

Dear Chiara Borrelli,

We would like to thank you and the two referees for their second review of the manuscript. We are particularly grateful that Referee #2 has spotted some minor technical and editorial inconsistencies that we have addressed in this revision. In our response, we have addressed each of Referee #2 concerns and questions in the following format: Each question or comment is re-stated as in the original review of the manuscript in **bold font**. Our response to each comment/question is written in normal black font. Specific changes made in the manuscript are **in blue font**. Line numbers refer to the revised track-changes version of the manuscript.

Referee #2

I thank the authors for their detailed responses and for considering all of my suggestions and comments. I confirmed that significant revisions were made to the manuscript according to my first review. I'm now overall satisfied with the manuscript, but I found several concerns, mainly on displaying the results and formatting. Sorry, I didn't notice these points at first review, but I think those are important to be clarified. Please consider the following comments and suggestions.

AC: We thank the referee for identifying these minor technical and editorial issues.

-Unit of size-normalized respiration rate

I found that the unit of size-normalized respiration rate is not correct at many points. In my understanding, it should be [pmol hr⁻¹ ind⁻¹ μm⁻¹] or [pmol O₂ hr⁻¹ ind⁻¹ μm⁻¹] (maybe ind⁻¹ is not necessary but I think it's better to include it). In section 3.2., the authors used [pmol hr⁻¹ ind⁻¹], and only at L350, [pmol hr⁻¹ ind⁻¹ μm⁻¹]. In Figures 5 and 6, the unit of the y-axis is [pmol hr⁻¹ μm⁻¹], but in the caption, it is stated as [pmol hr⁻¹ ind⁻¹]. Likewise, in Figure 7, the y-axis label is [pmol O₂ hr⁻¹ μm⁻¹], but in the caption, it is [pmol O₂ hr⁻¹ ind⁻¹ μm⁻¹].

AC: Throughout the revised manuscript and supplementary materials, we have changed the unit for size-normalized respiration rate to **pmol O₂ hr⁻¹ ind⁻¹ μm⁻¹** as suggested by the referee. The only exception is Figure 7 where we are using biovolumes to size-normalize respiration rates. As a result, units in Figure 7 are reported in **pmol O₂ hr⁻¹ ind⁻¹ μm⁻³**. Please also see response below.

I'm also getting confused by the term they used for normalized respiration. For size-normalized respiration (SNR), the "size" is the cube root cavity volume [μm], right?

AC: Yes, this is correct, except for figure 7 where we are using the cavity volume (= biovolume). Specifically, the formulae for size normalization for each individual measurement used are:

- (1) $SNR = (\text{Respiration rate} / \sqrt[3]{\text{cavity volume}}) * (\text{average } \sqrt[3]{\text{cavity volume}})$
- (2) $SNR = (\text{Respiration rate} / \text{cavity volume}) * (\text{average cavity volume})$

Where the "average $\sqrt[3]{\text{cavity volume}}$ " was determined by taking the average $3\sqrt[3]{\text{cavity volume}}$ for each species. As suggested by the Referee (see below) we included the equation and these additional detail in the methods in [ll. 333-335](#) of the track changes manuscript.

But in Table S10, "blank-corrected respiration rate" (probably [pmol hr⁻¹ ind⁻¹]) and "SNR" (I assume [pmol hr⁻¹ ind⁻¹ μm⁻¹]) are quite similar. Normalized for a specific-sized individual?

AC: We apologize for a transcription error in Table S10, which has now been corrected. In the course of revising the table and adding uncertainties to the reported values (see also comment below), we identified five respiration measurements for *N. pachyderma* that did not meet the limit of quantification and were therefore excluded from the analyses. Recalculation of the affected analyses resulted in only minor quantitative changes and does not alter the interpretation or conclusions of the study. All changes to values are visible in the track-changes document.

To facilitate access of the data in the supplementary materials, we have also amended the table headers for Table S10. Instead of "Log Biovolume" we now specifically state that these are "log $\sqrt[3]{\text{Cavity}}$

Volumes". The normalization was calculated using the average size for each species. This is now clearly stated in the methods (see also response above) in [ll. 333-335](#) of the track changes manuscript

In 3.3, Rbiovolume appears, and by definition in L435, it is the respiration rate normalized by biovolume. Is this normalized by [μm^3] or cube root volume [μm]? If the latter, it is the same as SNR. In fact, in the caption of Figure 7, the unit is [$\text{pmol O}_2 \text{ hr}^{-1} \text{ ind}^{-1} \mu\text{m}^{-1}$], representing that it is normalized by cube root volume. However, the plotted values (ranging ca. -6.5–0) are totally different from the ones in Figure 5(a), which is also the Log10 SNR (ranging 1–2.5).

AC: Yes, we are using Rbiovolumes (μm^{-3}) instead of $\sqrt[3]{\text{Cavity Volumes}} (\mu\text{m}^{-1})$ in figure 7. And the Referee is correct; in this instant the respiration rates are normalized by volume (μm^3). As a result, the units for Figure 7 should be $\text{pmol O}_2 \text{ hr}^{-1} \text{ ind}^{-1} \mu\text{m}^{-3}$. The difference in the plotted range between figures 5a and 7 occurred because the units in figure 7 were reported in μmol rather than pmol. In the revised manuscript we have corrected the units and axis titles to **pmol** for Figure 7. The ranges now match figure 5a.

Please note that the change in unit also changed the absolute values in the respective equations for R_4 , R_{15} and R_{24} and Table S12. The changes made to values are clearly shown in the track changes document.

Please check the unit carefully, and I recommend to include the equation of size normalization, which would avoid confusion.

AC: We agree with the reviewer and have added the equation for size normalization to the methods in [ll. 234](#) of the track changes manuscript. We have also thoroughly checked the units for each figure and within the manuscript.

-Precision of measurement and significant digits

The authors seem to pay little attention to significant digits. When the measured size (max Φ) is XXX.XX μm , the calculated value from that should be the same number of significant digit (for example, the root cavity volume in Table S8 is displayed to seven decimal places, but the latter part is almost meaningless). Likewise, in Table S10, since the max Φ has three significant digit XXX μm , the volume should be XXX μm^3 . For Table S11, the sizes of Lombard et al. (2009) are, e.g., 347.00 μm , but this should be 347 μm . So please check and correct the supplementary Tables carefully.

AC: We thank the reviewer for pointing out these inconsistencies. We have revised the tables in the supplementary material and standardized all significant digits.

Besides, what about the precision of respiration rate measurement? It is also related to the significant digit to be used. Given that the blank variability is large ($14.83 \pm 11.50 \text{ pmol h}^{-1} \text{ ind}^{-1}$), the resulting uncertainty in the corrected respiration rates could be substantial and should be explicitly accounted for (e.g., by propagation of errors).

AC: We thank the reviewer for highlighting the importance of measurement precision and uncertainty reporting. In the revised version of the manuscript, we now report respiration rates as mean $\pm 1\sigma$ **standard deviation** based on triplicate measurements ($n = 3$), which reflects the experimental precision of the assay. Blank correction was applied prior to analysis, and only values exceeding the limit of quantification (LOQ) were retained. The LOQ is derived from the variability of blanks and therefore accounts for background analytical uncertainty at low signal levels. As such, explicit propagation of blank error beyond this threshold is not applied to individual reported values. In the revised manuscript we clarify how the blank correction was applied and specific $\pm 1\sigma$ standard deviation for each measurement are reported in Table S10. These revisions ensure that both analytical detection limits (via LOQ) and measurement precision (via replicate variability) are transparently represented. We have clarified this point in section 2.3 in [ll. 205-211](#) of the track changes manuscript.

Although the blank corrected respiration rate is displayed to three decimal places in Table S10, considering the large variability in the blank, I suggest rethinking to which decimal places should be displayed, and please clarify how uncertainties were treated after blank subtraction.

AC: We have standardized the decimal places across all tables in the supplementary materials.

-Minor points:

- **Caption of Figure 7: the 95% confidence bounds ---> the 95% confidence interval**
- **The label of y axis for Figure 4 and Figure 5: SNrespiration ---> SN Respiration**
- **L259, L263: 5 °C should be 4 °C, isn't it?**
- **L277: 1.65-7.4 4 µm --> 1.65–7.44 µm**
- **L340: Hoogakker et al. (2022) ---> remove parentheses**
- **L341: none of the specimens analysed in this study bore symbionts. ---> How was this confirmed? If possible, please add a short explanation.**
- **Table S8: “Armitage et al.” ---> “This study” would be better (as used in Table S11)**
- **Unit of cube root cavity volume should be [µm], not [µm³].**

AC: All these minor points have been corrected in the revised manuscript.