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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 #1

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This manuscript describes the effect of different environmental parameters (salinity, light and food source) on the carbon (C) and nitrogen (N) uptake by a benthic kleptoplastic species: Elphidium excavatum. Individuals collected from Kiel fjord were incubated with different type of algae enriched in 13C and 15N, and subsequently analyzed by EA-IRMS to estimate their C and N uptake. Introduction and Material and method sections read well. I have only minor comments for those. However, one of my main concern is the section 4.2 about influence of light, where you seem to assume that kleptoplasts in E. excavatum are photosynthetically functional while this has never been proven. You even cite Lopez (1979) "Lopez (1979) detected functional chloroplasts in E. excavatum after 7 days of starvation.". This is wrong, he did detected chloroplasts

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in E. excavatum, but he did not see any C uptake and conclude they are unlikely to be functional and are even merely food items digested by the foraminifera. Please refer to my comment below. If that matter is changed in the text and it is clearly indicated that, yet, there are no proof that chloroplasts from E. excavatum are photosynthetically active, actually it seems from Lopez' (1979) study that chloroplasts in that species are inactive, then I would recommend publication. Despite this concern, I found the study well designed and the manuscript well written. Conclusions are supported by the results presented in the manuscript, and statistical analysis are adapted to the data. Therefore, I recommend publication after some revisions detailed below.

Main comments:

Results

Overall, the result section lacks numbers, i.e. you do not cite numbers to illustrate your description. I would prefer the numbers to appear in the text, especially since you chose to use graphs as figures. We can only infer very approximative values from these. Therefore, I think integrating the exact numbers in the text would ease the reading and the understanding of your data. Also, I found the description sometimes a bit messy and confusing and I recommend rewording some parts (detailed below). Finally, I have a few very minor comments (mainly cosmetics, detailed in the second part of my comments). Line 194: You state that "after 3 days of feeding, E. excavatum showed the lowest pC values at salinities 15 and 25 PSU". While if I look at figure 1, the value corresponding to 25 psu seems higher, even equal to the value of 20 psu. Please clarify. Lines 203 – 204: "After 7 days ($p<0.01$), the maximum of pN was observed at 20 PSU and decreased at 15 PSU and further at 25 PSU.". I found this sentence a bit confusing, the word "decreased" suggest that the N uptake decreased after 7 days for 15 psu and 25 psu, which is not the case for 15 psu. I think you meant that the value at 15 psu was lower and that the value at 25 psu is even lower. Please clarify. Lines 243-245: Wording is confusing, I suggest: "The negative effect of continuous darkness was also observable on pN ($p=0.026$). Despite this negative effect, pN tended to increase

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with time (p=0.091), particularly so under 16:8 h light:dark cycles." Lines 255 – 259: I found it disturbing that you first state that food uptake increase but that you say the line after that C uptake showed no significance time effect. I think it's misleading in the way that the reader will remember the first key sentence "there is a food uptake increase", while according to your statistical analysis this is not so clear. I agree that from the data it seems that there is a "tendency" of increased C uptake, that could eventually be checked with a longer incubation time (which you could suggest). Please consider rewording this section.

Discussion

The discussion is well written but, in a few occasions, I found that it lacks comparison with existing literature. You keep referring to the same references, papers from your lab, which is fine. But there is other existing literature to be taken onto account. Also, Section 4.2 is very speculative but should remain in the manuscript, but as I mentioned in the introduction, please specify that kleptoplasts from E. excavatum were never proven to be photosynthetically active. Some sentences have thus to tone down. Please find below some suggestions and some references to be added in the text. Lines 287 – 290: Here you mention that C can be transferred to the test and therefore lead to an underestimation of C uptake. I agree that this is important to take into account. Please consider commenting existing publications that recorded a transfer of C from the food source into the calcified test. For example, Lekieffre et al. 2017 (PloS One), measured the assimilation of 13C in the calcite test during a similar experiment involving feeding with 13C-labeled diatoms. Lines 293 – 301: in this section you talk about "reduced metabolism" and stress from different salinity levels. I strongly suggest that you elaborate on that. There are already quite a few examples of reduced metabolism due to stressful conditions in the literature (see Bernhard and Alve, 1996 Marine Micropal, Ross and Hallock, 2016 JFR, etc.). Bernhard, J.M., Alve, E., 1996. Survival, ATP pool, and ultrastructural characterization of benthic foraminifera from Drammensfjord (Norway): response to anoxia. Marine Micropaleontology 28, 5–17. LeKieffre, C., Spangen-

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berg, J.E., Mabilleau, G., Escrig, S., Meibom, A., Geslin, E., 2017. Surviving anoxia in marine sediments: The metabolic response of ubiquitous benthic foraminifera (Ammonia tepida). PLOS ONE 12, e0177604. https://doi.org/10.1371/journal.pone.0177604 Ross, B.J., Hallock, P., 2016. Dormancy in the Foraminifera: A review. Journal of Foraminiferal Research 46, 358–368. https://doi.org/10.2113/gsjfr.46.4.358

Lines 293 – 301: Also, I think it would be very interesting to add a small paragraph about the increase of lipid droplet accumulation observed in benthic foraminifera that encounter stressful conditions. Indeed, you state that "low salinities (...) may lead to a generally lower activity of foraminifera, which reduces their cell respiration and results in a lower C output." (line 296). This lower C output could be linked to the accumulation of lipid droplets which seems to be a common response of benthic foraminifera in response to stressful conditions such as anoxia or heavy metals contamination. Here are some references: Frontalini, F., Curzi, D., Cesarini, E., Canonico, B., Giordano, F.M., Matteis, R.D., Bernhard, J.M., Pieretti, N., Gu, B., Eskelsen, J.R., Jubb, A.M., Zhao, L., Pierce, E.M., Gobbi, P., Papa, S., Coccioni, R., 2016. Mercury-pollution induction of intracellular lipid accumulation and lysosomal compartment amplification in the benthic foraminifer Ammonia parkinsoniana. PLOS ONE 11, e0162401. https://doi.org/10.1371/journal.pone.0162401 Frontalini, F., Curzi, D., Giordano, F.M., Bernhard, J.M., Falcieri, E., Coccioni, R., 2015. Effects of lead pollution on Ammonia parkinsoniana (foraminifera): ultrastructural and microanalytical approaches. European Journal of Histochemistry 59, 2460. https://doi.org/10.4081/ejh.2015.2460 Koho, K.A., LeKieffre, C., Nomaki, H., Salonen, I., Geslin, E., Mabilleau, G., Søgaard Jensen, L.H., Reichart, G.-J., 2018. Changes in ultrastructural features of the foraminifera Ammonia spp. in response to anoxic conditions: Field and laboratory observations. Marine Micropaleontology 138, 72–82. https://doi.org/10.1016/j.marmicro.2017.10.011 Le Cadre, V., Debenay, J.-P., 2006. Morphological and cytological responses of Ammonia (foraminifera) to copper contamination: Implication for the use of foraminifera as bioindicators of pollution. Environmental Pollution 143, 304–317. https://doi.org/10.1016/j.envpol.2005.11.033

Section 4.2: Finally, as I mentioned in my general comments, I found disturbing that in some places you seem to assume that kleptoplasts in E. excavatum are photosynthetically functional while this has never been proven. For example, line 341: “chloroplasts may therefore have become inactive”. Please state clearly throughout the ms where necessary that kleptoplasts from E. excavatum were never proven to be photosynthetically functional. Therefore, all assumptions based on that are purely speculative. It could also be added that one of the main. Line 331: “Lopez (1979) detected functional chloroplasts in E. excavatum after 7 days of starvation. Âż This statement is wrong. If you read carefully his manuscript, he says twice that chloroplasts are inactive (once in the result section: “The slopes of the lines indicate that Elphidium williamsoni on an average takes up inorganic carbon at a rate about 5 times that in Nonion germanicum, and that the chloroplasts in E. excavatum are inactive with respect to primary production.”, and in the discussion: “In accordance with this, no light-induced uptake of 14C-HCO$\sim$ by the chloroplasts in E. excavatum could be detected, which means that they are probably merely food items being digested by the foraminiferan.”. To my opinion, regarding Lopez’ results and your own statement that your specimens were yellowish, your second hypothesis is unlikely. I am fine with you keeping it in your manuscript but Lopez (1979) should be cited correctly and it should be mentioned that E. excavatum chloroplasts were never proven to be functional, and that is rather unlikely.

Minor comments:

Introduction

Line 51: “Under anoxic conditions” is not relevant in this sentence. Lines 57 and 58: I assume “Thierry et al. 2016” refer to “Jauffrais et al. 2016”, please make the modifications. Lines 63 – 64: Use conditional here, there is no evidence in the literature yet that this is the case or that kleptoplasts found in foraminifera from below the photic zone are functional. I suggest the following modifications: “These aspects show SUGGEST that foraminifera can not only incorporate chloroplasts for photosynthetic activity, but MAY also benefit from other catabolic mechanisms (LeKieffre et al., 2018)”. Lines 97

– 102: I suggest to remove this paragraph as environmental impacts induced by anthropogenic activities is not the subject of your study and is not discussed in the later sections.

Material and methods

Lines 152 – 153: Please specify how many foraminifera were put per tin capsule. Lines 151 – 157: I assume you weighted your capsules before putting the foraminifera inside but this is not mentioned in the text. Please clarify.

Results

Line 199: “while at 25 PSU C 200 uptake (. . .)”, I think you meant “while at 25 PSU N 200 uptake (. . .)”. Please make the modification. Figure 1: Please remove the half days on the x axis (1.5, 3.5, etc.) as you did for the other figures. All graphs: I would suggest to increase the size of the axis legend. It is yet quite small and it would be nicer for the reader to know immediately which graph is pC or pN.

Discussion Line 262: As you also discussed the effect of the type of food, I suggest to add it in the section title.

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-306/bg-2020-306-RC1-supplement.pdf

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