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Effects of grazing, phosphorus and light on the growth rates of major bacterioplankton taxa in the coastal NW Mediterranean

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Summary

Estimation of growth rates is crucial to understand the ecological role of prokaryotes and their contribution to marine biogeochemical cycling. However, there are only a few estimates for individual taxa. Two top-down (grazing) and bottom-up (phosphorus (P) availability) manipulation experiments were conducted under different light regimes in the NW Mediterranean Sea. Growth rate of different phylogenetic groups, including the Bacteroidetes, *Rhodobacteraceae*, SAR11, Gammaproteobacteria and its subgroups *Alteromonadaceae* and the NOR5/OM60 clade, were estimated from changes in cell numbers. Maximal growth rates were achieved in the P-amended treatments but when comparing values between treatments (response ratios), the response to predation removal was in general larger than to P-amendment. The *Alteromonadaceae* displayed the highest rates in both experiments followed by the *Rhodobacteraceae*, but all groups largely responded to filtration and P-amendment, even the SAR11 which presented low growth rates. Comparing light and dark treatments, growth rates were on average equal or higher in the dark than in the light for all groups, except for the *Rhodobacteraceae* and particularly the NOR5 clade, groups that contain photoheterotrophic species. These results are useful to evaluate the potential contributions of different bacterial types to biogeochemical processes under changing environmental conditions.

Introduction

The rate of growth is an important ecological trait characteristic of any living organism. In marine ecosystems, growth along with mortality rates will determine the structure of bacterioplankton communities, i.e. the dominant types of organisms and their relative proportions. Growth rates thus govern to a certain extent which taxa are abundant members of bacterioplankton and which belong to the so-called rare biosphere (Pedrós-Alió, 2012; Kirchman, 2016). Since bacterioplankton play major roles in ocean biogeochemical processes, including organic matter utilization, determining the growth rates of the bulk community as well as that of individual bacterioplankton species or groups is necessary to understand their contribution to energy and matter cycling.

Bulk bacterial growth rates for the total community represent the average of a wide spectrum of organisms, from cells that are almost inactive or dormant to cells growing very rapidly (Del Giorgio and Gasol, 2008; Campbell *et al.*, 2011; Kirchman, 2016). Bulk bacterial growth rates are in general low, ranging between 0.05 and 0.10 day^{-1} in oligotrophic regions of the oceans, which corresponds to one division every one or two weeks (Ducklow, 2000). Nevertheless, maximal growth rates of individual taxa can be much higher, equivalent to generation times of a few days or hours (Teira *et al.*, 2009; Ferrera *et al.*, 2011; Kirchman, 2016). Previous studies have shown that in addition to differences in activity between individual cells (Sintes and Herndl, 2006), the percentage of active cells can vary enormously within broad taxonomic groups (Kirchman, 2016) which may explain the enormous differences observed between bulk and taxon-specific growth rates estimates. Unfortunately, there are only a few estimates available for ecologically relevant individual taxa (see the recent review by Kirchman, 2016).

Growth rates can be estimated following a variety of procedures, being the production-per-unit-biomass

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approach the most common one. Monitoring cell abundance of different taxa with specific fluorescence *in situ* hybridization (FISH) probes in seawater cultures allows the estimation of their growth rates. In most studies, growth rates have been calculated either in dilution or in grazer-free experiments, a strategy that allows the estimation of the gross but not the net growth rates of individual groups. In a previous study, we used manipulation experiments to determine the net and gross growth rates of different bacterioplankton groups at the Blanes Bay Microbial Observatory (BBMO) (Northwestern Mediterranean) and examined the effects that top-down and bottom-up controls play in limiting growth (Ferrera *et al*., 2011). By using the dilution approach we were able to estimate the effect that overall resource availability has on determining the growth of different groups (Ferrera *et al*., 2011). It is well known that often the primary limiting resource in the Mediterranean is phosphorous (P) availability (Thingstad *et al*., 1998; 2005; Sala *et al*., 2002; Pinhassi *et al*., 2006). A recent report revealed heterogeneity in the activity of different bacterial taxa in response to P-amendments in the Mediterranean (Sebastián and Gasol, 2013), but the effects on their growth rates were not evaluated. Thus, to determine the role of phosphorous on the growth rates of distinct bacterial phylogenetic groups further experiments are required.

Another important environmental factor that can modulate growth is natural sunlight (see review by Ruiz-González *et al*., 2013). The sensitivity to ultraviolet radiation (UVR; 320-400 nm) of bacterioplankton has been thoroughly studied, but less attention has been paid to the effect of photosynthetically available radiation (PAR; 400–700 nm) on natural communities. Considering that marine photoheterotrophic bacteria are more the rule than the exception in nature (DeLong and Béjà, 2010), PAR needs to be considered a relevant environmental factor controlling the dynamics of heterotrophic bacteria when building carbon flow models (Gasol *et al*., 2008). Whereas SAR11 bacteria and some taxa within the Gammaproteobateria or the Bacteroidetes among others can use light through proteorhodopsins (PR) (Pinhassi *et al*., 2016), aerobic anoxygenic photrotrophic (AAP) bacteria can do so using bacteriochlorophyll *a* reaction centres (Koblížek, 2015). Current knowledge on the growth of photoheterotrophs indicates that while the most abundant PR bacteria (the SAR11 clade) grow slower than other taxa (Kirchman, 2016), AAP bacteria grow faster than average marine bacteria (Koblížek *et al*., 2007; Ferrera *et al*., 2011). Yet, the role that PAR light plays on the growth of different taxa under natural conditions is less clear. Ruiz-González *et al*. (2012) evaluated the short-term (hour) responses in activity of different phylogenetic groups to *in situ* PAR radiation levels in the BBMO and found that SAR11 was consistently inhibited by PAR exposure, whereas the *Rhodobacteraceae* and the gammaproteobacterium NOR5 clade (both including AAP species) were occasionally stimulated, but these experiments did not examined the role of light on their growth rates.

In order to investigate the role of top-down control and nutrient limitation, in particular that of phosphorous, in combination with the effect of light, i.e. PAR, we designed manipulation experiments and evaluated the growth rates of distinct bacterial phylogenetic groups. The results represent a progress in our understanding of the magnitude of growth of marine bacteria and the effects that top-down and bottom-up controls play under different light regimes.

Results and discussion

Two manipulation experiments were conducted in May 2010 (Experiment 1) and July 2011 (Experiment 2). In each experiment, Mediterranean coastal seawater from the BBMO (41°40′N, 2°48′E) was subjected to three different treatments both in continuous dark (D) and in PAR/dark cycles (L): (i) whole unfiltered seawater (controls KD and KL), (ii) seawater prefiltered through 1-μm filters in order to reduce grazers while keeping most bacteria (FD and FL), and (iii) seawater prefiltered through 1-μm filters with 0.2 μM phosphorus amendment (PD and PL). Seawater in each treatment was distributed in 2-liter Nalgene bottles and incubated in duplicate for 3 days in large water baths with circulating seawater. Light treatments were maintained under natural light conditions (approximately 15 h-9 h light-dark cycles) using a net that reduced irradiance to mimic light conditions at 3 m water depth. UVR was excluded using two layers of Ultraphan URUV Farblos to prevent from cell damage. Experiments were conducted in May and July to cover the period of the year when day length is longer, and thus potentially detect an effect of light, and at the same time use contrasting initial communities in terms of productivity, nutrient limitation and phytoplankton community structure (see Gasol *et al*., 2016).

Abundances of Eubacteria, and the groups Bacteroidetes, Gammaproteobacteria and its subgroups *Alteromonadaceae* and the NOR5/OM60 clade, as well as the alphaproteobacterial *Rhodobacteraceae* and SAR11, were determined by catalyzed reporter deposition (CARD)-FISH as previously described (Pernthaler *et al*., 2002) using the FISH probes reported in Ferrera *et al*. (2011). Growth rates were subsequently calculated based on the slope of time course measurement of cell abundances during the exponential growth phase. The effect of top-down and bottom-up processes was estimated by comparing the growth rates in the filtered and

Table 1. Physicochemical and biological parameters of the initial samples in both experiments.

	Experiment	
Variable[a]	1 (May 2010)	2 (July 2011)
Temp (°C)	15.2	22.4
Secchi depth (m)	8	16
Chlorophyll *a* (μg liter^{-1})	0.93	0.2
[PO_4^{3}] (μM)	0.091	0.076
[NH_4^{+}] (μM)	1.151	0.029
[NO_2^{-}] (μM)	0.140	0.010
[NO_3^{-}] (μM)	0.430	0.423
[Si] (μM)	1.561	0.404
Leucine incorporation rate (pM h^{-1})	21.7	51.9
Bacterial abundance (10^5 cells/ml)	9.8	7.6
%HNA prokaryotic cells	46.0	52.7

a. Water temperature was measured *in situ* with a CTD (conductivity, temperature, and depth) probe, and water transparency was assessed using a Secchi disk. PAR was measured with a multichannel filter radiometer (PUV-2500; Biospherical Instruments Inc.). The concentration of inorganic nutrients was determined spectrophotometrically with an Alliance Evolution II autoanalyzer according to standard procedures (Grasshoff *et al*., 1983). Chlorophyll *a* was measured from acetone extracts by fluorometry. Leucine incorporation rate was estimated by using the [^{3}H]leucine incorporation method (Kirchman *et al*., 1985), modified as described previously (Smith and Azam, 1992). Bacterial abundance was enumerated by epifluorence microscopy of 4', 6-diamidino-2-phenylindole (DAPI)-stained cells. Flow-cytometric analyses were performed on a FACS-Calibur (Becton–Dickinson) flow cytometer and discrimination of populations with high percentage of nucleic acid content (% HNA) was done as described previously (Gasol and del Giorgio, 2000).

P-amended treatments, and the light effects, by comparing light and dark treatments (Figs 1 and 2). Our estimations (response ratios), however, indicate only their relative effect particularly for top-down processes, since these comparisons suffer from some methodological limitations (see Ferrera *et al*., 2011). For example, in the predator-reduced treatments, the removal of particle-attached or large bacteria when removing predators by filtration could have introduced changes in the initial bacterial community structure. Nevertheless, at least in the percentage of CARD-FISH-counts for the enumerated bacterial groups, we did not detected significant changes between treatments at time 0 (ANOVA, $p > 0.05$).

Initial environmental conditions and bacterial community composition

The physicochemical and biological parameters of the original seawater samples are shown in Table 1. Chlorophyll *a* and inorganic nutrients concentrations, including the inorganic Nitrogen:Phosphorous (N:P) ratios, were higher in Experiment 1, while bacterial production was more than twofold higher in Experiment 2. The initial bacterial community was dominated in both cases by the SAR11 group, followed by Bacteroidetes and Gammaproteobacteria, while the *Rhodobacteraceae*, NOR5/OM60 clade, and *Alteromonadaceae* were present at lower abundances (Table 2). Overall, the contribution of each phylogenetic group at time 0 was similar between experiments, except for the SAR11 group, which accounted for a larger proportion of the total prokaryotic community in May (62% *vs*. 49%). These values are in agreement with previous studies from the same location (Alonso-Sáez *et al*., 2007; Ferrera *et al*., 2011).

Effect of treatments on bacterial heterotrophic production, cell abundance and %HNA of total prokaryotes

Bacterial heterotrophic production (measured as Leucine incorporation rates, LIR) displayed similar changes in both experiments, even though some differences were

Table 2. Average contribution to total bacterial abundance ± standard deviation of the different bacterioplankton groups represented as percentages of DAPI-positive cells in this work and in other studies at the BBMO.

Group	Exp 1[a] (May 2010)	Exp 2[a] (July 2011)	BBMO[b] (June 2009)	BBMO[b] (July 2009)	BBMO[c] (2003–2004)
Eubacteria	86 ± 5	61 ± 8	87 ± 7	72 ± 5	73 ± 10
Rhodobacteraceae	4 ± 0	2 ± 0	6 ± 2	4 ± 2	4 ± 2
SAR11	62 ± 4	49 ± 2	33 ± 5	45 ± 5	22 ± 9
Gammaproteobacteria	6 ± 2	9 ± 0	12 ± 3	10 ± 1	4 ± 2
Alteromonadaceae	1 ± 0	0.3 ± 0	2 ± 1	1 ± 1	1 ± 1
NOR5/OM60	2 ± 1	3 ± 0	3 ± 2	3 ± 1	2 ± 2
Bacteroidetes	14 ± 1	10 ± 1	17 ± 3	12 ± 1	11 ± 3

a. For bacterial abundance determination, CARD-FISH was performed as described by Pernthaler *et al*., (2002) using the following probes: a mixture of Eub338 I, II, and III for Eubacteria (Amann *et al*., 1990; Daims *et al*., 1999); CF319a (Amann *et al*., 1990) for Bacteroidetes; Gam42a (Amann *et al*., 1990) for Gammaproteobacteria; Alt1413 (Eilers *et al*., 2000) for Alteromonadaceae; NOR5-730 (Eilers *et al*., 2000) for NOR5/OM60; Ros537 (Eilers *et al*., 2000) for *Rhodobacteraceae*, and SAR11 411R (Morris *et al*., 2002) for SAR11. Counterstaining preparations were done with DAPI (final concentration 1 μg mL^{-1}). DAPI and CARD-FISH stained cells were counted by fully automated microscopy (Zeder and Pernthaler, 2009; Zeder *et al*., 2011) with a Zeiss Axio Imager.Z2M using the automated image analysis software ACMEtool Tool (www.technobiology.ch).

b. Data from Ferrera *et al.* (2011).

c. Data from Alonso- Sáez *et al*. (2007) (average data of 14 month sampling).

observed in the values measured *in situ* at the time of sampling (Supporting Information Fig. S1). Leucine uptake rates exhibited an immediate increase after filtration and when P was added under both light regimes. However, P-addition resulted in a much higher promotion of bacterial production in both experiments. After this initial increase, LIR underwent a steady decrease until the end of the treatment. Overall, no significant differences in LIR between dark and light controls were found; yet a small inhibition in LIR in the light exposed treatments was observed towards the end of the experiments as compared with the dark control, particularly in the P-amended treatments. This inhibition could be the result of prolonged exposure to light but, overall, PAR caused no significant inhibition of bacterial heterotrophic activity.

The manipulation of top-down and bottom-up factors produced an increase in total prokaryotic abundance. However, the magnitude of the increase differed between experiments. In Experiment 1, P-addition had a much stronger effect than only removal of predators whereas, in Experiment 2, filtration and P-amendment had similar effects having P-addition in consequence a weaker effect (Supporting Information Fig. S2) despite the initial P concentration *in situ* was similar. A possible explanation is that bacteria could have also been limited by nitrogen in Experiment 2, since its concentration was much lower than in Experiment 1 (Table 1) (N:P ratio was 6:1 in Experiment 2 as compared to 13:1 in Experiment 1). A strikingly similar trend was found regarding the percentage of cells with high nucleic acid content (HNA) (Supporting Information Fig. S3). Nucleic acid content can be used as a single cell-based proxy of cell activity. Various studies have associated in some way or another the HNA cells with the most active members of the bacterial community, those in the process of replication and thus having more DNA (Gasol *et al*., 1999; Bouvier *et al*., 2007). Yet, they also represent versatile bacteria with larger and more flexible genomes (Vila-Costa *et al*., 2012). In Experiment 1, this increase was particularly remarkable in the P-amended treatments, reaching values above 80% of HNA (Supporting Information Fig. S3). In contrast, the increase was similar for the filtrated (F) and P-amended treatments in Experiment 2. While the response in cell abundance and %HNA followed the same trend in Experiment 2, the response in bacterial production to P-addition was clearly higher than to only predator removal, as in Experiment 1. A possible limitation by N would imply that cells were not able to divide at a higher rate despite the reduction in P-limitation. In any case, the increase in the fraction of HNA cells in both experiments could also be related to the increase in the abundance of taxa containing cells with large genomes, like the members of the *Alteromonadaceae* (Ivars-Martinez *et al*., 2008) and *Rhodobacteraceae* (Newton *et al*., 2010), as seen by CARD-FISH enumeration (Supporting Information Fig. S2). In Briefly, bacterial heterotrophic production, cell abundance and %HNA of total prokaryotes increased in response to top-down and bottom-up manipulation by increasing, whereas they hardly responded to light exposure.

Effect of top-down and bottom-up manipulation treatments on growth rates

In addition to the abundance of the total prokaryotic community (estimated from DAPI counts), changes in abundance of Eubacteria and six distinct phylogenetic groups were monitored over time using CARD-FISH. In general, there was an increase in the abundance of all bacterioplankton groups tested during the first 2 (Experiment 1) or 3 days (Experiment 2) of incubation (Supporting Information Fig. S2) after the manipulation of top-down and bottom-up factors. Overall, the response to treatments was higher in Experiment 1 than in Experiment 2, which could be related to a potential N limitation as mentioned above. Nevertheless, the removal of predators led to a rapid increase of various taxa in both experiments, remarkably of the Gammaproteobacteria and its subgroups the *Alteromonadaceae* and the NOR5. The SAR11, the *Rhodobacteraceae* and the Bacteroidetes also increased in the first experiment. In general, growth in the P-amended was higher than in the filtration treatments, particularly in Experiment 1.

Changes in abundance were used to estimate the net growth rates and rates close to gross values for the different prokaryotic groups (Supporting Information Figs S4 and S5). Net growth rates correspond to measurements carried out in the control treatments (i.e. with predators). Overall, the values found here are within the range reported from the same location for all groups as shown in Table 3. We estimated higher values for the net growth rate of the total prokaryotic community in Experiment 1 [0.43 and 0.33 day^{-1} mean values for treatments KD and KL respectively] compared with Experiment 2 [0.24 (KD) and 0.18 day^{-1} (KL)]. The bacterial community, determined with the eubacterial probes, displayed lower growth rates than the total prokaryotic community in Experiment 1 (0.27 and 0.21 day^{-1} for KD and KL treatments respectively), but were similar to the whole community in Experiment 2 (0.24 and 0.19 day^{-1} for KD and KL). Concerning the net growth rates of the two alphaproteobacterial groups, as expected the *Rhodobacteraceae* grew much faster than SAR11 (Hamasaki *et al*., 2007; Teira *et al*., 2009), particularly in the second experiment. The maximal net growth rates corresponded to the Gammaproteobacteria

Table 3. Summary of minimal and maximal growth rates (day^{-1}) for the different bacterioplankton groups investigated in this study and in a previous work at the same location.

Probe	Exp 1[a] (May 2010)	Exp 2[a] (July 2011)	BBMO[b] (June 2009)	BBMO[b] (July 2009)
Eubacteria	0.2–1.5	0.2–0.7	0.7–1.7	0.4–1.6
Rhodobacteraceae	0.5–3.7	0.4–1.8	0.9–2.9	0.3–1.9
SAR11	0.5–1.5	0.1–0.5	0.1–1.8	0.8–1.5
Gammaproteobacteria	1–2.7	0.5–1.5	1–3.6	1–3.4
Alteromonadaceae	1–4.7	0.4–3.4	2.3–5.4	1.4–5.8
NOR5/OM60	0.6–2.3	0.2–1.3	1.7–2.8	1.3–2.9
Bacteroidetes	0.2–2.2	0.2–0.6	0.7–1.5	0.5–1.6

a. Growth rates of the bacterial groups were estimated based on the slopes of the cell abundance vs. time curves during the exponential growth phase in the first 24 and 36 h for experiments 1 and 2 respectively.

b. Data from Ferrera *et al*. (2011).

and its subgroups *Alteromonadaceae* and NOR5, and to the *Rhodobacteraceae* group in both experiments (Supporting Information Figs S4 and S5).

Manipulation experiments where grazing was reduced and P was added allowed the estimation of growth rates closer to gross values than in the control (i.e. with neither limitation by P nor by predators). However, we would have to account for losses associated to viral lysis to obtain actual gross growth rates (see Ferrera *et al*., 2011). Nevertheless, the ratio between filtered *vs*.

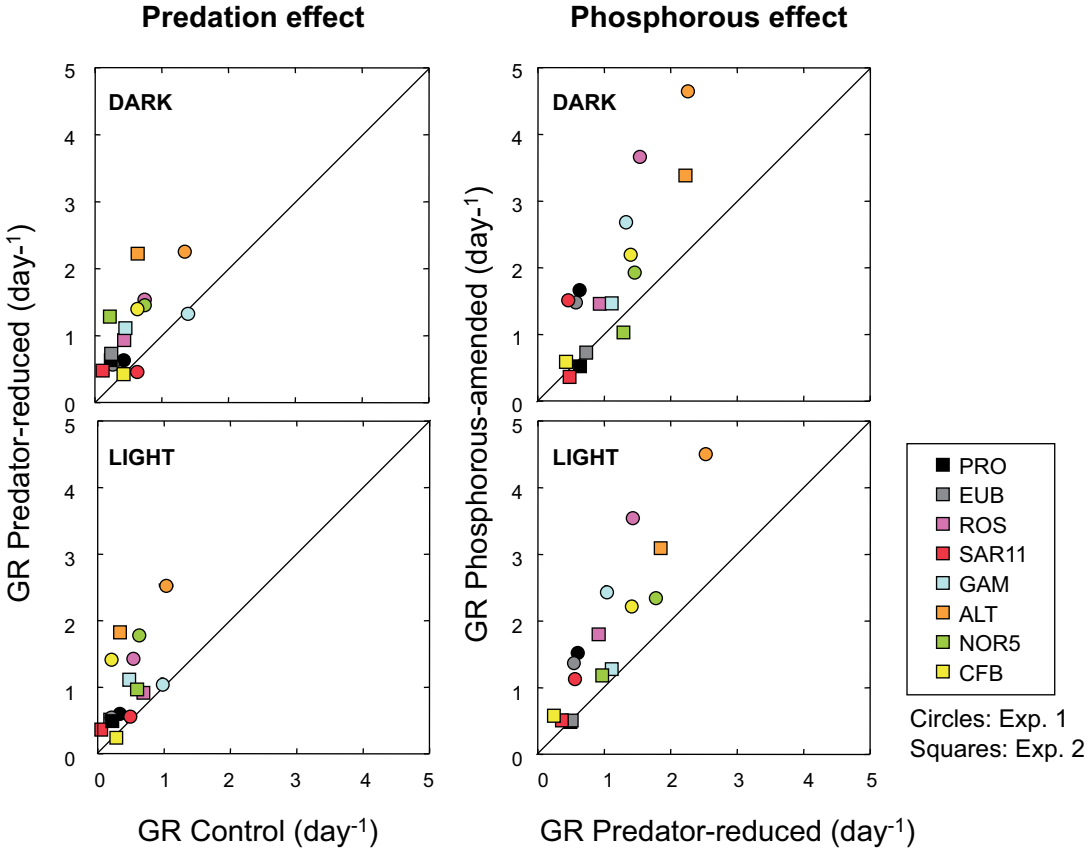

Fig. 1. Left panel: Mean growth rates of the different phylogenetic groups in Experiments 1 and 2 in the F (predator-reduced) *vs*. K (control) treatments. Right panel: growth rates of treatment P (predator-reduced and phosphorus-amended) *vs*. F treatments for the same groups in both experiments. Dots falling on the 1:1 line indicate that the growth rates have the same magnitude for both treatments; if dots are above or below the line, it indicates that the growth rates in the *y* treatments (F or P) are higher or lower than those in the *x* treatments (K or F) respectively. PRO, total prokaryotes as measured by DAPI staining; EUB, *Eubacteria*; ROS, *Rhodobacteraceae*; SAR11, SAR11 clade; GAM, Gammaproteobacteria; ALT, *Alteromonadaceae*; NOR5, NOR5/OM60 clade; CFB, Bacteroidetes.

control treatments provides insights on the relative effects of top-down control whereas comparison in growth rate in P-amended *vs*. filtered treatments indicates the relative effects of bottom-up control, in this case, of phosphorous. The response ratios indicate that the manipulation treatments resulted in increases in the growth rates of virtually all groups studied (Fig. 3), corroborating that both top-down and bottom-up factors interact in regulating population growth. Nevertheless, the magnitude of the increase differed among treatments and among the different groups (Fig. 3). Maximal growth rates were achieved in the P treatments, once grazers were reduced and P became a non-limiting nutrient. In particular, the *Alteromonadaceae* displayed the highest rates in both experiments, growing at up to 4.65 day^{-1} (Experiment 1, PD) and 3.38 day^{-1} (Experiment 2, PD), followed by the *Rhodobacteraceae*, which reached maximum values of 3.66 day^{-1} (Experiment 1, PD) and 1.80 day^{-1} (Experiment 2, PL) (Supporting Information Figs S4 and S5). However, when calculating the ratios between treatments, we observed that all groups largely responded to both filtration and P-amendment (Fig. 3) being the response to predation removal, in general, stronger than to P-amendment (Fig. 3). For example, in the case of the NOR5, the response to filtration was almost twofold that to P-addition. Noteworthy, the *Alteromonadaceae* showed the highest response to filtration and the *Rhodobacteraceae* to P-amendment but the SAR11, which presented the lowest growth rates, exhibited comparable responses to those groups displaying the highest growth rates.

In summary, we observed that, as seen in our previous study (Ferrera *et al*., 2011), abundance and growth rate are inversely correlated, being the groups that represented a lower percentage of the initial prokaryotic community those that exhibited the highest growth rates (Tables 2 and 3). Nevertheless, the experiments clearly show that, regardless of the magnitude of the growth, both bottom-up and top-down factors have an important role controlling the growth rates of all examined groups.

Effect of light on growth rates

Photoheterotrophic microbes are highly abundant in the oceans. These organisms capable of gaining energy from light are hypothesized to have an advantage over other heterotrophs. Evidences of faster growth with light than without it in laboratory experiments exist only for a few AAP and PR-containing isolates (Gómez-Consarnau *et al*., 2007; Kimura *et al*., 2011; Tomasch *et al*., 2011; Feng *et al*., 2013; Palovaara *et al*., 2014). However, the role that light plays on the growth of heterotrophic bacteria under natural conditions remains largely unknown (Fuhrman *et al*., 2008). Here, we compared the growth rates in light *vs*. dark treatments to obtain clues on their effect on the growth of different bacterial taxa, including those that contain photoheterotrophic members (Figs 2 and 3). The presence of PR-containing bacteria is expected since SAR11 and Bacteroidetes were abundant in all treatments and both taxa contain a large proportion of photoheterotrophic members (see review by Pinhassi *et al*., 2016). The relevance of AAP bacteria has been estimated in the BBMO in our previous

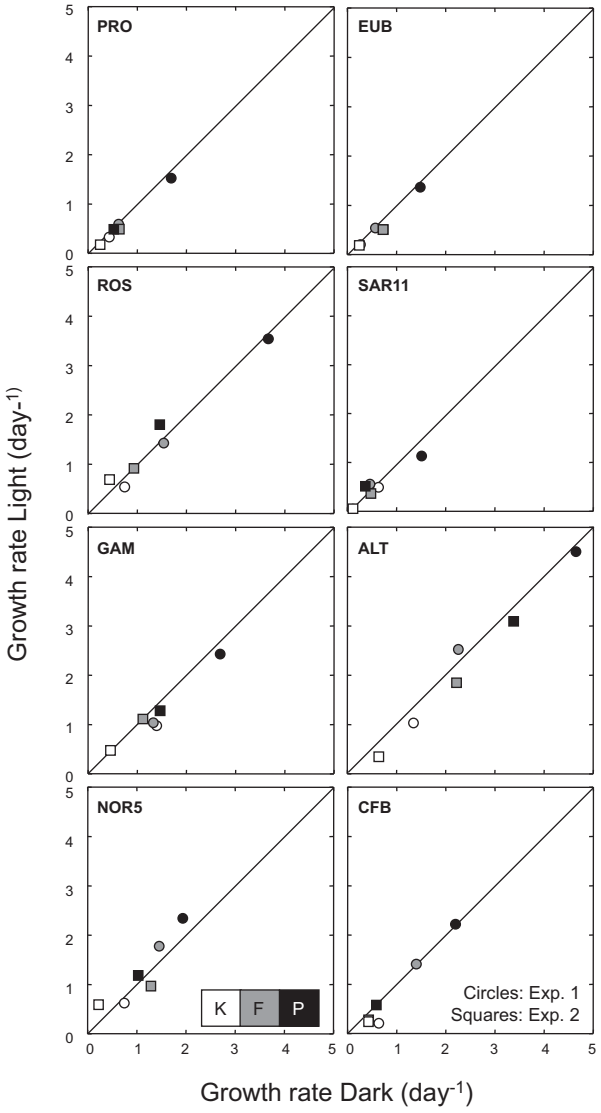

Fig. 2. Mean growth rates of the different phylogenetic bacterial groups in the treatments under light (PAR) *vs*. growth rates in the dark. Dots falling on the 1:1 line indicate that growth rates are equal under both light regimes; if the dots are above or below the line, it indicates that growth rates under PAR are higher or lower than growth rates in the dark respectively (K: control, F: predator-reduced, P: predator-reduced ± and phosphorus addition). PRO, total prokaryotes as measured by DAPI staining; EUB, *Eubacteria*; ROS, *Rhodobacteraceae*; SAR11, SAR11 clade; GAM, Gammaproteobacteria; ALT, *Alteromonadaceae*; NOR5, NOR5/OM60 clade; CFB, Bacteroidetes.

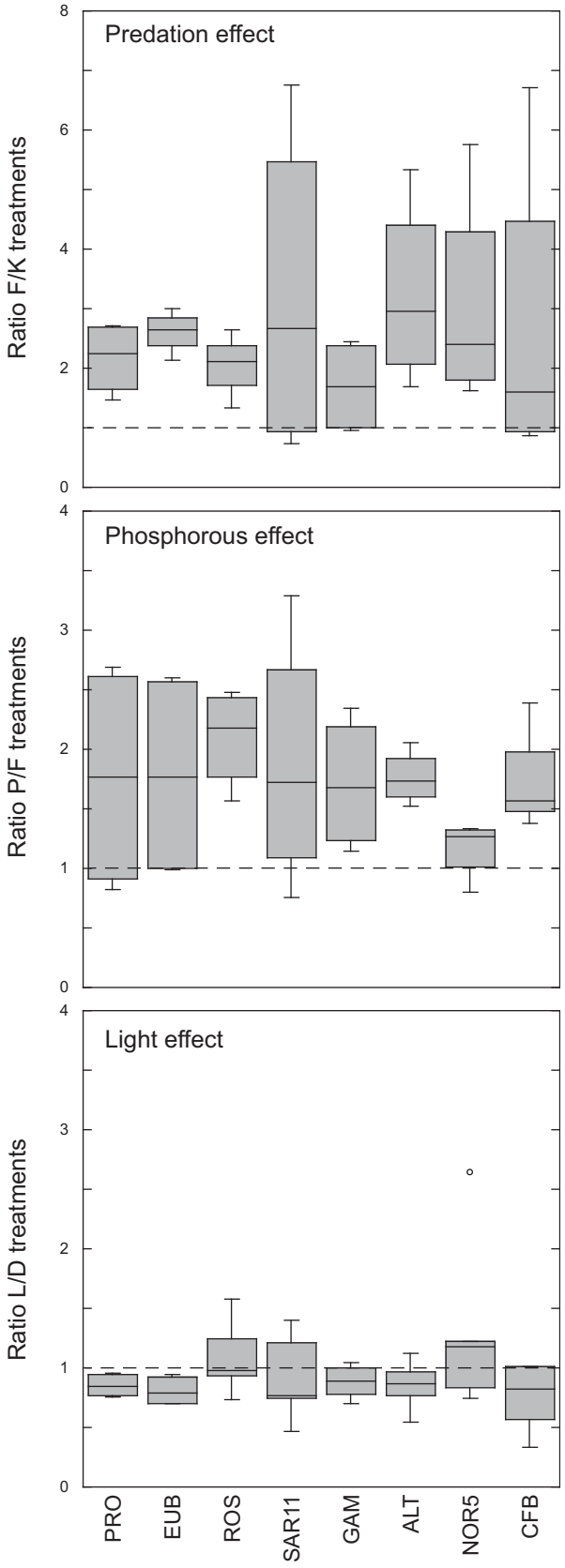

Fig. 3. Boxplots showing the relative effects of predation (upper panel), phosphorous (middle panel) and light (lower panel) on the growth of different taxa, as shown by the growth rate ratio between F/K, P/F and L/D treatments respectively. Values of all replicates and experiments are included. From top to bottom, the horizontal lines of the box represent the upper-quartile, median and lower-quartile. Whiskers extending from top and bottom of the box represent the largest and the smallest non-outlier in the data set. Points plotted separately in the chart represent outliers. The 1:1 dashed lines indicate that the growth rates have the same magnitude in both treatments. K: control, F: predator-reduced, P: predator-reduced and phosphorus addition, L: light, D: dark treatments. PRO, total prokaryotes; EUB, *Eubacteria*; ROS, *Rhodobacteraceae*; SAR11, SAR11 clade; GAM, Gammaproteobacteria; ALT, *Alteromonadaceae*; NOR5, NOR5/OM60 clade; CFB, Bacteroidetes.

studies (Ferrera *et al.*, 2011; 2014; Hojerová *et al.*, 2011). In addition, the *Rhodobacteraceae* and NOR5, both taxa containing AAP members, were also quantified in these experiments.

Despite no overall significant differences were found when comparing all growth rates under the different light regimes (ANOVA, $p > 0.05$), when comparing values between dark and light treatments for the different groups, we did observe an interesting trend (Fig. 3). Values were on average equal or a little higher in the dark than in the light for all groups, except for the *Rhodobacteraceae* and particularly the NOR5 clade, both groups containing AAP species, in which these differences were statistically significant ($p < 0.05$). Contrarily, no light-enhanced growth was observed for putatively PR-containing taxa (i.e. SAR11 and Bacteroidetes) (Fig. 3). Kirchman and Hanson (2013) provided theoretical calculations of the bioenergetics of phototrophy in marine bacteria and concluded that in spite of implying higher costs, phototrophy provides much more energy for AAP than for PR bacteria. Although our results represent indirect evidences, they would be in agreement with these theoretical calculations. In conclusion, we found no major effects of light on the growth rates of common bacterioplakton groups but a minor enhancement was observed for groups containing AAP members (*Rhodobacteraceae* and NOR5) in contrast with a small inhibition observed in the other taxa.

Concluding remarks

Manipulation of top-down and bottom-up pressures as well as light exposure in two different experiments in NW coastal Mediterranean waters revealed contrasting responses to the different treatments between experiments and among bacterial groups. Maximal growth rates were achieved after reduction of both grazing and P-amendment, however, when comparing response ratios, reducing grazing pressure had a stronger overall effect than P-addition. The initially low abundant *Alteromonadaceae* displayed the highest rates, followed by

the *Rhodobacteraceae* clade. Nevertheless, the abundant SAR11 group exhibited comparable response ratios to those groups displaying the highest growth rates. The experiments clearly show that both top-down and bottom-up factors have an important role controlling the growth of all examined groups, regardless of the magnitude of their growth rates. Interestingly, despite no overall major response was found to light exposure, the clades *Rhodobacteraceae* and NOR5, which contain photoheterotrophic AAP species, presented higher growth rates under PAR, a result that indirectly evidence a possible role of light in the growth stimulation of these groups in the NW Mediterreanean.

Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1. Bacterial activity measured as rates of leucine incorporation during the incubations for the two experiments. Values for the two bottle replicates (1 and 2) of each treatment are displayed. KD, control in the dark; KL, control under PAR; FD, predator-reduced treatment in the dark; FL, predator-reduced treatment under PAR; PD, predator-reduced plus phosphorus addition treatment in the

dark; PL, predator-reduced plus phosphorus addition treatment under PAR.

Fig. S2. Mean abundances over time of each phylogenetic group in the different treatments. PRO, total prokaryotes as measured by DAPI staining; EUB, *Eubacteria*; ROS, *Rhodobacteraceae*; SAR11, SAR11 clade; GAM, Gammaproteobacteria; ALT, *Alteromonadaceae*; NOR5, NOR5/OM60 clade; CFB, Bacteroidetes; KD, control in the dark; KL, control under PAR; FD, predator-reduced treatment in the dark; FL, predator-reduced treatment under PAR; PD, predator-reduced and phosphorus addition treatment in the dark; PL, predator-reduced and phosphorus addition treatment under PAR.

Fig. S3. Percentage of High Nucleic Acid content (HNA) cells for each treatment in the two experiments. Values for the two bottle replicates (1 and 2) of each treatment are displayed. KD, control in the dark; KL, control under PAR; FD, predator-reduced treatment in the dark; FL, predator-reduced treatment under PAR; PD, predator-reduced and phosphorus addition treatment in the dark; PL, predator-reduced and phosphorus addition treatment under PAR.

Fig. S4. Growth rates of the different phylogenetic groups in Experiment 1 (May 2010). Single values for each of the two bottle replicates (white dots) as well as mean values (bars) are displayed. KD, control in the dark; KL, control under PAR; FD, predator-reduced treatment in the dark; FL, predator-reduced treatment under PAR; PD, predator-reduced and phosphorus addition treatment in the dark; PL, predator-reduced and phosphorus addition treatment under PAR.

Fig. S5. Growth rates of the different phylogenetic groups in Experiment 2 (July 2011). Single values for each of the two bottle replicates (white dots) as well as mean values (bars) are displayed. KD, control in the dark; KL, control under PAR; FD, predator-reduced treatment in the dark; FL, predator-reduced treatment under PAR; PD, predator-reduced and phosphorus addition treatment in the dark; PL, predator-reduced and phosphorus addition treatment under PAR.

Supplementary Materials

Effects of grazing, phosphorus and light on the growth rates of major bacterioplankton taxa in the coastal NW Mediterranean

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Running title: *growth rates of bacterioplankton*

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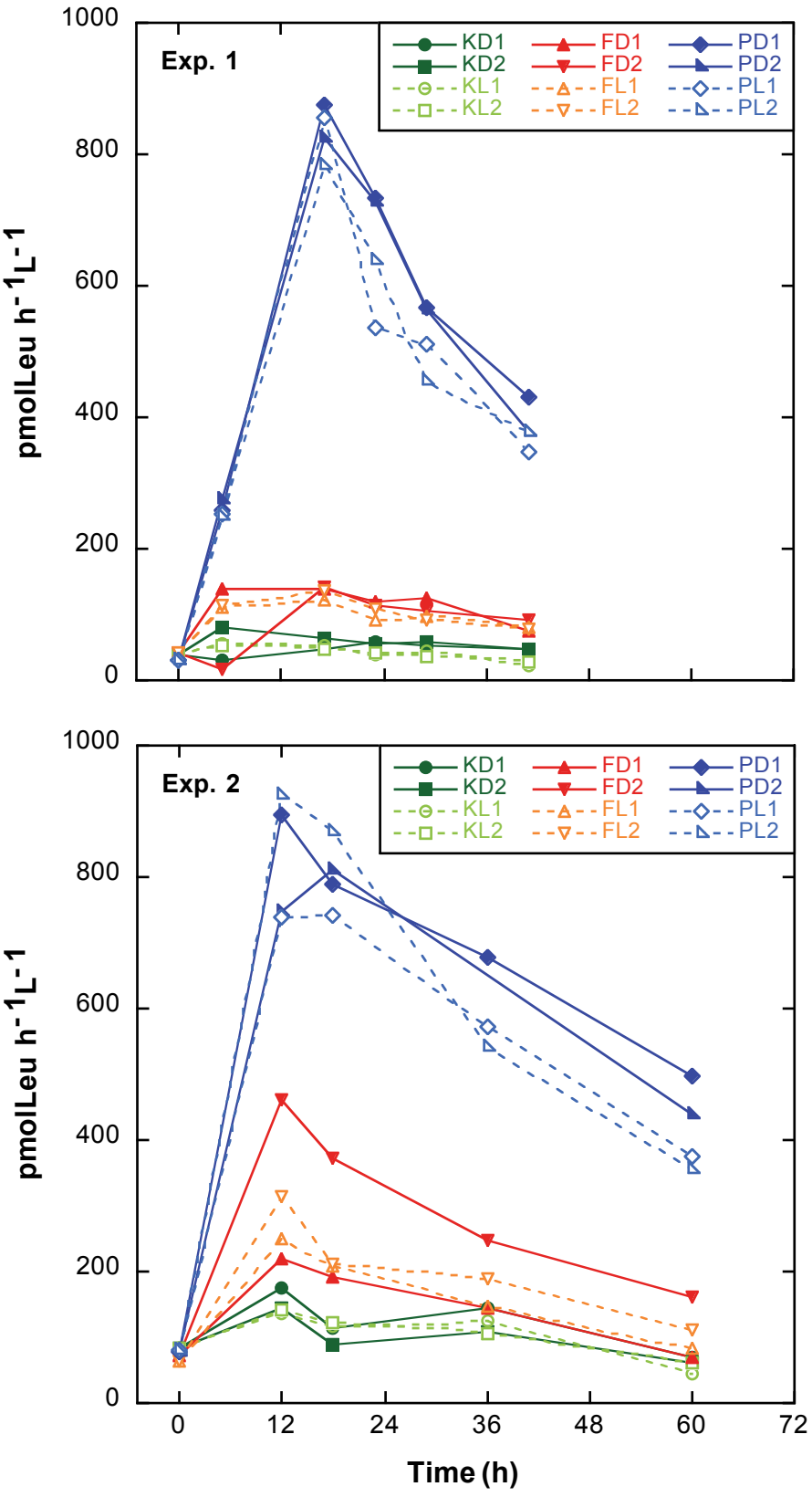

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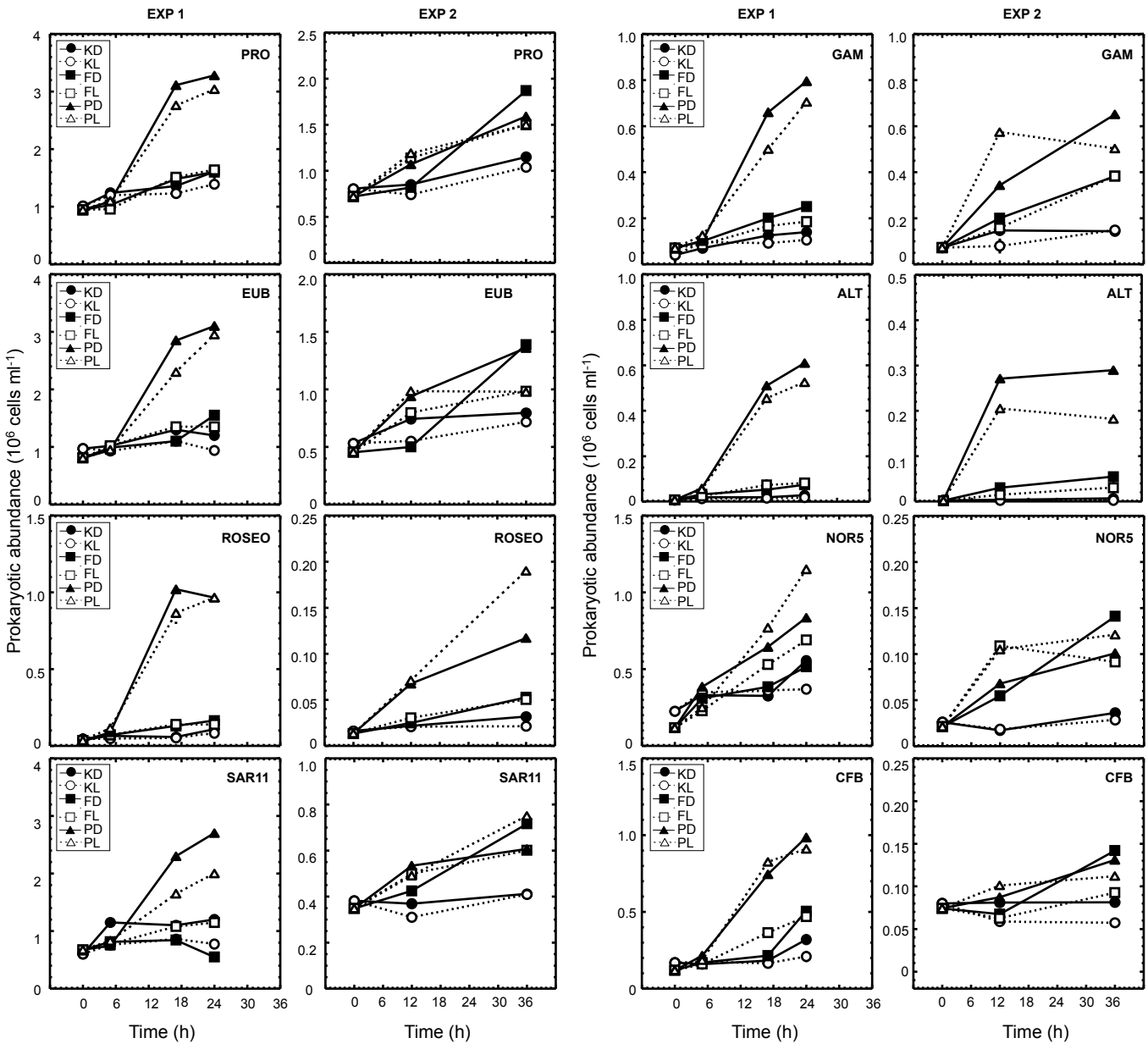

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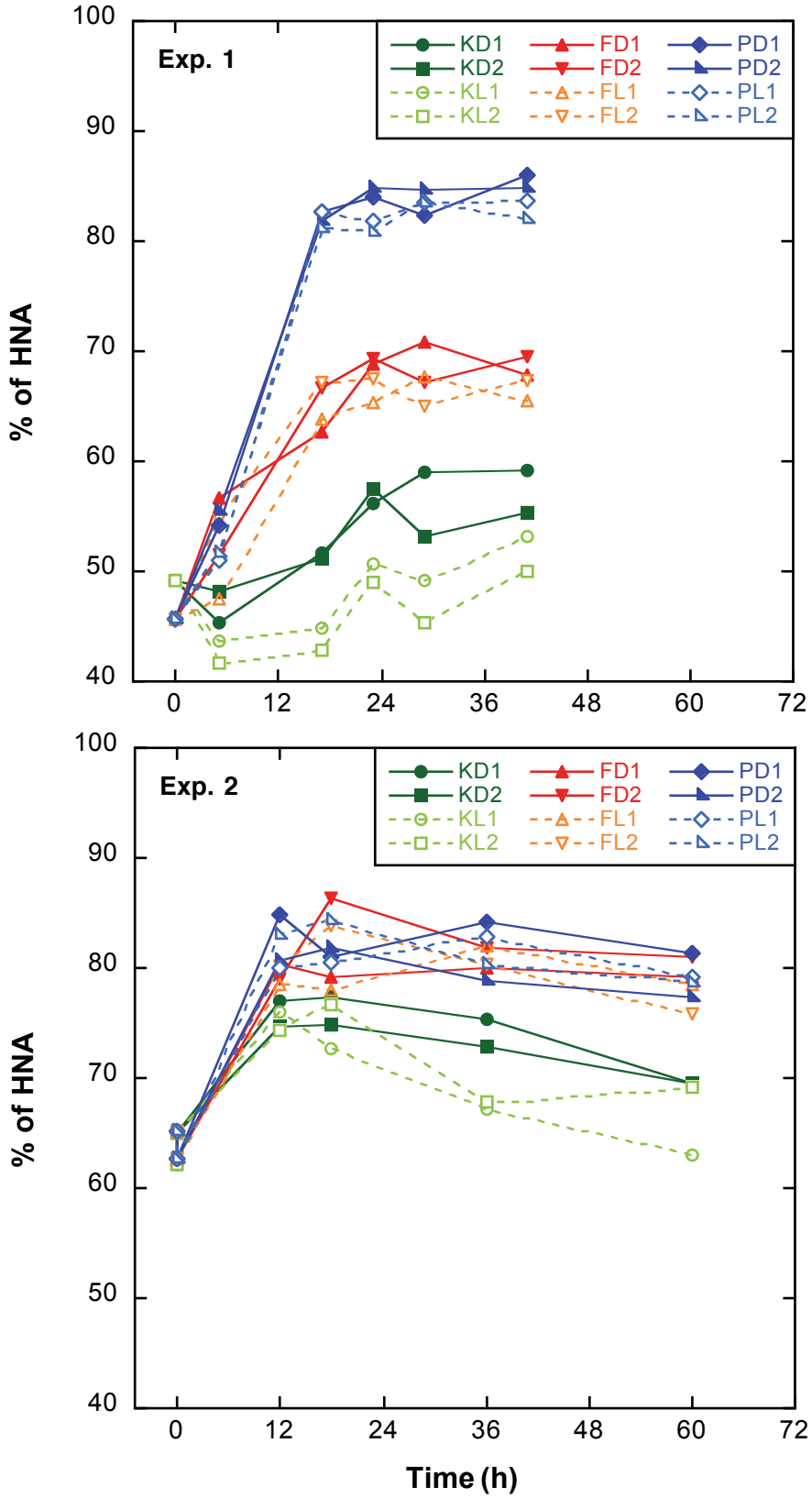

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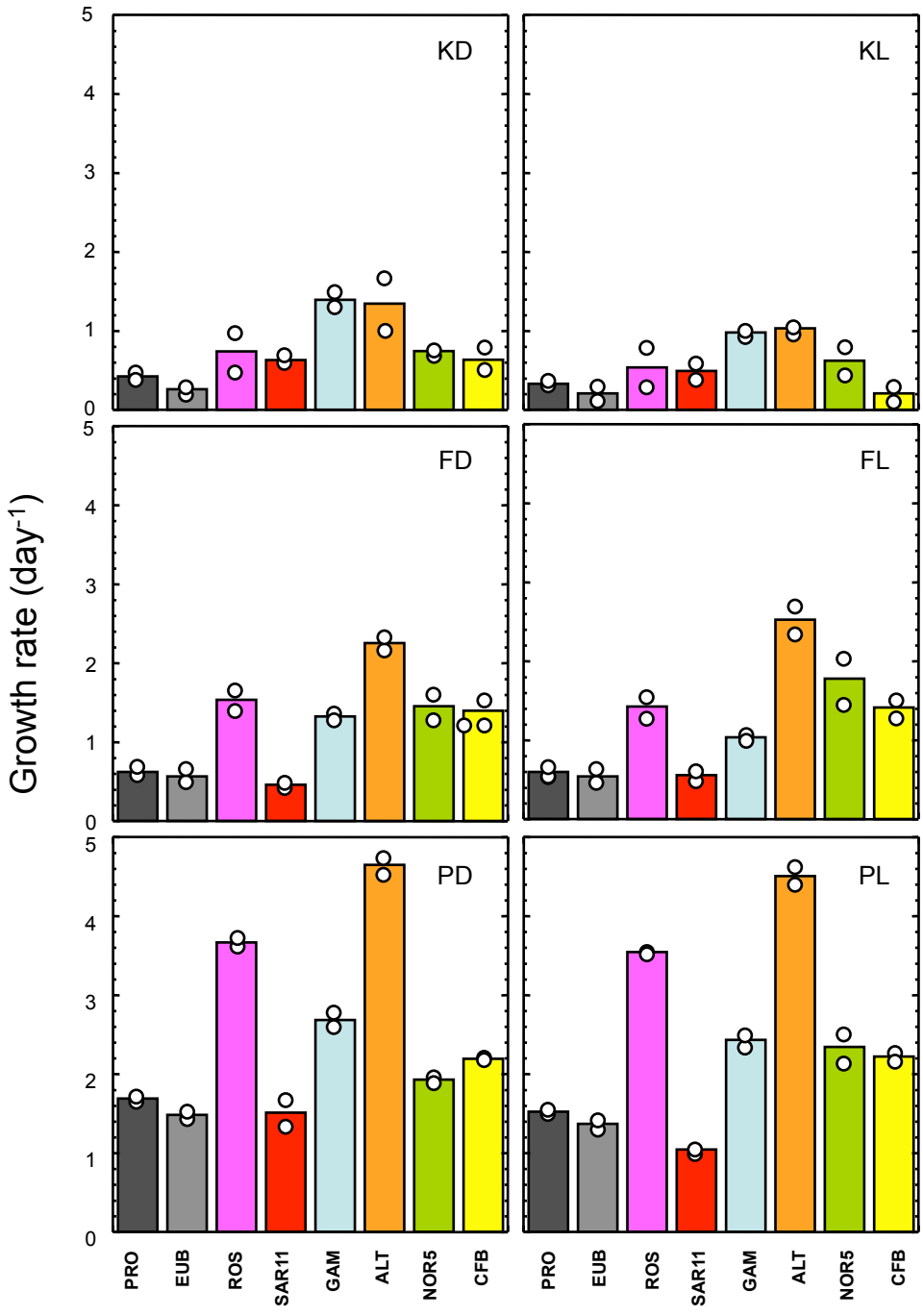

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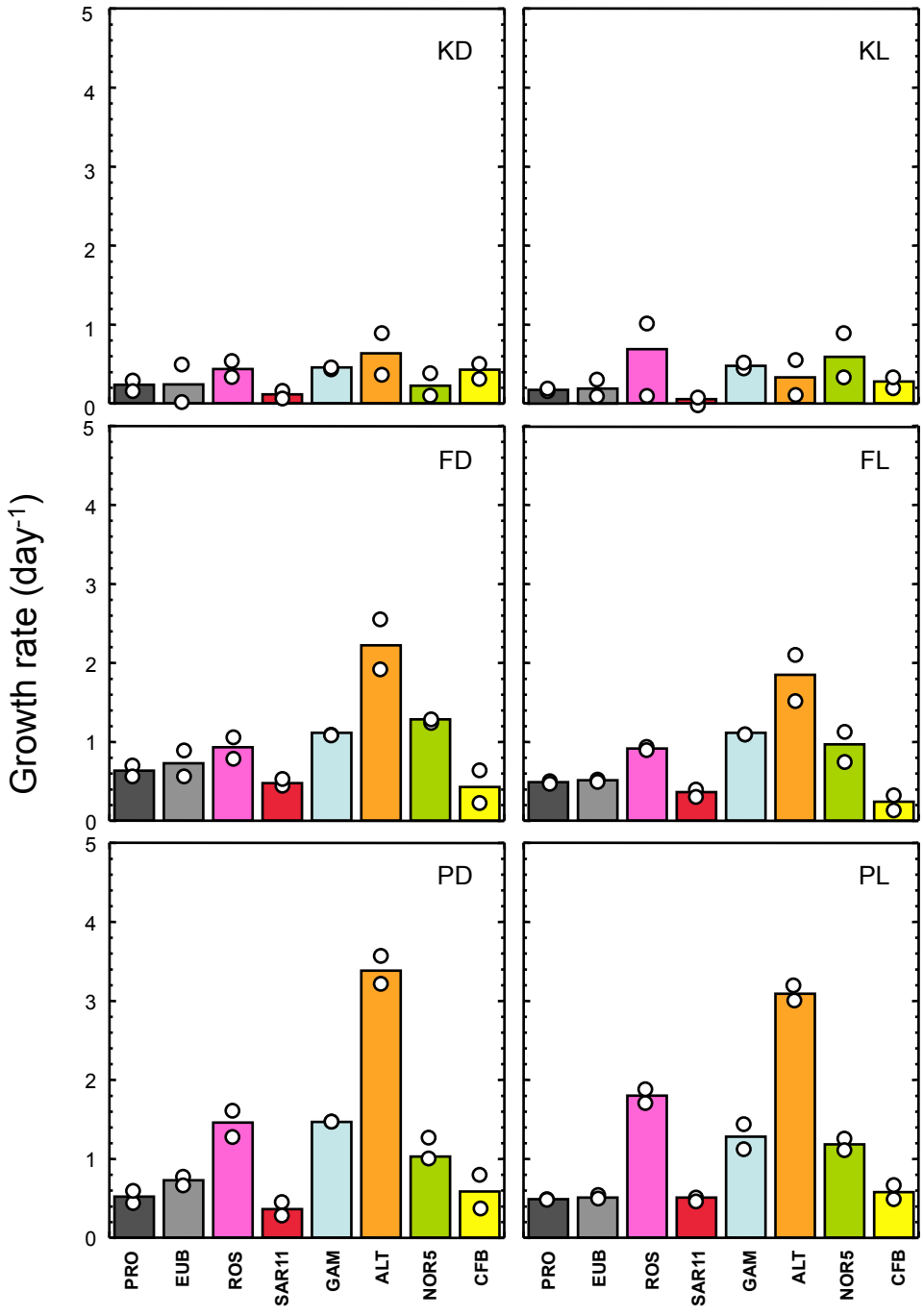

Fig. S5. Growth rates of the different phylogenetic groups in Experiment 2 (July 2011). Single values for each of the two bottle replicates (white dots) as well as mean values (bars) are displayed. KD, control in the dark; KL, control under PAR; FD, predator-reduced treatment in the dark; FL, predator-reduced treatment under PAR; PD, predator-reduced and phosphorus addition treatment in the dark; PL, predator-reduced and phosphorus addition treatment under PAR.