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Discussions

Interactive comment on "Spatial patterns of biphasic ectoenzymatic kinetics related to biogeochemical properties in the Mediterranean Sea" *by* France Van Wambeke et al.

Anonymous Referee #2

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The paper "Spatial patterns of biphasic ectoenzymatic kinetics related to biogeochemical properties in the Mediterranean Sea." by France Van Wambeke et al., reports prokaryotic ectoenzymatic activity, abundance and heterotrophic production in the epipelagic and the upper part of the mesopelagic layers in the Mediterranean Sea. In this study, the Vm and Km of the 3 enzymes (alkaline phosphatase (AP), aminopeptidase (LAP) and β-glucosidase (βGLU)) were determined using 2 series of substrate concentration. The paper points out that the choice of substrate concentrations affect the results and their interpretation.

Strength points

The paper presents an impressive quantity of data on ectoenzymatic activity of 3 enzymes: alkaline phosphatase (AP), aminopeptidase (LAP) and β-glucosidase (βGLU). The data are of good quality and I think they deserve publication. The methods are detailed and well described. This paper has the potential to be a reference work for future studies on enzymatic activity, since the authors showed that the use of different ranges of concentrations of substrates give different results and therefore lead to bias in the interpretation of the results. I think this is a very important point, since the measure of enzymatic activity is crucial to get insights into the main biogeochemical fluxes in the oceans. Often the published data are difficult to compare due to the different concentrations of substrate used leading to contrasting interpretations.

Weak points

The main shortcoming is the language, often due to language issues, I did not understand what the authors means and the paper is confusing in many points. There are also some grammatical mistakes and typos. I think that there are too many details in the results making them hard to follow. The discussion is not focused, there are too much information that confound the reader. My main recommendation is to deeply revise the English, to simplify the results and to rework the discussion, in order to make the paper easier to follow and to highlight its main message.

Specific comments are reported below.

Introduction

All the introduction would benefit of a deeply revision of the English. The text does not flow well and there are some grammatical issues making hard to read it.

L38-40, P1. "Most of the organic matter being in the state of high molecular weight material, its hydrolysis by ectoenzymes plays an important role in the degradation, utilization and mineralization processes in aquatic environments, but also in nutrients regeneration (Hoppe, 1983; Chróst, 1991)." I think there is a grammatical issue in this

sentence.

L47 Seldomly?

L49-51, P2. “Within these 5 studies for the concentration kinetic the minimum 50 concentration used was 50 nM at the lowest, ...” I think there is a grammatical issue in this sentence.

L66, P2. “among 44 isolated strains”, strains of what? Bacteria?

L95-97, P3. “The interaction between different enzymes has been largely studied in the Mediterranean Sea (Zaccone and Caruso, 2019) due to the particular role of this elemental stoichiometry.” I do not understand this sentence. What do you mean with “interaction among enzymes”? How can it affect the C/N/P ratios of nutrients and organic matter?

L107-109, P3. “Our aim was to study the effects of the respective activities of the ectoenzymes in relation to the quality of the organic matter present, below the productive layer and above the deep Mediterranean waters” How was the quality of the organic matter investigated? I did not find anything about it in the paper.

L104-107, P3. “In this study, we investigated in the Mediterranean Sea, the kinetics of three series of enzymes targeting proteins, phospho-mono esters and carbohydrates (aminopeptidase, alkaline phosphatase 105 and β-D -glucosidase respectively) in relation to the elemental stoichiometry of particulate and dissolved organic matter.” There is only 1 line (L333, P8) about stoichiometry data in the results and few lines (L572-573 and 592-593) in the discussion, so I think that this cannot be one of the main goals of the paper.

L106-107, P3. “We have paid particular attention to the use of a wide range of substrates concentrations to evaluate potential multiphasic kinetics.” and L116-117, P 3 “Finally, we discuss the biases in interpretation of past and current enzymatic kinetic, potentially induced by the reduced range of used substrates concentration.” I think

that the use of different concentration ranges of substrate for the 3 enzymes leading to different results is the main message and I would focus the manuscript on it.

2. Materials and Methods

L143, P4. “Heterotrophic prokaryotic production (BP), heterotrophic prokaryotic abundances (BA)” I would use HPA and HPB as abbreviations.

L147, P4. Replace ectoenzymatic activities by EEA, since the abbreviation is defined at L143-144.

L153, 154, 156 and 157, P4. I would use upper case letters for the abbreviations, in particular for DCM, LIW and MDW.

L156-157, P4. “and second sampled at 1000 m (the limit between meso and bathypelagic waters), except at 2 stations (FAST, 2500 m; ION, 3000 m) named ‘mdw’ “ Why did you select 1000 m as depth representative of deep waters and not a sample collected close to the bottom? How do you think that the different sampling depth at station FAST and ION can affect the results? This should be discussed in section 4.2 and 4.3.

L192-193, P5. Please add the batch of the CRM you used for DOC analysis, its nominal and measured concentration.

L217, P5. “Bacterial production (BP, sensus stricto referring to prokaryotic heterotrophic production)”, BP was already defined at L143, P4.

L225, P6. “On 9 occasions”, do you mean replicates? Samples?

Results

The results are very heavy to read, in section 3.3 there are too many comparisons, too many details that are not relevant for the main message of the paper. I recommend to simplify this section and to avoid details not relevant for the main message of the paper.

3.1 Hydrological situations.

The title should be changed, what does “Hydrological situations” mean? I suggest Physical properties

This section should cite some papers reporting the circulation of the Med Sea and the main physical properties of the water masses.

L294-295, P7. “The sampled stations have basins and latitude characteristics that were superimposed on a changing the seasonal pattern”, I do not understand this sentence.

L299, P7. “Modified Atlantic Waters (MAW) are characterized by low salinity below the seasonal thermocline” Do you mean above the seasonal thermocline?

L299-301, P7. “this property is stretched in the westernmost stations, then progressively relaxes on eastern station, revealing an eastward circulation in the Algerian Basin and a dispersion in the connected basins (northwestern Mediterranean, Tyrrhenian, and Ionian Seas).” And L303-305, P7. “This property is pronounced in the eastern stations and progressively lowered on the western stations, revealing an opposite circulation pattern to the MAW. “ It is not possible to infer the water mass circulation from the T/S graphs. Please add references and rework the sentences.

L305-308, P7. Please add references.

L308-310, P8. Please add references.

L310-311, P8. “The core of LIW is characterized by lower oxygen content than its surrounding water masses, shallower (MAW) and deeper (WMDW and EMDW)”. Looking at figure 2a, this observation is not always true, for example at stations ION and 6, LIW has higher oxygen than deep waters.

L312-314 “We thus presented all the figures/tables in the order ST10, FAST, ST1, ST2, ST3, ST4, ST5, TYR, ST6 and ION, according to the expected circulation of the LIW (from the right to the left).” If you want to follow the LIW path, I think it is better to invert

the order of the stations, since LIW move from ION to St.10.

3.2 Biogeochemical situation. I would replace situation with properties.

In this section, the values of DOC, DON and DOP should be reported not only their change with depth.

Section 3.3. Ectoenzymatic activities – kinetic trends

This section is really heavy and in some parts there is not correspondence between the number in the text and in the tables. The authors should carefully rework this section deleting all the details that are not relevant and checking the correspondence between the number in the text and in the tables.

L352, P8. “The ectoenzymatic activities were determined using large trophic conditions”, I do not understand this sentence.

L368-L374, P9. Is this paragraph relevant? Looking at table 2, I find different numbers.

L376-380, P9. “For LAP (Fig. 4), Vm50 was on average 3 times higher than Vm1 in ‘surf’ and ‘dcm’ layers, but the differences between these two rates increased with depth (x9 in ‘liw’, x12 in ‘mdw’). Vm50 decreased from epipelagic to mesopelagic waters by a factor of 8 on average, (ratio ‘depth variation factor’ – DVF), but by a factor x19 for Vm1 (Fig. 4a).” It is very hard to see these differences in Figure 4. I think you should also refer to Table 2. Looking at table 2 the number are different. As an example: Vm50 was 10-times not 12 higher that Vm1 in mdw. “Vm50 decreased from epipelagic to mesopelagic waters by a factor of 8 on average,”, if I consider dcm epipelagic and LIW mesopelagic Vm50 decreases by 4.6 times, if I consider surf as epipelagic the decrease is of 3.8, so I don’t understand how the authors calculated a value of 8, the same for Vm1.

L383, P9. I think there is a typo SD10, SD2 and SD6 should be St10, St.2 and St.6. St. FAST and St.1 are missing, they also show Km50 of LAP lower at the dcm than in the surf.

L395-396, P9. Also here there is not correspondence among the numbers in the text and the numbers that I can calculate from the table. Please check.

L410-411, P10. “Average Km50/Km1 ratio for βGLU was 320 and average Km1/Km50 ratio for LAP was 118.” My calculation looking at table 4 indicate 240 instead of 320 and 79 instead of 118. Please check.

L436, P10. I think you should cite Fig.7c,d not Fig.7a,b.

Discussion

The discussion is confounding, there are a lot of interesting ideas, but they are lost in the text. The discussion would strongly benefit of deeply revision of the English. I think that section 4.1 should be the focus of the paper, but I miss a conclusion. From your data do you suggest to use Vm1 or Vm50? Km1 or Km50? Or since they give different information does the use of one or the other depend on the goal of the work? From my understanding the use of a not-appropriate range of substrate determine bias in the results and in the interpretation and data, obtained using different range of substrate, are not comparable. I think these points should be better stressed in this section. I also think that the other sections should support this one, showing how the different ranges may affect the interpretation of trends with depth and regional variability.

L481, P11. “The biphastic factor as defined in Tholosan et al (1999). “Please define it in the text to help the reader.

L505-509, P12. Please rework, I do not understand this sentence.

L535-539, P13. This paragraph should be moved in section 4.1.

L569-571, P13. “With concentration kinetics ending at 50 μM of MUF-P, the specific activities of AP reached using per cell Vm50 or per cell Vm1 were not so different and their trend with depth were similar (Fig. 8). “ It is really hard to see these trends in Fig. 8.

L572-573, P14. “whereas DOC/DOP ratio decreased (from 2200-2400 to 1500-1200), suggesting a preference for heterotrophic prokaryotes to use dissolved organic phosphorus as substrate of AP.” Usually DOC/DOP ratio increases with depth due to the preferential removal of P. Are these ratios calculated using data collected in this cruise? The removal of DOP by heterotrophic prokaryotes should increase the DOC/DOP so, this sentence has no sense to me.

L574-587, P14. This paragraph should be moved in section 4.1.

L595-611, P14. This paragraph is not clear to me.

4.3 Regional variability

L613-615, P14. “In epipelagic waters, both AP maximum rates (Vm1, Vm50) significantly increased from the Algerian/Ligurian Basins to the Tyrrhenian Basin (t test, p = 0.002 and p = 0.02, respectively), and reached maximum values at ION.”. In Figure 6 this pattern is not very clear.

L658-663, P15-16. This paragraph should be moved in section 4.1.

L675-687, P16. This paragraph should be moved in section 4.1.

Figure 2. Please enlarge the name of water masses. I think the value of this figure would strongly increase if you mark on the T/S graphs where the samples for enzymatic activity were collected. You could also add a zoom of intermediate and deep waters.

Figure 4-6. Please add the legend.

Figure 8. This figure is really confuse and hard to understand, I suggest to remove it.

Figure 9 and 10. I suggest to report in the caption how bacterial nitrogen demand and bacterial carbon demand was calculated.

Table1, Please check the title of the columns.

Table 2, line 6 column 5, I think the lines are inverted, if not please check the number

in the text.

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