

# **Review of Wukovits et al. Effect of increased temperature on carbon and nitrogen uptake of two intertidal foraminifera (*Ammonia tepida* and *Haynesina germanica*).**

## **General Comments**

The manuscript by Wukovits et al. describes a series of feeding experiments designed to test how warming affects the metabolism of two common coastal foraminifera. The experiments themselves are relatively well designed and provide useful information on how climate warming will affect the role of foraminifera in benthic carbon and nitrogen cycling. The paper represents a potentially important contribution to our understanding of foraminiferal physiology and their role in coastal biogeochemistry. I would, therefore, recommend its publication in *Biogeosciences* subject to minor revision. The paper is technically sound, although I think the authors need to clarify their statistical treatment of the data, which was rather confusing. A relatively large number of different tests were employed during data analysis and I believe this could be streamlined. There is quite a lot of discussion at present regarding the use of p-values, and I would urge the authors to reflect on this. They have an excellent dataset, and in many ways the results are evident even without recourse to inferential statistical tests. Thus simplifying the approach could be beneficial.

In places the paper is somewhat verbose, and also the authors are prone to using rather conversational language in their text. I would urge the authors to revise the manuscript to try and make the results in particular more concise and to adhere more strictly to the formal rules, which underlie academic writing. Particular attention needs to be paid to sentence structure to ensure the manuscript is logical and easily understood by the reader.

A number of methodological points also concern me. Firstly, I can find no justification for the range of temperatures at which these experiments were conducted. How do these compare with the temperatures typically recorded in temperature coastal sediments? This information is key to the study into a wider environmental context. Secondly, why did the authors use a 12 hour Light:dark cycle.

Given that these forams are heterotrophs living in the sediment is light likely to influence their activity, and if so how?

Aside from these points and the specific comments raised below, I would be happy to see this paper published in the near future.

## **Specific Comments**

### *Abstract*

Pg 1 Line 3-4: “Factors limiting the abundance of specific foraminiferal species can be temperature related stress tolerant or food source processing efficiency” – Firstly, this paper does not strictly discuss changes in the foraminiferal assemblage, but rather the physiological responses of two species to changes in temperature. Given that temperature is the key parameter that defines the physiology of all ectotherms I think this needs to be given considerably more weight.

Pg 1 Line 6: “phytodetritus ingestion” I would suggest that you are not specifically measuring ingestion, given the time-frame of the experiment. Instead “retention” given that you are measuring changes in  $^{13}\text{C}$ -enrichment over different time periods.

### *Introduction*

Page 1 Line 20: replace “benthic species” with “benthic organisms”.

Page 1 Line 21-23: “Along with future environmental changes...” This sentence is not particularly elegant, please revise and simplify the structure.

Page 2 Line 1: What do you mean by “competitive drawbacks?” Please Clarify.

Page 2 Line 4-2: “smaller benthic foraminifera can contribute up to 80 %...” revise this sentence along the following lines – smaller benthic foraminifera contribute up to 80 % of the protest biomass (refs!) and are an important component of the food web (refs!). In the current draft both “can” and “considered” are superfluous words. Also when writing a paper with a major biochemical / geochemical theme try not to use “element” in a non-chemical sense.

Page 2 Lines 23-24: “Research about coupling of food derived carbon...” Move both references to the end to read (Enge et al., 2014; 2016).

Page 3 Line 4: replaces “therefore aimed” with simply “aims to investigate...”

Page 3 Line 7-8: “This study also tests the question...” revise to “This study also tests which species shows...” Try not to overuse “therefore”.

Page 3 Line 20-21: “This helps to interpret nutritional demands...” Please clarify this sentence.

Page 3 Line 22: “Studies aiming to develop new foraminiferal proxies...” would be a more elegant way to phrase this.

Page 3 Line 24: Replace “distinct observations” with “unique dataset”.

### *Methods*

Page 6 Line 20-23: Revise to “This test is recommended for sample sizes  $< 10$  and is robust against heteroscedacity within the data (Moser and Stevens 1992; McDonald, 2014; Ruxon, 2006).

Page 6 Line 23-26: I am confused by the statistical methods described here. To compare within species differences you used a two-way ANOVA – why then did you then use either pairwise t-testing or a one-way ANOVA as post-hoc tests. Surely with the two-way ANOVA you can then use Tukey HSD or other post-hoc tests to test for the significant interactions, and the ANOVA reveals the significance of any independent effects? Please clarify this.

Page 7 Lines 1-4: I am not sure that you can design a biological experimental and assume any data distribution. You are correct that ANOVA is relatively robust against departures from normality. Heteroscedacity is, however, often an issue in biological experiments with small samples sizes and I would strongly advocate Zuur et al ‘s (2009 – Methods in Ecology and Evolution) approach to this, which calls for visual exploration of the data residuals. In any case this section of the text is rather poorly written, please revise and consider looing at how your data fits the assumptions of homoscedacity visually.

### *Results*

Page 7 Line 13 (and elsewhere in the results): Delete “Remarkably” – restrict interpretive language to the discussion.

Page 7 Line 31: I believe you mean “Temperature effects cause...”

Page 9 Line 4: Delete “Same as for the  $\delta^{13}\text{C}$  values” Poorly written sentence, please revise. Please concentrate on describing the results. It would be simpler to simply state the trend for the  $\delta^{15}\text{N}$  values.

Page 10 Line 6: What do the authors mean by a diffuse fluctuation? This is not clear.

Page 10 Lines 7-8: Sentence is incomplete, how does *H. germanica* contrast with *A. tepida*.

Page 10 Line numbers do not appear to match up with the text.

Page 11 and 12. Line numbers do not match up with the text.

Page 11-12 Section 3.3. There is a lot of descriptive language here, which is quite confusing. The graphs probably provide a better summary of the data trends, please revise this section to make the trends clearer.

### *Discussion*

Page 13. Line 3-5: What are the effects on *A. tepida*? The key findings of the paper are about the different responses to temperature between the two species. This needs to be highlighted early in the introduction.

Page 13. The line numbers do not match up again, there appear to be 2 line 5s?

Page 13 Line 5 (II). New paragraph "In general..."

Page 13. Why did you not control for microbial respiration within your experiment. You could have run a set of control incubations with the forams absent.

Page 14. Again the line numbering system is a mess.

Page 15 Line 24. "112 cells mm<sup>2</sup>" Superscript missing.

Page 15 Line 27-28: The aging of the phytodetrital food source could have been controlled for within the experimental design. I think this may require further discussion. How does the phytodetritus quality change with aging?

Page 16. Line numbering is a again a problem here.

Page 16, 2<sup>nd</sup> paragraph: The author's discuss the low affinity of *H. germanica* to *D tertiolecta* as a food source. How representative is *D tertiolecta* as an algal food source. In intertidal sediments? diatoms represent the primary microalgal constituent of the microphytobenthos and coastal phytoplankton communities, wouldn't a diatom have provided a better POM source? Also could the production of MPB by the microphytobenthos not represent a major foram food source? I think these questions need to be addressed or alluded to.

Page 16, final paragraph: How do these results advance the potential use of forams as proxies for environmental monitoring. This is alluded to in the introduction and subsequently ignored.

