Biogeosciences Discuss., 10, C8216–C8223, 2014
www.biogeosciences-discuss.net/10/C8216/2014/


Biogeosciences
Discussions
Open Access



# *Interactive comment on* "Non-invasive imaging methods applied to neo- and paleontological cephalopod research" *by* R. Hoffmann et al.

**R. Hoffmann et al.**

rene.hoffmann@rub.de

Received and published: 24 January 2014

We thank the anonymous referee #1 for their constructive comments. We are responding to the main issues raised by the referee in the following, in each case we include the referee comment followed by our response.

1. Diversity and disparity are not addressed or discussed at all in the paper, and functional morphology is only inferred by the measurement of shell volume.

For each of the presented methods a potential application was provided. Within our manuscript we mentioned these potential applications but focus on one of these namely buoyancy calculation using a combination of non-invasive methods. Some of the mentioned applications will be dealt with in future contributions e.g. FEA or "diversity and



Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

disparity". We will rewrite the part of the introduction and the abstract accordingly to clarify that point.

2. In the text, no attempt is made to describe or discuss how volume is used in a biomechanical context, except the authors do cite Anderson et al. 2012 with obliquely mentioning, but not explaining, how to use imaging techniques with regard to, for example, finite element analysis. This is true, unfortunately the process of transferring CT data into meshes usable for FEA, CFD, MBD etc. is complex and would involve much more data processing which is beyond the scope of the paper. In this case we direct the reader to examples such as Rayfield 2007 for a review of FEA in palaeontology, Shiino et al. 2012 for an example for CFD, Bates & Falkingham 2012 for an example of MBD. 3. How is buoyancy related to a dynamic behaviour such as swimming (including propulsion) vs. a static behavior such as floating?

In this case we are simply addressing the question whether the shell can provide sufficient values for buoyant force to support the weight of the animal and the shell. This specific question does not involve dynamic processes, i.e. we are modelling the animal floating in a column of still water. While it is undoubtedly true that swimming behavior has to be taken into account in order to accurately understand life-habits, this will be addressed in future work that focuses on such questions. However, we will rewrite this part and present a more complete discussion as to how buoyancy is calculated including the equation we used to make this clear. No complex equations are needed through this method because we no longer need to estimate shell volume which is a focus of the mentioned Raup and Chamberlain 1967 paper. The volume of the shell and soft body come from the CT data. The density of the shell is taken from the literature. We can therefore calculate mass: mass = density * volume. With mass we can calculate weight: weight = mass * gravitational acceleration. The value of the buoyant force is equivalent to the weight of the volume of displaced water (Archimedes principle). The volume of displaced water is equal to the total volume of the animal. With the volume of the water and the density of sea water we can calculate the weight of the water using



BGD

10, C8216–C8223, 2014



the above equations. The buoyancy (which is the effective weight or the weight of the animal under water) is: Buoyancy = Buoyant force - Weight of the animal.

4. Concerning the morphological species concept, how does using imaging techniques "sharpen," "contribute to," "improve " (the authors' words) the "morpho-species" concept? The imaging techniques have nothing to do with the concept; rather, the methods are another way to describe morphological attributes or characters or enable calculations for morphometry.

Species description in paleontology is based on the morphology of preserved hard parts. Due to their accretionary growth conserving ontogenetic changes molluscs are ideal candidates to study ontogenetic change, intraspecific variability, and macroevolutionary patterns. Ammonite species were usually differentiated from each other utilizing a static ("Linnean") rather than a dynamic ("Darwinian") approach both representing the morpho-species concept. The static method does not account for intraspecific variation, co-variation, and ontogenetic changes. Many species were thus validated on the grounds of more or less subtle morphological differences of the adult stage. During the last decades the way of species description has changed significantly, regarding ontogeny as well as the use of intraspecific variability analyses. More recent studies of Mesozoic ammonoids document a wide intraspecific variability in conch parameters and ornamentation, when a sufficient amount of specimens were available. Application of non-destructive methods like surface scans and x-ray computed tomography became increasingly important for the study of morphology. A surface scan allows for a detailed morphological description of the most important parameters (conch geometry: general shape and coiling rate, conch ornament including direction and course of the growth lines, ribs, and constrictions). Computed x-ray tomography allows studying the complex internal structure of chambered cephalopods (e.g., septal spacing, suture line). Combined, the two methods are powerful, non-invasive tools used in comprehensive studies of fossil shapes open a new path to improve the paleontological species concept. The techniques can also be applied to



BGD

10, C8216–C8223, 2014



study very rare material, such as holo-, para- or neotypes. The detailed morphological description of shapes and the increased number of available features for species characterization enable subsequent cladistic analyses to test existing phylogenetic hypotheses of the studied groups.

5. However, when it comes to actual application to research, deciding which imaging method to use depends on the question of interest by the researcher, the scale of the morphology of interest, and the particular resolution and specifications of the instrument rather than making a comparison of multiple kinds of instrumentation. For example, the authors obtained the best images for the morphology of septa and spacing with micro-CT (resolution of 7.5$\mu$m), moment of hatching with nano-CT (resolution of 1.0 $\mu$m), and secondary calcite crystals with SR$\mu$CT (resolution of 0.74 $\mu$m). This illustrates that three different research questions are at work here, and three different instruments were appropriately used. One could argue that an actual comparison among methods when specifically applied to research is non-existant.

The paper is divided in two major sections in order to avoid confusion between addressed research questions. One section deals with the methods themselves and the second section shows the potential applications the methods can be used for in cephalopod studies (part 4). We do not present three different research questions - instead different application examples are presented to illustrate the usage of the methods. With the separation we give the reader the opportunity to decide either if they want to read about the available methods or about the application of these methods.

6. I think a more accurate assessment of the contributions of this paper would be that the authors compared various imaging instrumentation specifications, showed how non-invasive methods could be used to acquire data on morphological features, and that volume can be calculated with the aid of such non-invasive techniques. I think the comparisons in terms of research are incidental and reflect more of an artificial framework in which to present results.



BGD
10, C8216–C8223, 2014

The different imaging specifications are given in the separate sections of part 3 (methods). In part 4 the applications in cephalopod studies are presented in order to show how non-invasive methods could be used to acquire data concerning specific questions. Volume is calculated as an application example how to process data from non-invasive methods. We understand that some confusion may occur between the intention of the paper and information that is presented. Certain parts will be restructured as suggested by the referee. Our paper compares the specifications of all instruments in order to provide the reader a guideline to clarify which method is the most promising concerning their particular research question. In order to present the intention of the paper more clearly a table will be added including instrumentation specification, characteristics of acquired data, limits of the applied methods and application example.

7. I would have like to have seen more quantitative information on 3D reconstruction and associated problems and error measurements for each of the imaging methods.

We present error calculations for the Nautilus shell using the micro-CT running at different resolutions and using different reference bodies (phantoms). We demonstrate an inverse relationship between the PVE and resolution, i.e. as the resolution of the scan decreases the error introduced by the PVE increases. Unfortunately comparison with micro-CT, nano-CT and SR$\mu$CT was impossible due the large size of the Nautilus shell. The aforementioned inverse relationship means that the increased resolution of the nano-CT and SR$\mu$CT data is coupled with a decrease in PVE induced error. Another point is the improved radiation quality with nano-CT and especially for the monochromatic SR$\mu$CT. This improved radiation quality means less scan artifacts which will improve the precision and accuracy of the reconstruction.

8. numbering of chambers in illustration 6A seems to be the opposite of what is used in Fig. 6D, which is a plot apparently using the numbering system from Fig. 6A.

In Fig. 6A we show a Nautilus shell covered with a 10° grid to demonstrate how the morphological data are obtained starting with one and going counter clockwise due to



BGD
10, C8216–C8223, 2014





the orientation of the shell. Fig 6D is not referring to that grid but showing the number of chambers on the x-axis and the volume of the chambers on the y-axis. We will rewrite that figure caption to make that clear and include the reference Hoffmann & Zachow 2011 for Fig. 6A-C. This confusion arises from the differences between data-acquisition, which starts with the adult/final/latest secreted part of the shell and the data-presentation which commonly starts with the most juvenile part towards the adult phase. To be consistent with other publications by ammonoid researchers we decided to create the figure in that way.

9. Why are the plots in Fig. 7 semi-log plots? Since the maximum diameter is 17 cm, is it necessary to have a scale on the x-axis to 100 cm?

Semi-log plots are used because most of the planispiral shells of shelled cephalopods grow as a logarithmic spiral. Further, using semi-log plots it is easier to add larger or smaller shells (a large range of values being covered) and a third reason was again to be consistent with published figures for easier comparison and not to cut down every image to a different size.

10. Both in the text and in the Fig. 7 legend, there is no discussion of isometry and allometry with respect to growth and ontogeny. We agree with the reviewer, since we developed a precise method to determine volumes we could add some additional information about growth and ontogeny. 11. How should these changes be quantified and compared between taxa? Between extant and extinct ammonoids?

From our point of view the easiest way of quantifying and comparing changes in chamber volumes between taxa (of extinct or extant cephalopods) is following their ontogeny i.e. starting with the very first chamber, the protoconch. However, an easy comparison is hardly possible due to the broad morphological spectrum, different number of chambers between taxa and in the case of fossil forms different preservation. Volume of the largest phragmocone chamber of the extant deep sea squid Spirula spirula is about 45 $mm^3$ while the Nautilus pompilius protoconch exceeds that value with a volume of



BGD
10, C8216–C8223, 2014

about 55 $mm^3$ (own data).

12. What assumptions and models of growth can be used to formulate changes in ontogeny with respect to isometry and allometry?

The aim of our paper was to summarize non-invasive methods and to show potential applications of these to cephalopod research. A discussion about growth models including allometric or isometric growth fall in the field of diversity vs. disparity and is beyond the scope of our contribution.

13. There was no direct discussion about how to use imaging techniques to quantify or characterize taphonomy. Taphonomic processes like sedimentary infill of the shell or crystal or pyrite growth inside the shell will largely affect a potential reconstruction of CT-images as already demonstrated by Hoffmann & Zachow (2011). Disarticulation as known from vertebrates does not occur in cephalopods except for shell breakage due to high pressure. However, potential application can be also seen in retro-deformation of fossils. Application of x-ray diffraction tomography will aid in the recognition of the original crystal pattern of the shell allowing a more accurate detailing of alteration processes. However, taphonomy is beyond the scope of our contribution. 14. How are non-invasive methods useful with respect to minimization of contamination of specimens? Is this so, or if not, why not?

Not much is known about the contamination of fossil specimens/rocks, and the reviewer does not refer to a certain kind of contamination. No long term observations about the influence of x-ray radiation to rock samples are available. The CT images derived from different absorption properties depending on chemical composition, density, and thickness resulting in gray scale images i.e. a certain amount of x-rays will be absorbed by the scanned material. Of course the dose necessary to scan rocks is hazardous for living tissue but not for rocks. Sutton (2008) mentioned for neutron tomography appropriate for the imaging of organically preserved fossils that a hazardous level of radioactivity can be induced due to intense neutron bombardment.



BGD
10, C8216–C8223, 2014





15. how would analyses using imaging and 3D reconstructions be done that would match or exceed the detailed results of work done by, for example, Klug (2001) or Kröger (2002), or for that matter, by those who do detailed traditional work? Normal procedure is to cross cut one specimen, polish the cutting plane, scan the cutting plan and measure the 2D distances of interest. Therefore data is only available for every 180°. Skilled researchers could do a second cut in order to obtain data for every 90°. This is an enormous improvement here compared to the older works representing just a single set of measurement for one point (mainly the adult stage at the position of the final septum) of ontogeny. By cutting and measuring, ontogenetic trajectories become available. We greatly appreciate this development just saying that with non-invasive data the specimen remains untouched and data becomes available for every single degree if necessary. Shell irregularities such as allometric growth can be observed more precisely. Ontogenetic shifts of these growth irregularities during phylogeny (heterochrony) can be observed more precisely. As this question also touches the field of disparity vs. diversity we could not discuss it at length in this paper but will be part of a forthcoming contribution.

---

Interactive comment on Biogeosciences Discuss., 10, 18803, 2013.