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Interactive comment on "The effect of salinity, light regime and food source on C and N uptake in a kleptoplast-bearing foraminifera" *by* Michael Lintner et al.

Anonymous Referee #2

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General comments

The manuscript entitled "The effect of salinity, light regime and food source on C and N uptake in a kleptoplast-bearing foraminifera" by Michael Lintner et al. examined the influence of changing salinity, food sources, light regime, and starvation duration on food uptake by Elphidium excavatum, a kleptoplast-bearing benthic foraminifera. The isotopically labeled food sources were used for the experiment, and C and N uptake were evaluated. This study has fundamental importance on understanding the effects of various environmental factors, especially salinity, on benthic foraminifera, and interpretation of population changes in a highly fluctuating environment like Kiel fjord.

The manuscript is written clearly overall, but the lacks of explanation and careless mistakes are here and there. I feel the experiments are not well designed to investigate some targeted factors. In addition, there found some inappropriate usage of statistics, thus misunderstandings of the results, which makes overinterpretation or inappropriate derivation in the discussion. Unfortunately, I would say this manuscript does not fit to be published in Biogeosciences. The paper would be more improved if the following points are fully considered.

Major points

1. The amount of food

The amount of food provided is not mentioned in the text, so it is hard to evaluate the results. How much was provided? For the time-series experiments (experiment i and iii), was the food enough through the experimental period? If the food availability changes over time, it affects the food uptake accordingly. This point is very important in the experimental design. I assume that the food was provided once (at the start of the experiment). If the experiment is designed to keep the constant food availability, which I think is required in these experiments, please explain how.

2. Food sources

Except for experiment ii (food preference experiment), green algae (D. tertiolecta) was used as a food source despite it is already known that diatom is the more preferred food for the foraminifera (L320–321). Why did you choose green algae as a basic food source in the first place? In addition, in the end, you mentioned that both D. tertiolecta and diatom L. arenaria were not preferable food for E. excavatum (L265, L372). It sounds that the whole experiment was conducted under unsuitable conditions. How should we interpret the food uptake experiment using unfavorable food? I think it's okay that it finally turns out that the foods you chose were not incorporated that much as you have expected (it is how this kind of experiment goes). However, at least you need to explain the reason why these food sources were selected.

3. Marginal significant effect

The term “marginal significant effect” is frequently used in the text. The significance level of 0.05 was set as a threshold as you said (L183). Then, the p-values higher than 0.05 should be treated as “not significant”. Of course, the threshold is artificial, but that is the way of statistical tests based on the probability of a null hypothesis. You may set the significance level at 0.1, then you can say the p-value of 0.08 is significant, although the significance level of 0.1 is not commonly used in the field of natural science, I believe. In any case, “marginal significant effect” sounds awkward. You can describe the tendency of the results, but if it is not statistically significant, you should say so.

4. Incorrect result description

There can be found some wrong description of the results as follows: L224–225: Across both food types pC was lower at 25 PSU than at 15 and 20 PSU. —> This is not correct. According to the results in Table 3, in D. tertiolecta, pC was higher at 25 PSU (0.0547) than at 15 PSU (0.0463). L234–235: The highest pC was. . .while at higher salinities. . ..the C uptakes was higher when fed with D. tertiolecta. —> Again this sentence is not correct. The data in Table 3 shows that pC in the salinity level 25 was higher in L. arenaria (0.0780) than in D. tertiolecta (0.0547). This kind of error is fatal even if it does not make any difference in overall interpretation or conclusion. Please be very careful when you read the data and describe it. In addition, this kind of description of the data should be accompanied by the numbers (numerical data) and statistical test results. When you say something is higher/lower or increasing/decreasing, please clarify whether it is supported by statistical testing as well.

5. Data representation

In the figures, the horizontal axes are all “time [d]”, but it seems not appropriate. I suggest to use “Days after food addition” for Figure 1 and 2, and “Starvation duration” for Figure 3. I think it’s better to show all three plots (three replicates) in the figures, instead of showing the means and 2σ. Alternatively, please provide all the data used

as supplementary material. For Table 3, I recommend showing the results in a figure (e.g., bar plot with error bars). Numerical information alone is not easy for the readers to read the trends or differences.

Minor points

Usage of PSU: Practical salinity unit (PSU) is not an actual unit of salinity. According to Unesco (1985), the practical salinity scale defined as conductivity ratio has no units. So using PSU as if it is a unit is not appropriate. In addition, L89 “1 practical salinity unit = 1g salt per liter of water” is, strictly speaking, not correct. It is not the definition of practical salinity nor the derivative of its meaning. I think just explaining that you used practical salinity is enough since it is a regularly used representation of salinity in this field. I recommend using the wording like “salinity levels of 15, 20, and 25. . .” to avoid using PSU.

L18: Leyanella arenaria should be italicized.

L24: light/dark rhythm of 16:8 h —> In the method section, the light regime is 18:6 h. Which is correct?

L47: . . .shows large intraspecific variability. —> Variability of what?

L54: individuum —> individual

L80: ssp. should not be italicized.

L104: Materials —> Materials and methods

L110–111: How about the light condition? Was in kept in dark or illuminated?

L124: ASW —> It appears once in the text, so it does not need to be abbreviated.

L126–129: Please briefly describe how these values (at% of the food sources and their C:N ratio) were determined.

L133: 20 foraminifera specimens —> How the specimens were selected? Did you use

a certain criterion for specimen selection such as test size, cytoplasm color, cytoplasm filling, activeness, etc... Please clarify.

L136: sea water —> seawater

L137: salt concentrations —> Since it is in practical salinity, salt concentration is not appropriate. How about saying “salinity” or “salinity level”.

L138: These salinities correspond to different areas.... ca. 20 PSU at sampling location. —> Please indicate which area corresponds to the salinity levels 15 and 25 as well.

L139: 18:6h light:dark cycle: What kind of light source was used? Did you measure PAR? If so, please include the information. Since the tested species hosts kleptoplasts, information on the light condition is important.

L140: labeled D. tertiolecta food was added. —> How much was provided?

L141: ...measured after 3, 5, and 7 days. —> In the other time-series experiment (experiment iii and iv), food uptake was measured after 1 day as well. Why day 1 was omitted in this experiment?

L150: ...fed for 24 hours with D. tertiolecta. —> Again, how much?

L144: light dark rhythm of 16:8h —> In the caption of Table 3, it says 18:6 h. Which is correct, 16:8 or 18:6 ?

L144: cells collected after 5d. —> Here, do “cells” mean forams? If so, please use “specimens” or “foraminifera” instead.

L152: washed three times with distilled water —> Washing with distilled water may cause the loss of cytoplasm. Was it okay?

L168–172: background value of foraminifera —> How many specimens were used to generate Xbackgroud? Were those foraminifera incubated for 24 h without food (the

time-zero specimens)?

L173–176: ind-1: Individual based values are not used throughout the text, so μg ind-1 and associating explanation can be removed.

L183: 95,0% —> 95.0%

L184: LSD —> Least Significant Difference

L191: marginal significant effect —> Please do not use this term. See the major comment 3.

L197: highly significantly —> Remove “highly”.

L199: At 15 and 20 PSU N uptake increased steadily from 3 to 7 days —> Is it supported by the post-hoc test? If so, please indicate the p-values for both salinity levels.

L199–200: ...while at 25 PSU C uptake remained... —> I assume “C uptake” should be “N uptake”, right?

L242, L244: 16:8 light:dark cycle —> In the method and the caption for Figure 2, it says 18:6. Which is correct?

L250–251: It is already explained in the method. To avoid redundancy, this part should be deleted.

L264–266: The low level of ingested D. tertiolecta in comparison to other studies... —> If you compare the results of this study to the others, please include the value or range of the food uptake for the referring studies.

L268: marginally significant preference —> If the statistical result is not significant, you cannot say any preference. Therefore, the following sentence, “It is therefore likely that ...”, would not be the inference from the results.

L272–274: A shift in food preference ... —> I doubt that their result of experiment ii shows any preference of algal types. In the first place, as I pointed out before, pC from

the green algae at salinity level 25 is lower than that from the diatom. So this sentence is not correct from this aspect. In addition, I wonder the discussion of “food preference” from the pC and pN is possible. Since the C and N content of the two food sources differ, the resultant pC and pN may not reflect the preference of food. Moreover, what they detect from their experiment was not the gross C and N incorporated from the diet, but the net C and N in the foraminiferal cytoplasm after respiration and metabolization, as you also mentioned in the discussion. If you really want to know the preference of the diet, different experiments should be designed. Here, maybe the word “preference” is confusing. I think rephrasing the word “preference” to just “uptake” would make sense for some parts. Please consider this point.

L275–276: . . .given that N retention was approximately 3-fold higher with diets of green algae. . .—> The difference in resultant pC:pN (2.2-2.7 for D. tertiolecta and 6.4-7.5 for L. arenaria) may not be accounted for the difference in N retention. Please consider that the original C:N ratio of the food differs for the green algae and diatom. It is shown that the green algae have a higher N (lower C:N ratio) compared to the diatom (L128–129). Therefore, the pC:pN may simply reflect the C:N of each food source.

L293: After 1 day, . . . —> I cannot find the result after “1 day” for the salinity experiment (Fig. 1). In the method, it says “Food uptake was measured after day 3, 5, and 7.” So it maybe “After 3 days”? Please confirm it.

L294: the 15 PSU series is. . .with a positive slope. . .—> Is the “positive” slope supported by statistics? According to Table 2, the results from the salinity experiments do not show any significant effect on pC.

L315, 321: Elphidia —> Elphidium?

Section 4.2: I failed to understand the points of discussion in this section. You pointed out the two possibilities to explain the difference in food uptake under different light conditions. One explanation is that under dark condition, foraminifera consumes their own chloroplasts, which results in low food uptake for this group. It is understandable.

As the second explanation (paragraph from L339), you say “indirect light effects”, which I assume is about the possibility of the inactivation of chloroplasts. However, how this possibly affects food uptake is not clearly explained. Please clarify this point.

L336: . . .these individuals contained fewer functional chloroplasts from the beginning. . . —> This sentence ruins the earlier discussion in this paragraph. It somehow sounds strange for me that this study focuses on kleptoplast-bearing foraminifera, whereas the specimen used contained fewer plastids (although whether the plastids content is enough cannot be evaluated). I think the discussion relating the function of kleptoplasts should be toned down if you think the specimens used do not represent the general feature of kleptoplastidy.

L357: ..(marginal) significant effect —> It should be treated as “not significant” as I noted before. Please reconsider the tone of the discussion based on such results (results representing some trends but with no statistical support).

L369: . . .we found a slight preference . . .for the tested diatoms . . .over green algae. . . —> Talking about “preference” is not appropriate. Please see the above comment for L272–274.

L378–389: In this part, the influences of high salinity on test morphology (size and abnormalities) are addressed. However, I failed to understand how it relates to the results of this study (i.e., the effect of salinity on food uptake). I assume that you try to relate the higher stress at higher salinity from the aspect of morphology and food uptake. However, since no test abnormalities was observed at the highest salinity level in this study, I suggest to tone down this part. I think it is also safer to tone down the last sentence, “E. excavatum was very good adapted to the brackish milieu of the Kiel Fjord (L389)”.

L393: shoes —> shows

Figure 2: In my understanding, the “light data” is the same as the one in Figure 1

(salinity level 20). Please clarify it in the caption. Moreover, the plots of the “dark data” and their error bars are not aligned vertically. Please correct it.

A schematic diagram illustrating the experiments is very helpful to understand this study. Please consider to add this kind of figure.

I hope my comments above would be helpful.

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