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Reply on RC1

Roberto Velázquez-Ochoa et al.

Author comment on "An analysis of the variability of $\delta^{13}\text{C}$ in macroalgae from the Gulf of California: indicative of carbon concentration mechanisms and isotope discrimination during carbon assimilation" by Roberto Velázquez-Ochoa et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2021-50-AC1>, 2021

This paper presents a large survey of macroalgal d^{13}C . The authors attempt to explain the wide variation in the observations by comparing d^{13}C with phylogenetic, morphological, and environmental parameters.

Such kind of study is not novel, and it could be argued that the authors chose to perform a study that was doomed to failure since it has been long known that macroalgal d^{13}C is widely variable due to many interplaying factors, and that simply collecting specimens from several different locations, albeit as many as they did, will in the end only once more confirm that this huge variability cannot be easily explained by a single factor alone. Because of that, there is a case to reject this paper.

On top of that, the paper is very poorly prepared. Some problems:

The authors did not take any care to write the paper in proper English. Some parts are still in Spanish (Tables 4 and 10).

R.The paper was carefully reviewed for English. Few missed Spanish words in Tables 4 and 10 and other language mistakes were corrected.

Results are described in the methods (lines 215-227), parts of the result dealing with different topics are all mixed (lines 301-306).

R.We moved the (old lines 215-227) from the Methods to Results section (3.1. Taxonomy and morpho-functional groups).

We are not sure about the comment "parts of the result dealing with different topics are all mixed (lines 301-306)." In the referred section we described the results of the multiple comparison analysis. In this revised version, this paragraph was rephrased for better compression.

Abbreviations and definitions are not properly presented to the reader (what is GCE? What is R, C and O forms?).

R.The reviewer is right. GCE was acronymous for the Gulf of California ecoregion,

however, we only use GC for the Gulf of California. GCE was removed from the paper. Also, we removed the letters R, C, and O, the acronyms for Phyla Rhodophyta, Ochrophyta, and Chlorophyta, respectively.

The discussion needs that the figures or tables supporting the interpretations are linked to the text, otherwise it is impossible to evaluate the authors' claims.

R. In the revised version, each Figure and Table is linked to the text. Few Figures were improved for a better interpretation of the data.

Statistics are very poorly presented throughout the results. For example, line 296-300; which test was used there to compare different groups? There is nothing. This is the rule through the results.

R. A detailed description of the statistical analysis is provided in the Method section (2.3. Analysis of $\delta^{13}\text{C}$ -macroalgal variability).

A basic statistical analysis of $\delta^{13}\text{C}$ values in different macroalgae groups was applied to distribute and calculate the arithmetic mean, standard deviation, minimum and maximum. Kolmogorov-Smirnov normality test was applied for all variables. Comparisons among morphofunctional groups and taxon collected in the same habitat (within-subjects factor) were conducted by multivariate analysis of variance (MANOVA), which is a procedure for comparing multivariate sample means. When differences were noted, a Tukey-Kramer HSD (Honestly Significant Difference) test was performed. Besides, variations of $\delta^{13}\text{C}$ macroalgal in specimens of the same morpho-functional and taxon collected in different habitats were also investigated with a Kruskal-Wallis test.

The relationships between $\delta^{13}\text{C}$ with each independent variable related to the inherent macroalgae properties (morphology and taxon), biogeographical collection zone (GC coastline and coastal sector), habitat features (substrate, hydrodynamic, protection, and emersion level), and environmental conditions (temperature, pH, and salinity) were examined through simple and multiple linear regression analyses. Analyses of simple linear regression were performed to establish the relationships between $\delta^{13}\text{C}$ -macroalgal with each environmental parameter analyzed as possible driving factors (e.g., temperature, salinity, pH). Multiple linear regression analyses were conducted to evaluate the combined effects of those independent variables (macroalgae properties, biogeographical collection zone, habitat features, and environmental conditions) on the $\delta^{13}\text{C}$ -macroalgal. For all statistical tests, a probability $P < 0.05$ was used to determine statistical significance. The statistical analysis of the results was done using JMP 14.0 software (SAS Institute Inc.).

Many times, the authors make affirmations without any support from literature. For example, line 478, where are the numbers for the conditions in GC waters? Line 485, who said that an efficient CCM helps productivity when the alga is growing under sub-optimal conditions? Line 527, how is that that pCO_2 and temperature depend on light?

R. The reviewer is right. In the revised version, all our affirmations were correctly supported by the literature. Many other documents were reviewed and considered for a better interpretation of our data. We appreciate the support of the Reviewer suggesting and providing specialized literature to consult.

In summary, I SUGGEST REJECTION, because the paper is not novel and is very poorly prepared. Comments above are for re-work and maybe resubmission to another journal, but the lack of novelty may render all the effort useless.

R. In our opinion, the revised version was substantially improved. Data were re-interpreted and the Discussion section restructured according to the three study objectives.

On top of all that, the authors need to completely re-interpret their results. It is very clear that their approach is inefficient. All environmental factors influencing $\delta^{13}\text{C}$ are non-significant, and, when are, very weakly. The authors need to do a more rational analysis of their results.

R. In agreement with the Reviewer's comments, and to obtain a better interpretation of our results, we revisited our complete dataset and statistical analysis, consulted a lot of specialized literature, and considered the comments of both Reviewers. The most significant modifications of the original version occurred in the Discussion section. We discussed the useful information and knowledge of our large macroalgal $\delta^{13}\text{C}$ database to explain their variations in the function of phylogenetic, morphological, and environmental parameters. We have a substantially improved paper.

Macroalgal $\delta^{13}\text{C}$ is actually two variables in one: DIC $\delta^{13}\text{C}$ plus fractionation. Part of the variability in their results is due to differences in DIC $\delta^{13}\text{C}$. For example, when salinity changes, DIC $\delta^{13}\text{C}$ changes, and consequently macroalgal $\delta^{13}\text{C}$ changes as well. I believe the authors did not measure DIC $\delta^{13}\text{C}$ (if they did, they should definitely include these data, it would make a mediocre paper become a great paper). But, for most of their samples, DIC $\delta^{13}\text{C}$ will probably be very uniform, so most variation in macroalgal $\delta^{13}\text{C}$ will probably reflect fractionation. Fractionation in macroalgae is largely influenced by photosynthetic rates. So, the authors should reframe their discussion taking photosynthetic rates into consideration, possibly as the main consideration. Therefore, the authors should re-interpret their results using what is currently known about fractionation, and, if they can, about DIC $\delta^{13}\text{C}$. If they don't have DIC $\delta^{13}\text{C}$ data, they can at least estimate probable values using historical data for the geographical region, taking into consideration seasonal variation, and, importantly, rain parameters, as the main factor here will be seawater and freshwater mixing.

R. We appreciate the time and talent of the Reviewer to conduct this careful review of our MS. We appreciate the gem of knowledge shared in the last paragraph.

In this study, composite water samples were collected for a complimentary analysis of nutrients, alkalinity (and their chemical components), and $\delta^{13}\text{C}$ -DIC. However, the dataset was non-included in the old version. In this revised version, the $\delta^{13}\text{C}$ -DIC values in the Gulf of California surface seawaters were included as Supplementary Information in Fig. S1. In agreement with the preliminary data, the $\delta^{13}\text{C}$ -DIC in the GC seawater averages $1.4 \pm 0.4\text{‰}$ (-1 to 4.9‰) (Fig. S1).

Because $\delta^{13}\text{C}$ -macroalgal depends on the isotope discrimination during carbon assimilation in the photosynthesis ($\Delta^{13}\text{C}$), we calculated the $\Delta^{13}\text{C}$ by subtraction of $\delta^{13}\text{C}$ macroalgae to $\delta^{13}\text{C}$ -DIC seawater in the GC. In our concurrent analysis we observed that $\delta^{13}\text{C}$ -DIC in the GC surface seawater is relatively constant and uniform, thus, the influence of the $\delta^{13}\text{C}$ -DIC variations to the $\Delta^{13}\text{C}$ -macroalgal variability was negligible. Based on the integrative discrimination factor against ^{13}C , five groups were identified: one for Chlorophyta ($\Delta^{13}\text{C} = 16.0 \pm 3.1\text{‰}$), two for Rhodophyta ($16.6 \pm 3.8\text{‰}$ and $34.6 \pm 1\text{‰}$), and two for Ochrophyta ($9.1 \pm 1.7\text{‰}$ and $15.7 \pm 2.7\text{‰}$). Values $\delta^{13}\text{C}$ -macroalgal reflect mainly the discrimination during carbon assimilation ($\Delta^{13}\text{C}$), attributable to the intrinsic properties related to the life form (e.g., taxonomy,

morphology) and influenced by environmental conditions controlling the photosynthetic DIC acquisition.

Our research is the first approximation to understand the $\delta^{13}\text{C}$ macroalgal variability in one of the most diverse marine ecosystems in the world, the Gulf of California. We did not pretend to resolve the intricate processes controlling the variations of $\delta^{13}\text{C}$ or $\Delta^{13}\text{C}$ -macroalgal during carbon assimilation and respiration and determine the isolated influence of each environmental factor. Controlled experiments in laboratory and mesocosm type in combination with field studies are required to elucidate the complex processes controlling the $\delta^{13}\text{C}$ -macroalgal.

In our study, the $\delta^{13}\text{C}$ -macroalgal was a good proxy to identify CO_2 or HCO_3^- -source in photosynthesis and to infer the presence or absence of CCM's, which is a good indicator of the physiological state of photosynthetic metabolism. Because the ocean acidification in progress and the bloom-forming macroalgae events that increase in México and worldwide, the analysis of $\delta^{13}\text{C}$ -macroalgal constitute an excellent tool to study acidification and eutrophication, however, the $\delta^{13}\text{C}$ -macroalgal must be first evaluated.