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Biogeosciences Discussions

Interactive comment on "Elemental composition of invertebrates shells composed of different $CaCO_3$ polymorphs at different ontogenetic stages: a case study from the brackish Gulf of Gdansk (the Baltic Sea)" *by* Anna Piwoni-Piórewicz et al.

Anna Piwoni-Piórewicz et al.

apiwoni@iopan.pl

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We are grateful for this review, that will help us to improve the manuscript. We carefully read the comments and tried to answer all questions in a clear and concise manner.

Major comments:

Comment: I was surprised to read about the cleaning methods used in this study.

When investigate chemical composition of foraminiferal shells, we use a much more intense cleaning method with oxidation and reduction steps (especially when specimens are collected in the field), to remove any organics as well as diagenetic coating (like MnâARFeâARoxide coatings). I wonder if e.g. only mechanical removal of any organisms present on the shell is enough to remove any (organic or chemical) trace completely, and if coatings are present on the shell after the described cleaning protocol. Are there studies comparing different cleaning techniques?

Response: Concerning any surface contamination, the difference between the foraminifera and mollusk shells is the specific surface area, which is low in case of relatively large shells. The argument against chemical cleaning protocols was based on the observed preferential leaching of Mg (and, therefore, potentially many trace elements) out of carbonate skeletons during chemical cleaning observed by Loxton et al. (2017).

Comment: Furthermore, the amount of organic inside the CaCO3 might have a huge effect on the elemental composition of the shell, e.g. on Na, and the contribution of organics might differ between species and CaCO3 polymorphs, as also acknowledge by the authors (in l.54: 'species-specific organic matrix'). With the method described in this study, the organic matrix will also be analysed. I would like to see this issue discussed in the revised version of the manuscript. Has there been any study on the chemical composition of the organics in different species of bivalves, albeit on the compounds of the matrix or by microscale-analysis of the shell with e.g. nanoSIMS on cross-sections? This should be at least mentioned in the discussion as a potential reason for the offset between species, if not discussed in full detail.

Response: Removal of organics without mobilisation of any trace elements associated with CaCO3 is not a task that is easy to achieve. There are good studies on this subject, e.g., Barker et al (2003), Holcomb et al (2015), see also Loxton et al (2017) for further discussion of this issue. However, we do not believe there is a single accepted protocol for bivalve shells that is tested and validated for a large range of trace ele-

ments. We, therefore, have opted for the analysis of the bulk composition instead of trying to analyse selectively the CaCO3 phases. We will make it more obvious and discuss further in the revised version of the manuscript. In the revised version of the manuscript, we will emphasise that the variation observed could also be due to presence of organic material within the carbonate structure. This contribution should be minor relative to the major influence of the carbonate shell, while bivalve and barnacle shells contain in general up to 5% organic matter (Bourget; 2004; Rueda and Smaal, 2004), yet some patterns were found eg. for Mg and Sr (Walls et al., 1977; Lorens and Bender, 1980; Takesue and van Geen, 2004).

Comment: The authors are comparing small and big adults and use the obtained data to look at ontogenetic effects. However, the authors also state this is a very variable environment, with big seasonal (and maybe yearly) changes. Overprinted on the size effect, there is a time effect: the bigger specimens have recorded events that the smaller specimens did not experience. I'm missing the longevity of the different species in the discussion of the results. For example: The size effect observed for A. improvisus (life span = 1 year) might simply be a seasonal signal in food supply (and thus maybe growth rate) or physio- or chemical parameters like seawater temperature. If the samples are taken in end of summer (sample date not mentioned in the manuscript, should be added), it would explain why the Mg of the larger specimens is lower: lower temperatures lead to lower incorporation of Mg and these larger specimens likely experienced the winter period, while the smaller specimens maybe spawned in spring. As for the species with longer lifespans (of 10-12 years), could the decrease in element incorporation with size (thus, for older specimens) be due to increased heavy metal output of the Vistula river over the last years? Is there any (historical) data on this?

Response: In this study, individuals were collected in a wide range of sizes from each station, representing different ages and various periods of time, living under the influence of seasonal changes. The idea was to find any patterns related with the biological effect of organisms. This part of the discussion should include a more detailed envi-

ronmental background, which we will introduce (based on literature data) to draw more certain conclusions about the biological effect. We will put more detailed sampling information.

Comment: The authors see some difference between size classes, they conclude that in general smaller specimens have increased trace metal incorporation. Can this also be due to absorption of elements or diagenetic precipitation on the outside of the shell, compared to the more pristine CaCO3 below the surface, leading to a surface/volume effect? E.g. larger specimens have thicker shells, and thus lower surface over area ratio?

Response: This may be one of the reasons for the variability of metal concentration in shells of different sizes and we will include this aspect in the discussion.

Comment: I would also like to see habitat depth included in the discussion. The authors are assuming the shell chemistry of the different organisms tested are all reflecting either food or ambient seawater chemistry, but some organisms are living on hard substrates, while other live a few cm in the sediment, and the most extreme one (Mya arenaria) can live 20-30 cm in the sediment. The latter species would have a totally different "ambient seawater conditions", as it is exposed to interstitial water that is very likely to have totally different chemical signature then the overlying water. It would probably be in contact with e.g. much higher Mn concentrations. This is not reflected in the shell chemistry, so maybe this species does not take up elements from the seawater, but more from food intake? For your purpose it would be best to have also some kind of idea of the (evolution) of trace metal concentrations in seawater over time. Is there any chemical data on the seawater available from this area? E.g. about the metal concentrations close to the Vistula river (l. 478)? Is there data showing that station GN is indeed increased in heavy metals compared to the other stations? These other stations are located in a bay area, making it possible the residence time of the water is higher, and there might be an actual increase in the metal concentration here.

Response: The sediment type, feeding strategy and environmental sources of metals are important factors affecting the concentration of metals in shells and should be discussed. In the revised version of the manuscript, this subject will be improve. We will add information about sediment type in study area in the context of metal bioavailability. In sandy sediments, elemental concentrations are even several orders of magnitude lower than in silty sediments (Kim et al., 2004). We will also put more emphasis on discussing feeding strategy on the concentration of metals in shells. As previously mentioned, we will introduce a more detailed environmental background (based on literature data).

Comment: In retrospect, for the main goal of the study, it maybe would have been better to not analyze full shells, but make small aliquots/subsamples by e.g. drilling the shell. Is there any data (in literature) on small-scale variation in the shells of (some of these) species?

Response: There are such datasets e.g. for Mya arenaria (Strasser et al., 2008) and we will discuss them in the manuscript. Our strategy of investigating the whole shells was a deliberate choice in order to achieve better detection limits that would be possible with the spatially resolved analysis (e.g. LA-ICP-MS) and to concentrate on analyzing many individuals of different species. We presented data on the level of 12 metals in shells of mussels and barnacles from the Baltic populations, which have not been previously reported. Furthermore, we found some patterns of biological and environmental control over for the concentration of metals in shells.

Comment: In my opinion, at the moment, you have a combination of too many variables: CaCO3 polymorph, different stations environmental variables (incl. unknown chemical compositions of the seawater), size effect and the vital effect (calcification pathway) of the organisms. It becomes very difficult to disentangle different drivers of shell chemistry, which means you have to be more careful in your conclusions, or at least convince readers which variables are minor/neglectable. I think some variables, like the different sampling stations, can be convinced as being minor, by showing chem-

ical variability or the hydrological situations between the stations. The authors often point out the strong seasonality in this region, section 2.1 and through the manuscript, e g. l. 481-483. Maybe it is possible to add a (supplementary) figure to section 2.1, if needed compiled from literature data, about the environmental variability in this area, to show differences in physio-chemical parameters. This way, readers, like myself, that are not familiar with the study area can have a good overview of the (yearly) environmental variability in this area.

Response: The manuscript will present the trace element concentrations in calcitic, aragonitic and bimineralic shells and the patterns governing bioaccumulation of metals in shells. To make the manuscript more accessible for readers, the discussion will be divided into the three parts focused, respectively, on the polymorphic form of calcium carbonate, on potential environmental factors (based on literature data), and on potential biological control based on metal variability in shell size classes. As conclusions, we will distinguish patterns of inter-species and inter-individual variations in the concentration of metals in studied shells, which are associated with biological and environmental control. There is literature data regarding the concentration of some studied metals (mainly in sediments) around the study area (such as Rainbow at al. 2000; Rainbow at al., 2004; Szefer at al. 2002) and we will include this into the manuscript.

Minor comments:

(Since I have a lot of major points for the discussion section, I give minimal textual changes, since I believe the manuscript, especially the discussion, will probably greatly change after revision.) Throughout the manuscript:

Comment: Change ‘Mg/Ca ratio’ to ’Mg/Ca’.

Response: This will be improved in the revised version of MS.

Comment: Check manuscript for (double) bracketing issues, for instance in l.207: ‘(Darwin, 1854) (Arthopoda, Maxillopoda)’ should be e.g. ‘(Arthopoda, Maxillopoda; Darwin,

1854). Also lines 217, 228, etc. For l.131 and l. 515: reference should not have brackets.

Response: This will be improved in the revised version of MS.

Abstract:

Comment: The abstract as it reads a bit stiff. Please consider rewriting this section. For example, l. 28-29 on sample location can be merged with the first sentences, while line 29-30 is an explanation of the method, which should be either removed, or shortened, in my opinion.

Response: This section will be rewritten to improve the structure and to better introduce the reader to the content of the manuscript.

Comment: l. 26-27: 'The potential impact of environmental factors on the observed elemental concentrations in the studied shells is discussed': Is this really the case? Since there is no data on the environmental parameters presented, it is difficult to discuss the data in this framework.

Response: While we did not measure the concentrations of elements in the environment, the discussion about their impact on the composition of the shell is challenging, yet very valuable. While we do not have our own data on seawater concentrations, in the revised manuscript we will place more emphasis on the environmental characteristics based on literature data.

Introduction:

Comment: l.64-65: 'crystal layers are precipitated successively at regular periodicities,' is not true for all marine calcifyers, like Foraminifera. make it clear when you switch from all marine calcifyers to marine invertebrates.

Response: Thanks for spotting this, this will be corrected.

Comment: l.88-90: maybe add Stanley, 2008 , it is a nice overview paper. Stanley, S.

M. (2008). "Effects of global seawater chemistry on biomineralization: past, present, and future." Chemical reviews 108(11): 4483-4498.

Response: We will include this article. Thank you for suggestion.

Comment: l.144: remove . after shells

Response: This will be improved in the revised version of MS.

Method section:

Comment: l. 263: When were the samples taken?

Response: The samples were collected in May 2013 and June 2014. These information have been presented in Table 1, but we will also introduce them to the Chapter 2.3. Sample collection and preparation.

Comment: l. 295 What was the Ca concentrations in the sample solutions? 100ppm or varying?

Response: Calibration of the ICP-OES analysis was performed using solutions that were matrix-matched to the high calcium concentrations in the samples at a ratio of 49:1 calcium to magnesium. We will update this in the manuscript to make it clear.

Comment: l.297: What were the accuracy and precision of the measured elements?

Response: We will add more details to the method section: The accuracy and reproducibility of the analyses were checked using two calcium carbonate-rich certified reference materials (CRMs): JLs-1 Limestone and JDo-1 Dolomite (both from the Geological Survey of Japan) prepared by total digestion method (using hydrofluoric acid). The reference materials were diluted to match the concentrations of Ca in sample solutions. Ca, Mg and Sr concentrations were found to be within the uncertainty (1 standard deviation) of the reported values (Imai et al. 1996). Limits of quantification (LOQ) in solution for ICP-MS were generally determined as a concentration corresponding to ten times standard deviation of the signal obtained by analysing 5% HNO3 solution

(6–7 times) in each individual run. ICP-MS was run in helium (He) mode (5 ml min−1 He, 99.9995% purity) for lighter trace elements (V, Mn, Cu, Y and Cd) to minimize the molecular interferences from plasma and solution components and Ca from samples. The accuracy and reproducibility was checked by analyses of JLs-1 and JDo-1 before and after every batch of samples. The results obtained for all elements were within the uncertainty (2.5 SD) of the recommended values (Imai et al. 1996). Accuracy of Pb determination cannot be checked using these CRMs because of the large spread of reference values probably due to insufficient homogeneity of Pb distribution in these samples. Based on the analyses of CRMs and matrix-matched solutions, the maximum analytical error for the typical range of concentrations in the shells can be estimated (in relative percentage) as 1.5% for Ca, Mg and Sr; 3% for Ba; 20% for Cu and U; and 4–10% for all other elements. This is generally similar to what was reported in our previous publication (Piwoni-Pióreweicz et al 2017).

Discussion section:

Comment: I would advise to divide the discussion session in smaller paragraphs to increase readability.

Response: The discussion will be divided into the three parts to make reading easier. Due to the large number of factors potentially controlling the metal concentrations in skeletons, the discussion will be first focused on the polymorphic form of calcium carbonate (with the context of the shell organic matter); then on potential environmental factors (based on literature data); and finally on a potential biological response based on tracking metal variability in shell size classes. The discussion will be focused on finding patterns of inter-species and inter-individual variations in the concentration of metals in studied shells.

Comment: I would like to see variables as life span, habitat depth and organic material in the shell (see above) included in the discussion.

Response: We will take these factors into account in the discussion section.

Comment: l. 372: You obtained specimens with the same polymorph from two contrasting temperatures: i.e. aragonite Cerastoderma glaucum (16.9 C), Limecola balthica (4.6 C), Mya arenaria (16.9 C), I would like to see a discussion on the (absence of) temperture effect Sr incorporation, which is currently lacking in the manuscript, while it is being discussed for Na and salinity.

Response: Two species: Cerastoderma glaucum and May arenaria were collected at 10 m depth from the environment affected by cyclic temperature variation. However, the rest of the species were gathered from 31 – 36 m depth where the yearly variation of water temperature was lower. This is an important factor that can affect the concentration of metals in shells and we agree that it should be discussed. In the revised version of the manuscript, this part will be added.

Comment: l. 478: ref? Are there any studies on this?

Response: As mentioned above, the literature data regarding the concentration of some studied metals around the study area (such as Rainbow at al. 2000; Rainbow at al., 2004) and sediment type will be included into the manuscript. This will allow us to compare the bioavailability of metals between stations in the context of their concentrations in shells.

Comment: l. 483: suggest to change ‘animal’ into ’organism’.

Response: This will be changed as suggested.

Comment: Fig 2 and 4: indicate which polymorph of CaCO3 is used, like Fig. 3.

Response: This will be indicated.

Comment: Fig. 3: where possible, please use the same scaling for the y-axis for comparability, e.g. y axis of Mg for aragonitic species.

Response: This will be improved.

References: Barker, S., Greaves, M., & Elderfield, H. (2003) A study of cleaning pro-

cedures used for foraminiferal Mg/Ca paleothermometry. Geochemistry, Geophysics, Geosystems, 4(9).

Bourget, E. In Barnacle Biology, Southward, A.J., Ed.; A.A., Balkema: Rotterdam, 1987, Rueda, J.L. & Smaal, A.C. (2004) Variation of the physiological energetics of the bivalve Spisula subtruncata (da Costa, 1778) within an annual cycle. Journal of Experimental Marine Biology and Ecology, 301: 141-157.

Holcomb, M., DeCarlo, T. M., Schoepf, V., Dissard, D., Tanaka, K., & McCulloch, M. (2015) Cleaning and pre-treatment procedures for biogenic and synthetic calcium carbonate powders for determination of elemental and boron isotopic compositions. Chemical Geology, 398, 11-21.

Imai, N., Terashima, S., Itoh, S. & Ando, A. (1996) Compilation of analytical data on nine GSJ geochemical reference samples, Sedimentary rock series, Geostand. Newsl., 20(2), 165–216.

Kim, G., Alleman, L.Y., & Church, T.M. (2004) Accumulation records of radionuclides and trace metals in two contrasting Delaware salt marshes. Marine Chemistry, 87: 87-96.

Lorens, R.B., & Bender, M.L. (1980) The impact of solution chemistry on Mytilus edulis calcite and aragonite. Geochimica et Cosmochimica Acta, 44: 1265-1278.

Loxton, J., Najorka, J., Humphreys-Williams, E., Kuklinski, P., Smith, A. M., Porter, J. S., & Spencer Jones, M. (2017) The forgotten variable: Impact of cleaning on the skeletal composition of a marine invertebrate, Chemical Geology, 474, 45-57.

Piwoni-Piórewicz, A., Kukliński, P., Strekopytov, S., Humphreys-Williams, E., Najorka, J., & Iglikowska, A. (2017) Size effect on the mineralogy and chemistry of Mytilus trossulus shells from the southern Baltic Sea: implications for environmental monitoring. Environmental Monitoring and Assessment, 189(4), 197.

Rainbow, P.S., Fialkowski, W., Sokolowski, A., Smith, B.D., Wolowicz, M. (2004) Geo-

graphical and seasonal variation of trace metal bioavailabilities in the Gulf of Gdansk, Baltic Sea using mussels (Mytilus trossulus) and barnacles (Balanus improvisus) as biomonitors. Marine Biology, 144, 271–286.

Rainbow, P.S., Wolowicz, M., Fialkowski, W., Smith, B.D., Sokolowski, A. (2000) Biomonitoring of trace metals in the Gulf of Gdansk , using mussels (Mytilus trossulus) and barnacles (Balanus improvisus). Water Research, 34, 1823–1829.

Strasser, M., Walensky, M., Reise, K. (1999) Juvenile-adult distribution of the bivalve Mya arenaria on intertidal flats in the Wadden Sea: why are there so few year classes? Helgol. Mar. Res. 33, 45.

Szefer, P., Frelek, K., Szefer, K., Lee, C.B., Kim, B.S., Warzocha, J., Zdrojewska, I., Ciesielski, T. (2002) Distribution and relationships of trace metals in soft tissue, byssus and shells of Mytilus edulis trossulus from the southern Baltic. Environmental Pollution, 120, 423–444.

Takesue, R.K., & van Geen, A. (2004) Mg/Ca, Sr/Ca, and stable isotopes in modern and Holocene Protothaca staminea shells from a northern California coastal upwelling region. Geochimica et Cosmochimica Acta, 68: 3845-3861.

Walls, R.A., Ragland, P.C., & Crisp, E.L. (1977) Experimental and natural early diagenetic mobility of Sr and Mg in biogenic carbonates. Geochimica et Cosmochimica Acta, 41: 1731-1737.

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