Dear Dr Damian-Serrano,

…

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. Both reviewers (and I) appreciate the extensive revisions based on the first round of reviews. However, there still remain a considerable number of areas needed revision. The reviewers provide clear and constructive feedback about which areas need additional work.

>We thank the Editor for her generous feedback.

I also emphasize the need to be clear about sample sizes (N, n, df, test statistics) when reporting the statistical results.

>We added details on sample sizes everywhere where p-values are reported across the results.

Also, there appears to be a misconception by the authors about why phylogenetic comparative methods are used. The most crucial reason is to address the non-independence of data points when species are more or less closely related (independence of data points is required of all the statistics used in this manuscript). Please fix that section of the manuscript and clearly state how violating the rule of non-independence of data points may have influenced your statistics-based findings. Or, include a phylogeny and perform the statistics correctly using appropriate statistical methods.

>Please see response to the last point raised by Reviewer 1.

Please make sure to plot all data points on the box plots (data transparency requirement of JEB).

>We added jittered data points to the boxplots in Figs. 3 and 7 and now all data points are plotted in all plots.

Lastly, I encourage the authors to upload their R code to accompany the data spreadsheet. The code would further enhance the replicability of the study.

>We have now included the R code in the resubmission.

Provided you are able to fully address the reviewers' comments, we hope you won't mind the extra work involved in revising your manuscript and adhering to our formatting instructions below. Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Track changes' in Word files as these are lost in PDF conversion.

I would be grateful if you would also list how you have dealt with the points raised by the editor and reviewers in the 'Response to Reviewers' box. Please attend to all of the editor's and reviewers’ comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

In order to promote timely publication, we generally ask that the revision be completed within 90 days from the date of this letter. However, we recognise that this may not always be possible so we will be happy to grant an extension where this is needed: please just contact the Editorial Office.

I look forward to receiving your revised manuscript.

With best wishes,

S. Patek

Handling Editor

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Comments from the Reviewers:

Reviewer 1: Damian-Serrano et al have considered a comparison of swimming and metabolic-rate measurements across numerous species of salp to address a hypothesis that differences in swimming speed are driven by frontal area differences between different colony formations. I reviewed an earlier version of this manuscript and find this version to be a great improvement. However, I still have number of suggestions for improving the presentation of this work. Most importantly, the statistical analysis requires greater transparency in its presentation in the methods and through reporting of sample size in the Results.

>We thank Reviewer 1 for their generous feedback and for their willingness to re-review our manuscript.

SPECIFIC COMMENTS

(Line numbers are the ones on the right)

L45 - Given the hypothesis, seems like you'd want to see how COT scales with frontal area too.

>Since COT was only different in a few of the architectures based on Tukey’s posthoc pairwise comparisons and was mostly unrelated to frontal area (Table S2B), we chose to focus on the more robust relationship between swimming speed and COT.

L49 - The statement about "due to their differential advantage in frontal area scaling" is in interpretation is written in the tone of an observation. Given the evidence, I think it would be more appropriate to say sometime like "We found that linear colonies generally swim faster, which supports the idea that . . ."

>We rephrased the statement to “We found that linear colonies swim faster, which supports idea that their differential advantage in frontal area scales with an increasing number of zooids.”

L63 - Maybe "drawing" and "ejecting" instead of "inhaling" and "exhaling"?

>We modified the statement to “Zooids in the colony feed and propel themselves by drawing water in through the oral siphon, using muscle contraction to compress their pharyngeal chamber, and ejecting a jet of water from their atrial siphon (Bone & Trueman 1983).”

L91 - I think Alexander and Vogel only makes sense as a citations if they offered a unique statement about drag in salps, which may be the case. They are not really strong sources on the origins of drag.

>We chose to omit this statement all together when we streamlined the Introduction in response to Reviewer 2’s comments.

L139 - Nothing against Vogel, but there many options in the primary literature, and reviews, to support this statement, which would more directly point to the source of the information.

>We replaced this citation with Andersen et al 2016.

Andersen, K. H., Berge, T., Gonçalves, R. J., Hartvig, M., Heuschele, J., Hylander, S., ... & Kiørboe, T. (2016). Characteristic sizes of life in the oceans, from bacteria to whales. Annual review of marine science, 8(1), 217-241.

L168 - The statement about "scaling drive disparities between colonial architectures" is important because it articulates a major aim of the study. So, I recommend using more precise language: disparities in what respect?

>We rewrote this statement to be more precise and concise: “…we investigate how swimming speed varies with the number of propeller zooids and differences in frontal area scaling between colonial architectures.”

I believe JEB requires that you provide the location of manufacturers.

>We added the locations of manufacturers to the products throughout the Methods wherever possible.

I do not think Eqns. 1 and 2 are necessary, but fine to include.

>We kept the equations for completeness.

L288 - Readers may wonder why dried mass was not selected as the means of normalizing the metabolic measurements by animal size, given the presumably large volume of these gelatinous organisms that is not comprised of living tissue. Isn't it possible that differences in the measurements could be due to differences in the proportion of metabolically-active tissue. A concise mention of these considerations would be helpful.

>We added the following explanation: "Biovolume was used instead of dry mass to normalize measurements due to the inherent difficulties of accurately measuring dry mass of these fragile gelatinous organisms in the field. Biovolume provides a consistent and reliable measure of the live size of the colony, which is directly relevant to the volume of water being displaced during swimming."

L323 - This section requires a more expansive description of the statistical analysis. What particular linear models? Which continuous variables, in particular? How are repeated measures taken into account? I'm not sure if testing relative to a zero slope makes sense (vs. comparing regressions between species), but I am not sure what variables are being referenced here.

>We added the following text in the Methods to state more precisely how we used statistical methods.

Linear model: “To test the relationships between pairs of continuous variables across architectures (e.g. swimming speed vs. number of zooids), we used linear regressions.”

Testing against zero slope: “We evaluated the significance of the slope parameter when compared against a flat slope (one-tailed t-test) to test whether changes in the independent variable (e.g. number of zooids) were associated with changes in the dependent variable (e.g. swimming speed).”

Repeated measures: “Owing to the patchiness of some species despite 80+ hours spent underwater (Table S1), we used replicate measurements (n) from each specimen (N) in swimming speed ANOVAs and regressions. We used an exponential regression to test the relationship between speed and COT. Specimen means (N) were used for all COT comparisons and regressions. Individual measurements (n) were used up to determine oxygen consumption rates. To evaluate the relative contribution of zooid size, pulsation rate, zooid number, and architecture type on swimming speed, we fitted a generalized linear model and evaluated the significance and proportion of variance explained by each factor using their partial R2.”

Sample sizes should be reported in the Results. It should be made clear when p-values are reported what the sample sizes are. Supplemental tables and mention in the methods is insufficient.

>We added sample sizes everywhere where p-values were reported throughout the ms, including in the figure legends.

Fig. 5 - zooids/pulse — Does this mean zooid length/pulse? Perhaps this could be phrased more specifically?

>We updated the y-axis label to read “zooid lengths per pulsation”.

L408-415 - These details should be offered in the Methods.

>We briefly describe the generalized linear model (GLM) in the Methods and then name the model variables again in Results-- salp swimming speed (U) from zooid length (L), pulsation rate (P), number of zooids (Z), and colonial architecture represented as frontal area scaling mode (A) -- so that the reader won’t have to backtrack to the Methods.

L439 - Paragraphs should be 3 or more sentences.

>We adjoined this paragraph to the next one, which is also about energetic investment.

L442-446 - Run on sentence.

>We split the sentence into three sentences. The new wording reads: “We then compared the proportion of energetic investment in swimming to the COT values across species (Fig. S3A,B). We found no relationship with absolute COT (N = 74, 14 species, p = 0.24). We found a positive relationship with zooid-length scaled COT (N = 74, 14 species, Swimming % = 0.11\*COT per zooid length + 34.4, adjusted R2 = 0.22, p < 0.001), indicating that species with more costly locomotion per zooid length invest a larger proportion of their energy budget in swimming.”

L479 - size, by what metric?

>We rephrased the wording to be more precise “...suggesting an underlying relationship between pulsation rate and zooid length…”

Please cite the results supporting all of the statements of findings in the Discussion. This appears more towards the end of the Discussion than at the beginning.

>We added relevant figure and table citations throughout the Discussion.

L497 - There is no such thing as a "less hydrodynamic configuration." L603 Says "highly hydrodynamic forms". These are misuses of the term "hyrodynamic". By analogy, one would not say that one animal is more mechanical than another.

>We removed these mentions of “more/less hydrodynamic” and replaced them with the term “streamlined”.

L631-634 - I do not follow the logic of the co-evolution of traits as reason for a phylogenetic analysis as being inappropriate. This merits a more clear explanation or perhaps it is a non-essential point that could be avoided.

>We thank the Reviewer for raising this important point. We understand the importance of addressing the non-independence of data points when dealing with species that are related to varying degrees and acknowledge that our statement was not sufficiently clear.

We have revised the text to clarify our reasoning and acknowledge the potential influence of phylogenetic history on residual variation as follows: “In the current study we did not use phylogenetic comparative methods in our analysis because like other investigators comparing biomechanical properties across species (e.g. Dabiri et al. 2010, Di Santo et al. 2021) we were interested in inherent mechanical relationships dictated by the colony architectures. For instance, a linear arrangement of zooids inherently reduces drag compared to a cluster arrangement, leading to faster swimming speeds and potentially higher efficiency regardless of phylogenetic history. In other words, any phylogenetic inertia is irrelevant in instantaneous relationships between traits (Felsenstein 1985). Moreover, independence of data is often incorrectly assumed to be an assumption of standard (nonphylogenetic) regressions (Uyeda et al. 2018), when in reality the assumptions relate to the independence and distribution of the error terms. Thus, when all the phylogenetic signal is present in the predictor, as it is in the case with colonial architecture (Damian-Serrano et al. 2022) and its associated characteristics, there is no need for any “phylogenetic correction” (Uyeda et al. 2018). However, there may be unaccounted factors explaining the residual variation in our analyses that may bear phylogenetic signal. For example, tunic stiffness, tunic smoothness, muscle band number, muscle fiber density, swimming behavior, as well as metabolic and physiological baselines may be more similar between more closely related species, potentially erasing some of the architecture-specific signal. Future studies could address the role of phylogeny and heritable factors in salp swimming speed and cost of transport using phylogenetic comparative methods. These analyses could reveal whether these factors have co-evolved with each other and/or with respiration rate or colonial architecture.”

Dabiri, J. O., Colin, S. P., Katija, K., & Costello, J. H. (2010). A wake-based correlate of swimming performance and foraging behavior in seven co-occurring jellyfish species. Journal of experimental biology, 213(8), 1217-1225.

Di Santo, V., Goerig, E., Wainwright, D. K., Akanyeti, O., Liao, J. C., Castro-Santos, T., & Lauder, G. V. (2021). Convergence of undulatory swimming kinematics across a diversity of fishes. Proceedings of the National Academy of Sciences, 118(49), e2113206118.

Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, *125*(1), 1-15.

Uyeda, J. C., Zenil-Ferguson, R., & Pennell, M. W. (2018). Rethinking phylogenetic comparative methods. Systematic Biology, 67(6), 1091-1109.

Damian-Serrano, A., Hughes, M., & Sutherland, K. R. (2023). A new molecular phylogeny of salps (Tunicata: thalicea: salpida) and the evolutionary history of their colonial architecture. Integrative Organismal Biology, 5(1), obad037.

Reviewer 2: The primary aim of the paper is to determine what effect, if any, the architectures of salps colonies have on the swimming speed and metabolic costs of the organisms. The stated motivation for the work is to provide insight into bio-inspired designs, such as for underwater vehicles.

The authors' experimental measurements of difficult-to-obtain quantities and their contribution to the body of knowledge regarding salps are impressive. They have also made a conscious effort to clarify and strengthen many points, which indicates a desire to be clear, open, and honest in their reporting. Below I have tried to balance recognition of the enormous challenges involved in obtaining the data and the limited number of available data points for analysis with gauging the appropriateness of the strength of the claims.

>We thank Reviewer 2 for their generous feedback.

There are two main outstanding issues of the paper as I read it. First is the number of predictions and expectations in the introductory part of the paper, which tend to take away from rather than strengthen the main hypotheses.

>We have eliminated the predictions and expectations in the Introduction and now focus on a single expectation based on frontal area (p. 3): “Salp colonial architectures differ in how the number of zooids in the colony scales with their frontal area relative to motion (Madin 1990). Some architectures (linear, bipinnate, and helical) have a constant frontal area, regardless of zooid number. These architectures may benefit from increased thrust delivered by larger numbers of zooids while maintaining a constant frontal area. However, the rest of the architectures (oblique, transversal, whorl, and cluster) have an increasing (directly proportional) frontal area as the number of zooids increases (Fig. 1). Therefore, we expect the latter architectures to not only obtain more thrust, but to also experience more frontal water resistance as zooid number increases. As a result, we anticipate that swimming speed will be greater in colonies that bear a larger number of zooids, but only (or more so) for species with architectures that have a constant frontal area.”

The second is a claim of a causal relationship based on indirect rather than direct evidence. It's possible these questions/issues could be addressed within the current architecture of the paper.

Statement of hypotheses. The introduction contains at least 12 expectations/predictions (Lines 84, 85, 90, 91, 98, 102, 106, 129, 137, 142, 146, 158, 162.)

> Instead of stating expectations based on previous literature in the Introduction, we now discuss the Results in light of previous literature in the Discussion. The paper reads more smoothly.

The last paragraph (Lines 309-318) of the introduction states that the following will be studied: how swimming speed varies with the number of propellers and whether there are differences in frontal scaling drive [which?] disparities between architectures, assessing how cost of transport varies, and how COT varies with swimming speed and pulsation effort.

We have edited the last paragraph in the Introduction to say: “In addition, we investigate how swimming speed varies with the number of propeller zooids and differences in frontal area scaling between colonial architectures. Finally, we compare cost of transport (COT) across salp species and assess how COT scales with swimming speed and pulsation effort.”

Is one or more of these the main hypothesis of the paper? Many measurements are made and many tests run, but they don't seem to address a single (or maybe two) clearly stated question or line of inquiry. This may be in part due to the fact that there is limited data in some cases, so there may be a desire to present the case from many different angles. And I think the paper does have an intention (see below), but the number of side predictions obscures it. I suggest moving the predictions/expectations to the discussion as part of the analysis if they support the central thesis while leaving the introduction clear to lay the groundwork for the primary aim. If the authors feel the predictions lay this ground work, they may have a different notion of how to tighten the focus.

>We have streamlined the Introduction and now have a single prediction regarding scaling of frontal area. After re-visiting the literature, we determined that there are not enough data on the relationship between swimming speed and COT to have an a priori hypothesis. We therefore have re-worded the Introduction such that studying COT is a research objective rather than addressing a specific hypothesis. The end of the Introduction now reads: “we compare cost of transport (COT) across salp species and assess how COT scales with swimming speed and pulsation effort.”

Statement of causal relationships. If a clear purpose were to be identified, it appears to be the main result from the abstract which reads (Lines 49-53): "We found that linear colonies generally swim faster due to their differential advantage in frontal area scaling with an increasing number of zooids." I agree with the findings to an extent, but the cause is less clear. The qualifier on lines 525-542 acknowledges that there may be confounding factors, but the strength of the statement in the abstract belies that notion.

>We rephrased this to: "We found that linear colonies generally swim faster, which is consistent with the hypothesis that their differential advantage in frontal area scaling contributes to their increased speed."

Focusing on Figure 5 A for example, I agree that this shows linear colonies tend to swim faster as the number of zooids increases when only considering linear colonies. However, it would be a strong statement to state that linear colonies swim faster than bipinnate and helical as number of zooids grow (line 503) based on these data because 1) there is only one helical specimen and 2) that pattern is not necessarily clear in the range where you have data for all three types of specimens, and you only have linear specimens in the region where you see a clear increase in speed on the right.

In figure 5 B, there is a similar concern with the oblique data point, but also the gap in colony numbers for the cluster data is concerning. Ignoring the point on the right, there appears to be an upward trend in swimming speed, even if more slight than the left. This could indicate the right-hand point is an outlier, or there is likely a non-monotonic relationship between swimming speed and a number of colonies in some of the architectures. This means there is possibly a region where larger frontal areas swim as fast or faster than architectures with lower frontal areas. The data presented here neither confirm nor deny this.

>We agree with the reviewer that there is patchiness in sample sizes and gaps in zooid numbers. And, we originally had a figure showing number of zooids vs. swimming speed for each individual species in the Supplemental section but we removed it to comply with JEB’s figure limits (the raw data are still available in Dataset S1). We added a sentence to the Results associated with Fig. 5 to acknowledge the uncertainties associated with low specimen numbers: "However, the limited sample sizes for helical and oblique chains prevent us from drawing firm conclusions about these architectures."

Questions about data comparison:

\*In table S1, there is a lot of variation in the mean length of zooids between species. Is there a lot of variation within a species? It might be appropriate to report the standard deviation.

>The raw data including all of the zooid lengths and showing the full variability is available now in Dataset S1A and S1B.

\*I couldn't open/find the Dataset S1, so this might have been addressed already. There is some discussion about the 2D versus stereo measurements and that the 2D measurements gave slower speeds. I didn't see that it was specified which species were measured with which apparatus. Using obtainable footage is reasonable, especially when the specimens are hard to collect/track/find. But if speed is a primary part of the argument, it should be obvious where the differences in speed measurements lie.

>We were sorry to hear that the reviewer couldn’t access Dataset 1; please do let us know of there are any challenges with access this time. Dataset S1A specifies each video file analyzed, species and camera system (3D or 2D). Both measurement types were used for *S. zonaria* and *S. maxima*. However, *Brooksia, Ritteriella* spp., *Pegea*, *I. cylindrica, M. hexagona, S. fusiformis, S. aspera, C. bakeri, C. polae*, and *C. sewelli* were 3D-only; whereas *Helicosalpa, Thalia, C. affinis, and C. quadriluminis* were 2D-only.

Minor things:

\*It might be more appropriate to refer to these as all of the known architectures throughout rather than all of the architectures unless it is definitively known that no other configurations are possible.

>We modified the wording in the Introduction to: “In this study, we compare swimming speeds across 17 salp species and energetic costs of swimming across 15 species, encompassing all seven known salp colony architectures”

\*Different marker point shapes would make the graphs more color-/resource accessibility friendly. The results would be hard to discern for someone with a different color perception or who can only access a black-and-white copy. Similarly, using different striping patterns on bar charts would make the graphs more accessible. This might be something for the publisher to address.

>We appreciate this point and agree that the different markers might be hard to distinguish in a greyscale print version of Figs. 4, 5 and 7. On the other hand, since electronic versions are more accessible and widely read, we will leave the plotting requirements up to the publisher.