. Ecol. 29:135-144.

Table 1 . Physical and microbiological characterization of study sites.

Environment	Date	Temperature (°C)	Bacteria (10^9 L^{-1})	Bacterial Production (pmol ~~Leu~~***Tdr*** L^{-1} d^{-1})	Viruses (10^9 L^{-1})
Coastal	July 17, 1998	24.4	1.1	1.26	10.0
	June 8, 1999	22.1	1.1	0.90	22.6
Offshore	July 22, 1998	24.5	1.0	0.95	11.5
	June 14, 1999	21.3	1.2	0.16	21.1

Table 2. Evaluation of life strategy traits of the two populations in different environments and comparison with communities.

Community /Population	Environment	Variability of abundance (% of total)[a]	Growth rate on virus-free TDOM (% of community)[a,b]	Growth stimulation by HMW DOM (%)[a,c]	Growth reduction by viral lysis (%)[a,c]	Growth stimulation by dilution (x-fold)[a,c]
Community	Coastal	NA[d]	NA[d]	64 – 156	30 - 36	3.9 – 7.5
	Offshore	NA[d]	NA[d]	39 – 51	5 - 20	3.6 – 4.1
SRF3	Coastal	3.2 – 5.8	34 - 38	13 – 27 (0.17 – 0.20)	4 - 9 (0.13 – 0.24)	2.0 – 2.1 (0.27 – 0.56)
	Offshore	4.1 – 6.3	41 - 60	26 – 57 (0.66 – 1.13)	4 - 5 (0.26 –0.83)	1.7 –1.8 (0.45 – 0.48)
Vibrio	Coastal	<1 – 6.2	66 - 98	12 – 13 (0.08 – 0.21)	2 - 11 (0.07 – 0.30)	15 – 21 (2.8 – 3.8)
	Offshore	<1 – 3.4	107 - 134	6 – 15 (0.11 – 0.39)	7 - 9 (0.47 – 1.40)	23 – 30 (6.5 – 8.0)

[a] Data are given as ranges from two consecutive years and data in parenthesis show the ratio of parameters in populations compared to the community.

[b] Growth rates of populations on virus-free TDOM are given as ratio of community growth rates times 100

5 [c] The ability to use HMW DOM, viral infection, and dilution effects on the growth of populations and communities were calculated as follows: $(\mu_{<100kDa}/\mu_{<1kDa,}-1)\times100$, $(1-\mu_{<0.2\text{-}\mu m}/\mu_{<100kDa})\times100$ and $\mu_{<100kDa\text{-}Undiluted}/\mu_{<100kDa\text{-}Diluted,}$ respectively.

[d] NA, not applicable

Table 3. Some life strategy ~~features~~ ***traits*** of SRF3 and *Vibrio* populations.

Trait	SRF 3	*Vibrio*
Variability of abundance	Low	High
Growth rate	Low	High
Ability to use HMW DOM	High	Low
Effect of dilution on growth rate	Weak	Strong
Susceptibility to infection	Weak	Weak
Substrate limitation	Unknown	Strong [a]
Defense mechanisms against grazing	Unknown	Variable [b]
Antagonistic interactions	Unknown	Strong [c]

[a] Data from literature (5, 9)

[b] Data from literature (15, 16)

[c] Data from literature (22)

Figure legends

Figure 1. Examples of the changes in concentrations of viral and prokaryotic communities incubated with different size fractions of DOM. Values are given as
5 means and error bars show ranges of duplicate incubations.

Figure 2. Growth rate of total bacterial communities and *Vibrio* and SRF3 populations in the presence of size fractions of DOM. Values are given as means and error bars show ranges of duplicate incubations.

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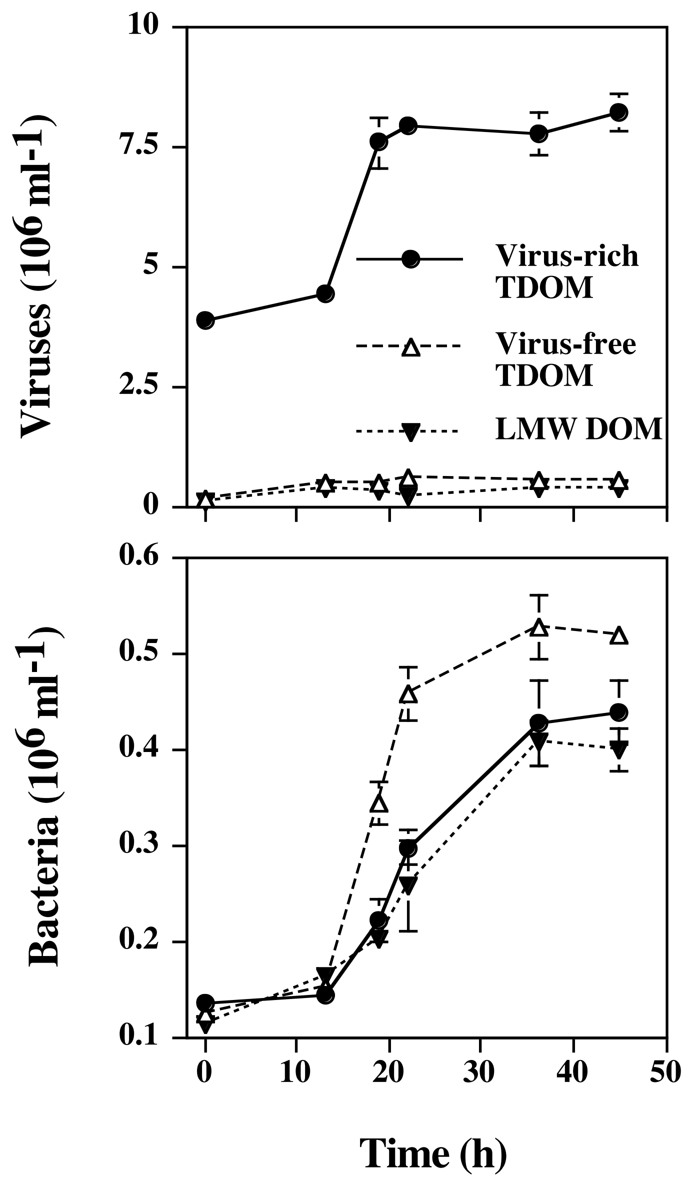
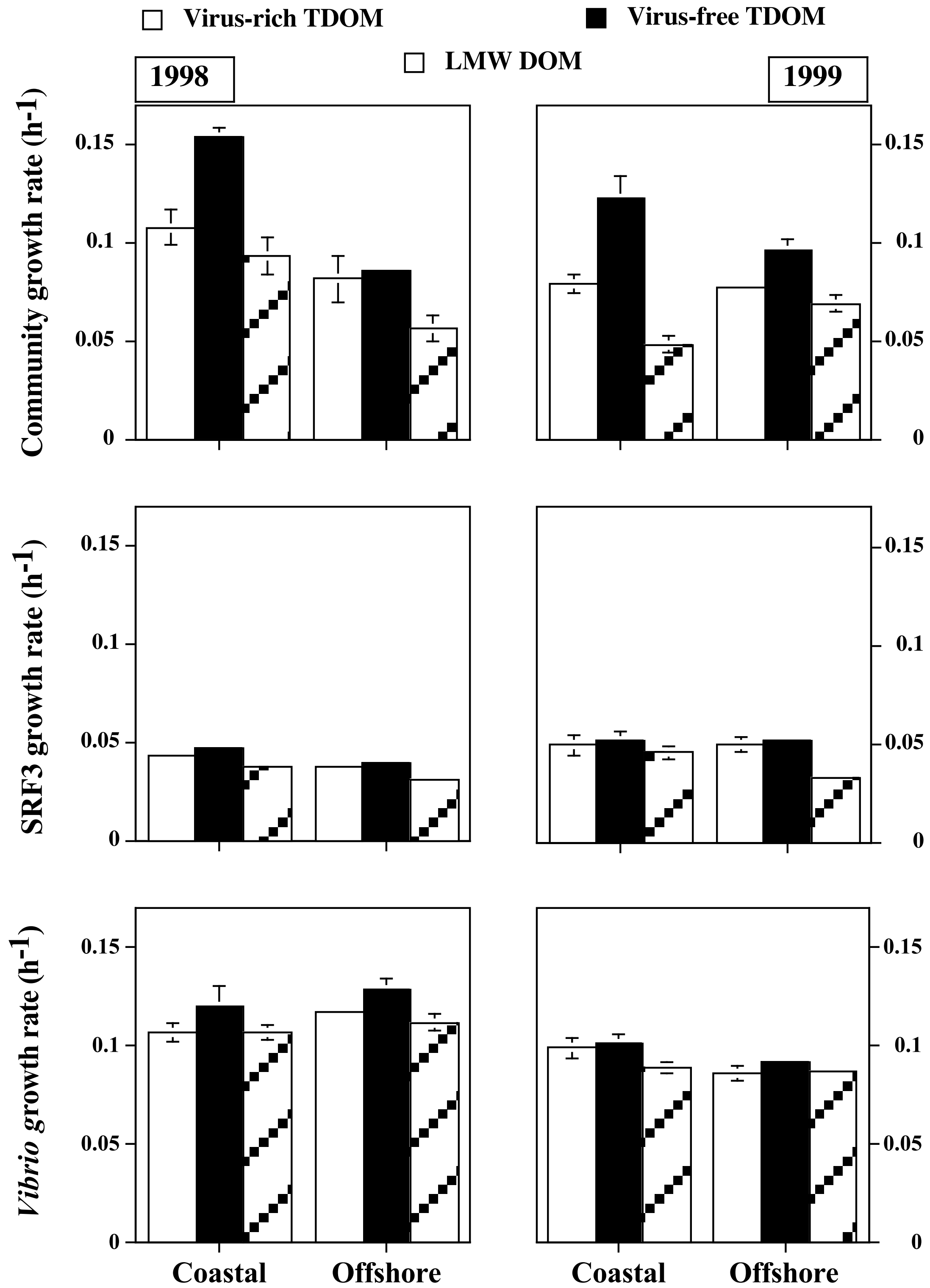
Figure 3. Changes in the concentrations of *Vibrio* (G V) and SRF3 populations incubated with different size fractions of DOM. Values are given as means and error bars show ranges of duplicate incubations.

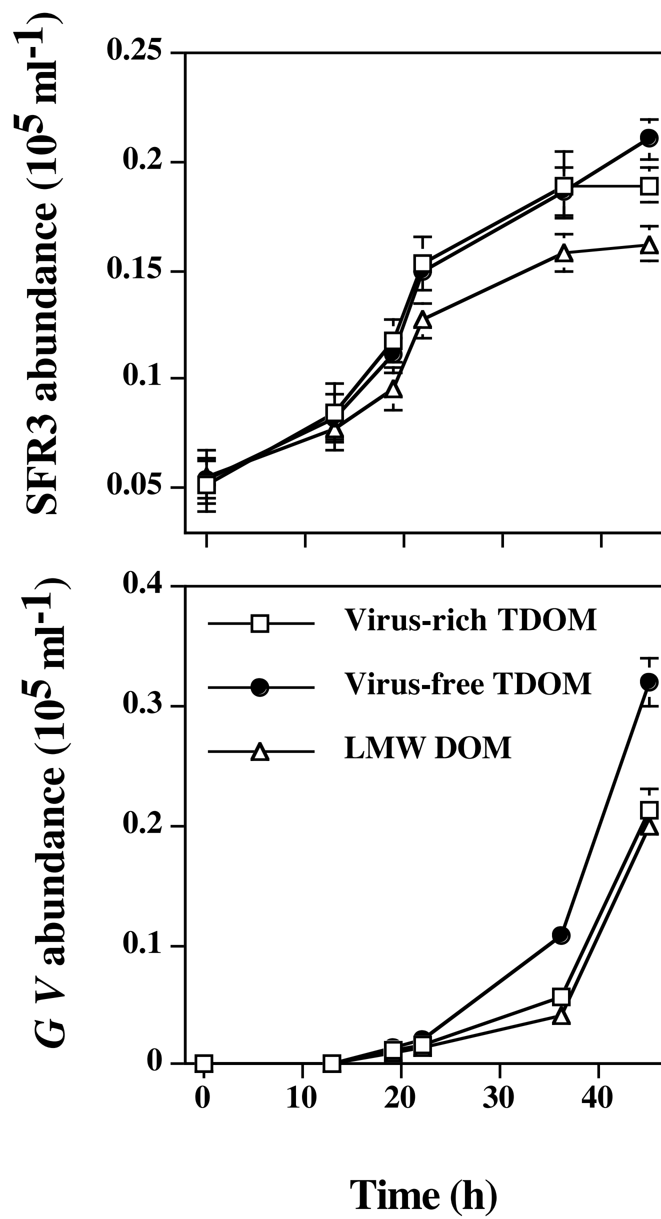
Weinbauer et al. Fig. 1



Weinbauer et al. Fig. 2



Weinbauer et al. Fig. 3



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