Growth rates of different phylogenetic bacterioplankton groups in a coastal upwelling system

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Summary

Microbial degradation of dissolved organic matter (DOM) in planktonic ecosystems is carried out by diverse prokaryotic communities, whose growth rates and patterns of DOM utilization modulate carbon and nutrient biogeochemical cycles at local and global scales. Nine dilution experiments (September 2007 to June 2008) were conducted with surface water from the highly productive coastal upwelling system of the Ría de Vigo (NW Iberian Peninsula) to estimate bacterial growth rates of six relevant marine bacterial groups: *Roseobacter*, SAR11, Betaproteobacteria, Gammaproteobacteria, SAR86 and Bacteroidetes. Surprisingly, SAR11 dominated over the other bacterial groups in autumn, likely associated to the entry of nutrient-rich, DOC-poor Eastern North Atlantic Central Water (ENACW) into the embayment. *Roseobacter* and SAR11 showed significantly opposing growth characteristics. SAR11 consistently grows at low rates (range 0.19-0.71 d⁻¹), whilst *Roseobacter*, has a high growth potential (range 0.70-1.64 d⁻¹). By contrast, Betaproteobacteria, Bacteroidetes, SAR86 and Gammaproteobacteria growth rates widely varied among experiments. Regardless of such temporal variability, mean SAR86 growth rate (range 0.1-1.4 d⁻¹) was significantly lower than that of Gammaproteobacteria (range 0.3-2.1 d⁻¹). Whereas the relative abundance of different bacterial groups showed strong correlations with several environmental variables, group-specific bacterial growth rates did not covary with ambient conditions. Our results suggest that different bacterial groups exhibit characteristic growth rates, and, consequently, distinct competitive abilities to succeed under contrasting environmental conditions.

1 **Introduction**

2 Microbial degradation of dissolved organic matter (DOM) in planktonic ecosystems is
3 carried out by diverse prokaryotic communities, whose growth rates and patterns of DOM
4 utilization modulate carbon and nutrient biogeochemical cycles at local and global scales
5 (Kirchman, 2004; Mou *et al.*; 2007, Gasol *et al.*, 2008). The complexity of DOM
6 composition and the variable DOM supply rate are likely the most important factors
7 determining the high prokaryotic diversity in the oceans (Nagata, 2008).

8 The prokaryotic community in ocean surface waters is often dominated by a few
9 major bacterial groups: Alpha-, Beta-, and Gammaproteobacteria, and the group
10 Bacteroidetes (Giovannoni and Rappé, 2000; Giovannoni and Stingl, 2005). Growing
11 evidence supports the view that members of these major phylogenetic groups could
12 actually represent functionally relevant units in terms of bacterial carbon cycling (Cotrell
13 and Kirchman, 2000; Elifantz *et al.*, 2005; Alonso-Sáez and Gasol, 2007; Teira *et al.*,
14 2008). On the other hand, within the Alphaproteobacteria, the groups SAR11 and
15 *Roseobacter* show contrasting i) spatio-temporal patterns, the latter dominating under
16 meso-or eutrophic conditions (Selje *et al.*, 2004; Wagner-Dobler and Biebl, 2006;
17 Fuhrman and Hagstrom, 2008); ii) DOM uptake patterns (Alonso and Pernthaler, 2006;
18 Alonso-Saez and Gasol, 2007); and iii) susceptibility to grazing and viral lysis (Suttle,
19 2007). Similarly, the SAR86 clade, which has not yet been cultivated, does not appear to
20 share the potential for explosively rapid growth of many other marine cultivable
21 Gammaproteobacteria such as *Alteromonas* and *Vibrio* (Eilers *et al.*, 2000).

22 The different ecological traits shown by distinct bacterial types, even belonging to
23 the same broad phylogenetic group, indicate that different groups of bacteria may greatly
24 differ in the key factors controlling their growth rates (Fuhrman and Hagstrom, 2008;

Gasol *et al.*, 2008). However, information about the environmental variables regulating the growth of individual bacterial groups under in situ conditions is scarce (Jürgens *et al.*, 1999; Yokokawa and Nagata, 2005, Simek *et al.*, 2006). Knowledge of the growth rates of potentially functional bacterial groups in relation to critical environmental biotic and abiotic factors is essential to achieve a reasonable understanding of bacterial regulation of carbon fluxes and for modeling bacterial dynamics in marine systems.

The availability of readily assimilated DOM has been found to most frequently limit bacterial growth in marine planktonic systems (Church, 2008). Bacterial grazing appears to be in balance with bacterial production, which explains the apparent relatively stable bacterial biomass found across systems, regardless of the high variability in growth and productivity (Jürgens and Massana, 2008). Contrary to what happen in freshwater systems, grazing mortality rates seem to vary little among different marine bacterial groups (Suzuki, 1999; Yokokawa and Nagata, 2005), although there have not been as many studies on this issue in marine compared to freshwater systems.

The coastal upwelling area of the Ría de Vigo (NW Iberian Peninsula) produces and processes large amounts of DOM (Álvarez-Salgado *et al.*, 2001). Lønborg *et al.* (2009) showed that DOM bioavailability in this highly productive system is coupled to seasonal variations in plankton biomass and activity. Teira *et al.* (2008) found a close link between the bacterial community composition, several environmental factors and the bacterial carbon cycling.

Within this context, we conducted nine dilution experiments to estimate growth rates of bacteria belonging to six phylogenetic groups (*Roseobacter*, SAR11, Betaproteobacteria, Gammaproteobacteria, SAR86 and Bacteroidetes) under contrasting hydrographic conditions in the Ría de Vigo. Then, the relative abundance and the growth

rates of the different groups were related with environmental factors (temperature, salinity, chlorophyll-a, inorganic nutrient concentrations), DOM optical properties and DOC concentration, in order to explore the linkage between bacterial community composition, growth rates, and environmental conditions in this highly dynamic ecosystem.

Results and discussion

Nine dilution experiments were conducted with surface water (5 m depth) from a fixed station in middle segment of the the Ría de Vigo (42°14.09' N, 8°47.18' W) from September 2007 to June 2008. The bacterial inoculum was obtained by filtering seawater through a precombusted GF/C filter (nominal pore size 1.2 µm) and incubated 0.9-diluted with 0.2-µm-filtered seawater from the same location and time. Incubations were done in the dark at 15°C. The abundance of *Roseobacter*, SAR11, Betaproteobacteria, Gammaproteobacteria, SAR86 and Bacteroidetes was estimated using CARD-FISH at day 0, 1, 2 and 4.

Initial environmental conditions

The hydrographic conditions at 5 meters depth during this study (Table 1S) were within the natural ranges of variability found in long-term studies conducted in the Ría de Vigo (Fig. 1a) (Nogueira *et al.*, 1997). During the autumn 2007 surveys, the Ekman transport (Table 1S) indicated an initial strong upwelling event. During this period, chlorophyll concentration kept constant at around 3 mg m⁻³, dissolved organic carbon (DOC) decreased from 93.8 to 74.8 µmol C L⁻¹ and, alongside, dissolved inorganic nitrogen (DIN) increased from 3 to 13 µmol L⁻¹ (Table 1S). During the winter 2008 surveys,

chlorophyll-a levels were $<1.5 \text{ mg m}^{-3}$ and DIN concentrations maintained above $8 \text{ } \mu\text{mol L}^{-1}$. Salinity reached its lowest level on 24-Apr-2008, when the high N/P molar ratio of the nutrients is indicative of continental inputs (Gago *et al.*, 2005). During the summer experiments, high chlorophyll and DOC levels and low inorganic nutrient concentrations were found.

Initial bacterial community composition

The hydrography and dynamics of the Ría de Vigo has been shown to affect biogeochemical cycling, phytoplankton size-structure and composition (Tilstone *et al.*, 1999; Álvarez-Salgado *et al.*, 2001; Cermeño *et al.*, 2006), and bacterial community composition (Alonso-Gutiérrez *et al.*, 2009).

The percentage of DAPI-counts (referred to as prokaryotic abundance, PA) detected by the mix of Eub338 probes (referred to as bacterial abundance, BA) was generally high (74-100%), except in winter (53-60%). The sum of the relative abundance of the six considered bacterial groups accounted, on average, for $86 \pm 7\%$ of the total BA. Temporal variability was relatively high for all groups (Fig. 1b), with variation coefficients in relative abundance ranging from 42% for *Roseobacter* to $>84\%$ for SAR11, SAR86, and Gammaproteobacteria. Previous studies in coastal waters have shown pronounced temporal changes in the abundance of *Roseobacter* and SAR11 (Schauer *et al.*, 2003; Mary *et al.*, 2006; Fuhrman *et al.*, 2006) and also of major phylogenetic groups of bacteria (Gammaproteobacteria or Bacteroidetes) (Alderkamp *et al.*, 2006; Alonso-Sáez *et al.*, 2007; Garcés *et al.*, 2007, Teira *et al.*, 2008).

During autumn, the bacterial community was initially dominated by Bacteroidetes (47% of DAPI-counts), but rapidly shifted to a community increasingly dominated by

SAR11 (up to 38% of DAPI-counts on 4-Oct-2007). In winter, the community was dominated by Bacteroidetes, except at the beginning of the period when SAR11 and Bacteroidetes co-dominated. The dominance of SAR11 over the other bacterial groups observed in autumn is striking in this highly productive ecosystem. In a previous work, the relative abundance of SAR11 in the Ría de Vigo never exceeded 5% of DAPI-counts (Teira *et al.*, 2008). SAR11 tends to dominate under oligotrophic conditions (Morris *et al.*, 2005; Alonso-Sáez *et al.*, 2007) being less important in meso-/eutrophic environments (Henriques *et al.*, 2004; Mary *et al.*, 2006). Given the highly productive nature of the Ria the Vigo, we hypothesize that the surprisingly high contribution of SAR11 observed in autumn and winter must be related to the entry of nutrient-rich, DOC-poor Eastern North Atlantic Central Water (ENACW) into the Ría de Vigo associated to upwelling-downwelling dynamics.

The spring bacterial community was clearly dominated by Bacteroidetes (40-60% of DAPI-counts), and in summer, *Roseobacter* (17% of DAPI-counts), SAR11 (16% of DAPI-counts) and CFB (22% of DAPI-counts) similarly contributed to the bacterial community. The dominance of the Bacteroidetes group has been repeatedly associated to phytoplankton blooms (Riemann *et al.*, 2000; Alderkamp *et al.*, 2006; Teira *et al.*, 2008). A relatively higher abundance of *Roseobacter* in spring and summer was also observed by Mary *et al.* (2006) in the western English Channel and agrees with a previous work in the Ría de Vigo (Teira *et al.*, 2008).

The groups Gammaproteobacteria and SAR86 showed a similar seasonal trend being relatively less abundant in winter and on 26-Jun-08, when a strong upwelling event occurred (Fig. 1b, Table 1S). Using the current rRNA database (SILVA) we found that the vast majority (98%) of sequences detected with SAR86/1245 are not detected with the

GAM42a, therefore we have separately considered both groups, despite SAR86 belongs to the Gammaproteobacteria subclass. The lower abundance of SAR86 in winter was previously found by Mary *et al.* (2006) in the western English Channel and by Alonso-Gutiérrez *et al.* (2009). We hypothesize that the abundance of this group in coastal marine ecosystems could be associated to the water column stability.

The group Betaproteobacteria showed very low relative abundances throughout the sampling period (0-3% DAPI-counts), which is in agreement with the reported dominance of this group in freshwater systems (Methé *et al.*, 1998).

Bacterial community dynamics during the course of the dilution experiments

The 90% dilution of the bacterial community in 0.2- μ m-filtered seawater promoted bacterial growth during the first two days. Then, bacterial abundance stabilized or decreased (Fig. 2). Maximum bacterial abundance ($> 20 \times 10^5$ cells mL⁻¹) was reached on 24-Apr-2008 and 26-Jun-2008. The percentage of DAPI-counts detected by the Eub338 probe mix (EUB) increased over 24 hours, reaching in all the experiments 90-100%.

Fuchs *et al.* (2000) pointed to systematic changes in bacterial community composition associated to the dilution culture method, leading to a dominance of Gammaproteobacteria, preferentially responding to an artificial enrichment of substrate. Therefore, they argued that dilution experiments do not measure in situ growth. We did not observe such a systematic preferential increase of a given group (Fig. 3). Moreover, the group *Roseobacter* is the one showing the largest increments in relative abundance (from 5-10% to 20-40%), but only in 4 out of 9 experiments (one in autumn and three in winter). Gammaproteobacteria reached a maximum relative abundance of 36% only in 1 out of 9 experiments. Only the groups *Roseobacter* and SAR11 showed significant

differences between the initial and final relative abundances during the incubations (T-test, $p = 0.021$ and $p = 0.044$, respectively). Yokokawa *et al.* (2004) also did not find such systematic changes in bacterial community composition during similar dilution experiments in both fresh and estuarine waters. Furthermore, these authors found similar production rates with the dilution and the thymidine (TdR) method, and did not find significant changes in average cell volume of bacterial cells, suggesting that physiological changes were minimal during the incubations.

We suggest that a careful manipulation of samples (gentle filtration, use of gloves and acid-ultra-clean and pre-combusted material), will minimize artificial enrichment and, therefore, the differences in net growth rates of different bacterial groups among sampling periods will reflect changes in the environmental conditions.

Growth rates of phylogenetic bacterial groups

Using the time-course measurements of the absolute abundance of each phylogenetic group (cells mL⁻¹), we estimated the growth rates of six single bacterial groups during the 9 dilution experiments (Fig. 4). Each growth rate represented in figure 4 derives from the slope of the corresponding regression of $\ln N$ vs. time, where N is the abundance of the corresponding group, for the time interval during which exponential growth was observed (0-2 or 0-4 days). Correlation coefficients ranged from 0.8 to 1. Given the low sample size ($n=3-4$), a power analysis was conducted using the GPower 3.1.0 software (Faul *et al.*, 2007). We computed the adequate significance level for each slope which balances the likelihood of type I and type II errors. The derived significance levels ranged from 0.004 to 0.195, therefore the power of the statistical analysis remained always >0.8 . All the regression slopes were significant except two cases for the group SAR11 and 3 cases for the group SAR86. Although grazing was eliminated in our experiments, we consider

1 these growth estimates as net growth rates (NGR) because viruses can pass through the
2 GF/C filter, and thus we can not rule out bacterial mortality due to viral lysis during the
3 incubation.

4 Relatively few studies have estimated in situ group-specific bacterial growth rates
5 in marine (Yokokawa *et al.*, 2004; Yokokawa and Nagata, 2005) and freshwater
6 environments (Jürgens *et al.* 1999, Simek *et al.* 2006) using time-course experiments.
7 Some other works provide data that also allow such estimations for some marine bacterial
8 groups (Fuchs *et al.*, 2000; Eilers *et al.*, 2000; Allers *et al.*, 2007). In situ group-specific
9 bacterial growth rates have been also estimated combining microautoradiography and
10 FISH techniques (Malmstrom et al 2005).

11 The seasonal and annual mean NGR for each individual bacterial group, for the
12 whole bacterial community (Eub338-positive cells) and for the whole prokaryotic
13 community (DAPI counts) are summarized in Table 1. On an annual basis, bulk
14 bacterioplankton growth rate was 0.82 d^{-1} , rendering a turnover time of 29 hours, which is
15 within the range of previously reported values for marine ecosystems ($0.4\text{-}3.5 \text{ d}^{-1}$, see
16 review by Yokokawa *et al.*, 2004). We only found significant differences between
17 bacterial and prokaryotic growth rates in winter (paired t-test, $p=0.005$) likely due to the
18 presence of inactive bacteria, not detectable by CARD-FISH, or Archaea in the initial
19 samples. Unfortunately we do not have estimates of archaeal abundance in the Ría de
20 Vigo. A low detectability of bacteria could have affected the estimates of specific
21 bacterial groups during winter. However, the changes in the percentage of Eub338-
22 positive during the first 24 h were much smaller (about 1.6-fold) than the corresponding
23 changes in the percentages of *Roseobacter* (range 4-12-fold) and Gammaproteobacteria
24 (4-15-fold). A similar discrepancy was observed by Yokokawa *et al.* (2004), who

1 concluded that relatively small changes in bacterial detectability must not seriously affect
2 NGR estimates of individual bacterial groups.

3 A certain degree of temporal variability in NGR was observed for the different
4 bacterial groups (Fig. 4), except for *Roseobacter* and SAR11 which showed a rather
5 constant growth rate (variation coefficient <30%). The highest NGR were measured in
6 autumn and summer for the groups Bacteroidetes ($>1.19\text{ d}^{-1}$) and SAR86 ($>0.97\text{ d}^{-1}$),
7 whereas the highest NGR were measured in winter and spring for the group
8 Betaproteobacteria ($>1.1\text{ d}^{-1}$). The lowest NGR estimates occur in autumn or spring for
9 most of the groups. Yokokawa *et al.* (2004) also found a great temporal and spatial
10 variability in the growth rates of different bacterial groups, which they speculated to
11 derive from changes in dissolved organic matter concentration and composition. Changes
12 in the species and strains of each phylogenetic group could also explain the observed
13 temporal variability in NGR. Undoubtedly, we would need periodical measurements over
14 several years in the same sampling point to identify consistent seasonal patterns in the
15 growth of distinct bacterial phylogenetic groups.

16 Regardless the observed temporal variability, NGR were statistically different
17 among groups (ANOVA F-test, $p=0.005$). The NGR of *Roseobacter* was significantly
18 higher than the NGR of SAR11 and Betaproteobacteria (Tukey test, $p<0.05$); and the
19 NGR of Gammaproteobacteria was significantly higher than the NGR of SAR11 and
20 SAR86 (Tukey test, $p<0.05$).

21 Contrasting growth rates were found for *Roseobacter* and SAR11, both belonging
22 to the Alphaproteobacteria subclass. The mean growth rate obtained for *Roseobacter*
23 (1.15 d^{-1}) is within the range obtained by Allers *et al.* (2007) using the same probe (0.69
24 to 1.25 d^{-1}) and compares well with Alphaproteobacteria growth rates measured in coastal

1 waters (1.2 to 5.5 d^{-1}) (Yokokawa *et al.*, 2004; Yokokawa and Nagata, 2005). On the
2 other hand, previously reported growth rates for Alphaproteobacteria in oceanic waters
3 (0.5 - 1.0 d^{-1}) agree better with the mean growth rate of the group SAR11 (0.51 d^{-1}).
4 Interestingly, the SAR11 growth rates measured in the eutrophic system of the Ría de
5 Vigo, agree very well with the rates derived from cultures (0.4 - 0.58 d^{-1}) (Rappé *et al.*,
6 2002) or with the in situ growth rates obtained by Malstrom *et al.* (2005) in the northwest
7 Atlantic Ocean (0.13 - 0.72 d^{-1}). Hamasaki *et al.* (2007) hypothesized that SAR11 could be
8 growing at extremely low rates in coastal waters, as they did not respond well to
9 bromodeoxyuridine. By contrast, our results and previous observations suggest, that
10 SAR11 populations exhibit a characteristic growth rate over a wide range of marine
11 ecosystems.

12 The group Gammaproteobacteria showed the highest mean NGR (1.30 d^{-1}),
13 implying mean turnover times of 18 h, which agrees with previous observations reporting
14 growth rates for this group ranging from 1.1 to 4.6 d^{-1} (Fuchs *et al.*, 2000; Eilers *et al.*,
15 2000; Yokokawa *et al.*, 2004; Yokokawa and Nagata, 2005). The maximum growth rate
16 of Gammaproteobacteria (2.1 d^{-1}), however, was considerably lower than those derived
17 from the studies by Fuchs *et al.* (2000) which may be related to differences in the
18 environmental conditions or to minimized artificial enrichment in our experiments.
19 Betaproteobacteria (0.77 d^{-1}) and Bacteroidetes (0.88 d^{-1}) showed similar mean NGR
20 (equivalent to a turnover time of 31 and 27 h, respectively), also in agreement with
21 previous observations (Jürgens *et al.*, 1999; Simek *et al.*, 2006; Yokokawa *et al.*, 2004;
22 Yokokawa and Nagata, 2005).

23 The group SAR86, showed a similarly low growth rate (0.61 d^{-1}) than the group
24 SAR11. Unfortunately, this group has not been yet cultivated; however, the calculated

mean growth rate agrees with that obtained in Eilers *et al.* (2000) (0.54 d^{-1}) in undiluted, grazing-free seawater samples.

Relationship between bacterial abundance and growth rates and, environmental variables

The correlation between the relative abundance and NGR of the different bacterial groups (dependent response variables, Y) and the environmental data (independent predictor variables, X) was explored using partial least square (PLS) regression (Abdi, 2007). This statistical tool combines features from principal component analysis and multiple regression. It can simultaneously handle two sets of collinear variables maximizing the covariance between datasets (X and Y). The two sets of variables showed a relatively good correlation ($r=0.93$). The loadings plot (Fig. 5) depicts the covariance of the variables, showing distinct clustering among the variables. The first two principal components explained 70% of the variance observed in the environmental data and 34% of the variance in bacterial abundance and growth rates.

The groups Roseobacter, Bacteroidetes and Gammaproteobacteria showed strong positive correlations with the fluorescence of protein-like dissolved organic matter (FDOM-T), indicative of labile substrates, total bacterial abundance, the concentration of dissolved organic carbon (DOC) and temperature; and strong negative correlations with the concentration of inorganic nutrients (nitrate and phosphate). On the other hand, SAR11 showed a positive correlation with ammonium and negative with DOC concentration, FDOM-T, and bacterial abundance, which confirm the tendency of this group to thrive in oligotrophic waters. Betaproteobacteria correlated positively with the fluorescence of humic-like dissolved organic matter (FDOM-M), indicative of refractory

1 substrates, and negatively with salinity; in accordance with Teira *et al.* (2009), who
2 linked the occurrence of this group in marine environments to freshwater influence.

3 By contrast, NGR of major phylogenetic groups did not correlated with any of the
4 considered environmental variables, although, there is an overall negative correlation
5 between the relative abundance of each bacterial group and its corresponding NGR. It is
6 very likely that changes in DOC composition, not reflected by the rather coarse
7 fluorescence indicators, are controlling the growth rates of distinct bacterial groups.

8 *Ecological implications*

9 *Roseobacter* and SAR11 groups showed a rather limited variability in their growth rates,
10 which could be related to the ability of using a wide variety of substrates that are supplied
11 at a relatively constant rate. Both *Roseobacter* and SAR11 have been found to use a wide
12 variety of low molecular weight substrates such as sugars, amino acids, or dimethylated
13 sulfur compounds (Moran *et al.*, 2003, Malmstrom *et al.*, 2004a; 2004b; 2005; Alonso
14 and Pernthaler, 2006; del Giorgio and Gasol, 2008). These compounds are continuously
15 supplied during phytoplankton growth in marine surface waters. SAR11 comprise a
16 rather high and stable fraction of the bacterial community under oligotrophic conditions
17 (about 33%), whereas *Roseobacter* accounts for about 20% of communities in coastal
18 waters (Fuhrman and Hagström, 2008). Such differences between these two groups with
19 similar substrate requirements might be partially explained by differential substrate
20 uptake patterns. It has been demonstrated that low ambient levels of substrates represent a
21 competitive advantage for SAR11 bacteria (Alonso and Pernthaler, 2006), which would
22 grow at low rates at the expenses of having a high affinity for substrates.

1 When a given population specializes in the use of a limited range of distinct
2 substrates, which are intermittently supplied, a certain degree of variability in growth
3 rates can be expected. Such temporal variability was observed for the groups
4 Betaproteobacteria, Gammaproteobacteria, SAR86 and Bacteroidetes. Bacteroidetes have
5 been repeatedly reported to be specialized in the use of polymeric organic substances
6 (chitin, protein) (Kirchman, 2002; Pinhassi *et al.*, 2004; Teira *et al.*, 2008). The group
7 Gammaproteobacteria, consistently shows the lowest percentages of active cells
8 incorporating different common substrates, including glucose, amino acids or protein (del
9 Giorgio and Gasol, 2008), which might be indicating that this broad phylogenetic group
10 includes a great diversity of specialized bacteria.

11 Mou *et al.* (2007) examined 46 marine bacterial genomes, including Alpha- and
12 Gammaproteobacteria and Bacteroidetes, for evidence of glycine betaine and vanillic acid
13 degradation pathways. The genetic ability to metabolize both compounds was present in
14 78% of the genomes belonging to the *Roseobacter* clade and also in *Pelagibacter ubique*
15 (SAR11). By contrast, none of the Gammaproteobacteria or Bacteroidetes genomes
16 showed the ability to degrade both compounds, and about 50% lacked the ability to
17 degrade any of the model compounds.

18 Both the Gammaproteobacteria and the Bacteroidetes groups showed high growth
19 rates. The temporal variability observed in their growth rates agrees with the observed
20 variability in their relative abundances. Although both groups are susceptible to predation
21 and viral lysis, they can temporarily escape predation by increasing their cell size, and
22 therefore, they can boom and bust and sporadically account for a significant fraction of
23 the total bacterial biomass (Pinhassi *et al.*, 2004, Teira *et al.*, 2008).

1 In summary, we have found statistically significant differences in the mean
2 growth rates of several relevant bacterial groups, which strongly supports the idea that
3 members of these phylogenetic groups might actually share physiological traits. Their
4 growth characteristics aid in explaining their global ecological significance and both
5 temporal and spatial distribution patterns. The reported differential growth rates provide
6 valuable information that can be incorporated into predictive models of bacterial
7 mediated carbon fluxes.

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1 Table 1. Mean ($\pm$ SE) seasonal and annual net growth rates (d^{-1}) for the individual
2 bacterial groups, for all bacteria and for all prokaryotes. N, number of growth estimates.

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	Autumn	Winter	Spring	Summer	All
	N=2-3	N=1-3	N=1-2	N=1	N=6-9
Roseobacter (N=9)	0.95 $\pm$ 0.17	1.45 $\pm$ 0.12	1.03 $\pm$ 0.33	1.09	1.15 $\pm$ 0.11
SAR11(N=7)	0.46 $\pm$ 0.15	0.59	0.54 $\pm$ 0.02	0.53	0.51 $\pm$ 0.06
Betaproteobacteria (N=9)	0.45 $\pm$ 0.09	1.06 $\pm$ 0.17	0.91 $\pm$ 0.56	0.57	0.77 $\pm$ 0.14
Bacteroidetes (N=9)	0.91 $\pm$ 0.23	0.83 $\pm$ 0.20	0.75 $\pm$ 0.41	1.19	0.88 $\pm$ 0.12
SAR86 (N=6)	0.51 $\pm$ 0.45	0.51 $\pm$ 0.23	0.22	1.42	0.61 $\pm$ 0.21
Gammaproteobacteria (N=9)	0.98 $\pm$ 0.25	1.72 $\pm$ 0.25	0.86 $\pm$ 0.58	1.82	1.30 $\pm$ 0.20
Bacteria (N=9)	0.73 $\pm$ 0.03	0.93 $\pm$ 0.07	0.49 $\pm$ 0.38	0.93	0.82 $\pm$ 0.08
Prokaryotes (N=9)	0.66 $\pm$ 0.01	0.67 $\pm$ 0.06	0.49 $\pm$ 0.38	0.88	0.70 $\pm$ 0.07

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Figure legends.

Figure 1. (a) Seasonal cycle of nitrate (thick lines) and chlorophyll-a (thin lines) concentration in the Ría de Vigo. Data represent mean (± 1 standard deviation) values for the period 1987-1996 (modified from Nogueira *et al.* 1997). (b) Initial composition of the bacterial assemblage during the different sampling periods. The relative abundance of each group (ROS, *Roseobacter*; SAR11; BETA, Betaproteobacteria; CFB, Bacteroidetes; SAR86; GAMMA, Gammaproteobacteria) is expressed as percentage of total DAPI-stained cells. The abundance of each group was estimated using CARD-FISH as described in Teira *et al.* (2008) using the following oligonucleotide probes: Ros537, for ROS (Eilers *et al.*, 2001); SAR11-441R, for SAR11 (Morris *et al.*, 2002); BET42a, for BETA (Manz *et al.*, 1992), CF319a, for the class Flavobacteria of phylum Bacteroidetes (CFB) (Manz *et al.*, 1996); SAR86/1245, for SAR86 (Zubkov *et al.*, 2001); and GAM42a, for GAMMA (Manz *et al.*, 1992).

Figure 2. Time course of prokaryotic abundance (PA) (cells mL⁻¹) (DAPI-stained cells) and percentage of prokaryotic cells detected with the mix of Eubacteria probes (EUB338, EUB338II, EUB338III) (Daims *et al.*, 1999) during the nine dilution experiments, grouped by seasons (autumn, winter, spring and summer). The error bars represent the standard error from two replicates.

Figure 3. Time course of the relative abundance of ROS, *Roseobacter*; SAR11; BETA, Betaproteobacteria; CFB, Bacteroidetes; SAR86; Gamma, Gammaproteobacteria expressed as percentage of total DAPI-stained cells during the nine dilution experiments, grouped by seasons (autumn, winter, spring and summer). The error bars represent the standard error from two replicates.

Figure 4. Net growth rates (in d^{-1}) of ROS, *Roseobacter*; SAR11; BETA, Betaproteobacteria; CFB, Bacteroidetes; SAR86; Gamma, Gammaproteobacteria derived from the slope of the regression $\ln$ (abundance) vs time, during the nine dilution experiments. Error bars represent the standard error of the slope in the regression model.

Figure 5. Loadings plot for the predictor variables (environmental variables) and dependent variables (relative abundance and growth rates of different bacterial groups) in the PLS regression model. ROS, *Roseobacter*; SAR11; BETA, Betaproteobacteria; CFB, Bacteroidetes; SAR86; GAMMA, Gammaproteobacteria. gROS, gSAR11, gBETA, gCFB, gSAR86, gGAMMA represent the corresponding net growth rates. Temp, temperature; Sal, salinity; DOC, dissolved organic carbon concentration;; FDOM-T, protein-like dissolved organic matter fluorescence; FDOM-M, humic-like dissolved organic matter fluorescence; Chl-a, chlorophyll-a concentration, BA, bacterial abundance, PA, prokaryotic abundance.. The fluorescence of dissolved organic matter was measured in a Perkin Elmer LS 55 luminescence spectrophotometer. Excitation/emission (Ex/Em) measurements were performed at peaks T (aromatic amino acids, average Ex/Em, 280/350 nm; termed FDOMt) and M (marine humic substances, average Ex/Em 320/410 nm; termed FDOMm), obtained from Nieto-Cid *et al.* (2006).

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5