UNIVERSIDAD NACIONAL
AUTONOMA DE
MEXICO

INSTITUTO DE CIENCIAS DEL MAR Y LIMNOLOGIA
UNIDAD ACADEMICA MAZATLAN
EXPLANADA DE LA AZADA Y CERRO DEL CRESTON
APARTADO POSTAL 811
MAZATLAN 82040, SIN. MEXICO.
TEL. (01)(669) 985-28-45 AL 48

June 23th, 2021.

Aninda Mazumdar
Editor
Journal Biogeoscience,

Dear Dr. Mazumdar:
We are writing in connection to the Research article: An analysis of the macroalgal $\delta^{13}C$ variability in the Gulf of California (bg-2021-50, Submitted on 24 Feb 2021). I would first like to thank the conscientious review work done by both Reviewers.

We attached a letter with the point-by-point responses to each Reviewers' comments. Data were re-interpreted, and the Discussion section restructured. Hopefully, our revised article meets the expectations of the reviewers and you as the editor. In our opinion, the revised version was substantially improved, with scientific merits to be published in the prestigious Journal Biogeoscience.

We appreciate all your support on this submission, best regards.

Dr. Martín F. Soto Jiménez
ICMYL-UNAM, México
Email: martin@ola.icmyl.unam.mx

Reviewer 1

This paper presents a large survey of macroalgal d13C. The authors attempt to explain the wide variation in the observations by comparing d13C 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 d13C 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 is 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 acronymous 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 δ^{13}C-macroalgal variability).

A basic statistical analysis of δ^{13}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 δ^{13}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 δ^{13}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 δ^{13}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 δ^{13}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 pCO2 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 interpterion 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 d13C 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 d13C database to explain their variations in the function of phylogenetic, morphological, and environmental parameters. We have a substantially improved paper.

Macroalgal d13C is actually two variables in one: DIC $\delta^{13}C$ plus fractionation. Part of the variability in their results is due to differences in DIC $\delta^{13}C$. For example, when salinity changes, DIC d13C changes, and consequently macroalgal $\delta^{13}C$ changes as well. I believe the authors did not measure DIC $\delta^{13}C$ (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 d13C will probably be very uniform, so most variation in macroalgal d13C 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 $\delta^{13}C$. If they don't have DIC $\delta^{13}C$ 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 $\delta^{13}C$-DIC. However, the dataset was non-included in the old version. In this revised version, the $\delta^{13}C$-DIC values in the Gulf of California surface seawaters were included as Supplementary Information in Fig. S1. In agreement with the preliminary data, the $\delta^{13}C$-DIC in the GC seawater averages 1.4±0.4‰ (-1 to 4.9‰) (Fig. S1).

Because $\delta^{13}C$-macroalgal depends on the isotope discrimination during carbon assimilation in the photosynthesis ($\Delta^{13}C$), we calculated the $\Delta^{13}C$ by subtraction of $\delta^{13}C$macroalgae to $\delta^{13}C$-DICseawater in the GC. In our concurrent analysis we

observed that δ^{13}C-DIC in the GC surface seawater is relatively constant and uniform, thus, the influence of the δ^{13}C-DIC variations to the Δ^{13}C-macroalgal variability was negligible. Based on the integrative discrimination factor against 13C, five groups were identified: one for Chlorophyta (Δ^{13}C=16.0±3.1‰), two for Rhodophyta (16.6±3.8‰ and 34.6±1‰), and two for Ochrophyta (9.1±1.7‰ and 15.7±2.7‰). Values δ^{13}C-macroalgal reflect mainly the discrimination during carbon assimilation (Δ^{13}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 δ^{13}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 δ^{13}C and Δ^{13}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 δ^{13}C-macroalgal.

In our study, the δ^{13}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 δ^{13}C-macroalgal constitute an excellent tool to study acidification and eutrophication, however, the δ^{13}C-macroalgal must be first evaluated.

Reviewer 2

Authors have collected and measured an impressive number of samples to establish the relationship between d13C-microalgal and biological as well as environmental parameters. The statistical analyses are robust and helps to understand the correlation. However, one of the major flaws of this work is that authors only focused on describing the numbers, but do not go beyond the dataset. The underlying processes behind the observed relationships are required to be explained to gain a bigger picture. I believe that the manuscript has a scope to improve and can provide useful concepts related to this field. Thereby, I suggest to accept the manuscript after moderate revision if authors agree to incorporate the given suggestion.

R.Reviewer criticisms referent to go beyond the dataset, is right. We appreciate the time and recommendations to improve our MS.

In this revised version we focused in three objectives. Based on a large inventory of specimens collected during five years along Gulf of California coastlines,

1) we pretended to explain the δ^{13}C-macroalgal variability in function of taxonomy (phylum, genus, and species) and morpho-functional groups (e.g., thallus structure, growth form, branching pattern, and taxonomic affinities), and with the interaction to environmental conditions in shallow marine habitats. The taxon-specific photosynthetic DIC acquisition properties related to the intrinsic characteristics of each morpho-functional group of macroalgae (e.g., thallus structure, growth form, branching pattern, and taxonomic affinities) are determinants for the δ13C-macroalgal signals. Changes in the habitat features and environmental conditions also influence on δ^{13}C signal. The full model considering the combined effect of the life form, coastline sector, and environmental conditions explain until 62 (morphological groups) and 72% (genus) of the variability. The effect of the coastal sector, pH ranges, and emersion level was significant, while for salinity and temperature negligible.

2). By using the δ^{13}C-macroalgal to infer carbon uptake strategies in macroalgal shallows communities of the Gulf of California. In agreement to the literature 4 carbon uptake strategies has been identified. We used our number to identify those strategies in each specimen. The facultative uptake of HCO_3^- and CO_2 (strategy 2: -10<δ^{13}C>-30‰) is the most common strategy identified in macroalgal shallow communities in the GC and worldwide. The carbon uptake strategy 1, that use only HCO3-, was the second in importance. The higher proportion of CCM species (HCO3- users) was expected, because we focused on intertidal and shallow subtidal habitats featured by high-light intensities. Only three non-calcifying species (*Schizymenia pacifica*, *Halymenia* sp., *Gigartina* sp.) belonging to Rhodophyta (3%)

were CO2 exclusive users (strategy 3: $\delta^{13}C$<-30‰). Calcifying macroalgae genera *Amphiroa* and *Jania* using HCO_3^- and diffusive CO_2 influenced by the calcifying process, represented the strategy 4.

3) we explored any geographical pattern in the $\delta^{13}C$ macroalgal along and between the GC bioregions. Literature report significant correlations between $\delta^{13}C$ signal and latitude, mainly related to the light and temperature. In the latitude range (21º-31ºN) in our study, the linear regression analyzes showed a low correlation for the δ13C macroalgal dataset. Because the shallow habitats occupied by macroalgal communities in the GC were high-light environments with narrow ranges in temperature, not clear patterns along the GC latitudes. However, detectable changes were observed in the $\delta^{13}C$-macroalgal and in the proportion of specimens with different carbon uptake strategies among coastal sectors.

Our research is the first approximation to understand the $\delta^{13}C$ 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 $\delta^{13}C$ or $\Delta^{13}C$-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 dilucidated the complex processes controlling the $\delta^{13}C$-macroalgal.

The minor comments are listed below:

Specific comments

Abstract

Line 18: Replace ‘C’ by carbon

R. Done.

Line 22 and throughout the text: The stable isotopic composition should be referred up to one decimal point.

R. Done.

Line 22 or 26: Kindly mention the environmental parameters included in this work.

R. Done.

Line 36: The ending of the abstract seems very abrupt.

R. Done.

Introduction

Line 42: The information should be substantiated by appropriate references.

R. Done.

Line 60: closing bracket is missing.

R. Done.

Line 71: The information should be substantiated by appropriate references.

R. Done.

Line 87: The information should be substantiated by appropriate references.

R. Done.

Line 88: The comparison between microalgal and terrestrial plant d13C is useless unless the authors describe the later with facts and references.

R. The sentence was deleted, it was not relevant for the MS.

Line 96-102: This section must come before the previous to justify the study site selection.

R. The paragraph was moved in agreement with the Reviewer's recommendation.

Study area

Line 111: Please mention a few of the endemic specimens to lure broader audience.

R. Examples of endemic specimens includes Chlorophyta (*Codium amplivesiculatum*), Rhodophyta (*Laurencia papillosa, Chondracanthus squarrulosa, Gracilaria spinigera, Gracilaria subsecundata*), and Ochrophyta (*Cutleria hancockii, Sargassum herphorizum, Sargassum johnstonii*). A paragraph mentioning these endemic specimens was included in the section 3.1. Taxonomy and morpho-functional groups

Line 158: Please maintain a consistency referring GC.

R. Done.

Methods

Authors have performed the statistical analyses in detail. Such great length of description actually helps to understand the work. Impressive! Just a soft suggestion, please refer the relationship only in terms of adjusted R2, else it would be difficult to follow at places.

R. Done.

Section 4.2

Line 552-554: Weird sentence construction. Please re-phrase the lines.

R. Done. The sentences were rephrased.

Just don't mention the correlation coefficient. Dig deeper to explain why does pH have a weaker relation with the measured d13C values.

R. Done. A poor but significant correlation was observed between δ13C and pH (R2 = 0.04) (Table 4).

Figures

Most of the figures are very difficult to follow. Authors must improve the representations in graphical format.

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, Figures were improved in graphical format and new Figures constructed.

Figure 6. Replace d13C by d13C in the y-axis legend.

R. Done.

Figure 6 and 7: It is not understandable why does the authors provide trendline for a near-zero correlation (R2=0.04 to 0.07). Kindly, remove them. Also, the inset texts are not readable.

R. Reviewer is right. The trendline was removed.