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Biogeosciences
Discussions

Interactive comment on "Assessing annual variability in the shell thickness of the pteropod *Heliconoides inflatus* in the Cariaco Basin using micro-CT scanning" *by* Rosie L. Oakes and Jocelyn A. Sessa

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Reviewer comments have been copied below and author responses are listed underneath them. Page and line numbers refer to the tracked changes version of the manuscript.

Reviewer #2 - K. Kimoto

This manuscript describes the biometrics of pteropod shell and its degradation based on sediment trap samples in the Cariaco Basin. This kind of works of pteropod shells

using the sediment trap samples are insufficient, so it is very important to trace the biological responses to the ocean acidification and related ocean environmental changes. Especially the information of tropical species is less. In this sense, this work has the potential to become the base and develop criteria for this kind of study. Below I pointed out some concerning issues for this ms and make the comments

Reviewer comment: The relationship between shell length and whorls. According to photos in supplemental document, the aperture of some shells was damaged or showed bad preservation. The author inferred that shell diameter and whorl does not show clear relationship, but if a part of aperture had lost by dissolution and/or physical damage, its relationship between shell length and whorl might become uncertainty. If the plankton tow samples are available, the authors should use those plankton samples, not sediment trap ones. Or at all possible, the authors should use the only perfect shell in order to interpret length-whorl relationship.

Author response: Thank you for pointing out this potential source of bias. The fragility of H. inflatus shells make them particularly susceptible to breakage, often during collection (Pg. 7, lines 7 – 8). Shell diameter and shell whorl measurements were made on CT-scanned specimens but analyses were also run on a subset of 29 unbroken specimens. There was a weak but statistically significant relationship between shell diameter and the number of whorls. The FDR corrected p value was identical in both the whole and the subset dataset, although the R2 was higher in the subset dataset (whole dataset: R2 = 0.074, p Bon. = 0.415, p FDR = 0.057; subset dataset (Table S1): R2 = 0.101, p Bon. = 0.513, p FDR, 0.057). We have added this information into the methods (Pg. 7, lines 8 – 9), and results (Pg. 10, lines 27 – 30) sections of our manuscript. Since there was no difference between the two analyses, Figure 6 in the main manuscript contains the full dataset. The same figure with only the unbroken specimens is reproduced in the supplemental materials (Fig. S4).

Methods: "Because H. inflatus shells are fragile, they often break at the aperture during collection and processing. Although shell diameter and number of whorls were mea-

sured on all CT-scanned specimens, a subset of 29 shells with complete apertures was created for further analyses (Table S1)."

Results: "There was a weak, but statistically significant correlation between shell diameter and the number of whorls which remained when analyzing the subset of complete shells (Table S1) (whole dataset: R2 = 0.074, p Bon. = 0.415, p FDR = 0.057; subset dataset (Table S1, Fig. S4): R2 = 0.101, p Bon. = 0.513, p FDR, 0.057)."

Reviewer comment: Shell dissolution: How and when? The authors described that preservation states of shells in the sediment trap was not related to the duration time, so it might be negligible dissolution in the sediment trap collection cups. If this is correct, shell dissolution occurred at the water column, and it was associated with microzoo/bacterial activity which was decomposing organic tissues. My questions are that 1) in this case, does shell dissolution occurred at the inside, and outside of shell is sufficiently preserved? Can the authors show this evidence? Based on the photographs on the supplement material, surface texture of some shells looks like cloudy and lost their luster, indicating dissolution of outer shell. I am wondering that the decision of less dissolution in the sediment trap collection cups based on the result of relationship between residence time and LDX might be insufficient. In other words, I infer that shell dissolution occurs not only by microorganisms/bacterial activity but also post depositional oxidization in the sediment trap cups, as authors mentioned. I am understanding that this certification is very difficult, but the authors are using SEM, so please show some possibilities from the direct observations of materials.

Author response: We agree that this is an important question and decided to investigate a subset of seven shells, ranging from the best to the worst shell condition, under the scanning electron microscope (Page 8, section 2.4, lines 1 – 7). Our investigations revealed that the majority of the dissolution at LDX scores below 2.5 occurs on the outside of the shell. At LDX scores of 2.5 or higher, there is dissolution on both the inside and the outside of the shell. The external dissolution could be attributed to either dissolution associated with decaying organic matter in the water column, or alteration

associated with the preservative in the sediment trap cup. Internal dissolution is associated with the decaying organic body of the pteropod and/or alteration in the sediment trap cup. We have added discussions of these possible scenarios in the manuscript (Pg. 12, lines 18 – 19): "Scanning Electron Microscopy reveals that the majority of this dissolution occurred on the outside of the shells (Fig. 2, Fig. S2).", and (Pg. 13, lines 2 – 3): "SEM images reveal that the internal shell walls were only impacted by dissolution at LDX values of 2.5 and higher (Fig. 2 d, h, l), indicating that the preservative did not cause dissolution." We have added a figure of a selection of the SEM images to the main manuscript (Fig. 2) and all the SEM images to the supplemental (Fig. S2).

Reviewer comment: Another possibility, is it available the comparison between organic carbon of the samples and LDX? Highly input of organic carbon flux induce carbonate dissolution at the inside of sediment trap cup.

Author response: Unfortunately, there are no measurements of the organic carbon content of the samples from the sediment trap cups. Particulate Organic Carbon was measured as part of the CARIACO time series hydrographic measurements; however, these values are only available for the first half of this study so we cannot make this comparison.

Reviewer comment: Relating to above, I understood this study is the first, and make the baseline of this kind of pteropod study, but it is bit unclear the main subject and purposes. If the authors interpretation is correct, does the pteropod shell of this species /or in this region not become an index of ocean acidification? I suppose that the authors want to make the criteria as OA index by using pteropod shell, but in this case, I think that shell preservation states indicate microorganisms activity in the pteropod shell.

Author response: Hopefully the restructuring of the abstract, and introduction have served to clarify the purpose of this study. From an ocean acidification perspective, we make the point that although many studies have focused solely on aragonite saturation, the availability of food and the collection and preservation methods used may also

affect shell condition (Pg. 14, section 4.3). We hope this research leads to a more holistic view of shell condition interpretations. We have rephrased the conclusion to clarify this point (Pg. 15, lines 27 – 32): "This demonstrates that in this aragonite-supersaturated setting, the availability of food has a greater control on shell formation than aragonite saturation. This pattern has been seen in other groups of molluscs, such as oysters and mussels and underlines the necessity of assessing pteropod shell parameters and dissolution in the context of multiple biotic and abiotic factors, not just aragonite-saturation. We hope that the baseline dataset of pteropod shell parameters presented in this study is the first of many focused regional studies around the world. These datasets will enable the quantification of the response of this sentinel group to ocean acidification."

Reviewer comment: I think it is very important finding that shell thickness does not have relationship with surrounding omega value (but still supersaturated). I strongly agree with the authors that they have resistance characteristics to small changes of saturation states and depends on available food to build their shells. However, the authors did not show what kind of food is important for their prey. If their main food is phytoplankton, please show their annual variations through a year instead of nutrient concentrations (Or is it possible to show the number of diatom bulbs in the sediment trap cups?) Because their food is particulate matters, not chemical component. It might be a good evidence to indicate their food availability.

Author response: Yes, good point. Although we don't have any diatom counts from these sediment trap cups, we have added references to two other studies conducted in the same basin which find that both organic carbon production (Thunell et al., 2000) and diatom populations (Romero et al., 2009) show strong increases at times of upwelling. Nutrient concentrations are good proxies for upwelling and therefore showing the nutrient changes through the year is a reasonable approximation for food availability (Introduction: Pg. 5, lines 21 – 24, Discussion: Pg. 14, lines 13 – 16).

Introduction: "Organic carbon fluxes in the basin vary in response to these hydro-

graphic changes, with one study reporting a tripling of primary productivity in response to upwelling (Thunell et al., 2000). Diatoms, a known food source for pteropods (Lalli and Gilmer, 1989), contribute to over 50% of this organic carbon flux, with their blooms coinciding with hydrographic and nutrient changes during times of upwelling (Romero et al., 2009)."

Discussion: "These upwelling-related nutrient changes in the Cariaco Basin have been shown to correspond with increases in organic carbon flux and diatom blooms (Thunell et al., 2000; Romero et al., 2009), indicating that pteropod food supply (Lalli and Gilmer, 1989) increases during upwelling conditions."

Pteropods eat diatoms, as well as dinoflagelletes and tintinnids. We have added a reference about this to the introduction (Lalli and Gilmer, 1989) (Pg. 4, lines 3 – 4): "Pteropods are also key components of the marine food web, feeding on phytoplankton and small zooplankton, such as diatoms, dinoflagellates, and tintinnids (Gilmer and Harbison, 1986, 1991; Lalli and Gilmer, 1989)".

Reviewer comment: The authors did not touch the phylogenetic variation of the species, but I am wondering the possibility of mixture of some lineages of this species. H. inflatus is certificated as a single-genetic species around the Cariaco basin? Or exists some cryptic species? If the author has this kind of information, please mention it for just confirmation. It is possible that the plasticity of shell (shell length, number of whorls) of this species that author mentioned is related with the phylogenetic variations.

Author response: There has not been any genetic work done on H. inflatus. Both Van der Spoel (1967) and Janssen (2004) noted that there was variability in the shape and location of the rib, however, there is no data to determine whether this in intra- or inter-specific variability. We have added this information to the discussion (Pg. 13, line 34 – Pg. 14, line 4): "Both Van der Spoel (1967) and Janssen (2004) have described variability in the shape and position of the aperture tooth in H. inflatus, which could be attributed to intraspecific or interspecific variations. As there has not been any genetic

work conducted on H. inflatus from the Caribbean, we cannot be sure that the variability we see in shell shape cannot be attributed to two or more genetically-defined species."

Reviewer comment: Can the author interpret about morphological implication from the microXCT analysis Because it is very powerful tool and shows huge possibility for morphological information. If the authors want to indicate some suggestive issues, please make comment for following researchers and future study.

Author response: We agree that the CT data offer the opportunity to do some really interesting geometric morphometric work. We have added a section entitled "Further Work" (Pg. 15, lines 1 – 9) where we discuss the challenges of the field of gastropod geometric morphometrics, and discuss two recent studies. Our CT data will be available on publication and so can be used in future geometric morphometric studies.

We would like to thank Dr. Kimoto for his constructive review. It has helped us improve the completeness and clarity of this work.

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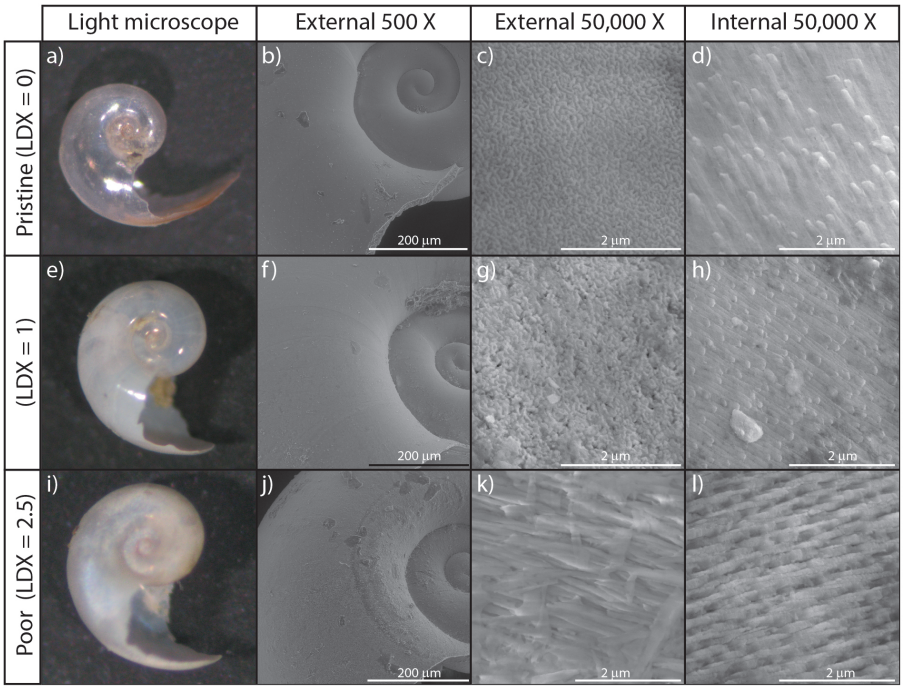

Fig. 1. Figure 2: Light microscope and SEM images showing dissolution on internal and external shell walls

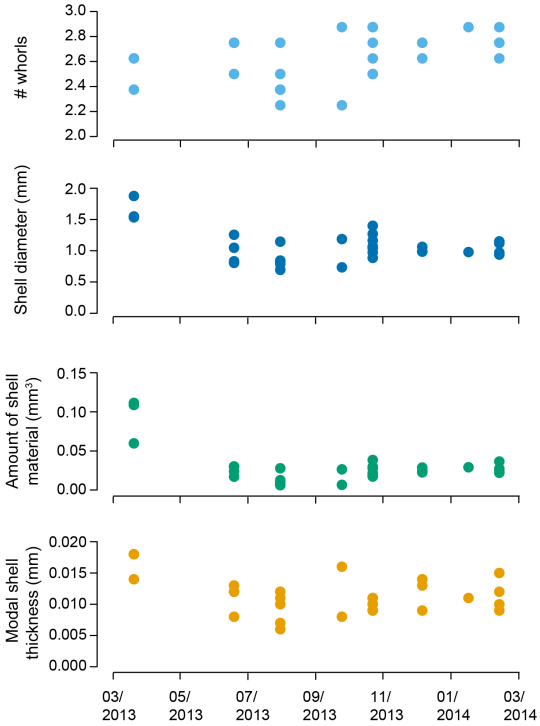

Fig. 2. Figure S4: Figure 6 from the main manuscript plotted with only the subset of 29 unbroken Heliconoides inflatus shells