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## ***Interactive comment on “Phosphate monoesterase and diesterase activities in the North and South Pacific Ocean” by M. Sato et al.***

**Anonymous Referee #2**

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General comments: Phosphate monoesterase activity (MEA) and its role in various areas of the Pacific Ocean have been assessed in several studies, including fairly recent ones (<5 years old). Nevertheless, little is known about phosphate diesterase activity (DEA). Improving our knowledge of the role of this enzyme in marine phosphorus (P) cycling is thus important and the research goal by Sato et al. is relevant. Unfortunately, I have major concerns with the approach used to measure diesterase activity and the interpretation of this dataset. Moreover, I find it disappointing that only monoesterase activity was measured in both the north and south Pacific since the only originality of this report resides in studying the contribution of diesterase activity.

Specific comments: In the introduction the authors wrote, “datasets on phosphorus cycling across different ocean basins are still lacking”. Nevertheless, they later cite

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Duhamel et al. 2011 and Mather et al. 2008 who exactly did that in the Pacific and Atlantic oceans, respectively.

The next sentence is not true either: "... no reports on cycling or utilization of phosphate diesters...": see for example the work of Björkman and co-authors at Station ALOHA in the North Pacific subtropical gyre. They might not have used the same approach as the one described in the present study but they report data on bioavailability of P diesters.

The sampling location is poorly described. Although a map is included (Fig. 1), information on location should be also provided in the text. More importantly, SCM sampling depths should be reported. Considering the wide range of environments sampled, I would expect the SCM depth to vary widely across stations and thus the light level and temperature to be largely different from incubation conditions. How would that affect the results considering a Q10 of 1.5-2.0 for alkaline phosphatase? Moreover in some environments there is no obvious SCM: how did the author determine the SCM (i.e., what criteria were used)?

As I said earlier I find the enzymatic assays far from straightforward, particularly for diesterase. The authors acknowledge various drawbacks: 1) because Bis-MUP can release 2 molecules of MUF, DEA can be overestimated by a factor of 2 2) MEA and DEA at SCM may be overestimated due to incubation temperature 3) DEA was not always measured at saturating concentration and some data might be underestimated 4) the kinetic experiments are based on only 3 MUP or Bis-MUP concentrations: how could the kinetic parameters be calculated from only 3 concentrations? Especially considering that  $0.05 \mu\text{M}$  was sometimes too low to measure significant change in fluorescence? This is really not reasonable and I am not surprised that some data would not fit the model and had to be discarded. The sentence "The DEA kinetics parameters at 10 m fluctuated greatly, sometimes with wide intervals of significance" says it all... I would be curious to see the plotted data that were used to estimate the kinetic parameters (hydrolysis rates vs. substrate concentration), particularly for DEA. Considering

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that  $V_m$  and  $K_m$  are roughly estimated, I definitely cannot trust the turnover time estimates and their interpretation 5) I am not convinced that the kinetic parameters should be calculated from the substrate concentration plus LDOP. Indeed this fraction of DOP is difficult to measure: high detection limit (in fact the authors measured several zero values, see table 1) and prone to analytical variability. Moreover most kinetic parameter data available in the literature do not use LDOP and comparisons would not be simple 6) using chlorophyll to normalize MEA and DEA is not ideal since a large fraction of these enzyme activities are due to heterotrophic bacteria. I wonder if that influences results presented in figure 5, particularly for the outliers. It might be useful to add non-normalized activities on figure 5. Moreover, considering that chlorophyll concentration must vary widely across sampling sites (these data would be great to present separately in the paper) and that non-normalized enzyme activities varied little (data not shown but stated in the result section), then the relationships presented in Fig. 5 could be due to the trend between chlorophyll vs SRP.

I am surprised that the ratio of dissolved to total enzyme activity is constant throughout the entire dataset considering the wide range of environment sampled. Previous studies reported large variability within much smaller areas. For example: 42–74% in the northern Red Sea (Li et al. 1998), 13–44% in the Bay of Biscay (Labry et al. 2005), and 6 to 30 % in the north pacific subtropical gyre (Duhamel et al. 2011). How do the authors interpret their result? Could it be an experimental artifact due to their filtration method? What is the relationship for MEA when removing the point at  $>0.5 \text{ nM h}^{-1}$ ?

The authors argue that dissolved MEA was lower at SCM than at 10m which is in contradiction with another study showing increase in dissolved activity with depth higher than 100 m (Baltar et al. 2010). Then they conclude that the relationship between dissolved MEA and depth is opposite in the photic layer vs. the aphotic layer. This conclusion goes pretty far considering that the authors only measured activities at 2 depths. Moreover, I would argue that they could be wrong because the variation of dissolved MEA throughout the euphotic layer (6 depths) was previously described in

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the North Pacific by Duhamel and co-authors (2011) and generally showed increasing rates with depth.

Because of all these points, I do not think that the dataset presented can support the conclusions reached in the summary.

Minor comments: Discussion page10108 line 10-18: why would the authors expect MEA and DEA to be controlled by DOP since phosphatase activity is controlled by SRP availability?

Table 1.: why SRP values >200 nM?

Fig. 3 is hard to read.

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