Response of *Alteromonadaceae* and *Rhodobacteriaceae* to glucose and phosphorus manipulation in marine mesocosms

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Summary

Microbial successions were studied in experimental mesocosms of marine water in the presence of additional organic carbon (glucose), phosphorus (P) or both. P addition lead to pronounced blooms of phytoplankton and to significantly enhanced bacterial production. Characteristic succession patterns were observed for two phylogenetic groups of bacteria that both transiently formed > 50% of total cells. An initial bloom of bacteria affiliated to the *Alteromonadaceae* could not be assigned to any specific treatment and was interpreted as a response to the manipulations during mesocosm set-up. These bacteria rapidly declined with the appearance of heterotrophic nanoflagellates, suggesting a negative effect of selective grazing. The persistence of *Alteromonadaceae* in the microbial assemblages was significantly favored by the presence of additional glucose. During the second half of the experiment, bacteria affiliated to *Rhodobacteriaceae* formed a dominant component of the experimental assemblages in treatments with addition of P. The community contribution of *Rhodobacteriaceae* was significantly correlated with chlorophyll *a* concentrations only in the P-amended mesocosms ($r^2 = 0.58$). This was more pronounced in the absence of glucose ($r^2 = 0.85$). The phylogenetic and morphological diversity among *Rhodobacteriaceae* was high, and treatment-specific temporal successions of genotypes related to *Rhodobacteriaceae* were observed. We suggest that the observed succession patterns reflect different niche preferences: *Alteromonadaceae* rapidly responded to disturbance and profited from allochthonous glucose input, whereas *Rhodobacteriaceae* benefited from the phytoplankton bloom.

Introduction

Recurring seasonal phenomena, such as phytoplankton spring blooms, are an important feature of coastal ecosystem dynamics that lead to predictable patterns of bacterial successions (Schauer *et al.*, 2003; Fuhrman *et al.*, 2006). In addition, some coastal environments such as lagoons and estuaries exhibit high and stochastic variability of, for example, salinity, nutrients or concentrations of dissolved organic carbon (DOC) (Kirchman *et al.*, 2005), potentially causing short-lived blooms of particular bacterial species (Piccini *et al.*, 2006). Succession within the bacterioplankton in coastal marine systems may thus be triggered by autochthonous processes, but also by other input of labile organic substrates, for example, the nutrient-rich run-off from rivers or organic contamination (Revilla *et al.*, 2000; Schendel *et al.*, 2004). Succession patterns are moreover shaped by selective mortality, as, for example, the grazing preferences of heterotrophic nanoflagellates (HNF) or selective viral lysis can affect the relative proportions of different microbial populations (Thingstad, 2000; Pernthaler, 2005).

Experimental manipulations of marine bacterioplankton assemblages suggest that there are at least two groups of planktonic bacteria that can rapidly respond to environmental changes, but that nevertheless appear to occupy different niches. On the one hand, the growth of bacteria from particular phylogenetic lineages is apparently stimulated by the input of certain labile substrates (e.g. glucose; Pinhassi and Berman, 2003) that might also be released during experimental manipulations due to disturbance of the autochthonous organic matter field (Kepkay, 1994; Azam and Worden, 2004). For example,

Gammaproteobacteria affiliated to the genera *Alteromonas*, *Pseudoalteromonas* and *Vibrio* spp. are typically rare *in situ* (Eilers *et al.*, 2000a), yet these bacteria are readily enriched during incubation of seawater in bottles or mesocosms with or without addition of substrates or after food web manipulation (Lebaron *et al.*, 1999; Pukall *et al.*, 1999; Eilers *et al.*, 2000b). At the same time, these bacteria appear to be particularly sensitive to selective HNF grazing (Beardsley *et al.*, 2003; Worden *et al.*, 2006).

On the other hand, some bacteria appear to be more tightly associated with blooms of phytoplankton: typically, the abundances of some *Bacteroidetes* and *Alphaproteobacteria* increase in the presence of natural and experimentally induced algal blooms (Riemann *et al.*, 2000; Zubkov *et al.*, 2001; Pinhassi *et al.*, 2004). In particular, bacteria from the physiologically diverse family of marine *Rhodobacteriaceae* specifically accompany artificially generated blooms of, for example, diatoms (Schäfer *et al.*, 2002; Grossart *et al.*, 2005). Such bacteria have been found to closely follow the seasonal development of primary producers in coastal North Sea waters (Eilers *et al.*, 2001). In contrast, no stimulation of *Rhodobacteriaceae* was observed during manipulations that did not trigger phytoplankton growth (Eilers *et al.*, 2000b).

Growth of bacteria from these two lineages in meso- or microcosms has been reported previously (Pukall *et al.*, 1999; Schäfer *et al.*, 2000; 2002; Pinhassi *et al.*, 2004). Currently it is unclear, however, how populations of *Alteromonadaceae* and *Rhodobacteriaceae* might interact in such systems. Specifically, it is not known if these groups form simultaneous blooms in experimental assemblages, or if there are distinct succession patterns that can be assigned to differences in their respective niche preferences as outlined above. Mesocosm set-ups seem to be particularly useful to study the relationship between these microbes in detail, because (i) the initial confinement in itself represents a disturbance of the original environment, (ii) mesocosms can be spiked with organic carbon (e.g. glucose), and (iii) sufficiently sized mesocosms allow the induction of phytoplankton blooms (by addition of nutrients).

We monitored the diversity and short-term temporal dynamics of bacteria related to *Alteromonadaceae* and *Rhodobacteriaceae* in mesocosms of coastal Mediterranean waters that were either amended with glucose (+G), phosphorus (+P), both (+GP) or left unmanipulated (control). This was studied in the context of changes in phytoplankton density and of the development of the HNF community.

Results

Dynamics of chlorophyll a *concentrations and HNF densities.*

Both P-amended mesocosms displayed pronounced maxima of phytoplankton biomass – as reflected by chlorophyll *a* (Chl *a*) concentrations – between days 4 and 5 (1.3 µg l^{-1} and 1.0 µg l^{-1} in +P and +GP respectively) (Fig. 1). The average Chl *a* concentrations in these treatments were significantly higher than in the other mesocosms (Fig. 1, Table 1). Interestingly, the addition of glucose also resulted in significantly elevated Chl *a* concentrations as compared with the control treatment (Table 1). A second maximum of Chl *a* was observed on day 8 only in +P.

The cell numbers of HNF during the first 6 days of the experiment generally followed the development of the bacterial assemblage with a time lag, and HNF reached their highest densities always at least 1 day after the first peak of bacterial abundances (Fig. 1). This maximum formed latest in the control mesocosms (day 5), when HNF densities in the other treatments had already started to decrease. HNF reached significantly higher average (Table 1) and maximal densities (day 4, 2.7×10^4 cells ml^{-1}) in +GP than in the control and +G. A second bloom of the flagellate population was observed in this treatment at the end of the experiment (Fig. 1). In all but the control mesocosms there was a highly significant positive correlation ($P < 0.001$) between HNF numbers and Chl *a* concentration (Spearman rank correlations, +G: $r_S = 0.78$; +P: $r_S = 0.88$, +GP: $r_S = 0.75$).

Table 1. Treatment-specific differences of Chl *a* concentrations, bacterial and HNF counts, bacterial production and of the community contributions of *Rhodobacteriaceae* and *Alteromonadaceae* in the experimental mesocosms as established by ANOVA and *post hoc* tests.

Treatments	Chl *a* concentration	Counts		Bacterial production	Relative abundance	
		HNF	Bacteria		*Rhodobacteriaceae*	*Alteromonadaceae*
Control	a	a	a	a	a	ab
+G	b	a	a	a	a	ab
+P	c	ab	b	b	b	a
+GP	c	b	b	b	b	b

Treatments denoted with 'a' are significantly different at $P < 0.05$ from 'b' and 'c', but not from 'ab', and treatments denoted with 'b' are significantly different from 'a' and 'c', but not from 'ab'.
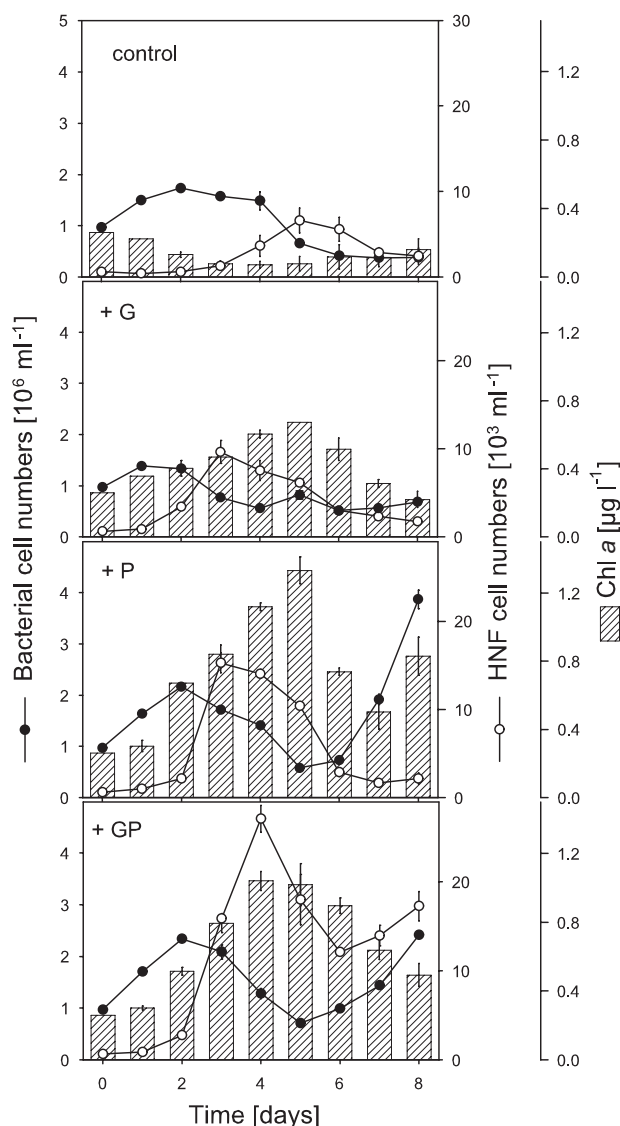
Fig. 1. Development of total cell numbers (solid circle), numbers of heterotrophic nanoflagellates (HNF; open circle), and of chlorophyll *a* (Chl *a*; columns) in the different experimental mesocosms (means and ranges of replicates). Control, unamended; +G, +glucose; +P, +phosphate; +GP, +glucose and phosphate.

Bacterial abundance and production

Initial bacterial cell numbers ranged around 1×10^6 cells ml^{-1} (Fig. 1). There was a significant difference both in cell numbers and in bacterial production between treatments with and without P addition (Table 1). Cell numbers in the control and the +G mesocosms less than doubled during the first 2 days of the experiment, whereas they rose to $> 2 \times 10^6$ cells ml^{-1} in +P and +GP. A second maximum of total abundances was observed towards the end of the experiment only in +P and +GP (Fig. 1). This increase of cell numbers was mirrored by higher bacterial production only in the +GP, but not in the +P variant (Fig. 2). Bacterial

production in the P-amended treatments increased by more than two orders of magnitude within 24 h (Fig. 2). This initial peak was less pronounced in the control and +G variants, and production in these treatments subsequently decreased to < 80 μg C l^{-1} day^{-1} (Fig. 2).

Temporal dynamics of Alteromonadaceae *and* Rhodobacteriaceae

Cell detection rates by fluorescence *in situ* hybridization and catalyzed reporter deposition (CARD-FISH) were $58 \pm 6\%$ (mean ± range) in the original water sample and $88 \pm 7\%$ in the mesocosms (data not shown). In both P-amended treatments there was a clear succession pattern of bacteria affiliated with *Alteromonadaceae* and *Rhodobacteriaceae* over the course of the experiment, which was less obvious in the control mesocosms and absent in +G (Fig. 3). In all mesocosms *Alteromonadaceae* accounted for a substantial fraction of the rapid initial change in cell numbers (Figs 1 and 3) (and thus probably also of bacterial production, Fig. 2). Within 48 h of incubation, bacteria targeted by probe ALT1413 multiplied from 7×10^4 cells ml^{-1} in the original water sample to $0.5–1.0 \times 10^6$ cells ml^{-1} in the various treatments. During that phase the relative contribution of *Alteromonadaceae* to the respective bacterial assemblages increased by five- to sixfold in +G and +P, and by eightfold in +GP and the control mesocosms (Fig. 3). A subsequent disproportional decline of these bacteria to 1% (+P) to 12% (+GP) of total cells was observed in all mesocosms except for +G. In that treatment *Alteromonadaceae* maintained a community contribution of approximately 20% between days 3 and 8, reflecting the stability of total cell numbers during that period (Fig. 1).
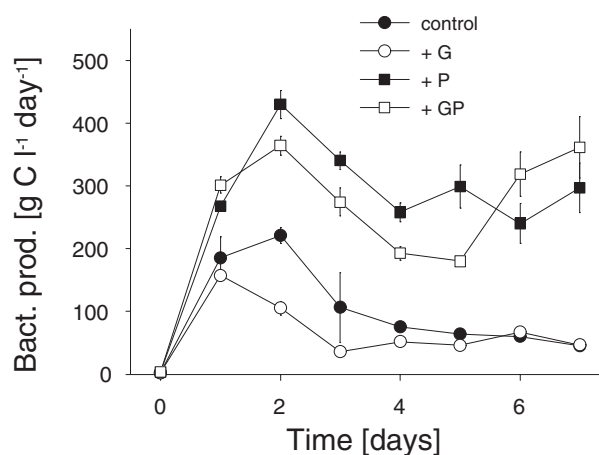

Fig. 2. Mean and range (error bars) of bacterial production in the different treatments. Control (solid circles), +G (+glucose; open circles), +P (+phosphate; solid squares) and +GP (+glucose and phosphate; open squares).
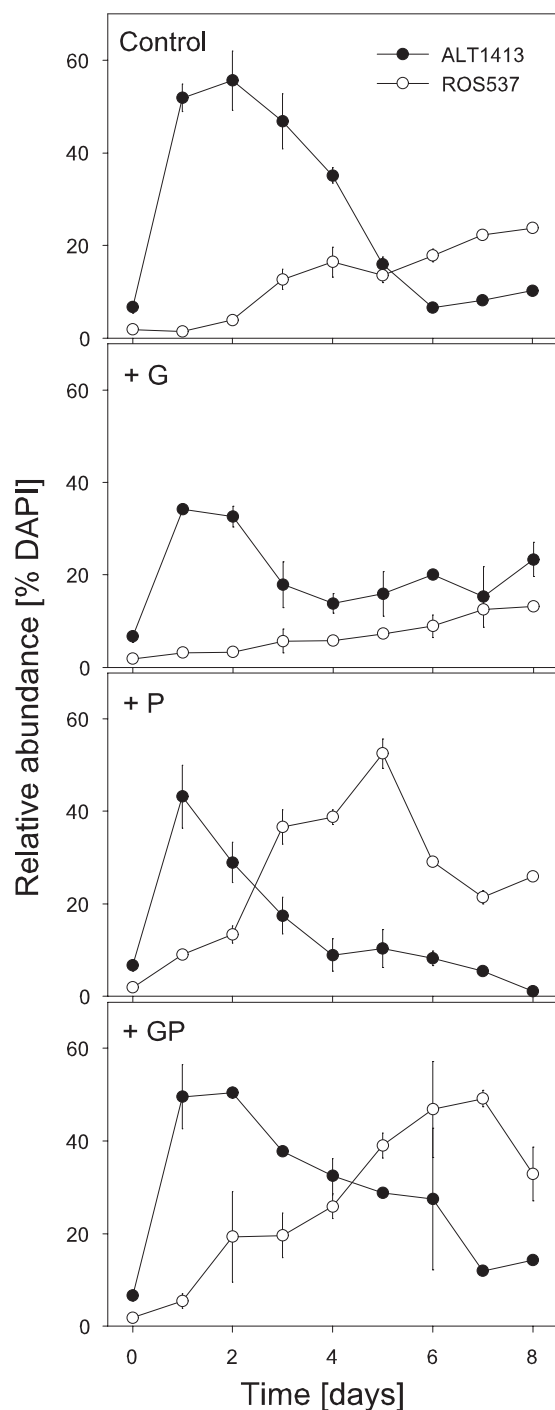
Fig. 3. Development of the relative abundances of *Alteromonadaceae* (ALT1413; solid circles) and of *Rhodobacteriaceae* (ROS537; open circles) in the different experimental mesocosms (means and ranges of replicates). Treatment designations are described in the legend to Fig. 1.

There was no significant relationship between the relative abundances of *Alteromonadaceae* and HNF numbers or Chl *a* concentration (data not shown). However, *Alteromonadaceae* and HNF abundances showed highly corre-

lated patterns of population dynamics with a time delay of 1, 2 and 3 days in the +G, the +P and the control mesocosms respectively (Spearman's rank correlation, data not shown).

Members of the *Rhodobacteriaceae* were rare in the original water (day 0, Fig. 3). Their community contribution increased most steeply in treatments amended with P, where they accounted for approximately half of total cells between days 5 (+P) and 7 (+GP) and subsequently declined. The +G treatment, which sustained a stable population of *Alteromonadaceae*, in turn, triggered no substantial bloom of *Rhodobacteriaceae*. In terms of absolute cell numbers (details not shown), a first maximum of these bacteria was observed after 48–72 h of incubation, which ranged between 4 and 6×10^5 cells ml^{-1} in +P and +GP, and approximately half as much in the control treatment. Cell numbers of *Rhodobacteriaceae* subsequently declined, but a second, even denser bloom (8–10×10^5 cells ml^{-1}) formed in the P-amended mesocosms on the last two sampling dates.

In the P-amended mesocosms, there was a significantly positive correlation between the relative abundances of *Rhodobacteriaceae* and Chl *a* concentrations, which was more tight in the +P ($r_S = 0.93$, $P < 0.001$) than in the +GP ($r_S = 0.62$, $P = 0.006$) mesocosms. While this correlation was most likely linear (Fig. 4, linear correlation coefficient, $r^2 = 0.58$), the relationship between *Rhodobacteriaceae* and HNF in these treatments was best modelled by a lognormal regression ($r^2 = 0.74$): the relative abundances of *Rhodobacteriaceae* reached a maximum at HNF densities $> 10^4$ ml^{-1} and declined thereafter (Fig. 4), reflecting the observation that the highest community contribution of *Rhodobacteriaceae* was found after the HNF bloom (Fig. 1 and Fig. 3).

Genotypic and phenotypic diversity of
Rhodobacteriaceae *and* Alteromonadaceae

The diversity of bacteria affiliated with the *Rhodobacteriaceae* was clearly reflected in denaturing gradient gel electrophoresis (DGGE) and partial sequence analyses of 16S rRNA genes. Altogether, 21 different genotypes related to this lineage could be obtained from various treatments and sampling dates either from excised DGGE bands or from isolates obtained on agar plates (Table 2). In contrast, only four genotypes affiliated with *Alteromonadaceae* (including one *Glaciecola*) were obtained by cultivation, two of which were closely related (sequence identity > 99%). No sequence related to *Alteromonadaceae* was obtained by DGGE. This is surprising, because the here used primer pair should theoretically also match to the vast majority of bacteria from this lineage, including our isolates (probe match against all 16S rRNA gene sequences with the primer target site in
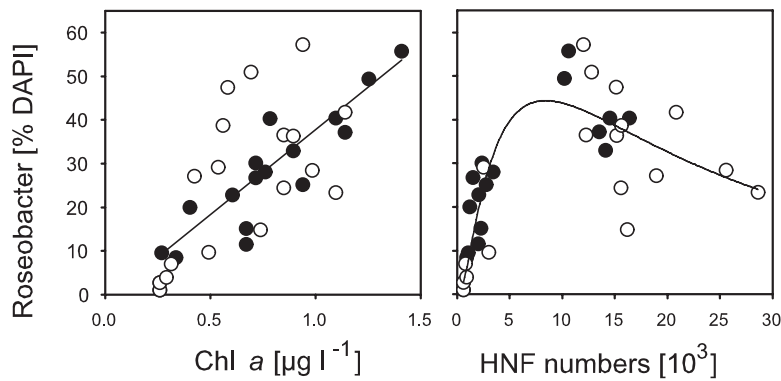
Fig. 4. Correlation between the community contribution of *Rhodobacteriaceae* and the concentration of Chl *a* (left) and HNF numbers (right) in the +P (solid circle) and +GP (open circle) treatments. The relationship of *Rhodobacteriaceae* and HNF in both P-amended treatments is best described by a non-linear pattern (regression type: lognormal, $r^2 = 0.74$), whereas it is a linear one for *Rhodobacteriaceae* and Chl *a* ($r^2 = 0.58$).

the database of the Ribosomal Database Project). DGGE bands that were related to genotypes targeted by probe ROS537 according to sequence analysis were submitted to cluster analysis (Fig. 5). In both P-amended treatments there was a clear distinction between the composition of sequence types related to *Rhodobacteriaceae* from the early and late sampling dates. During the initial bloom, very similar genotypes of *Rhodobacteriaceae* were present in +P and +GP (+P, days 2 and 4; +GP: day 2, Fig. 3), yet these bacteria were clearly distinct from the *Rhodobacteriaceae* in the original sample (Fig. 5). In contrast, during the second bloom (days 6–8), the assemblages of *Rhodobacteriaceae* in +P and +GP were clearly separated, both from the assemblages at the earlier dates and from each other.

Both *Rhodobacteriaceae* and *Alteromonadaceae* increased in cell size during the experiment in +GP. On day 8, cells of both populations were on average almost twice as long as in the beginning (Fig. 6). Additionally, a parameter for cell curvature (the ratio between the cell width and the width of a box enclosing the cell, i.e. the minimal Feret dimension) indicated the presence of at least one additional morphotype affiliate with *Rhodobacteriaceae* at the end of the experiment that was not present or rare during the initial bloom. In contrast, such morphological diversification was not observed for *Alteromonadaceae* (Fig. 6).
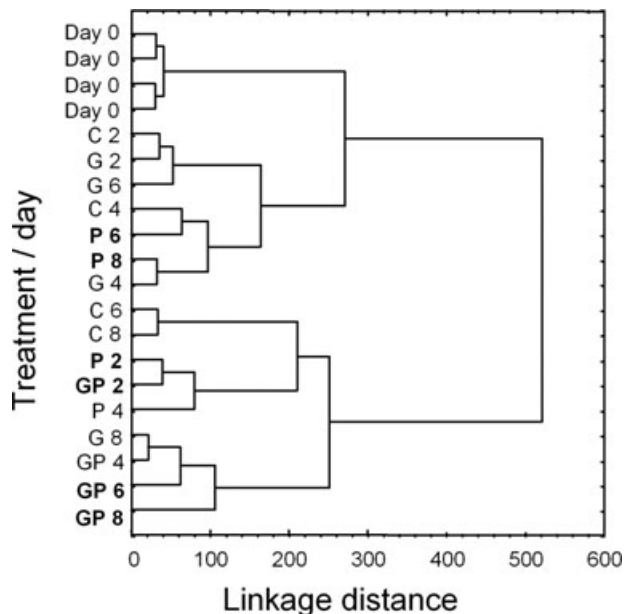
Discussion

Population dynamics of Alteromonadaceae

Our results suggest that the initial rise of *Alteromonadaceae* (Fig. 3) was a treatment-independent response to the disturbance caused by the transfer of seawater into the mesocosms. A rapid enrichment of these bacteria upon manipulation has been observed before during bottle incubations of North Sea water (Eilers *et al.*, 2000b; Beardsley *et al.*, 2003). *Alteromonadaceae* have also been detected in mesocosms of Mediterranean Sea water irrespective of nutrient addition, but only during the initial successional stages of the experimental assemblages (Schäfer *et al.*, 2001).

It is unclear what might have caused this conspicuous reaction to confinement. The mere handling of seawater might cause a disruption of the natural continuum of dissolved to particulate organic matter (Azam and Worden, 2004), e.g. the regular stirring of the mesocosms might cause a flocculation of colloidal material due to turbulence (Kepkay, 1994). This would favour bacterial taxa such as the *Alteromonadaceae* that can thrive on solid surfaces (Dang and Lovell, 2000) or particles (Acinas *et al.*, 1999) as well as in the water phase (Eilers *et al.*, 2000b; Beardsley *et al.*, 2003). Moreover, some *Alteromonadaceae*

Fig. 5. Similarities of the *Rhodobacteriaceae* communities in the different treatments at the beginning and at days 2, 4, 6 and 8 of the experiment in the different treatments. The dendrogram depicts the results of cluster analysis (Ward's method) of those bands in DGGE gels that were related to *Rhodobacteriaceae* according to sequence analysis. Treatment designations are: C, control; G, +glucose; P, +phosphate; GP, +glucose and phosphate. Highlighted in bold: similarity of the *Roseobacter* community in +P and +GP at day 2 and their contrasting development (days 6–8).

Table 2. Genotypes and isolates related to *Alteromonadaceae* (in bold) and *Rhodobacteriaceae* lineages that were retrieved from different mesocosms and different times.

	Retrieved from	Designation	Closest genotype/ closest isolated relative	Identity (%)
Control	C 8 (C 6, P 2, GP 2)	Mes36	Uncultured alphaproteobacterium clone PI_4a9f (AY580451)	98
			Roseovarius sp. DFL-35 (AJ534219)	98
	C 8 (C 6, P 2, GP 2)	Mes15	Uncultured alphaproteobacterium clone PI_4a9f (AY580451)	98
			Roseobacter gallaeciensis (AY136134)	96
	C 8 (C 6)	Mes37	Uncultured marine bacterium D015 (AF177555)	95
			Roseobacter algocolus (X78315)	94
	C 8 (C 6)	Mes38	Bacterium K2-53B (AY345413)	97
			Phycococcus omphalius (AB193438)	97
	C 8	M625*	*Nereida ignava* (AJ748748)	99
			Thalassobacter oligotrophus (AJ631302)	95
	C 8	**M620***	**Uncultured marine eubacterium HstpL30 (AF159672)**	**94**
			***Glaciecola punicea strain* ANT9087 (AY167279)**	**92**
+Glucose	G 8	Mes47	*Roseobacter* sp. HYL-SA-18 (DQ008594)	97
			Agrobacterium gelatinovorum (D88523)	97
	G 8 (GP 6)	Mes26	*Agrobacterium gelatinovorum* (D88523)	98
			Thalassobacter oligotrophus (AJ631302)	98
	G 8 (P 2, GP 2, GP 8)	Mes49	*Rhodobacteraceae* bacterium CL-TA03 (AY962292)	97
			Jannaschia cystaugens (AB121782)	96
+Phosphate	P 4 (GP 4)	Mes8	Uncultured alphaproteobacterium clone JL-ECS-X8 (AY663968)	99
			Jannaschia cystaugens (AB121782)	96
	P 6	Mes9	Uncultured *Roseobacter* sp. clone (AY573530)	100
			Roseobacter gallaeciensis (AY136134)	97
	P 6	**M604***	**Uncultured *Alteromonas* sp. clone JL-ESNP-I29 (AY664213)**	**99**
			***Alteromonas macleodii* (AMY18231)**	**98**
	P 6	**M609***	**Uncultured gammaproteobacterium clone JL-ETNP-R11 (AY726784)**	**99**
			***Alteromonas alvinellae* (AF288360)**	**99**
	P 6	Mes11	Uncultured bacterium clone J-154 (AY600952)	96
			Agrobacterium gelatinovorum (D88523)	96
	P 8	M612*	Roseobacter sp. DSS-8 (AF098493)	98
			Agrobacterium gelatinovorum (D88523)	96
	P 8	M613*	Uncultured alphaproteobacterium clone JL-ECS-X8 (AY663968)	99
			Jannaschia cystaugens (AB121782)	96
	P 8	**M614***	**Uncultured gammaproteobacterium clone JL-ETNP-R11 (AY726784)**	**99**
			***Alteromonas alvinellae* (AB121782)**	**99**
+Glucose and phosphate	GP 2 (C 0, P 0, P 2)	Mes16	Marine arctic deep-sea bacterium HD9 (AJ557871)	98
			Agrobacterium gelatinovorum (D88523)	96
	GP 4	Mes23	CVSP bacterium CV919-312 (AF114484)	98
			Phycococcus omphalius (AB193438)	97
	GP 6	M602*	Uncultured alphaproteobacterium (AJ633943)	99
			Roseobacter gallaeciensis (RG16SRR)	98
	GP 6	M605*	*Thalassobius mediterraneus* (AJ878874)	99
			Agrobacterium gelatinovorum (D88523)	97
	GP 8	Mes28	*Roseobacter* sp. JL-126 (AY745859)	98
			Alphaproteobacterium MBIC1887 (AB026492)	98
	GP 8	Mes48	*Roseobacter* sp. HYL-SA-18 (DQ008594)	98
			Agrobacterium gelatinovorum (D88523)	97
	GP 8	M606*	*Roseobacter* sp. JL-126 (AY745859)	99
			Ruegeria atlantica (AF124521)	99
	GP 8	M618*	Uncultured *Roseobacter* sp. clone JL-ECS-X3 (AY663966)	97
			Roseobacter gallaeciensis (AY136134)	97

Genotypes were identified either by sequencing or by comparison with position of sequenced bands in the DGGE (reported in brackets). Isolates are indicated by asterisks. C, control; G, +glucose; P, +phosphate; GP, +glucose and phosphate.

apparently maintain high levels of ribosomes during extended periods of non-growth, which would allow these bacteria to rapidly initiate growth (Fig. 2) at changing environmental conditions (Eilers *et al.*, 2000b; Pernthaler *et al.*, 2001). This would also match the short estimated doubling times of this particular population during the first 24 h of the incubations, which varied between 7 (+P and +GP) and 12 h (control). Alternatively, the addition of ammonium to all treatments (to avoid N limitation, see *Experimental procedures*) might have also influenced community composition. High ammonium concentrations significantly favored the growth of colony-forming *Gammaproteobacteria* from North Sea waters (Eilers *et al.*, 2001).
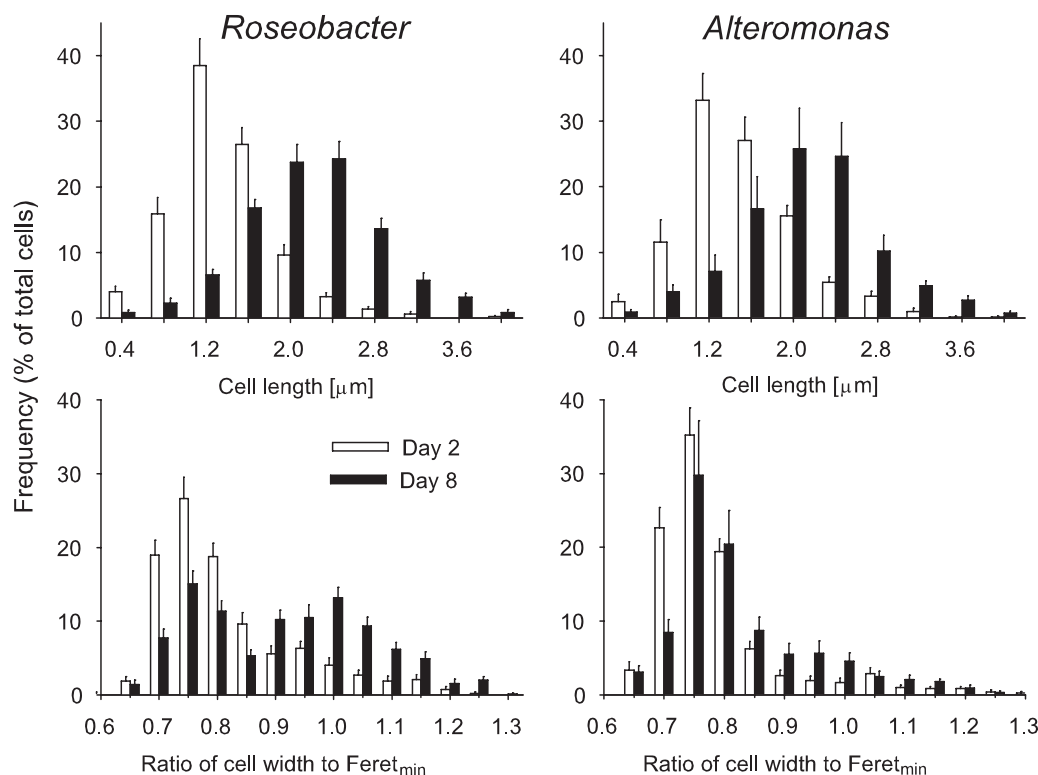
Fig. 6. Distributions of cell size and shape of *Rhodobacteriaceae* (left) and *Alteromonadaceae* (right) in the glucose- and phosphate-amended (+GP) treatment on days 2 (open bars) and 8 (closed bars). Top panels: cell length. Bottom panels: cell shape, expressed as the ratios of cell width to the minimum Feret dimension. Note the presence of two distinct morphotypes in *Rhodobacteriaceae* on day 8.

During the second half of the experiment the relative contribution of *Alteromonadaceae* to the microbial assemblages disproportionally declined in all treatments, concomitant with a rise of HNF numbers (Figs 1 and 3). The time delay between the two blooms suggests that the bacterial population was negatively affected by selective flagellate grazing (Pernthaler, 2005). A similar succession of *Alteromonadaceae* and HNF was observed during bottle incubations of North Sea water (Beardsley *et al.*, 2003). Our results thus support the conclusion that *Alteromonadaceae* are rare in coastal surface waters because their rapid growth response to changing environmental conditions is counterbalanced by high predation mortality. In addition, *Alteromonadaceae* might have been selectively reduced by viruses. As such a viral 'killing the winner' scenario (Thingstad and Lignell, 1997) is based on host specificity, it would require a low genotypic diversity within the *Alteromonadaceae*. The results of cultivation support this assumption: only four different genotypes affiliated with this lineage could be retrieved at various time points, and three of those isolates were phylogenetically closely related (identity, 93–99%, Table 2). As *Alteromonadaceae* are known to be readily culturable (Pinhassi and Berman, 2003) it is likely that the diversity of these bacteria was indeed low. On the other hand, the total abundances of viral particles changed little in mesocosms without P addition (M. Weinbauer, unpubl. data), suggesting that protistan predation probably represented the dominant mortality source for *Alteromonadaceae* in these treatments.

An initial stimulation by confinement followed by top-down control alone cannot provide an exhaustive explanation for the observed dynamics of the *Alteromonadaceae*. For example, the abundances of HNF declined in all treatments towards the end of the experiment (Fig. 1), probably due to predation by omnivorous, oligotrichous ciliates (J. Masmitjà and D. Vaqué, unpubl. data). However, there was no corresponding second bloom of *Alteromonadaceae* (Fig. 3), hinting at the presence of other competitors during the decline of the phytoplankton bloom, e.g. members of the *Bacteroidetes* (Riemann *et al.*, 2000; Pinhassi *et al.*, 2004). Moreover, the *Alteromonadaceae* maintained relative abundances of approximately 20% of total bacterial counts in the +G and the +GP treatments during the second half of the study, whereas they decreased to 10% or less in +P and in the controls (Fig. 3). This significant difference between treatments with and without glucose (days 5–8: Mann–Whitney *U*-test, $n = 16$, $P < 0.001$) indicates that the presence of an additional source of simple carbon positively influenced the persistence of *Alteromonadaceae* in

the respective microbial assemblages. Thingstad and colleagues (2005) suggested that some bacteria might utilize excess carbon to increase cell size in order to obtain full or partial resistance against HNF predation. Members of the *Alteromonadaceae* indeed formed significantly larger cells at the end of the experiment (Fig. 6). It is, however, unclear if a mean cell length of 2–3 μm (Fig. 6) would be sufficient to substantially reduce grazing mortality. Microscopic inspection showed that some of the dominant flagellate predators themselves were between 1.5 and 3 μm in size (K. Simek, unpubl. obs.), and thus were probably not able to feed on such large cells. Alternatively, glucose might have provided additional metabolic energy to compensate for high losses by higher growth rates than other bacterial groups. *Alteromonas* and related genera represented a large fraction of colony-forming units in glucose-amended dilution cultures of Mediterranean Sea water (Pinhassi and Berman, 2003).

The ubiquity of *Alteromonadaceae* and closely related genera in coastal marine habitats (Acinas *et al.*, 1999; Dang and Lovell, 2000; Garcia-Martinez *et al.*, 2002; Yoon *et al.*, 2003), their typical rarity in bacterioplankton communities (Eilers *et al.*, 2000b; Beardsley *et al.*, 2003; Alonso-Sáez *et al.*, 2007) and their characteristic response upon changes in growth conditions (Fig. 3) raises the question whether these bacteria might qualify as indicators of disturbance events in coastal surface waters. It is, for example, conceivable that blooms of these bacteria might be related to point sources of allochthonous organic carbon. In this context, it is intriguing that a pronounced bloom of bacteria related to *Alteromonadaceae* (> 30% of total cells) was observed in July 2003 at the site of our sampling (Alonso-Sáez *et al.*, 2007).

Temporal dynamics of Rhodobacteriaceae

The physiologically highly diverse *Rhodobacteriaceae* are one of the major groups of marine bacteria, comprising up to 20% of coastal and 15% of mixed-layer ocean bacterioplankton communities (Buchan *et al.*, 2005 and references therein). Between late autumn and spring (November to April) these bacteria constituted approximately 5% of total cells at our sampling site, whereas they were relatively rare during summer (Alonso-Sáez *et al.*, 2007).

In contrast to the development of the *Alteromonadaceae* the relative contribution of *Rhodobacteriaceae* rose slowly, but these bacteria continuously gained importance over a period of several days (Fig. 3). During the second half of the investigation period, *Rhodobacteriaceae* transiently became the dominant bacteria in treatments amended with P. On the one hand, this conspicuous proliferation of *Rhodobacteriaceae* after P addition might have been induced directly by the nutrient.

Low P concentrations can limit bacterioplankton growth in the Northwestern Mediterranean Sea during large parts of the year (Pinhassi *et al.*, 2006). Moreover, some *Rhodobacteriaceae* contain bacteriochlorophyll *a* (Shiba, 1991; Buchan *et al.*, 2005). It is thus conceivable that these bacteria might be able to increase growth at sufficient concentrations of P irrespective of DOC levels.

However, entirely phototrophic growth of these bacteria so far has not been shown, and is unlikely in view of recent genomic information (Swingley *et al.*, 2007). Therefore, it is likely that *Rhodobacteriaceae* in the +P and +GP mesocosms benefited from the more pronounced blooms of phytoplankton (Fig. 1) rather than directly from the nutrient. This is suggested by the positive correlation between Chl *a* concentrations and the community contribution of *Rhodobacteriaceae* in these treatments (Fig. 4), which was moreover much tighter in the absence of an extra carbon source. *Rhodobacteriaceae* are typically abundant in bacterial communities that are associated with algae, including both natural (Eilers *et al.*, 2001; Zubkov *et al.*, 2001) and induced phytoplankton blooms (Pinhassi *et al.*, 2004). During such periods these bacteria may increase in abundance by up to 2.5 times (Moran *et al.*, 2003 and references therein). Some *Rhodobacteriaceae* can moreover prosper on phytoplankton-derived DOC, e.g. glucose (Alonso and Pernthaler, 2006) or the algal osmolyte dimethylsulfoniopropionate (Zubkov *et al.*, 2001; Vila *et al.*, 2004).

The high (morphological and genotypic) diversity of *Rhodobacteriaceae* in the experimental mesocosms might be another indication of the dependence of these bacteria on the phytoplankton (Table 2; Figs 5 and 6). Algal-derived DOM from exudates and cell lysis products consists of a variety of readily degradable substrates, such as mono- and polysaccharides, amino acids and proteins, etc. (Myklestad, 2000), potentially providing different niches for coexisting strains. In addition, the taxonomic composition of the phytoplankton assemblages differed in the various experimental treatments (P. Gasol, unpubl. obs.), and various algal species might have favoured specific bacterial genotypes (Schäfer *et al.*, 2002; Pinhassi *et al.*, 2004). A succession of ecophysiologically specialized genotypes of *Rhodobacteriaceae* is suggested by community fingerprinting (Fig. 5): during the first 48 h of the experiment the communities of *Rhodobacteriaceae* were highly similar in the +P and +GP treatments (Fig. 5), whereas they significantly differed at the end of the incubations. This implies that there were one or more genotypes from this family that acted as primary colonizers in both treatment types (Mes15, Mes16 and Mes49, Table 2). In contrast, the genotypes that dominated the respective assemblages during the later phase of the study apparently differed, for example, in their preference for high glucose concentrations.

A second aspect that might have led to the apparent success of *Rhodobacteriaceae* in the P-amended mesocosms could be a lower than average grazing rate on these bacteria at low to medium densities of the predators. This is suggested by the non-linear relationship between the community contribution of *Rhodobacteriaceae* and HNF abundances in the +P and +GP treatments (Fig. 4): at low HNF densities (i.e. before and after the HNF bloom) the fraction of *Rhodobacteriaceae* tended to increase with flagellate numbers, whereas the opposite was true at HNF densities of 1.5×10^4 ml^{-1} or above. A smaller effect of HNF on *Rhodobacteriaceae* than on *Alteromonadaceae* (at protistan densities $< 10^4$ ml^{-1}) has also been observed during short-term bottle incubations (Beardsley *et al.*, 2003).

Conclusions

One might envisage heterotrophic picoplankton communities in coastal waters as being composed of rather stable populations and others with rapid temporal fluctuations. The former would include bacterial taxa that are adapted to constantly low substrate concentrations, e.g. members of the SAR 11 clade (Morris *et al.*, 2002; Alonso and Pernthaler, 2006). The more variable components appear to fall into different categories, two of which were analysed in our study: (i) phylogenetic lineages such as the *Alteromonadaceae* harbour bacteria that respond to irregular disturbances by external events and to allochthonous DOC input, but do not maintain large population sizes in the plankton, and (ii) bacteria from lineages such as the *Rhodobacteriaceae* are related to more predictable autochthonous events, specifically phytoplankton blooms (Eilers *et al.*, 2001; Zubkov *et al.*, 2001). Obviously, mesocosms are extremely simplified systems that moreover greatly exaggerate the instable aspects of picoplankton assemblages. Nevertheless, our experimental incubations allowed distinguishing between the specific niches of two groups of planktonic marine bacteria that both can rapidly respond to a changing environment.

Experimental procedures

Experimental set-up and sampling

Water was collected from 1 m depth at the Blanes Bay Microbial Observatory, approximately 1 km off the Port of Blanes, Catalunya (Western Mediterranean Sea 41°40′N, 2°48′E) on 19 October 2004. Eight transparent rectangular polyethylene tanks were each filled with 200 l. These mesocosms were maintained for a period of 8 days at *in situ* temperature and in a 12:12 h light : dark cycle. All mesocosms were amended daily with 2 µM NH$_4$ (NH$_4$Cl) in order to prevent N limitation. In addition, two mesocosms were amended with 50 nM of PO$_4$ (KH$_2$PO$_4$) (treatment designation: +P), two mesocosms with

13.25 µM of glucose (treatment designation: +G), and two with both glucose and PO$_4$ at the same concentrations as in the separate additions (treatment designation: +GP). One set of two mesocosms was left untreated (treatment designation: control). All mesocosms were mixed twice a day by hand-held stirring, and samples were taken once every day.

Chlorophyll a concentrations

Chlorophyll *a* concentrations were determined after Parsons and colleagues (1984). Subsamples of 150 ml were filtered through glass fibre filters (GF/F, Whatman) and subsequently extracted in 90% acetone overnight at 4°C in the dark. Fluorescence was measured with a Turner Designs fluorometer.

Bacterial production, bacterial and flagellate cell numbers

Bacterial bulk growth activity during the enrichments was estimated from the incorporation rate of tritiated (^{3}H) leucine (Leu) as described by Kirchman and Ducklow (1993). Samples were incubated with 40 nM of ^{3}H Leu in microcentrifuge tubes in the dark for 1 h at ambient temperatures (Smith and Azam, 1992; Gasol and Morán, 1999). Trichloroacetic acid-killed samples were used as controls. Bacterial heterotrophic production was calculated as Leu incorporation rate times the standard 3.1 kg C mol Leucine^{-1} conversion factor (Gasol *et al.*, 2002).

Total bacterial cell numbers were counted with a Becton Dickinson FACScalibur benchtop flow cytometer (BD Bioscience, Franklin Lakes, NJ, USA). Daily samples from each mesocosm were processed as previously described (Gasol and del Giorgio, 2000). Portions of 1 ml were fixed with 1% buffered paraformaldehyde solution (PFA, pH 7.0) plus 0.05% glutaraldehyde, incubated for 10 min at room temperature and then stored in liquid nitrogen. For total bacterial cell counts, 200 µl of these subsamples were stained with a DMSO-diluted SybrGreen I stock solution (10:1; Molecular Probes, Eugene, OR, USA) at a final concentration of 2.5 µM. The staining was carried out for 10 min in the dark. For flow cytometric analysis 10 µl of a solution of yellow-green latex beads (size, 1 µm; final concentration, 10^6 ml^{-1}; Polyscience, Washington, PA, USA) was added to each sample as an internal standard. Bacterial cell numbers were determined from the ratios of cells to beads after Gasol and del Giorgio (2000).

For the enumeration of non-pigmented HNF, subsamples (100 ml) were preserved according to Simek and colleagues (1995). Portions of 5 ml from these samples were stained with 4′,6-diamidino-2′-phenylindol (DAPI, final concentration, 1 µg ml^{-1}) and passed through 0.8 µm Poretics polycarbonate filters (GE Osmonics, Minnetonka, MN, USA) by gentle vacuum filtration. Non-pigmented nanoflagellates were enumerated via epifluorescence microscopy. All samples were analysed within 24 h after preservation.

Temporal dynamics of Alteromonadaceae and Rhodobacteriaceae

Subsamples (50 ml) were fixed with 1% PFA for 24 h at 4°C for analysis of the bacterial community by CARD-FISH

(Pernthaler *et al.*, 2004). Subsamples were filtered onto white membrane filters (GTTP, 0.2 μm pore size, 47 mm in diameter, Millipore, Eschborn, Germany), and bacterial cells were fixed onto the filter by embedding the surface into 0.2% agarose (MetaPhor, Cambrex Bio Science, Rockland, USA). Before hybridization, cells were permeabilized by treatment with lysozyme and proteinase K as described previously (Teira *et al.*, 2004). Proteinase K concentration was adjusted to 0.075 μl ml^{-1} (2129 U mg^{-1}, 9.5 mg ml^{-1}, Fluka) and samples were incubated for 15 min. Filter sections were hybridized with horseradish peroxidase (HRP)-labelled oligonucleotide probes (Biomers, Ulm, Germany) as previously described (Pernthaler *et al.*, 2004). Probes were targeting members of the *Bacteria* (EUB I–III; Daims *et al.*, 1999), several genera within the *Rhodobacteriaceae* (ROS537; Eilers *et al.*, 2001), and bacteria affiliated to *Alteromonadaceae* and *Colwelliaceae* (ALT1413, genera *Alteromonas*, *Colwellia*, *Glaciecola*; Eilers *et al.*, 2000a). Stringent hybridization conditions were achieved by 55% Formamide (FA) in the hybridization buffer for EUB I–III and ROS537, and 60% FA for ALT1413 respectively. After 2.5 h of hybridization the probe-delivered HRP was detected with tyramides (diluted 1:700 in amplification buffer) that were custom labelled with fluorescein (Molecular Probes, Eugene, USA). The preparations were subsequently embedded onto glass slides in a previously described mountant mix containing 1 μg ml^{-1} DAPI (Pernthaler *et al.*, 2004). The fractions of CARD-FISH-stained cells of all DAPI-stained objects were determined by epifluorescence microscopy and semi-automated image analysis (Pernthaler *et al.*, 2003). Negative controls were routinely determined with the EUB I–III antisense probe NON338 (Wallner *et al.*, 1993).

Determination of cell morphology

Images of cells hybridized with probes ROS537 and ALT1413 in the +GP treatment at days 2 and 8 were acquired at blue excitation (CARD-FISH staining) from randomly selected filter areas with a ZEISS Axio Imager 1.1 equipped with a VDS COOL-1300Q digital camera (Vosskühler, Osnabrück, Germany) and image analysis software (LUCIA, G, Laboratory Imaging, Prague, Czech Republic). Morphological parameters (area, perimeter, length, width, Feret dimensions) of > 500 cells were determined in 10–25 images per preparation by previously described procedures (Posch *et al.*, 1997).

Extraction of microbial community DNA

Samples of microbial community DNA for subsequent analyses by DGGE, cloning and 16S rRNA gene sequencing were collected at the beginning of the experiment (day 0) and on every second day until day 8. Microbial biomass from approximately 700 ml of sample was collected onto 0.2 μm-pore-size polycarbonate filters (diameter, 47 mm, Durapore, Millipore). Filters were stored frozen at −70°C in sucrose buffer (0.75 M sucrose, 40 mM EDTA, 50 mM Tris pH = 8.3). DNA was extracted using a combined treatment with enzymes (lysozyme, proteinase K) and phenol-chloroform as described by Riemann and colleagues (2000).

DNA was re-suspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and quantified fluorometrically (PicoGreen; Molecular Probes).

Diversity analysis

Bacterial 16S rDNA was amplified by PCR using a bacterial primer complementary to position 341–358 with a 40 bp GC-clamp (GC341F; Muyzer *et al.*, 1993) and a universal primer complementary to position 907–927 (907RM; Muyzer *et al.*, 1998). Initial denaturation was at 95°C for 2 min followed by a thermal cycling programme as follows: denaturation for 30 s at 94°C; annealing for 30 s at an initial 63°C, decreasing 1°C every two cycles to a final of 53°C; extension for 90 s at 72°C. Ten cycles were run at 53°C for a total of 30 cycles followed by final 7 min of incubation at 72°C. The quality and size of PCR products were verified by agarose gel electrophoresis.

Sixty nanograms of PCR product was analysed by DGGE using the D Gene System (Bio-Rad) at 60°C for 6 h at 150 V. DGGE bands were excised using a sterile razor blade and eluted in 20 μl of MilliQ water overnight at 4°C, followed by a freeze–thaw cycle. A total of 5 μl of the eluate was used for re-amplification with the original primer set. A part of the PCR product was analysed by DGGE together with the original sample to verify the correct position of the band, and in cases where more than one band was present the target band was processed again as described above. PCR products were purified with the QIAquick PCR-Purification Kit (Qiagen) and quantified fluorometrically (PicoGreen; Molecular Probes).

Sequencing reactions were carried out using the DYEnamic™ ET terminator cycle sequencing kit (Amersham Biosciences) and primer 907RM as described by the manufacturer. The obtained partial 16S rRNA gene sequences were compared with existing prokaryotic sequences in GenBank (NCBI) using BLAST (Altschul *et al.*, 1990). Isolates were obtained by plating samples from dilution to extinction cultures (most probable number approach) or by direct plating from the mesocosms onto Zobell agar plates. The isolates were pre-screened according to colony morphology, and representative strains were identified by 16S rRNA gene sequencing as previously described (Pinhassi and Berman, 2003). All sequences were submitted to GenBank and their accession numbers are provided in Table S1.

Statistical analyses

Digitized DGGE images were analysed with the Quantity-one software (Bio-Rad). The software allows identification of different bands and calculating the contribution of each band to total intensity in each lane. Cluster analysis of banding patterns based on Ward's method was used to obtain a dendrogram using Statistica 6.0 (StatSoft, Tulsa, OK, USA).

The relationship between the relative abundances of *Alteromonadaceae* and *Rhodobacteriaceae* with HNF abundances, Chl *a* concentration and bacterial production were tested separately for each treatment type by pairwise correlations (Spearman's rank sum correlations, r_S and/or Pearson product moment correlations, r^2). Data from the two replicates

were pooled. In order to compensate for the chance of producing spurious significance by multiple correlations the critical alpha level required for significance was adjusted to $P < 0.01$.

Treatment-specific differences in bacterial abundance and relative abundances of *Alteromonadaceae* and *Rhodobacteriaceae,* HNF abundances, Chl *a* concentration and bacterial production were established by one-way ANOVA. In order to account for the different data distributions the relative abundances of *Alteromonadaceae* and *Rhodobacteriaceae* were arcsine transformed prior to analysis, whereas the other variables were log transformed. Subsets of treatments that were statistically indistinguishable for a particular parameter were determined by *post hoc* pairwise comparisons (Scheffé method). Analyses were performed using the software SPSS (SPSS, Chicago, IL, USA)

References

Acinas, S.G., Antón, J., and Rodríguez-Valera, F. (1999) Diversity of free-living and attached bacteria in offshore Western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. *Appl Environ Microbiol* **65:** 514–522.

Alonso, C., and Pernthaler, J. (2006) *Roseobacter* and SAR11 dominate microbial glucose uptake in coastal North Sea waters. *Environ Microbiol* **8:** 2022–2030.

Alonso-Sáez, L., Balagué, V., Sà, E.L., Sánchez, O., González, J.M., Pinhassi, J., *et al.* (2007) Seasonality in bacterial diversity in NW Mediterranean coastal waters: assessment through clone libraries, fingerprinting and fluorescence in situ hybridization. *FEMS Microbiol Ecol* **60:** 98–112.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J Mol Biol* **215:** 403–410.

Azam, F., and Worden, A.Z. (2004) Oceanography: microbes, molecules, and marine ecosystems. *Science* **303:** 1622–1624.

Beardsley, C., Pernthaler, J., Wosniok, W., and Amann, R. (2003) Are readily cultured bacteria in coastal North Sea waters suppressed by selective grazing mortality? *Appl Environ Microbiol* **69:** 2624–2630.

Buchan, A., Gonzalez, J.M., and Moran, M.A. (2005) Overview of the marine *Roseobacter* lineage. *Appl Environ Microbiol* **71:** 5665–5677.

Daims, H., Bruhl, A., Amann, R., Schleifer, K.H., and Wagner, M. (1999) The domain-specific probe EUB338 is insufficient for the detection of all *Bacteria*: development and

evaluation of a more comprehensive probe set. *Syst Appl Microbiol* **22:** 434–444.

Dang, H., and Lovell, C.R. (2000) Bacterial primary colonization and early succession on surfaces in marine waters as determined by amplified rRNA gene restriction analysis and sequence analysis of 16S rRNA genes. *Appl Environ Microbiol* **66:** 467–475.

Eilers, H., Pernthaler, J., Glöckner, F.O., and Amann, R. (2000a) Culturability and *in situ* abundance of pelagic bacteria from the North Sea. *Appl Environ Microbiol* **66:** 3044–3051.

Eilers, H., Pernthaler, J., and Amann, R. (2000b) Succession of pelagic marine bacteria during enrichment: a close look on cultivation-induced shifts. *Appl Environ Microbiol* **66:** 4634–4640.

Eilers, H., Pernthaler, J., Peplies, J., Glöckner, F.O., Gerdts, G., and Amann, R. (2001) Isolation of novel pelagic bacteria from the German Bight and their seasonal contribution to surface picoplankton. *Appl Environ Microbiol* **67:** 5134–5142.

Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M.V., and Naeem, S. (2006) Annually reoccurring bacterial communities are predictable from ocean conditions. *Proc Natl Acad Sci USA* **103:** 13104–13109.

Garcia-Martinez, J., Acinas, S.G., Massana, R., and Rodriguez-Valera, F. (2002) Prevalence and microdiversity of *Alteromonas macleodii*-like microorganisms in different oceanic regions. *Environ Microbiol* **4:** 42–50.

Gasol, J.M., and del Giorgio, P.A. (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Scientia Marina* **64:** 197–224.

Gasol, J.M., and Morán, X.A.G. (1999) Effects of filtration on bacterial activity and picoplankton community structure as assessed by flow cytometry. *Aquat Microb Ecol* **16:** 251–264.

Gasol, J.M., Comerma, M., Garcia, J.C., Armengol, J., Casamayor, E.O., Kojecka, P., and Simek, K. (2002) A transplant experiment to identify the factors controlling bacterial abundance, activity, production, and community composition in a eutrophic canyon-shaped reservoir. *Limnol Oceanogr* **47:** 62–77.

Grossart, H.P., Levold, F., Allgaier, M., Simon, M., and Brinkhoff, T. (2005) Marine diatom species harbour distinct bacterial communities. *Environ Microbiol* **7:** 860–873.

Kepkay, P.E. (1994) Particle aggregation and the biological reactivity of colloids. *Mar Ecol Prog Ser* **109:** 293–304.

Kirchman, D.L., and Ducklow, H.W. (1993) Estimating conversion factors for the thymidine and leucine methods for measuring bacterial production. In *Handbook of Methods in Aquatic Microbial Ecology*. Kemp, P.F., Sherr, B.F., Sherr, E.B., and Cole, J.J. (eds). Boca Raton, FL, USA: Lewis Publishers, pp. 513–517.

Kirchman, D.L., Dittel, A.I., Malmstrom, R.R., and Cottrell, M.T. (2005) Biogeography of major bacterial groups in the Delaware Estuary. *Limnol Oceanogr* **50:** 1697–1706.

Lebaron, P., Servais, P., Trousellier, M., Courties, C., Vives-Rego, J., Muyzer, G., *et al.* (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. *Aquat Microb Ecol* **19:** 225–267.

Moran, M.A., González, J.M., and Kiene, R.P. (2003) Linking

a bacterial taxon to sulfur cycling in the sea: studies of the marine *Roseobacter* group. *Geomicrobiol J* **20:** 375–388.

Morris, R.M., Rappe, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, C.A., and Giovannoni, S.J. (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420:** 806–810.

Muyzer, G., de Waal, E.C., and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59:** 695–700.

Muyzer, G., Brinkhoff, T., Nübel, U., Santegoeds, C., Schäfer, H., and Wawer, C. (1998) Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In *Molecular Microbial Ecology Manual.* Akkermans, A.D.L., Van Elsas, J.D., and De Bruijn, F.J. (eds). Dordrecht, the Netherlands: Kluwer Academic Publishers, pp. 1–27.

Myklestad, S.M. (2000) Dissolved organic carbon from phytoplankton. In *Mar Chem.* Wangersky, P. (ed.). Berlin, Germany: Springer Verlag, pp. 111–148.

Parsons, T.R., Maita, Y., and Lalli, C.M. (1984) Fluorometric determination of chlorophylls. In *A Manual of Chemical and Biological Methods for Seawater Analysis.* Oxford, UK: Pergamon Press, pp. 107–109.

Pernthaler, J. (2005) Predation on prokaryotes in the water column and its ecological implications. *Nat Rev Microbiol* **3:** 537–546.

Pernthaler, A., Pernthaler, J., Eilers, H., and Amann, R. (2001) Growth patterns of two marine isolates: adaptations to substrate patchiness? *Appl Environ Microbiol* **67:** 4077–4083.

Pernthaler, J., Pernthaler, A., and Amann, R. (2003) Automated enumeration of groups of marine picoplankton after fluorescence *in situ* hybridization. *Appl Environ Microbiol* **69:** 2631–2637.

Pernthaler, A., Pernthaler, J., and Amann, R. (2004) Sensitive multicolour fluorescence *in situ* hybridization for the identification of environmental organisms. In *Molecular Microbial Ecology Manual*, 2nd edn. Kowalchuk, G.A., De Bruijn, F.J., Head, I.M., Akkermans, A.D.L., and van Elsas, J.D. (eds). Dordecht, the Netherlands: Kluwer Academic Publishers, pp. 711–726.

Piccini, C., Conde, D., Alonso, C., Sommaruga, R., and Pernthaler, J. (2006) Blooms of single bacterial species in a coastal lagoon of the southwestern Atlantic Ocean. *Appl Environ Microbiol* **72:** 6560–6568.

Pinhassi, J., and Berman, T. (2003) Differential growth response of colony-forming alpha- and gamma-proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. *Appl Environ Microbiol* **69:** 199–211.

Pinhassi, J., Sala, M.M., Havskum, H., Peters, F., Guadayol, O., Malits, A., and Marrase, C.L. (2004) Changes in bacterioplankton composition under different phytoplankton regimens. *Appl Environ Microbiol* **70:** 6753–6766.

Pinhassi, J., Gómez-Consarnau, L., Alonso-Sáez, L., Sala, M.M., Vidal, M., Pedrós-Alió, C., and Gasol, J.M. (2006) Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW Mediterranean Sea. *Aquat Microb Ecol* **44:** 241–252.

Posch, T., Pernthaler, J., Alfreider, A., and Psenner, R. (1997) Cell-specific respiratory activity of aquatic bacteria studied with the tetrazolium reduction method, cyto-clear slides and image analysis. *Appl Environ Microbiol* **63:** 867–873.

Pukall, R., Päuker, O., Buntefuß, D., Ulrichs, G., Lebaron, P., Bernard, L., *et al.* (1999) High sequence diversity of *Alteromonas macleodii*-related cloned and cellular 16S rDNAs from a Mediterranean seawater mesocosm experiment. *FEMS Microbiol Ecol* **28:** 335–344.

Revilla, M., Iriarte, A., Madariaga, I., and Orive, E. (2000) Bacterial and phytoplankton dynamics along a trophic gradient in a shallow temperate estuary. *Estuar Coast Shelf Sci* **50:** 297–313.

Riemann, L., Steward, G.F., and Azam, F. (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl Environ Microbiol* **66:** 578–587.

Schäfer, H., Servais, P., and Muyzer, G. (2000) Successional changes in the genetic diversity of a marine assemblage during confinement. *Arch Microbiol* **173:** 138–145.

Schäfer, H., Bernard, L., Courties, C., Lebaron, P., Servais, P., Pukall, R., *et al.* (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater. *FEMS Microbiol Ecol* **34:** 243–253.

Schäfer, H., Abbas, B., Witte, H., and Muyzer, G. (2002) Genetic diversity of 'satellite' bacteria present in cultures of marine diatoms. *FEMS Microbiol Ecol* **42:** 25–35.

Schauer, M., Balagué, V., Pedros-Alio, C., and Massana, R. (2003) Seasonal changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic system. *Aquat Microb Ecol* **31:** 163–174.

Schendel, E.K., Nordström, S.E., and Lavkulich, L.M. (2004) Floc and sediment properties and their environmental distribution from a marine fish farm. *Aquaculture Res* **35:** 483–493.

Shiba, T. (1991) Roseobacter litoralis gen-nov, sp-nov, and Roseobacter denitrificans sp-nov, aerobic pink pigmented bacteria which contain bacteriochlorophyll a. *Syst Appl Microbiol* **14:** 140–145.

Simek, K., Bobkova, J., Macek, M., Nedoma, J., and Psenner, R. (1995) Ciliate grazing on picoplankton in a eutrophic reservoir during the summer phytoplankton maximum: a study at the species and community level. *Limnol Oceanogr* **40:** 1077–1090.

Smith, D.C., and Azam, F. (1992) A simple, economical method for measuring bacterial protein synthesis rates in seawater using ^{3}H-leucine. *Mar Microbial Food Webs* **6:** 107–114.

Swingley, W.D., Sadekar, S., Mastrian, S.D., Matthies, H.J., Hao, J., Ramos, H., *et al.* (2007) The complete genome sequence of *Roseobacter denitrificans* reveals a mixotrophic rather than photosynthetic metabolism. *J Bacteriol* **189:** 683–690.

Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G.J. (2004) Combining catalyzed reporter deposition-fluorescence *in situ* hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean. *Appl Environ Microbiol* **70:** 4411–4414.

Thingstad, T.F. (2000) Elements of a theory for the mecha-

nisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnol Oceanogr* **45:** 1320–1328.

Thingstad, T.F., and Lignell, R. (1997) Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. *Aquat Microb Ecol* **13:** 19–27.

Thingstad, T.F., Ovreas, L., Egge, J.K., Lovdal, T., and Heldal, M. (2005) Use of non-limiting substrates to increase size; a generic strategy to simultaneously optimize uptake and minimize predation in pelagic osmotrophs? *Ecology Lett* **8:** 675–682.

Vila, M., Simo, R., Kiene, R.P., Pinhassi, J., González, J.A., Moran, M.A., and Pedros-Alio, C. (2004) Use of micro-autoradiography combined with fluorescence *in situ* hybridization to determine dimethylsulfoniopropionate incorporation by marine bacterioplankton taxa. *Appl Environ Microbiol* **70:** 4648–4657.

Wallner, G., Amann, R., and Beisker, W. (1993) Optimizing fluorescent *in situ*-hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry* **14:** 136–143.

Worden, A.Z., Seidel, M., Smriga, S., Wick, A., Malfatti, F., Bartlett, D., and Azam, F. (2006) Trophic regulation of *Vibrio cholerae* in coastal marine waters. *Environ Microbiol* **8:** 21–29.

Yoon, J.-H., Kim, I.-G., Kang, K.H., Oh, T.-K., and Park, Y.-H. (2003) *Alteromonas marina* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Bacteriol* **53:** 1625–1630.

Zubkov, M.V., Fuchs, B.M., Archer, S.D., Kiene, R.P., Amann, R., and Burkill, P.A. (2001) Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulfoniopropionate in an algal bloom in the North Sea. *Environ Microbiol* **3:** 304–311.

Supplementary material

The following supplementary material is available for this article online:
Table S1. Genotypes and isolates and their corresponding GenBank accession number.

This material is available as part of the online article from http://www.blackwell-synergy.com