

## ***Interactive comment on “Vertical partitioning of phosphate uptake among picoplankton groups in the P-depleted Mediterranean Sea” by A. Talarmin et al.***

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These comments were valuable to improve the critical points to be discussed and highlight the most relevant data. The reviewer should know that conclusions and abstract were entirely re-written, the discussion deeply re-structured and reinforced with comparisons and references. Thank you for these helpful insights.

P3L7. As widely known, these are reports on utilization of organic phosphorus by marine plankton after enzymatic hydrolytic actions, not direct uptake of organic phosphorus. And the utilization of phosphomonoesters after hydrolysis catalyzed by alkaline phosphatase had been known earlier. These precedent reports should be included in

C7824

references.

I agree, alkaline phosphatase activities have been reported in Pi-limited environments and in cultures a long time ago. Here we actually cite references with actual uptake of DOP compounds, apart from Glibert et al 2004 who measured uptake of DON compounds but monitored DOP utilization by measuring AP activities. With reports of high alkaline phosphatase activities linked to other origins than strict Pi limitation [Hoppe, 2003; Karl and Björkman, 2002] and the presence of this enzyme being subjected to various interpretations [Duhamel et al., 2011], we wanted to avoid focusing this point onto enzymatic activity. Uptake of ATP: Bjorkman et al 2012, Casey et al 2009, Duhamel et al 2012, Lomas et al 2010, Sebastian et al 2012, Fu et al 2006 Uptake of ATP and numerous other compounds: Bjorkman & Karl 1994

P3L20. “realize a significant fraction of their P” I was not able to understand what this phrase means.

Indeed, this was changed for clarity. “Some eukaryotes possess mixotrophic capabilities and grazing on P-richer prokaryotes can fill most of their requirements in P”.

P4L14. “significantly higher” Is this true for all the 6 experiments conducted ever? This description gives us the impression that it is an established fact. Thank you for pointing this out, I will rephrase it with a little more flexibility. In the few studies providing measurements in both non-pigmented and pigmented prokaryotes, per cell uptake rates of heterotrophs are systematically lower than Syn’s, and comparable to or lower than those measured for Proc cells. Text was changed into: “When looking into the contribution of picoplanktonic groups to total Pi uptake, prokaryotes are better competitors than eukaryotes. Among prokaryotes, taxon-specific uptake rates (per cell) of Pi higher for Syn compared to Hprok and Proc in the Sargasso Sea [Michelou et al., 2011], and higher per cell rates were measured for Proc than Hprok in the North Pacific Subtropical Gyre [Björkman et al., 2012], especially during light incubation [Duhamel et al., 2012].”

C7825

P5L5. In this section, it is not clear where and from which depths samples were collected for some parameters (FCM, Pi uptake and nutrient concentrations).

Further details are added to the text to improve clarity. "Pi turnover times were measured at each of the 30 stations while group-specific processes were assessed at 3 long duration stations (St. A, B, C) and at 4 short duration stations (St. 5, 9, 21, 25). Vertical Pi uptake profiles in sorted groups at stations 9, 21, A and 25 are presented here, and concentration bioassay experiments conducted at stations C, 5, B and A."

P6L15. What is the material of the bottles?

Polycarbonate

"Clean 30-mL polycarbonate Nalgene bottles were filled with 10 mL of seawater samples."

P7L10. Why did the authors choose the depth of 15 m above the DCM? Due to the decreasing hot/cold isotopic ratio inherent to concentration kinetic experiments, they often require an increased  $^{33}\text{P}$  spike and a higher amount of sorted cells compared to regular  $^{33}\text{P}$  uptake experiments. This could only be achieved (and as you can see not 100% with the below detection data) a lot deeper than the surface layer with non-preconcentrated samples.

"Surface experiments carried out between stations B and C led to unsatisfying results where signals were too weak for Pic and unstained Proc cells could not be detected. The upper deep chlorophyll maximum (DCM) depth was then chosen as a biogeochemically consistent level, knowing that the depth of the DCM and nutriclines was expected to vary considerably along the transect. Concentration kinetics experiments were conducted at stations A, B and C by adding increasing quantities of a cold  $\text{KH}_2\text{PO}_4$  solution (0, 4, 8, 10, 15, 20, 40, 60, 80, 100 nmol L<sup>-1</sup> added concentration, Sa)."

P9L6. Do these observations emphasize the strong P-deñiciency?

Indeed it does not. Changed to: "emphasizing the strong oligotrophic state of the  
C7826

eastern waters."

P9L10. "possibly due to mesoscale variability" It does not explain the reason or mechanism for the high values in the western basin. Why or how was the mesoscale variability formed?

A companion paper from the BOUM cruise indicated that the mesoscale eddies encountered along the transect and located by the long-duration stations A, B and C could have been formed the previous winter [Moutin and Prieur, 2012]. Following this comment, we deem it was not relevant to develop this detail in our study, as we do not have sufficient data to compare to physical measurements. Therefore it has been re-stated as: "possibly due to incomplete stratification of the water column and the proximity to the Rhone River."

P9L19. "below" should be replaced by "over"? Done.

P10L17. I did not understand how the authors obtained kinetic parameters from Fig. 5. In Fig. 5, the fitting to Michaelis-Menten curve seems to be unsuccessful for the bulk community at St. C. However, the kinetic parameters are described in Table 2. How were these values obtained?

The kinetic parameters were obtained as described in [Thingstad et al., 1993], which is an approach detailed by [Wright and Hobbie, 1966] for glucose and acetate uptake when  $S_n$  was unknown. In our case, this was used due to uncertainties related to the reliability of  $S_n$  determination.  $S_n$  was low, but certainly not negligible compared to the maximum  $S_a$  of 100 nM, which is one requirement of application of the Michaelis-Menten equation extrapolated to uptake kinetics. Also, the application of M-M is only valid if  $K_m$  is large. [Björkman et al., 2012] for this reason chose not to discuss the  $K_m$  parameter, and it is partly why we chose to use an alternative option with  $K_t + S_n$ . The other reason is that  $K_t$  is supposedly a good proxy of the affinity for the substrate. In the reference paper by [Wright and Hobbie, 1966], they develop the Michaelis-Menten equation in order to calculate kinetic parameters independently from the ambient sub-

strate concentration  $S_n$ :  $(K+S_n)/V_{max}+S_a/V_{max}=T_t$

The turnover time of Pi in low-Pi environment always increased linearly with the addition of Pi in our experiments. Therefore, when plotting the concentration of added substrate  $S_a$  versus the Turnover time at a given concentration (independent from the in situ concentration), the linear regression gives:  $T_{tx} = \alpha \times S + \beta$ . intercept with the Y axis gives an estimated turnover time in the sample at ambient concentrations ( $h$ ), the inverse of the slope is the estimated  $V_{max}$  and  $K + S_n$  is the intercept of the regression with the X axis, i.e. when the turnover time tends to zero, i.e.  $K+S_n = -\beta / \alpha$ . As explained in the legend of Figure 5, the added lines are the estimated  $K+S_n$  and  $V_{max}$ .  $S_n$  was those parameters in the Michaelis-Menten equation, we drew the fit curves

Kinetic experiments from St. 5 and St. B are now removed. From Fig. 5, Table 2 and the text, as well as the plotted estimated  $V_{max}$  and  $K+S_n$  values for Proc and Hprok at St. C. Results and discussion were modified accordingly.

P12L6. Does higher taxon-specific Pi uptake rate by *Synechococcus* just reflect their higher biomass, or higher affinity to Pi or both, compared to picoeukaryotes? I understand this refers to volumetric rates at Station 25 compared to the small contribution of Syn to the bulk Pi uptake at other stations. This has been further developed in the text as follows: Paragraph 4.1. ends with

“The dominance of Syn in Pi uptake fluxes above the DCM at St. 25 was likely due to their higher affinity for Pi compared to Pic cells, because their biomass was 50 times lower than Pic.”

P12L7. Figs. 4 and 5 seem to show me that Hprok-specific rates of Pi uptake was not always lower compared to cyanobacteria.

That is correct when looking at what we defined as volumetric rates (in  $\text{nmol P L}^{-1} \text{h}^{-1}$ ) and contributions. The text has been modified to specify per cell rates vs volumetric rates. Per cell rates of Pi uptake for Hprok are consistently lower than cyanobacteria's

C7828

per cell rates, with the exception of one depth at St. B out of 24 Hprok rates measured.

P12L26. “found no difference” sounds too strong. “found no clear difference” or “found no significant difference” may be more appropriate.

Done.

P13L12. What do the authors think caused the difference found among the areas? Based on our small data set and the limited amount of environmental variables to compare them to, we did not have a chance to run multivariate analyses to point out one or more variables explaining most of the differences in Pi uptake along our transect. Therefore, we can only speculate about differences across regions, and this was added to the discussion in section 4.1.

End of paragraph 4.1. We suggest 4 main reasons to explain differences across regions: i) different composition of the cyanobacterial community between the Sargasso Sea and the Mediterranean, ii) the very low proportion of *Prochlorococcus* cells (<10%) able to oxidize Pi in the subsurface layers of the Sargasso Sea [Martínez et al., 2012], iii) different proportions of live versus dead cyanobacterial cells across oceanic regions and depths [Agusti, 2004], and iv) measurements conducted on fixed samples in the mentioned studies from the Sargasso Sea, possibly involving a significant leakage of intracellular Pi [Talarmin et al., 2011].

P13L14. Theoretically, the community maximum uptake rate should be the sum of each population, thus this description seems no wonder.

Indeed. This formulation was not adequate, the point was to mention that the missing  $V_{max}$  to add up to the sorted groups could be  $V_{max}$  from larger organisms that are not considered in our study.

Summed volumetric  $V_{max}$  of sorted groups added up to the community  $V_{max}$  or below. This missing fraction might belong to unsorted larger protists which do not contribute highly to bulk Pi uptake fluxes ( $> 2 \mu\text{m}$ , data not shown), but may have the ability to store

C7829

large amounts of Pi in case of upwelled or deposited inputs. Our kinetic experiment results shall be interpreted very cautiously due to their scarcity, and they should serve as a starting point to infer Pi uptake strategies with regard to environmental conditions.

P13L20. Is there any evidence or reference to support the low uptake by larger populations?

During the BOUM cruise, we also measured size-fractionated Pi uptake rates. Those data will not be presented in the paper because the point was to bring more details using cytometric groups rather than size fractions, and also because those measurements were often below detection limit and therefore no entire profile of size fractionated rates could be obtained. Nevertheless, data from stations 1 (5m), 5 (50m), 9 (5 and 100 m), C (40 and 100 m), 3 disrupted profiles at St. B (75, 100, 120, 140, 160 m), 14 (125 m) and A (25, 100, 130 m) gathered in supplementary figure S1 showed that the  $>2\mu\text{m}$  size fraction contributed the least to total Pi uptake fluxes. cf attached Fig. S1.

It does however show that, in the  $> 2 \mu\text{m}$  size fraction, contribution to bulk Pi uptake increases linearly with increasing turnover time ( $r^2=0.6$ ). A study in the North Atlantic showed that under SRP concentrations below  $10 \text{ nmol P L}^{-1} \text{ h}^{-1}$ , per cell rates of Pi uptake in pico and nanoeukaryotes are higher than for cyanobacteria [J. R. Casey et al., 2009], but the total contribution to Pi uptake fluxes is lower for larger cells.

P14L1. The authors can estimate cell volume of phytoplankton measured by a flow cytometer, if they obtained scatter (FSC or SSC) data. The data can be calibrated against standard beads, and converted to cell diameter.

This could indeed have been done if calibration had been conducted to process all cruise samples using different sets of beads. However, the counts presented here were acquired using only  $1 \mu\text{m}$  fluorescent beads and no size calibration at the time was conducted. Plus, counts were conducted on PFA-fixed cells, which volume likely shrunk compared to live cells [Sherr and Sherr, 1993]. We are aware that this is a

C7830

major missing information in our data set, we discuss the issue in more details and do suggest that more size/biovolume analyses are conducted along with uptake rate measurements as recommended by [John R. Casey et al., 2013].

P14L9. This discussion seems to contain some leaps in logic. I was not able to understand why the vertical partitioning of Pi uptake in the present study may show that Pi concentration was a major factor explaining the distribution of osmotrophs. Additionally, does "distribution" in this sentence mean vertical distribution or horizontal distribution?

This paragraph and the entire discussion have been largely remodeled. "The vertical partitioning of Pi uptake observed in the present study suggested that different nutrient uptake strategies and capabilities may contribute to explain the vertical structure of microbial communities throughout the water column."

P15L10. "the spatial distribution . . . was partly attributable to their respective capabilities to take up Pi" I do not fully agree to this idea. As mentioned in the comment above, this is not sufficiently supported by observations. The spatial distribution of plankton taxa seems to just reflect their Pi uptake traits. Indeed, thank you for this comment. The idea was not expressed as it was meant. The Conclusion was rewritten and starts as: "While a few taxon-specific Pi uptake rates from various areas were published in the past 7 years, our study was the first focusing on the Mediterranean Sea and uncovering a vertical partition of Pi uptake fluxes among microbial groups. Each group studied in this survey seemed to have a key role in Pi cycling under given environmental conditions, whether it through high affinity for Pi at low concentrations (Hprok and Proc), or the ability to take up Pi at high rates (Syn and Pic). The variability observed within and across sorted groups seems to reflect different kinetic abilities ranging along a continuum of Pi uptake strategies as well as phylogenetic diversity within cytometric groups."

P15L18. "While a few taxon-specific . . ." This sentence should appear on the top of this paragraph. Done

C7831

Figure 5. As mentioned earlier, more than half of the fittings were insignificant. Is it appropriate to include kinetic parameters from insignificant fittings?

Indeed, based on these and another referee's comments, we decided to take out 2 stations (5 and B) where no fitting was significant. It is reported in the results Data from St. C and A only could be used to explore kinetic characteristics, while no response to Pi addition was observed at St. 5 and B.

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C7832

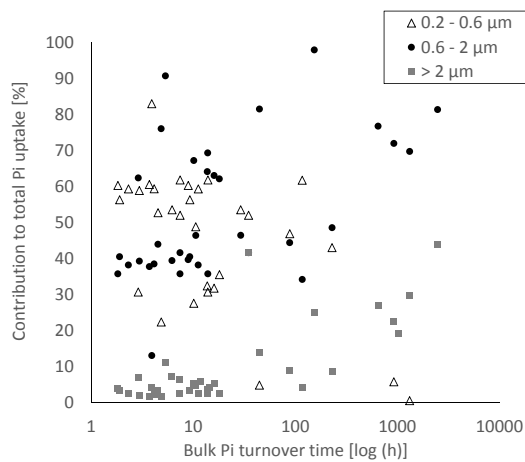


Fig. S1. Contribution of size fractions to total Pi uptake as a function of bulk Pi turnover time at various stations and depths

Fig. 1.

C7833