**Editor: As you will see, the reviewer gave a favourable report but raised some critical points that will require amendments to your manuscript.   I appreciate the extensive work to revise the manuscript for the second round of reviews.  Unfortunately, there remain many issues with the manuscript.  Most of these issues relate to attending to important details that can be addressed without too much effort (although, I will add that these types of issues do lead to reviewer fatigue and I encourage the authors to please carefully read and check the manuscript before submitting it to JEB again).**

**>We thank the editor for their persistence and help with the manuscript. We have addressed the comments of the reviewer and overhauled the statistical approach to use a mixed models approach and then compared models using AIC to prevent statistical issues with pseudo-replication.**

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Comments from the Reviewers:

Reviewer 3: I have read through the manuscript again. Here are, hopefully, the final comments needed.

>We thank the reviewer for their significant efforts and appreciate their feedback which has greatly improved the manuscript.  
  
Fig 1: In the figure caption, refer to the rows by name to reduce confusion. This is particularly important for the row labeled "scaling". As written is it not clear how scaling relates to this statement "expected frontal area increase factor". Please ensure that the figure caption is clearly written and explanatory.

>We changed the row label “scaling” to “expected frontal area increase factor” so that the language in the caption matches the row labels. To improve clarity, we also added the following text to the Fig. 1 caption: “In colonies with an expected frontal increase factor of 1 (helical, linear, bipinnate), frontal area stays constant as zooid number increases.”  
  
Figure 2: It is not clear how the view could be dorsoventral - this seems impossible. Please label dorsal, ventral, anterior, posterior, distal, proximal, lateral, etc., as appropriate. Fix the blue dashed line so that the arrow is pointing to the blue angle wedge. Otherwise, it appears that this dashed line is the angle being indicated. This could also be achieved by stating in the figure caption that the zooid-colony rotation angle is formed by the colony and zooid axe and is indicated by the blue wedge. It is also not clear whether this is an actual rotation or just an angle. Could the word "rotation" be removed for clarity?

>Since multiple zooids are involved in any colony orientation, the zooids don’t all have the same orientation, i.e. half have a dorsal orientation and half have a ventral orientation. We updated the figure caption as follows: “dorsoventral perspective (dorsal for the top row, ventral for the bottom row)”. We flipped the arrow orientation. The word “rotation” refers to the zooid rotation that occurs during development, but we left this word out for simplicity, as suggested. Describing the different orientations and arrangement and how they change during development is complex, which is why we dedicated a separate publication to this topic (see below) and appreciate the comments to help us streamline such descriptions in the current paper. Damian-Serrano A, Sutherland KR. 2023. A developmental ontology for the colonial architecture of salps. The Biological Bulletin. 245(1):9-18.

Line 84-85: Please change to, "to our knowledge, the effect of varying numbers…has never been investigated.." It is not possible to know with certainty that a topic has or has not been investigated throughout scientific history and throughout international journals.

>We made the change.

Line 106: This paragraph spans multiple topics and is difficult to follow, especially given that the topic sentence includes "linearity of colonies" and "pulsation rates" which have not been defined yet and are not clearly defined at all (linearity of colonies) or not until later in the paragraph. I recommend splitting this paragraph into multiple paragraphs so that these terms and their relevance can be clearly explained to the reader.

>We split this paragraph into three shorter paragraphs explicitly addressing linearity of colonies (which we now define), size and pulsation rates which we define as “frequency that zooid ejects water from the posterior atrial siphon”.  
  
Line 107: Define degree of linearity and degree of parallelism or rephrase so that this is more clear. This is not made more clear in Figure 2. If the goal of Fig 2 is to explain these terms, then please include those terms, define them, and illustrate them in Figure 2. One way to do this is, if I understand this correctly, to simply state that linearity increases as the zooid-colony rotation angle decreases. It is not clear how parallelism could be achieved or how it could express linearity. These are different terms with different meanings.

>We have omitted the use of the term parallelism throughout the manuscript.  
  
Line 109: State which angle is being referred to as "this angle". Explain "developmental dorsoventral rotation". It is not clear how this relates to the topic of this paragraph.

>We clarified to say: “This zooid-colony angle” and the entire sentence reads: “This zooid-colony angle is determined by the degree of dorsoventral zooid rotation during development … While all blastozooid buds start their development in a transversal position, most species undergo this rotation largely during the first day of colonial development.

Line 199-207: Define the variables t and n and connect them to your dataset (i.e., frame number, duration). Ensure that subscripts are correctly coded and formatted. Note that in lines 166-168 n was defined as sample size.

>We omitted equation 1, which was not necessary (according to a comment from a previous round of review) to omit the usage of “n” in two places. We defined t in equation 2 (now Eq. 1) and explain how it was calculated: “….t refers to instantaneous time points. Time points were extracted from the frame rate of the video and the spacing between chosen frames for analysis.” We also provided descriptive statistics on the variability of frames per second across the videos used:

>Minimum = 28, 1st quartile = 60, median = 120, 3rd quartile = 97, maximum = 240

>And the variability in temporal spacing between measurements:

>Minimum = 0.07s, 1st quartile = 0.16s, median = 0.17s, 3rd quartile = 0.25s, maximum = 0.5s

Line 211: Ensure that in the preceding paragraphs that the resolution of the ruler used to make these measurements is included and then ensure that the accuracy of the measurements are correctly reported relative to the resolution to the calibration ruler. If the accuracy is low relative to the measurements, then include a statement of measurement uncertainty that carries through to the reported calculations, such as speed (line 206).

>We added the following statement: “Increments on the scale ruler were 1 mm and we estimate that measurements made in ImageJ are accurate to the tenths place.” [Line 182]  
  
Line 216: State the temporal resolution of the respiration measurements based on image sampling and ensure that reporting of these measurements reflects this accuracy.

>We added the following temporal resolution information: “The exact interval time for each measurement was variable but recorded (Dataset S1B, mean time interval spacing = 11.1 min, standard deviation = 14.6 min, median = 7.3 min).” We did not use images to determine respiration rates, so we were unclear on this part of the suggestion, however we did provide descriptive statistics on the temporal spacing of frame measurements (see response above).

Results:  
  
Include all test statistics and df. For example, in a linear regression, R^2 values should be included as the test statistic. This is missing on line 318 but present on line 320. Test statistics are also missing on lines 337 and 339, and subsequent paragraphs. Line 379, etc. All reported statistics are missing degrees of freedom, which obscures the actual tested sample sizes. Throughout the study, the issue of lumping all data together looms large as contributing to many statistically significant findings which may simply be due to type I error - inflated sample sizes and pseudoreplication.

>We have added R2 values and test statistics in the places the reviewer highlights and elsewhere where appropriate. We have also added degrees of freedom and note that sample sizes are included in each figure.

Line 318: Ensure that resolution of values matches resolution of instruments. Then, represent that resolution in the graphs and tables.

>We have updated all of the tables and mentions of values to match the resolution of the instruments, in most cases, down to the tenths or hundredths place.

Figure 3: The graphs are not labeled with A,B,C,D which makes it difficult to use the caption to understand the graphs. Ensure that the y-axis label for Species is aligned such that it does not look like the title for the lower graphs.

>The labels have been added.  
  
Lines 347-340: Following on from early comment for Introduction and Figure 2, please use one term, e.g., zooid rotation angle or just zooid angle, and avoid switching between degree of linearity and parallelism (which seem to me to be addressing a more perpendicular position, not parallel).

>We now use zooid rotation angle throughout the manuscript, and we have omitted use of the term “zooid angle”. We have omitted the use of the term “parallelism” throughout the manuscript.  
  
Figure 4: Missing A, and B labels.

>The labels have been added.  
  
Figure 5: explain "scaling frontal motion-orthogonal frontal area scaling modes" - this does not make sense to me and needs clarity and explanation here and in the text.

>We updated the relevant part of the figure caption as follows: “(B) shows species with a frontal area that is expected to scale with the number of zooids in the colony (N=29).”  
  
Lines 388-399: Statistical modeling should be presented in Methods in the statistics section. Please include individual, colony and species in this model. Otherwise, it seems likely that the analysis will yield a statistically significant result due to type I error (pseudoreplication). Applying the AIC approach would work well here, because the models could be compared across these factors. This will also allow for or not allow for later claims that species is irrelevant.

>We have overhauled the statistics to use a mixed models approach and Akaike’s Information Criterion (AIC) to identify the best fitting model. We explored the following: a model with only the primary predictors’ fixed effects (fixed-effects model, a mixed model that also includes species random effects (species model), a mixed model with specimen random effects (specimen model), and to the mixed model that contains the nested random effects of species and specimen (mixed model). Please see Statistical Analysis section on pages 10-11 for a full description.  
  
Line 448: Take care with phrasing - "linear species" is nonsensical. Presumably, the intent here is that species with a low colony-zooid angle (or perhaps rotation angle? See comment relative to introduction and fig 2).

>We have eliminated any instances of the phrasing “linear species”.  
  
Line 539: Define "autoecology" or just use "ecology".

>We eliminated “autoecology”.  
  
Line 550-551: Define "biological carbon pump".

>We now include a definition for the biological pump: “the process by which biological activity (such as the feeding and defecation of salps) in the ocean’s water column transports carbon from the atmosphere into deep water.” [Line 566-568]

Line 616: Some of this issue would be addressed if species and individual colony were included as predictors in the linear models of the present study. If they do not improve the statistical model, then, at the very least, it would offer support for this statement even if the phylogenetic analyses were not performed at all. There seems to be a conflation of issues here.

>As described above, we have now included both species and individual colonies as predictors. We explored the following: a model with only the primary predictors’ fixed effects (fixed-effects model, a mixed model that also includes species random effects (species model), a mixed model with specimen random effects (specimen model), and to the mixed model that contains the nested random effects of species and specimen (mixed model). Please see Statistical Analysis section on pages 10-11 for a full description.  
  
Please clarify in this statement that Uyeda et al (2018) addresses the problems of applying comparative methods to singular events in evolution, not comparative methods, in general. The present study is not about singular events in evolution.

>We added the following statement: “Though Uyeda et al. (2018) focuses on singular-event evolution, which is not the nature of the present study, the statement is broadly relevant to comparative regressions.”

Separately, the lumping of data together for many of the statistics in this study, violates many statistical assumptions, regardless of discussions about phylogenetic comparative methods.

>As described above we now use a mixed models approach and have included models that use species and individual colonies as predictors.   
  
Lastly, these papers may be more useful for this section of the paper"  
Autumn, K., Ryan, M. J., & Wake, D. B. (2002). Integrating historical and mechanistic biology enhances the study of adaptation. The Quarterly Review of Biology, 77(4), 383-408.  
  
Taylor, G. K., & Thomas, A. L. R. (2014). Evolutionary Biomechanics. Oxford University Press.  
  
Moen, D. S., Cabrera-Guzmán, E., Caviedes-Solis, I. W., González-Bernal, E., & Hanna, A. R. (2022). Phylogenetic analysis of adaptation in comparative physiology and biomechanics: overview and a case study of thermal physiology in treefrogs. Journal of Experimental Biology, 225(Suppl\_1). [https://doi.org/10.1242/jeb.243292](https://urldefense.com/v3/__https:/doi.org/10.1242/jeb.243292__;!!C5qS4YX3!A1QNMEfPj4cZVYYAzpS0wTuduaPJ_9N12Dp5VcNg4q8GhMd1nJEbIMsZLhzQwDQS-8dbv-ovQtQbD4Qz$)  
  
>We thank the reviewer for pointing out these papers and have incorporated Autumn et al 2002 and Taylor & Thomas 2014 into the Discussion.