Biogeosciences Discussions

Biogeosciences Discuss., author comment AC2
https://doi.org/10.5194/bg-2021-74-AC2, 2021

Reply on RC2

Nicolai Schleinkofer et al.

Author comment on "Host-influenced geochemical signature in the parasitic foraminifera *Hyrrokkin sarcophaga*" by Nicolai Schleinkofer et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-74-AC2, 2021

Reply to Reviewer 2 (Inge van Dijk)

We want to thank the Reviewer Inge van Dijk for her thorough review, that helped us greatly in improving our manuscript by providing useful remarks and comments.

Mayor Comments

Reviewer 2:For the EPMA analysis, I believe some more investigation is necessary to support the claims of the authors on the co-variation of certain elements (e.g. high Mg corresponds with high Na and S.) This can be done several ways, e.g. plotting the map counts of Mg versus other elements, or calculating line transects of the different elements (see for example my own protocol for foraminifera in van Dijk et al., 2019, https://doi.org/10.3389/feart.2019.00281).

Answer: We agree with the reviewer's statement, regarding the EPMA analysis, which was not adequately described and lacked information. We have added a further figure as proposed by the reviewer and added more information to the results section to better explain the results (L.255-270).

Reviewer 2: For the mixing model, I would like to see some changes in the model parameters, mainly for Mn and Sr. The chosen DMn from Mucci, 1988 is based on inorganic precipitation, and DSr from Raitzsch et al., 2010 is from a high-Mg species, which in general have higher D values. I would propose to use DSr derived from a mid-Mg species like Amphistegina, which is in the same range of Mg/Ca. This will likely greatly change the results of Fig. 6 and the current interpretation and discussion. The model is a bit to simplified in my opinion, and it ignores a lot of observations on foraminiferal calcification of direct uptake of the elements during calcification through trans membrane transport (Nehrke et al., 2013; Toyofuku et al., 2017). Even when following seawater endocytosis model of Erez, 2003, the seawater is not unmodified when it is available for the foraminifer, as the model assumes. For example, Mg ions are likely pumped out of the seawater, before it is available at the site of calcification as calcifying fluid. I understand that there are still a lot of unknowns in foraminiferal calcification processes and the authors cannot include all in their mixing model, but maybe they can add a step/fractionation from seawater to calcifying fluid (which can be based on e.g. the offset between distribution coefficients observed for inorganic calcite and foraminifera).

Answer: In addition to the distribution coefficients we initially used, we also added plots

with the distribution coefficients suggested by the reviewer (Fig. 7). As the reviewer says, changing the distribution coefficients used in the model results in different absolute values that are calculated. However, it does not change the statement we want to make with the model, namely presenting a possible explanation why we do see changes in the Sr/Ca and Mn/Ca ratios of *H. sarcophaga* influenced by the host, but not in the Mg/Ca and Na/Ca ratios (see also our answer to Reviewer 1).

Considering the composition of the initial calcifying fluid, it might be appropriate to use an initial composition that is different from seawater with regards to Mg. The reviewer states that this could be based on an offset between inorganic and foraminifera distribution coefficients. Again, this would not change the main point of the model. We think that such a correction would make the model less "simplified" but not necessarily more realistic. As the reviewer says, there are a lot of unknowns in the calcification mechanism of foraminifera and we think that if we make the model less simplified, we are introducing more assumptions. Nevertheless, we added a section which states the possible modification of the seawater composition and how it influences the outcome of the model (L.452- 461).

Specific Comments:

Reviewer 2: Figure 1: Was this specimen embedded in resin and cut/polished? Please give some details about the preparation/treatment of the sample.

Answer: Yes, the specimen was embedded in resin, cut and polished. We added the information to the text (L129)

Reviewer 2: L89: This is a bit unclear for me. What does HAO stand for, and why is HAW (with callus according to L88) included in HA, which are H. sarcophaga that infested A. excavata without callus formation.

Answer: HAO stands for *Hyrrokkin* on *Acesta* "ohne" (german for without) callus. HAW on the other hand for *Hyrrokin* on *Acesta* with callus. HA is used for samples that grew on Acesta but without a further distinction if a callus was formed -> HAW +HAO = HA (L89)

Reviewer 2: L91-93: For this study, you picked/selected only specimen that were firmly attached to the host?

Answer: Yes, every specimen was still attached to the host when we picked them for analysis. We added this information to the manuscript (L92).

Reviewer 2: L94-113: I prefer to put sample preparation with the paragraph of the method, e.g. L94-98 in 2.4. It makes the methods section much easier to read.

Answer: We agree with the reviewer and have included the sample preparation in the methods section.

Reviewer 2: L96: Which type of resin was used and was the embedding done in vacuum to avoid air bubbles etc.? Where the shells cleaned in anyway?

Answer: We used Araldite 2020 in a vacuum. The shells were ultrasonically cleaned in MilliQ-water prior to embedding. We added this information to the manuscript (L103).

Reviewer 2: L97: I am normally polishing until 0.2 um using a diamond emulsion. With 3 um, is the surface smooth enough? Was a previous method followed for the preparation?

Answer: The sample polish can have significant effects on EPMA measurements. Very

rough ground samples (140 µm) can lead to intensity deviations of up to 20 %, which, however can be accounted for by choosing the right measurement parameters (Rönnhult et al., 1987). When using high acceleration voltages (15 keV) the effects of rough surface polish are already minimized (Merlet and Llovet, 2012). "Out of focus" effects are only evident when using grits above 20 µm and rough surface polish does also mostly affect L intensities, whereas K intensities are very tolerant towards surface roughness (Rönnhult et al., 1987). In our case, only Sr was measured using L intensities. As our results considering Sr (and Na and Mg) are consistent with the results from ICP-OES we do not expect that the surface polish has significant influences on the results. Finer grid polishing can lead to problems as well, especially in porous biogenic samples, like diamond or corundum grains embedded in the sample.

Reviewer: L103 and again at 108: No kind of e.g. reductive and/or oxidative cleaning?

Answer: We cleaned the samples ultrasonically, but did not perform oxidative/reductive cleaning on the samples. We added this information to the manuscript (L. 112)

Reviewer: L105: Were the Eppendorfs acid cleaned?

Answer: Yes, every vial in contact with the samples was acid cleaned with 5% HNO_3 at 45°C (L. 131).

Reviewer 2: L125: type of microscope?

Answer: The microscope used was a KEYENCE VHX-S660E (L. 99)

Reviewer 2: L135-142: Samples were carbon coated? How many maps of how many specimens were taken? What kind of areas were selected?

Answer: Yes, the samples were carbon coated. We added this information to the manuscript (L 115) We only took maps of two separate specimen (which are both in the manuscript). The maps were taken in the area of the callus to get a better understanding about their composition.

Reviewer 2: L141: You preformed maps, lines or point measurements, and how many? With maps, how did you exclude resin / pores?

Answer: We made two maps of the callus area of two specimen of *A. excavata* that are included in the manuscript. We excluded resin/pores by excluding areas under a certain Ca cps level (7000 cps). In addition, we performed point measurements (n = 49) in certain points of interest (fluorescent, non-fluorescent layers, foraminifera) to provide quantitative measurements in addition to the semiquantitative maps. These measurements are the basis for Fig. 2. We added the number of samples to the figure caption.

We also did point measurements on the samples far qualitative measuring (Fig 2) in the different shell regions with n=11, 5, 17, 16 for calcite, aragonite, SRZ and *H. Sarcophaga*, respectively (L. 225)

Reviewer2: L149-157: Sample were diluted to solutions of e.g. 10 ppm Ca?

Answer: Yes, sample material was diluted to reach a concentration of 25 ppm Ca (L. 137).

Reviewer 2: L152 and 159/160: What was the accuracy of the standards?

Answer: The accuracy of the ICP-OES measurements, reported as %-deviation from

standard reference materials MACS-3 and JCP (Jochum et al., 2005) was better than 1% for Mg/Ca and Sr/Ca, 3% for Mn/Ca and 5% for Na/Ca (L.138-142)

Accuracy of The Mn/Ca ICP-MS measurements is 7% (%-deviation from standard material ECRM 752 (Greaves et al., 2005)). We added the information to the manuscript (L140 & L 154)

Reviewer 2:L159: What was the measurement precision?

Answer: Precision of the Mn/Ca ICP-MS measurements, reported as relative standard deviation (RSD%) is better than 1%. We added this information to the manuscript (L156)

Reviewer 2:L187: What test was used to compare the point measurements by EPMA (Fig. 2)

Answer: We added a table with the results of a Wilcoxon-Mann-Whitney Test, to investigate if the different regions have significantly different means (Table 1, L. 227).

Reviewer 2: L190: Was a Bonferroni correction applied to the obtained p-values?

Answer: Yes, Bonferroni correction is applied during calculation of the p-values (L. 203).

Reviewer 2: L197: Supplement S1, not S2.

Answer: Corrected

Reviewer 2: L203: You mean especially in the foraminifer?

Answer: No, we mean foramen. The organic matter is mainly concentrated in the openings of the test. We adapted the wording to "test apertures" to avoid potential misreading (L205)

Reviewer 2:L205: Is there also a high magnification image available of the SRZ? Maybe this can be added as an insert in Figure 1, to show the thickness of the organic layers.

Answer: No, there is no higher magnified picture. We added the original .CR2 and .tiff pictures to the supplement that have a better resolution

Reviewer 2: L206-211: The authors describe in detail the shape and size of e.g. the bore canal and layering in SRZ. Did they also obtain images from other SRZ and canals on other specimens to check the variability?

Answer: We did also obtain picture from other specimens, where we observe no strong variability. We kept this section rather short, because the morphology of traces produced by H*. sarcophaga* on the host is already extensively covered in Beuck et al. (2008)

Reviewer 2:L223: How many measurements per sample? – could be added to the method section

Answer: Each sample was measured three times. The reported values are the means of these three measurements. We added this information to the method section (L131 & 162)

Reviewer 2:L225, L228, 235 and in general throughout 3.3. as well as 3.4.: Are these similarities or differences significant or not? These statements need to be supported by statistics (e.g. Kruskal-Wallis test with obtained (Bonferroni corrected) p values).

Answer: We added a table, displaying the results of a Wilcoxon-Mann-Whitney test to show if the differences are significant (L227)

Reviewer 2:L231: Na/Ca in both region E1&2 is exactly 14.8 mmol/mol? Or on average for both area?Please clarify, or better, give values for both regions and show they are not significantly different.

Answer: We want to thank the reviewer for that remark. The mean value of 14.8 mmol/mol for Na/Ca is only true for the microgranular calcite layer (E_2). We have not measured the fibrous shell layer by EPMA, because it is basically completely dissolved by the foraminifera. The fibrous shell layer is commonly slightly depleted in Na/Ca in comparison to the microgranular layer (Schleinkofer et al., 2021)

Reviewer 2: 3.3.: Could you add the standard deviation to the mean ratio in the text? For comparison with other studies this comes in handy.

Answer: We added standard deviations in the text.

Reviewer 2: 3.4.: There is a lot of information in the EPMA maps, but the authors use merely 2 sentences to describe the results. Please add more information, like what is the width of the high intensity layers, and do the correspond exactly to the fluorescence image (I presume Fig 1 and Fig3A are the same specimen?). What are min and max values of the different elements and values of the high and low intensity bands. Also: how were porous areas removed from the calculations, e.g. observed with Ca counts in Fig.3 panel A.

Answer: We agree with the reviewer that this section is too short. We have added information about the different composition of fluorescent and non-fluorescent layers, size of the layers and correlations between the elemental ratios. Generally, the fluorescent layers are enriched in Mg, Sr and S and are congruent with the layers revealed by fluorescence microscopy. Max Na concentration are also higher in the fluorescent layers than in the non-fluorescent layers. We observe significant correlations between Mg and S and Na and S in the fluorescent layers, indicating that these elements are incorporate into the bivalve's organic phase. We also added an additional figure to better present these results (L. 255- 270, Fig. 4).

Reviewer 2: L247: The covariation or colocation of elemental bands is difficult to distinguish by eye. Authors claim Na and S are enriched at the same location, but looking at Na and S of Fig. 3, I disagree For instance, Na and S of Panel A: the green band of S in the middle of the map seems to correspond to the blue band (low intensity) in the Na map. How did the authors check the covariation of elements? Merely by eye is not sufficient in my opinion.

Answer: We added a Figure (Fig. 4.) that shows the correlation between the measured elements as well as extending the results section for the EPMA maps. Covariation plots show a significant correlation between Mg and S and Na and S in the fluorescent layers. The reviewer is right with the statement that the described layers in Panel A apparently do not follow the described distribution. However, on close examination one can see that the described bands (green S band, blue Na band) are not exactly congruent. The blue Na band starts slightly above the green S band. These layers are the first layers of the callus, we expect this to be an effect of the disturbed calcification process.

Reviewer 2: L253: Again, how many measurements are included in fig 4?

Answer: The number of samples used is stated in the method section (L. 128). We added information about repeated measurements on the same samples (L. 131).

Reviewer 2:L261: Maybe add d18O and d13C values as a line to Fig. 4

Answer: We added lines that show the isotopic composition of the ambient water to the figure.

Reviewer 2: L324: how many specimens and host?

The amount of specimens is stated in the method section (L128 &149 & 159). We added more information about the number of host specimen (L143)(n= 3)

Reviewer 2: L329: Any idea if this is a chemical or a mechanical penetration of the host shell?

Answer: An extensive discussion of the traces produced by *H. sarcophaga* can be found in Beuck et al. (2008). These authors state that there is indication for chemical penetration indicated by the xenoglyphic surface texture of the boring and variability with the penetrated host microstructure (Beuck et al., 2008). We added this information to the manuscript

Reviewer 2: L369/Table 3. The authors use partitioning coefficient of inorganic precipitated calcite. Please use published values of foraminifera i.e. 1) for small benthic foraminifera see Barras et al., 2018, or 2) for Amphistegina lessonii (which has similar Mg/Ca as the studied species), which can be calculated from van Dijk et al.,2020 Frontiers in Marine Science 8 (DMn ~1). Also, it should be noted that the partitioning of Mn is not stable and increases with decreasing seawater Mn/Ca (e.g. Barras et al., 2018).

Answer: We agree with the reviewer about the problematic distribution coefficient for Mn. We have chosen a distribution coefficient from inorganic calcite due to the high range of distribution coefficients reported for benthic foraminifera (Barras et al., 2018; Groeneveld and Filipsson, 2013 and refernces therein). We added the distribution coefficients suggested by the reviewer to the figure to present an alternative. However, different distribution coefficients do not change the message of this model. Even with the adapted distribution coefficient the model predicts changes in the Mn/Ca ratio of *H. sarcophaga* influenced by the host organism.

Reviewer 2: L549-551: Is this confirmed by the EPMA measurements of this study?

Answer: Yes, it is, we observe a significant correlation (r^2= 0.34, p=0.014) between Mg/Ca and S/Ca in the callus region as well as a significant inverse correlation (r^2= 0.5, p=0.0018) between Mg/Ca and Ca wt%. We added a new figure to show this information (L. 267)

Reviewer 2: L560: As mentioned before, in e.g. Fig3A I could not clearly see this simultaneous increase in Mg with other elements. Consider making line transect over this area to see and compare the location of the high intensity bands of different elements. This is also necessary to understand if S is increased in the carbonate, or present in organics between carbonate layers (The authors did not perform EPMA on samples which were oxidative cleaned for comparison?)

Answer: We added a Figure (Fig. 4.) that shows the correlation between the measured elements as well as extending the results section for the EPMA maps. We observe a significant correlation between Mg/Ca and S/Ca ratios as well as significant correlation between Na/Ca and S/Ca indicating the presence of Na and Mg in the organic phase of the bivalve.

Reviewer 2: L551: Again, did the authors made this comparison by eye, or by e.g. plotting

them overlapping?

Answer: We added an additional figure (L. 267) to show the stated correlations.

Reviewer 2: L617: How will the data be made available? Through doi / supplement?

Answer: The data will be included in the supplements.

Figures/Tables:
Reviewer 2: S1_Meigen Test. Please add a proper caption to this image, in the same style as the other figures.

Answer: We added a proper citation to the supplement image (S1)

Reviewer 2: Fig. 2 and 4: Maybe add the n above the boxplot. Explain that we are looking at boxplots, e.g. Boxplot distributions (line = median, boxes = interquartile range, whisker = min/max values). Are the measurements on different samples significantly different or not?

Answer: We added further descriptions about the boxplot in the figure caption. We also added a table with the results of a Wicoxon-Mann-Whitney test to show if the measurement results from different regions differ significantly from each other.

Reviewer 2: Fig. 3: Include more details in the caption. These are SEM images? What does A and B stand for (I presume different specimens)? Elements are in counts? What were the min and max counts per element (maybe give each of the their own quantified scale bar.

Answer: Yes, A & B are two different specimens of *A. excavata.* We added the counts to the caption of the figure.

Reviewer 2:Fig. 4&7: Please consider choosing another color for either HAW or L.pertusa.

Answer: We changed the colors to improve visibility.

Reviewer 2: Table 2/3. Tables and Figure should be readable without the main text. So please add the full names oF HAW, HAO, D etc in these captions.

Answer: We added descriptions of the abbreviations to the captions (L. 323 & 411).