Interactive comment on “Rates and drivers of Red Sea plankton community metabolism” by Daffne C. López-Sandoval et al.

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Reviewer 1 General comments: The authors describe a dataset of environmental variables related to the metabolism of planktonic communities along a depth and latitudinal gradient in a seasonal resolution in the Red Sea. The authors conclude that gross primary production relates positively to sea surface temperature and nutrient availability. The dataset is extensive, and the research questions (for this part of the Red Sea), to my knowledge, are novel and worthy of publication. The abstract is clear and reads well, but shows a different narrative than the rest of the manuscript. Thus, I suggest for the authors to consider rewriting the manuscript. As mentioned in the author contributions, the manuscript is written by several people and this is noticeable (see specific comments). The abstract mentions the latitudinal gradient but the manuscript introduces two more variables, i.e. depth and seasonality. While interesting variables, they make the story confusing at times and harder to disentangle the story the authors want to tell (according to the abstract). Concerns about the methods used are mentioned in the specific comments and need to be addressed first. Proper description of statistical analyses is lacking. My recommendation is that the manuscript needs major revisions, but only if methodological concerns can be addressed adequately. Then, I suggest a complete overhaul of the manuscript's narrative by focusing on 1 or 2 of the 3 major variables (latitude, water depth and seasonality) and stick with these in the entire narrative. Also, there needs to be a clear description of used statistics in the M&M section and figures and tables should be cut back and/or improved. Consistency in the presentation of the results (including the statistics) and the use of abbreviations (as well as changing them) is recommended.

Reply to General Comments:

We sincerely appreciate the thorough revision, comments and the devoted time to review our manuscript in such a rigorous manner, as the help us to greatly improve our manuscript. We have addressed all the reviewer's comments, and significant changes were done to the figures and tables so that they all consistently provide information from the depths we measured metabolic rates. Also, we have included a detailed description of the statistical analyses. The changes can be tracked in the new version of the manuscript in Figures 2, 3, and Tables 1, 2, and in the methods section 2.4. We summarised the information shown in table 3 in a new figure and moved Figures 4–6 as supplementary information.

Regarding the main concerns about the methodology used to measure planktonic metabolic rates, we think there is a misunderstanding. The reviewer indicated (RC10) that a major potential flaw in our study is the method we used to measure net photosynthesis. We agree with the reviewer that to measure net photosynthesis (i.e., the net organic carbon production in the light, \( P_n = \text{gross photosynthesis - respiratory losses in the light} \)) (Falkowski and Raven 2007), a 24-hour incubation can carry potential bias.
However, in our study, we are not measuring nor reporting net photosynthetic rates. We stated in our introduction and methodology that one of our goals was to quantify the metabolic balance of the planktonic community and from there determine the net community production (NCP) and not Pn. NCP is the organic matter remaining after consumption of the GPP through respiration by plants (autotrophs), microbes (either autotrophs or heterotrophs), and animals (heterotrophs) (Ducklow and Doney 2013), and can be derived from the equation GPP = NCP + CR. This equation describes the balance between the photosynthetic process and respiration (Ducklow and Doney 2013). We also would like to highlight that the methodology used to quantify planktonic metabolism is based on the dark and light method in combination with the Winkler titration method (as mentioned in our reply AC5) and not optodes (as the reviewer stated), and that the procedure we followed was explained in detail in the methods section 2.3. The selected methodology based on oxygen measurements is primarily used since the earliest studies of H. H. Gran, and there is a vast amount of literature available where planktonic metabolism is quantified by this method, on a 24-h period, and the results are reported in oxygen units, as it is what is measured.

Reply to specific comments:

Reviewer Comment (RC) Authors Reply (AC)

RC1.- Title: Says Red Sea but Gulfs are not included.
AC1: We agree with the reviewer that our study does not include data from the Gulf of Aqaba nor it does include the western half of the Red Sea. However, this detail is carefully described in the methods section and Fig.1, so the reader will not be misled. Our study focusses on testing the changes across the latitudinal gradient in the Red Sea, where the samples extend between 17–27 °N (while the Gulf of Aqaba, for instance, extends along 28–29 °N). Browsing through the 2018 papers published in Biogeosciences that have one sea or ocean named without any additional qualifications in the title, we can find studies like the one of Sargeant et al., 2018. Their work was entitled Basin-scale variability of microbial methanol uptake in the Atlantic Ocean. Their work covered a transect from 50° N to 30° S, and therefore missed a large proportion of the Atlantic Ocean, without this generating confusion as to what the results show. We, therefore, prefer to leave the title as it is and provide the details on the sampled areas in the manuscript.

RC2.- Abstract: Line 10: Mentioning “Low productive waters” immediately brings down the importance of the story.
AC2: We modified the text accordingly

RC3.- Page 2: The first paragraph is loaded with self-referencing while many others are not or less.
AC3: There is no intent to load the paper unnecessarily with self-references, but it turns out that the co-authors of this paper have published a large number of relevant articles on the topic. We have now, however, added additional works, by other researchers, to the 1st paragraph.

RC4.- Page 2, line 4-5 and 11: Introduce abbreviations once (see technical corrections) and use them consistently throughout the ms.
AC4: Thank you for your comment, we modified the text accordingly

RC5.- Page 2: The abbreviations of GPP, CR and NCP are presented with units of daily oxygen produced or used. However, these abbreviations are normally used for daily production and use of carbon. I suggest the authors change the abbreviations for these processes and/or use a conversion factor to present daily carbon production and use.
AC5: This and other comments from the reviewer, led us to believe that there is a misunderstanding as they seem to suggest that our work was focus on primary production, which is performed by the photosynthetic components of the plankton community during the daytime and that has gross and net components (as phytoplankton excrete and
respire carbon). However, our paper focuses on the entire plankton community, both photosynthetic and heterotrophic (e.g. bacteria), where the net community production (NCP) represents the organic matter remaining after consumption of the GPP through respiration by plants (autotrophs), microbes (either autotrophs or heterotrophs), and animals (heterotrophs) (Ducklow and Doney 2013). Hence, it is the balance of the photosynthetic processes (GPP) and respiratory activity of the entire plankton community (not just phytoplankton) measured during 24 h periods. Studies that focus on the photosynthetic component of the plankton community (e.g. Net Primary Production, NPP) report values for the daylight period only. Whereas studies, such as this, report (24 h) rates. For instance, published synthesis of community metabolism rates report values per day (24 h), e.g., Robinson and Williams 2005, Regaudie-de-Gioux 2012, 2013).

Reports of daily GPP, NCP and CR rates in oxygen units has remained the case since the Haaken H. Gran, used the light and dark bottle method in combination with the Winkler method to report community metabolic rates (Gaarder and Gran 1927; Gran 1927). The reviewer can find other examples in McIntire et al. 1964; Williams et al. 1979; Williams et al. 1983; Owens and Crumpotm 1995; Robinson and Williams 1999; López-Urrutia et al. 2006; Stanley et al. 2010; García-Martín et al. 2017. The use of 24 h to report rates is indeed not just a tradition but is justified as the metabolic budget need be resolved over 24 h to be completed, for photosynthesis this does not proceed at night, but respiration, which is necessary to define net community production, occurs at night as well. Moreover, all of the above papers report rates based on oxygen units, since this is the property measured and converting these to C requires some assumptions, such as a theoretical PQ value. However, to allow comparisons with another component of carbon cycling, we have provided an estimate, as an indication, of what the mean GPP reported here represents in terms of carbon production, assuming a PQ = 1 (please refer to this between lines 202-204 in the new version of the manuscript)

RC6.- Page 4, line 7-8: There are plenty of references that describe metabolism in the northern part of the Red Sea (e.g. Rahav et al. 2015 MEPS, Tilstra et al 2018 Frontiers, Levanon-Spanier et al 1979 Deep Sea Res.)

AC6: Thank you for the references, we included them in our manuscript.

RC7.- Page 5-6: Silicate is measured, mentioned in the results and in many figures/tables (with significant interactions) but nowhere mentioned in the Discussion. If not important, mention briefly in Discussion. Page 6-7, line 20 and 1 (resp.): Was NH4 determined? If not, then you have NOx values, not DIN.

AC7: Thank you for pointing this out. We have changed DIN by NOx through the manuscript. Regarding the lack of discussion about the specific relationship between metabolic rates and silicates, we would like to mention that we did not discuss in detail the relationship between metabolic rates and any of the inorganic nutrients measured, as we aimed to discuss the overall patterns found among all variables (i.e., nutrients, temperature, autotrophic biomass). However, we agree that it would be worth exploring these relationships in detail in future work, perhaps a manuscript mostly centred towards that goal.

RC9.- Page 7, line 10: provide actual depths of PAR measurements. Also, I am confused about the use of 100%, 60-20 and 8-1 as table 1 and 2 give different ranges. Is the data comparable if different depths of sampling were used?

AC9: The reviewer is absolutely right, and we understand the reviewer’s confusion. We have modified the text, tables and figures to clarify this point. During our surveys, the samples to quantify planktonic metabolic rates were consistently taken within the first optical depth (zeta), towards the end of the photic layer (3-4.6 zeta), and one intermediate sample either taken where we found the max. Chl-a fluorescence, or in case the Chl-a max was at the surface or the bottom layers, the intermediate sample was collected towards the middle of the euphotic layer (approx. 2.3 zeta). Therefore, our measurements are comparable. We chose the sampling based on the optical depths instead of physical depths (i.e., m) as they are biologically relevant to describe
metabolic processes such as GPP. In Red Sea waters, there is a marked latitudinal and seasonal variability in the apparent optical properties. For example, during March 2018, the vertical attenuation coefficient of downward photosynthetically available radiation (Kd PAR) changed across the basin, from 0.062 m⁻¹ towards the southern region to 0.051 m⁻¹ approx. 21° N, while in the northern area, the Kd was 0.059 m⁻¹. Due to this variability, the physical depths (“actual depths”) corresponding to the sampled optical depths varies within cruises and regions.

RC10. Page 7: Were samples for metabolic rates filtered? Does the planktonic community include both single and multicellular organisms? Were the optodes adjusted for salinity? A major, potential, flaw in the methods used for metabolic rates is that it appears as though net photosynthesis was measured for 24h. If correct, this includes an approx. A 12-hour period of darkness and thus results in data that cannot be used for calculations for gross photosynthesis, i.e. O₂ measurements will be severely lower due to dark respiration. net photosynthesis should have been measured only during daylight and respiration rates should have been measured in 2 phases; during the daytime and during nighttime, so approximately 12:12 h as respiration rates can have a diurnal rhythm. So extrapolating these data to daily rates could result in a wrong estimation of gross photosynthesis Also, how was O₂ production data extrapolated to per day? I suggest authors stick to hourly values for oxygen rates. If methods are used correctly, carbon budgets can be calculated using a conversion factor. If net photosynthesis was measured during daylight and respiration for 24 hours, the authors need to state assumptions of the values to their manuscript (potential over- or underestimation of rates)

AC10: We agree with the reviewer that to measure net photosynthesis (i.e., the net organic carbon production in the light), a 24-h incubation can carry potential bias. However, in our study, we are not measuring nor reporting net photosynthetic rates. As clarified in our AC5 and also stated in our introduction and methodology, our goal was to quantify the daily net community production. Therefore, we aimed to estimate the metabolic contribution not only from the autotrophic community but the entire planktonic community (i.e., the balance between the production and respiration of organic material). The selected methodology to quantify planktonic metabolism is based on the extensively used dark and light method in combination with the Winkler titration method (as mentioned in our reply AC5) and not optodes (as mentioned by the reviewer), in a 24 h incubation period, hence that we report our results as daily rates.

RC12.- Page 9, statistics: Need to be expanded with actual models used.

AC12: We included an extended explanation of the statistical analyses

RC13.- Page 9, line 11: NOx, not DIN.

AC13: We modified the text accordingly

RC14.- Page 10, line 17: 56% of heterotrophs suggests dominance of this trophic strategy

AC14: We modified the text to clarify this point.

RC15.- Page 11, line 4: What models were used to test this?

AC15: We determine if plankton metabolism and nitrate +nitrate were correlated by using a Pearson's correlation. We clarified this point in the new version of the manuscript now between lines 261–262.

RC16.- Page 11, line 8: Which analysis?

AC16: OLS linear regression. This information is now been stated in section 2.4 (statistical analyses) and was in the associated figure (Figure 8).

RC17.- Page 11, line 11: Introduction of this statistical method should be in the appropriate section

AC17: We included this information as suggested in section 2.4.

RC18.- Page 11: How were AE values calculated? - Page 11: AE are presented as
negatives, are they? Next page the authors mention a positive value.

AC18: The procedure to obtain the activation energies were explained in the methods section 2.3 (page 8 between lines 16–21). That been said, we determined the activation energies by fitting an OLS linear regression to the relationship between the natural logarithm of Chl-a specific metabolic rates and the inverse of the absolute temperature. The slopes of these so-called Arrhenius plots represent the average activation energy.

The negative values seen in Figure 10, resulted from the way we plotted the normalised metabolic rates against the inverse of the absolute temperature times the Boltzmann’s constant (from 38 to 39.5 eV-1, lower x-axis), the slope of the resulting relationship is negative. However, please note that the relationship with the temperature (upper x-axis) would yield a positive slope if plotted from 20.7 to 32.3 °C.

RC19.- Page 13, line 14: GPP is said to be low, compared to what?

AC19: We clarified this point in the new version of the manuscript. Now in line 328.

RC20.- Page 15, line 18: How was AE standardized to Chl-a?

AC20: We understand the confusion, we clarified this point as we were talking about the AE obtained from the Chl-a normalised GPP.

RC21.- Page 16, line 2: What is “the ocean”?

AC21: We modified this noun, as we meant open oceanic waters.

RC22.- Page 16: Opens with “Surprisingly” and a discussion, then the next paragraph mentions a contradiction that is not surprising. What is the contradiction exactly the authors mean?

AC22: Thank you for highlighting this point, we agree with the reviewer, we deleted this adverb, as it is misleading.

RC23 Page 16, line 6-7: Authors compare results with other references but need to mention actual values.

AC23: We added the relevant information. The changes can now be tracked between lines 387–390 in the new version of the manuscript.

RC24.- Figure 3: Thickness of the pink or green seems to say something about how significant it is but this is said nowhere. In line with this, the diagonal dark green lines seem to signify extreme significance instead of same variable and thus not tested. DIN is NOx. Are variables tested at different depths than metabolic rates of plankton? If so, how can you relate the 2?

AC24: We agree with the reviewer’s comments. Therefore, we decided to modified this graph to present information relevant to the depths we analyse metabolic rates. Also, we changed the type of graph and explained the colour code.

RC25.- Figure 4-6: Lots of white space and hard to see with tiny colored dots anyway. Revise these figures. I suggest to distill from them the most important results you want to show and add the rest to the supplementary section.

AC25: We present this information in the supplementary section.

RC26.- Figure 7: could be mentioned with text in the results section. Suggest moving figure to supplements.

AC26: The information regarding this figure is mentioned in the results, but as it also provides information to derive the GPP threshold we prefer to keep as the main result.

RC27.- Figure 9: Same as Figure 7, B is missing a parenthesis on the y-axis - Figure 10: Same as Figure 7.

AC27: Noted, we corrected the typo. However, we prefer to keep the figure as the main result.

RC28.- Please use continues line numbers for the manuscript
AC28: We followed the format designated by the journal. However, in the new version of the manuscript, the changed to continues lines as suggested by the reviewer.
RC29.- Page1, line 8-9: Please rewrite, it reads as if you want to understand their variability and their present and their future but you want to understand their variability in the present and the future
AC29: Noted, this has been modified as suggested
RC30.- Page 2, line 4-5: Add community
AC30: Done as suggested
RC31.- Page 2, line 11: First mention of NCP, introduce abbreviation.
AC31: The term NCP was mentioned for the first time Page 1 line 14. Therefore, we are not modifying the line.
RC32.- Page 3, line 1: “The Red Sea is a semi-enclosed”
AC32: Noted
RC33.- Page 3, line 3-5: Consider merging this sentence with the previous one
AC33: Done as suggested
RC34.- Page 3, line 9: “throughout the year”
AC34: Noted
RC35.- Page 3, line 10: Delete the dot before the references
AC35: Noted
RC36.- Page 4, line 12: Add “relatively” to “unproductive waters”
AC36: Done as suggested
RC37.- Page 4, line 18: Add "latitudinal gradient" to the sentence
AC37: Done as suggested
RC38.- Page 10, line 8-10: I suggest to start the Results section with this sentence
AC38: thank you for the suggestion but we prefer to keep the sentence as a closing sentence.
RC39.- Page 10, line 16: net autotrophic?
AC39: The sentence was modified accordingly
RC40.- Page 12, line 8: Please stay consistent, use R2.
AC40: Noted
RC41.- Page 13, line 9: Heterotrophic suggest no autotrophs, add “net”
AC41: Done as suggested
RC42.- Page 15, line 6-9: Please rewrite.
AC42: Modified as suggested
RC43.- Page 16, line 4: Add i.e. or parentheses after 2.5 _C
AC43: Changed as suggested.
RC44.- Page 19, line 6: Heterotrophic
AC44: Noted
RC45.- Figure A1: Add axis titles to every part of the figure, having double axes without titles is confusing, especially since the 27 _N axis title (Temperature) is not on any axis.
AC45: Changed as suggested
RC46.- Table 1: Add Silicate to the table description. Also, it is unclear which header belongs to which environmental variable. Also, I fail to see the benefit of the min and max values
AC46: We modified the table accordingly.
RC47.- Present data as mean +/- SE
AC47: Noted
RC28.- Table 2: N does not need decimals
AC48: Noted
RC29.- What does “rank” mean? % PAR differs from Table 1
AC29: Noted, we modified the table.
RC30.- Table 3: Upper part are, what seems to be, Pearson rank coefficients, not the units given in the description. The lower part seems to be p-values, mention this in the description. A hyphen is not the same as a blanc.
AC30: Thank you for pointing this out. It is indeed a correlation matrix, with the r coefficients and p-values. We decided to summarised the table in a new figure

References:
Duarte, C. M., and A. Regaudie-de-Gioux. 2009. Thresholds of gross primary pro-
Robinson, C., and P. J. I. B. Williams. 1999. Plankton net community production and


