RC1:

This study examined the ETS (electron transport system) method, which has gained traction as a tool for estimating respiration in marine plankton communities, with a focus on prokaryoplankton. The authors measured the INT_R and oxygen consumption (Winkler titrations and optodes) simultaneously on a wide range of relevant marine prokaryoplankton to establish the empirical equations between O_2C and INT_R. They examined whether it is constant within species and whether it can be extrapolated to natural plankton communities. Overall, this study is of significant necessity, serving as an essential reference for refining the ETS method. Also, the manuscript is well-written. I have several comments for further improving this manuscript.

Thank you for the constructive comments and suggestions. Find here below our changes based on your comments in bold font.

Line 15: Spell out the full name for "prokaryo-, zoo- and phytoplankton".

Agreed

Line 31: Also, use "prokaryoplankton, zooplankton" instead of "bacterio-, zoo-".

Agreed

Line 37: add reference for 0.8 μm. Some studies used 1 μm for prokaryoplankton.

The reference is at the end of the sentence but added it again after the 0.8 μm for clarity.

Line 137: How are the incubation times "between 5 and 20 minutes" determined?

Explained now: "based on the optimum incubation time prior to INT induced toxicity obtained from time series experiments used for each culture"

Line 191: use "FC" instead of flowcytometry to be consistent with previous text.

Agreed

Table 1: It is better to add one column to show the experiment number to distinguish the two experiments.

Added

Fig. 2: Add mean values to the boxplot to illustrate the data distribution more visually. Also, I suggest putting all the figures of single-cell respiration, including O2C, into Fig. 2. All the figures support the data grouping into "copiotrophs" and "oligotrophs".

Mean values added to the boxplots. We have combined all the previous figures of single cell respiration into Figure 2 and therefore removed them from the second appendix. Grouping is already mentioned in the text and represented in the plots as warm colours

for copiotrophs (red, orange, yellow) versus cold colours for oligotrophs (blue, green, purple), and I added this text to the figure's capture as well.

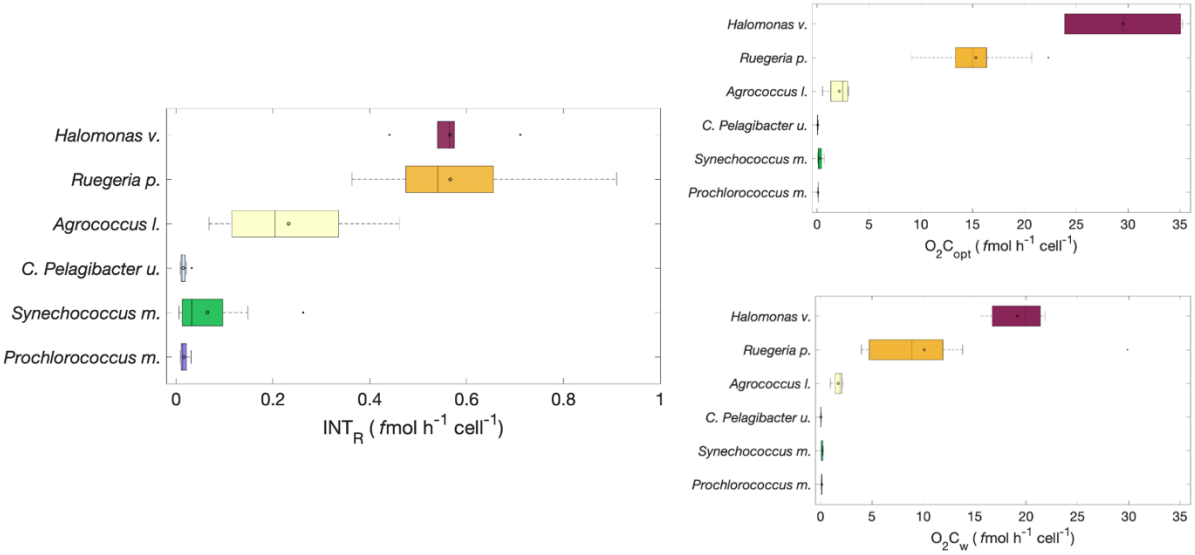

Figure 2. Cell specific rates of formazan production (INT_R (fmol h^{-1} $cell^{-1}$) and oxygen consumption (O_2C_{opt} and O_2C_w (fmol h^{-1} $cell^{-1}$)) ; n = 12. of *Prochlorococcus marinus*, *Synechococcus marinus, C. Pelagibacter ubique*, *Agrococcus lahaulensis, Ruegeria pomeroyi* and *Halomonas venusta*. The boxplot central mark (—) indicates the median, the circle the mean (∘), the side edges of the box indicate the 25th and 75th percentiles, respectively, the whiskers (⊤) extend to the most extreme data points, and the outliers are plotted using the (•) symbol. Copiotrophs are shown with warm colours (red, orange, yellow) and oligotrophs with cold colours oligotrophs (blue, green, purple).

Line 249: Pooling the data from both methods (i.e., Winkler titrations and optodes) to build a linear regression requires the assumption that there are no significant differences between the two methods. Otherwise, it is preferable to use a single dataset.

We tested for significant differences and overall, we found that O_2C_{opt} and O_2C_w agreed very well ($R^2 = 0.95$; $p < 0.01$) (Table 1) and therefore decided to combine these data. This is stated two sections above (line 218), and therefore, to make it clearer, we added the following text (line 252): "Since there was no significant difference between O_2C_{opt} and O_2C_w ($R^2 = 0.95$; $p < 0.01$), we combined the datasets to construct the relationship between formazan and oxygen consumption.

Fig. 3: Add p-value to each sub-figure.

Added to each sub-figure

Line 266: Which statistical method was used for the covariance analysis? Please specify it.

The Analysis of Covariance (ANCOVA) was performed with the Matlab function aoctool, this analysis includes an interaction term, and accounts for the differences in

the slopes. We specified the inclusion of an interaction term in the text to clarify that is not a one-way ANCOVA (line 269)

Fig. 5 and Fig. 4 are similar. It seems not necessary to use two figures. I suggest removing Fig. 4 and moving Fig. 5 forward.

Although figures 4 and 5 look similar, they represent different things and removing figure 4 would make the results section unclear. Figure 4 shows the results of this study while figure 5 is part of the discussion and compares our results with previous experiments with autotrophs. We therefore would like to maintain Fig 4 to not include previous studies within the results section and include Fig 5 for clarity and better flow of the discussion.

Line 424: Which equation was used for this calculation? Please specify it.

(y = 0.72x + 0.44) added to the text (line 432)

Conclusion: Can you draw more specific findings from your study or suggestions for the experiments in natural waters? For instance, within what time frame can safety be guaranteed without triggering toxicity?

We decided to do not suggest specific time frames in the conclusion because although our laboratory experiments show the large differences between species, they don't necessarily indicate the exact time frame for incubations in natural waters. That is why we recommend performing a time series experiment in the area of study first to find out the safe time frame for respiration incubation measurements.

However, we have added further explanation within the discussion, in section 4.2, line 401, we say "These higher rates suggest that toxicity times would also be faster in cultures than in natural waters. Previous studies found toxicity times between 30 minutes and 2 hours in bacterial batch cultures and experiments (Martínez-García et al., 2009; Baños et al., 2020), and between 30 minutes and 5 hours in bacterial assemblages in natural waters (Martínez-García and Karl, 2015; García-Martín et al., 2019b). Our results suggests that the time at which toxicity occurs can vary from 20 min to 5 hours. It is very challenging to simulate natural oceanic conditions, especially oligotrophic ones, in the laboratory, therefore the results of laboratory experiments should only be extrapolated to natural populations with caution. Similar toxicity times are only expected in cultures growing at optimum conditions or in highly active natural eutrophic systems."