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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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The discussion has been modified significantly in order to add more comparisons and support to our hypotheses. Our limited data set does require cautious though regarding conclusions and interpretation, which is what we have mostly worked on, and these comments were very helpful in doing so.

1. You state that the Mediterranean Sea is P-deĭňĄcient, implying that cells were P-limited while in fact the data presented in Figure 5 clearly show that most groups were taking up Pi at saturating concentration. Instead, I suggest using the term low-Pi, which only refers to Pi concentrations and not to the physiological state of microorganisms.

Please consider this aspects in the discussion and conclusion. I agree that part of the results do not support a Pi-limitation like it is currently thought and this is now further discussed in the last parts of the article. The system itself, though, the stratified Mediterranean Sea, is clearly Pi-depleted. The short Pi turnover times and low levels of TChl a, as well as the possible multiple nutrient limitation suggested by on board microcosm experiments, do suggest a Pi-limitation. The statements P-limited and P-deficient were modified for more clarity into low-Pi, or explicitly developed.

2. Concentration bioassays: how did you calculate K+Sn for HProk and Proc since on Fig. 5 it appears to be impossible (i.e. no dose response)? I think that this is a critical aspect of this paper and the authors need clarify their approach and results. 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 Sn was unknown. In our case, this was used due to uncertainties related to the reliability of Sn determination. Sn was low, but certainly not negligible compared to the maximum Sa 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 Km is large. [Björkman et al., 2012] for this reason chose not to discuss the Km parameter, and it is partly why we chose to use an alternative option with Kt+Sn. The other reason is that Kt 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 substrate concentration Sn: (K+Sn)/Vmax+Sa/Vmax=Tt

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 Sa versus the Turnover time at a given concentration (independent from the in situ concentration), the linear regression gives: Ttx = $\alpha \times$ S + β. 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 Vmax and K + Sn is the intercept of the regression

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with the X axis, i.e. when the turnover time tends to zero, i.e. K+Sn = - β / α. As explained in the legend of Figure 5, the added lines are the estimated K+Sn and Vmax. Sn 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 Vmax and K+Sn values for Proc and Hprok at St. C. Results and discussion were modified accordingly:

3. I ïňĄnd puzzling that the bulk and the group speciïňĄc kinetic constants could only be measured at St. A where the turnover times of Pi were the longest: how do the authors explain that? I understand the question as: how come that only at Station A the M-M model explains the kinetic uptake curves? At St. A, abundances, and possibly biomass were very high for each group of interest. Longer Pi turnover time in the microbial community suggested that Pi was more available, maybe along with other sources of P, and it is likely that cells were not P-stressed with saturating uptake rates.

SpeciïňĄc comments: 1. P14642 L9-10: I would remove “as shown by. . ..since 2007” since there are a few missing references and this does not add to the point made. Done. The previous list of 33P coupled with cell sorting was however updated with the very recent paper showing taxon-specific Pi uptake rates of phytoplankton groups in the Sargasso Sea by Lomas et al.

2. P14642 L21: “Pi-depleted surface waters”: do you mean euphotic layer as in the method section? To me surface is the top 5-10 m but not down to 200m. Maybe you mean upper water column? Indeed, this was incorrect, thank you for noticing. Changed to:” The present study investigates the contribution of sorted picoplankton groups to total Pi uptake flux in the Pi-depleted stratified upper water column of the Mediterranean Sea (down to 200 m).”

3. P14644 L27-28: this sentence is not grammatically correct Changed to:” A cold Pi solution was added to blank samples (final concentration of 0.1 mmol L-1) 15 minutes prior to radiolabeling and processed like other samples.”

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4. P14645 L3: I would remove “embarked” and say “The radioactivity was counted onboard. . .” Changed to:” The radioactivity was counted onboard within 5 hours after addition of the scintillation cocktail using a Packard LS 1600 liquid scintillation counter.”

5. P14645 L10-11: justify why you chose to conduct concentration kinetic experiments at 15m above the DCM: that sounds random to me. Rewritten as: “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 KH2PO4 solution (0 – 100 nmol L-1 added concentration, Sa).” Due to the decreasing hot/cold isotopic ratio inherent to concentration kinetic experiments, they often require an increased 33P spike and a higher amount of sorted cells compared to regular 33P 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.

6. P14647 L6-7: you say that because the integrated chlorophyll concentration decreases west to east that “emphasize” the strong Pi deïňĄciency: I don’t see why. Improve or remove. Based on your 1st comment, this was modified to: “Total chlorophyll-a concentrations integrated over 150 meters were up to twice as high in the western basin (e.g. St. 25) compared to the Levantine basin (e.g. St. 9; Table 1), emphasizing the strong oligotrophic state of the eastern waters.”

7. P14647 L15-16: I would replace “extreme values” by ranges Done.

8. P14647 L19: “below 50m”: you mean above? Yes, thank you. This was fixed.

9. P14648 L5 please give average +/-SD and n values When all available, summed contributions of the 4 sorted groups to Pi uptake under ambient Pi concentrations represented 83.3 $\pm$ 30 % of the bulk signal (n=8).

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9. P14648 L10: please give average +/-SD and n values Syn cells contributed to bulk signal by 53 % at the coastal station, where they reached the highest abundance measured during the cruise, while their average contribution over the cruise was of 16.3 $\pm$ 14 % (n=18).

10. P14648 L20: "not signiïňĄcant": I would say "below the detection limit of our method" Done.

11. P14649 L1: "higher than for other groups" but I still cannot understand how you could measure K+Sn for other groups than Syn When using the turnover approach and extrapolating a K+Sn, values can always be obtained, unless the Vmax is extremely high, in which case the half-saturation constant is rarely relevant. Considering that our SRP concentrations are not as low as the turnover times could suggest, we could never have estimated a Km from our experiments. When kinetic parameters are estimated based on turnover times, the dilution effect of the radio-isotope with cold Pi is strong and not corrected for like uptake rates are. This is why we could still obtain K+Sn values.

12. P14650 L9-10: this sentence needs to be rewritten. Done. The discussion has been entirely re-structured and in large parts re-written based on the reviewers' comments and new elements added.

13. P14651 L14: "bulk community were 2 to 40 times higher": Does that mean that larger phytoplankton and aggregates missed in the cell sorting group-analyses present higher Vmax than the small cell groups studied here? This should have been reported as a missing fraction rather than a ratio. Please find below the entire paragraph. I think it is indeed a possibility that larger organisms may have a high Vmax, considering the different systems involved for algae (Pit) and bacterioplankton (high levels of Pst) in low-Pi environments. Upregulation of RNase and de novo production of phosphatases has been shown for eukaryotic algae in low Pi conditions (Dumont et al 1993, Matagne 1976), supposedly allowing the release of phosphate within the cells, thereby increas-

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ing gradients between the outside and the inside of the cells. We found that cells >2 um were not contributing a lot to bulk Pi uptake fluxes (cf. Fig. S1). However, we can see that the contribution of this size class to bulk Pi uptake increases linearly (when plotted on a linear scale) with increasing turnover time (r2=0.6). This last observation is consistent with a non-saturating uptake, i.e. possibly a high Vmax. Please refer to attached Figure S1

14. P14652 L9: I would replace “detected” by “measured” Done.

15. The last paragraph of the discussion just throws ideas: the authors should develop those ideas or remove this paragraph which does not bring much to the paper at this stage.

Indeed, most of it was removed, or changed: “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. In the surface where SRP concentrations are the lowest, only organisms with the lowest K+Sn can utilize Pi efficiently, i.e. Proc and Hprok cells. The cyanobacterial contribution to Pi uptake possibly decreased below the DCM because of light limitation [Duhamel et al., 2012]. During this cruise, a mesocosm study showed that surface communities were submitted to N and P co-limitation or N limitation, but no strict P-limitation [Tanaka et al., 2011], and no nutrient (N, P) limitation was found at St. A. Dust deposition were found to be 89% from anthropogenic sources at this station [Ternon et al., 2011], which may provide more or different P sources than in the more eastern basins isolated from all inputs. A larger effort in measuring environmental data, combined to phylogenetic analyses of the sorted groups would help to further link the diversity of microbes to their Pi uptake performances. Such experiments have been conducted in mesocosm conditions and showed that different bacterial taxa responded to Pi additions with different strategies in the Mediterranean Sea [Sebastián et al., 2012]. The concept of competition among microbes for a limited resource in natural environments is challenged by the numerous potential sources of

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growth limitation and the high diversity of cytometric groups (e.g. [Kashtan et al., 2014; Marie et al., 2010]). “

16. Conclusion: avoid making conclusions based on the half saturation constant if this parameter could not be properly measured. I would also avoid concluding about bacteria carbon limitation unless there are any data to prove this. Finally, the term “biodiversity” is out of place in the last sentence.

“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 is for potential Pi storage capacities (Pic), possibly high affinity for Pi at low concentrations (Hprok and Proc), or the ability to take up Pi at high rates (Syn). 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. We found that different groups were dominating bulk Pi uptake fluxes at different depths, with Hprok contributing the most in the surface, subsurface layers and very likely bottom layer of the euphotic zone, while cyanobacteria were dominating fluxes around the DCM zone. Multiple nutrient and energy limitations need to be further investigated to better understand this vertical partition of Pi uptake in oligotrophic waters.”

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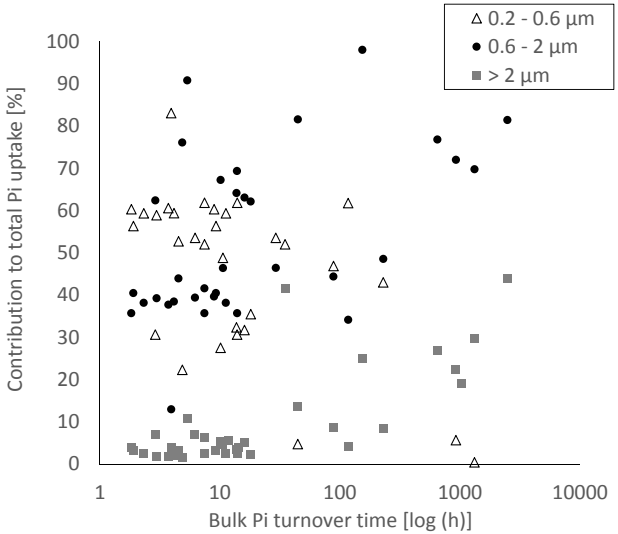

Fig. S1. Contribution of size fractions to total Pi uptake as a function of bulk Pi turnover time at various stations and depths

Fig. 1.

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