Response to Reviewers

Schwemmer et al. “Attributing hypoxia responses of early life *Menidia menidia* to energetic mechanisms with Dynamic Energy Budget theory”

ECOMOD-24-322

*Original reviewer comments are provided with the original and new line numbers, and our responses are italicized. Line numbers pertain to the “track changes” version of the revised manuscript, but we have also provided a “clean” version.*

Reviewer #1

Reviewer #1: The manuscript by Schwemmer et al provides an excellent case study on the application of simplified DEB models to analyse and understand the impact of environmental stressors. This is a very interesting study, and a good example of how various sources of information can be tied together to form a DEB representation of an organism, and to analyse stress responses. Overall, the manuscript is well written and a very useful contribution to the field. However, I do have a few problems. My first issue is with the size of the manuscript and the number of references (I counted 128). The manuscript almost seems to be a combination of a review paper and a modelling paper. I would suggest reducing the text considerably, focussing on the essentials of the modelling exercise, reducing the wider context and some of the speculations. Also, the authors can consider moving some parts to an SI (or to a separate review paper).

* *We appreciate the constructive feedback on the length and focus of the manuscript and have taken your suggestion to move some of the wider context to the SI (Line 86-153) while also making much of the introduction, methods, and discussion more concise. We now refer to this in the Methods at Line 401-405, and further justify the utility of this information in the Introduction at Lines 146-151. We do believe that laying out the potential mechanisms of inhibition or damage to synthesizing units is important to justify our interpretation of parameters on which we focused. Our aim was to provide biological insight behind the synthesizing unit concept with the modeling exercise, rather than simply running the estimations to see which parameters provide the best fit to the data, and this approach sets the paper apart from many other modeling studies. The readers of Ecological Modelling have a common background in modeling but not necessarily in physiology, so the biological background we provided is useful context. We agree, however, that the necessary details can be more briefly summarized and we have moved the bulk to the SI as it is a collection of information that some readers may find useful.*

A second (potential) problem lies in the early life history of the species. I assume that this species has a yolk-sac larva, like most fish species? In that case, the assumption that hatching equals depletion of the egg buffer is invalid. I go into a bit more detail below on the potential issues for the model analysis. I am not sure how big of a problem this is, but I would like to ask the authors to consider the implications of a yolk-sac larval stage carefully, and discuss it in the text. In conclusion, I would advice that moderate-major revisions are needed before this paper can be accepted for publication.  
  
Yolk-sac stage issues  
  
Please reconsider Line 196-197 (*now Line 217-218*): "Hatching occurs when the egg buffer is fully depleted." This is not correct: it should be "Birth occurs ...". In many species with eggs, hatching and birth (i.e., start of exogenous feeding) occur (almost) simultaneously. However, for fish this is not typically the case as hatching precedes birth. The yolk sac larval stage, in DEB terms, would be an embryo since it does not feed exogenously. This would imply that the assumption of 'hatching time equals egg buffer mass of zero' (e.g., Table 3, Line 222-224, Line 254) is highly questionable. If this species indeed has a yolk-sac stage of non-negligible duration, this would require a number of modifications in the analysis (e.g., in the data set for the egg buffer mass).

* *Thank you for bringing up this important concern.* M. menidia *is different from typical fish species because it has little to no yolk sac larval period. We have edited the text at Lines 318-322 to explain this with references to studies that noted the short to nonexistent yolk sac larval period and the need to begin feeding the day of hatching (Bayliff, 1950; Bigelow and Schroeder, 1953; Middaugh and Lempesis, 1976). We have also replaced “hatching” with “birth” at Line 217-218 and stated our assumption that birth happens upon hatching at Line 302 when discussing the data we used. The hatch timing data has a resolution of 1 day, so even if there is a slight delay before feeding begins later the same day this would not be picked up in the data and would not affect the model. Finally, we have added in the discussion at Line 834-839 that a consequence of this assumption is that the model cannot as readily be used for other fish species with longer yolk sac larval durations and longer delays to the start of feeding.*

Related to the previous point, also the calculations in Line 226-233 (*now Line 260-277*) require a closer look. Is the relationship between length and dry weight also valid for (yolk sac) larvae? The estimated dry weight at hatching of 0.18 mg is larger than the dry weight of the fresh egg (W\_B0 in Table 1 of 0.15 mg). This seems like an impossibility already. If we can ignore maintenance losses during the embryonic stage, we would expect the structural mass at birth (when yolk runs out) to be W\_B0 x y\_VA x kappa (and minus the weight of the chorion etc.). With the values in Table 1, that should lead to a much lower dry weight at birth than the value of 0.18 mg. In particular, the low value of y\_AV seems inconsistent. As I already noted, hatching does not necessarily equal birth for fish; at hatching there may still be quite some yolk present, and yolk may have a different density than structural tissues. Further, the dry weight density of 0.40 mg/mm3 seems quite high to me. In some species, dry-weight density decreases rapidly after hatching, which may relate to yolk absorption (see the paper of Jager et al DOI 10.1016/j.ecolmodel.2022.110005, Fig. 2).

* *We have re-examined the egg dry weights, dry weight to length relationship, dry weight at hatching, and dry weight density. We agree that it makes sense for the dry weight at hatching to be much smaller, and as the data used in the dry weight to length relationship started at 6.2 mm total length, this relationship is probably best suited for larvae that are a couple days post-hatching and have been feeding for a while. We found a paper on the closely related* M. peninsulae *that measured length and dry weight directly after hatching and used that to fit a new function. This gave us a much lower dry weight of 0.046 mg for a hatch length of 5.3 mm, which is also much closer to the anticipated value of 0.04 mg from multiplying W\_B0 x y\_VA x kappa.*
* *Using a dry weight of 0.046 mg to calculate dry weight density resulted in a very low d\_V of 0.1 mg mm-3. As you stated, this may be due to the recent depletion of the yolk. When fitting the whole-life dataset with this value, we obtain poor fits relative to using d\_V=0.4 mg mm-3, either underestimating growth or overestimating time to birth. It seems likely that dry weight density increases soon after the early larval stage, and a d\_V value of 0.4 mg mm-3 provides the best fit to the whole life growth and reproduction. Unfortunately, we have not been able to find data on volume of* M. menidia *at other stages, but 0.4 mg mm-3 is close to d\_V values that have been used for fish, such as 0.28 mg mm-3 for lumpfish in Jager et al. 2022 (Table 1, DOI 10.1016/j.ecolmodel.2022.110005). We therefore decided it is justified to use the original length to dry weight conversion for* M. menidia *(which is based on the larval to adult stages) instead of borrowing from* M. peninsulae *(and using values only appropriate just after hatching) and continue to use the d\_V value of 0.4 mg mm-3. Importantly, when applying the hypoxia-based correction factor to the parameters following the procedure laid out in Section 2.5, we obtain the same results regardless of which of the two values of d\_V we use. In both cases, y\_VA, mu\_emb, and mu\_lar were the best parameters to which to apply the correction factor according to AICc.*
* *We have added further clarification of the two ways in which we calculated d\_V in the SI, with our explanation of why we chose the greater value (Lines 44-77 of the SI), and briefly state the justification at Lines 275-277: “This is slightly higher than the dV values used for other fish species (e.g. Jager et al., 2022), but the overall results were not sensitive to this parameter and it allowed for a good fit to growth data across all life stages.”. We have also changed “egg” to “embryo” at Lines 266, 272, and 278 of the main text to more accurately reflect the fact that we used diameters of embryos without the chorion, via microscope images, to estimate embryo volume immediately before hatching.*

Minor comments:  
- In Table 1, L\_Vp is specified as 'Total length at puberty', but what is it exactly? Is it physical length or volumetric length? In Table 2, it is used as volumetric length in the specification of J\_J, but as physical length when specifying W\_Vp. Please check. The value in Table 1 suggests that it is physical length (which has a different symbol in Table 2).

* *We have changed the equation for J\_J to use (W\_Vp/d\_V) instead of L\_Vp^3 as this makes it clearer that the equation is using the volume at puberty. As L\_Vp^3 is not mentioned elsewhere in the paper, we removed its row from Table 2. The remaining uses of L\_Vp are total physical length at puberty, and we have added “physical” to Table 1 to clarify that.*

- In Table 1, y\_AV is defined as the 'Yield of assimilates on volume'. Probably better to replace volume by structure. This parameter is relevant for starvation situations only. Is that relevant for this manuscript?

* *Thank you for pointing out this error. We have corrected it to say “structure”. Reviewer 2 pointed out that starvation could occur under hypoxia and suggested we add a brief description of how the model handles it, so we have done so at Lines 254-259 and left y\_AV in Table 1.*

- In Table 1, it would be good to specify whether the grammes are dry or wet. This could also be done in the caption as they are all dry weights.

* *Thank you, we have taken this suggestion and updated the caption.*

- In Table 1, the mortality rates for embryos and larvae need a unit (1/d).

* *Thanks for catching this, we have added the units.*

- In Table 2, the specification of volumetric length L is completely trivial. You could define it using the structural dry mass and the dry weight density, for example.

* *We have changed it to show how it relates to both the physical length and dry mass.*

- In Line 216-217 (*now Line 242*), you could add for clarification that the non-somatic fraction is dissipated and therefore does not contribute to biomass.

* *We have added this clarification at Line 242, thank you for the useful suggestion.*

- Line 236-241 (*now Line 282-288*): it would not be strange to see delta\_M change from (yolk-sac) larvae to juveniles as they can look quite different. Would a change in shape over ontogeny be an explanation for this apparent misfit?

* *This is a great point, and likely explains why the delta\_M we calculated with embryo volume does not allow as close of a fit as the slightly lower value. We have added a sentence acknowledging this at Line 263-265 and 283-286*)*. Unfortunately, we were unable to find data for the structural volume of* M. menidia *later in life and could only calculate volumetric length for embryos the day before hatching (we have images of embryos from which we can estimate volume as a sphere not including the chorion), but future work on this species should try to include this measurement. Because delta\_M is used to calculate length, which is then used in JA and JV, too great of a delta\_M value did not allow us to obtain a reasonable fit to both growth and egg buffer depletion at the same time.*

- Line 254 (*now Line 301*): it is not really 'extrapolated'; the data comprise initial egg mass and the assumption that the egg buffer is depleted at hatching (which is questionable, as already noted above).

* *We have changed the wording here to be more accurate and mention our assumption that egg buffer mass is zero at hatching. The information we added supporting this assumption comes later in this section (Line 318-322).*

- Line 312-314 (*now Line 365-367*): this could use a bit more explanation, perhaps in the SI (with a figure), as it is not a trivial calculation.

* *We have added the methods for this calculation in the Supplementary Methods (Line 22-47 of SI), including the equation used to calculate mass-specific dry weight lost over time and how we used d\_V to convert it to J\_M^v. Rather than a figure, we included a table of the relevant values because we did not fit a curve to the weight loss, but rather calculated it using one mean initial and mean final dry weight as reported in the study.*

- Line 389 (*now Line 118 of SI)*: fluxes in DEBkiss are not in carbon units but in biomass units (mg of structure or assimilates).

* *We have made this correction, and this section is now in the Supplementary Methods at Line 123.*

- Line 466 (*now Line 528*): "exponential" does not seem to be a correct term for Z.

* *This was an error and we have removed it.*

- Line 492-495 and Line 632 (*now Line 555-558* *and Line 697*): Ja\_Am and y\_AV are not multiplied directly. Please add that they are only multiplied (and cannot be independently identified) when the maintenance flux J\_M is negligible (which is very likely the case for the early life stages).

* *This is a good point. We have changed the phrasing at Line 555-558 in the methods and Line 697 in the discussion to say they both contribute to J\_V and that they are directly multiplied when J\_M is negligible as it likely is in early life stages.*

- Line 537 (*now Line 600*): should "increasing" be "decreasing" here?

* *Yes, thanks for catching that. We have corrected it.*

- Line 677 (*now Line 747*): the insensitivity of JV\_M should not come as a surprise. For very small individuals (far away from their asymptotic size), maintenance is only a small part of the total energy budget (in DEB, at least).

* *We agree that it is not surprising given the model equations and maintenance’s relation to volume rather than surface area. We have added a clarifying sentence at Line 750-753 about the relative role of maintenance as the surface area to volume ratio decreases with growth, to add some insight as to why maintenance had little effect: “Because maintenance is dependent on volume, it is a relatively small portion of the energy budget in the very small early life stages but increases substantially relative to the surface area-specific assimilation when larger sizes are reached, increasing its relative role in determining growth rate and, indirectly, all size-specific fluxes.”*

- Line 746-749 (*now Line 826-828*): why is this "suggesting"? If hypoxia reduces gonad development, this might simply imply less and/or delayed reproductive output. A reduction in reproduction does not "require" energy to be redirected from the soma.

* *We have rephrased it at Line 822-824 to avoid speculating and use the fact that hypoxia can impact gonad development to highlight that measuring how hypoxia affects reproductive investment could improve the model: “For example, hypoxia can reduce gonadosomatic index and gonad development in fish (Wu et al., 2002; Thomas et al., 2006; Landry et al., 2007), but we do not have data on gonad development or reproductive output after rearing* M. menidia *in hypoxia, which would allow us to investigate if* κ *is an affected parameter.”*

Editor/Reviewer #2 - Dina Lika

Due to the extensive delay of the second reviewer, I have personally reviewed the paper and provided some additional suggestions to the authors. The manuscript presents an interesting study on the effects of stressors, specifically hypoxia, on the energetics of Menidia menidia, with a focus on early life stages, using a simplified DEB model. The paper is well-written but requires revisions before it can be accepted for publication. The reviewer #1 suggests moderate to major revisions, and I concur. Below are specific comments:

Figure 1. lines 161-162 (*now Line 172*) suggest that the organism undergoes 3 life stages embryo, larval, and adult. Is larva modeled different from juvenile? In the text (line 197; *now Line 219*) you state after hatching juveniles feed. Do larvae also feed or use the yolk-sac? Please clarify the stages you are using and the way they are model. Also, in figure 1 (left) you should highlight J\_M instead of “maintenance”.

* *To make it clearer that the post-hatch mortality rate also applies to juveniles, we have added them to the figure and caption of Figure 1 (Line 181).*
* *In the text at Line 218, we have added the word “larvae” and clarified that larvae and juveniles are treated identically in the model. This is because* M. menidia *larvae start feeding on the day of hatching, and they hatch with little to no yolk sac. A similar statement was made at Line 251 but moving it to this earlier paragraph will help readers understand this important point before reading the details of the model. In response to comments from Reviewer 1, we have added further information at Line 318-322 to justify the assumption that the larval stage begins at hatching and address the implications of the assumption at Line 834-839.*
* *Thank you for catching the inconsistency in Figure 1. We have moved the red box to “J\_M” as suggested.*

Lines 197-198 (*now Lines 219-220*). Juveniles feed and mature while adults feed, do not mature any longer, and reproduce. All stages pay maturity maintenance as shown in Table 2. Please explain the energy allocation clearer. Also explain how the model handles starvation. Hypoxia combined with food limitation may lead to this situation.

* *Thank you for these suggestions to describe the energy allocation more clearly and accurately. We have added this information and rephrased some of the existing text to improve the explanation (Lines 213-222, 247-251).*
* *We have added a short paragraph describing starvation at Line 254-259, following the detailed description of other fluxes: “Starvation is defined in two stages, with the first stage being insufficient flux of assimilates to the somatic fraction to meet maintenance requirements so that energy is diverted from the flux to maturation or the reproduction buffer. In the second stage, when the flux to both the somatic and reproductive branches is insufficient and the reproduction buffer is empty or puberty has not been reached, structure is converted to assimilates with conversion efficiency yAV to go towards maintenance costs (Jager, 2018).”*

Table 1 has a parameter “yield of assimilates on volume” (volume of what), but it is not explained how it is used. The term volume is used in several definitions, and you should explain in the text its connection with structural mass.

* *We have changed it to “yield of assimilates on structure”, as we wrote “volume” in error. This parameter is now defined in the paragraph about starvation at Line 258.*
* *We have added a sentence at Line 261-263 explaining how length, volume, and mass are connected through the parameters* dV *and* δM*.*

Table 2 (Fluxes). “Flux to maturity” should be “Flux to maturity maintenance”. This formula has the parameter J\_J^v (volume-specific maturity maintenance costs). What is its value? If a value is not given because you only consider early stages, you should mention it.

* *We set the volume-specific maturity maintenance costs by assuming the value is connected to the somatic maintenance cost parameter through the* κ *value rather than being estimated: J\_J^v = (1-κ)/κ \* J\_M^v. According to Jager (2018) this allows the investment in maturity to be independent of food availability. Given a calculated J\_M^v of 0.0214 mg mm-3 d-1, J\_J^v = 0.00535 mg mm-3 d-1. We have added the equation to Table 2 and included a brief description in the text at Line 247-251 and details on the calculation at Lines 41-44 of the SI.*

Table 2 (State variable). “Structural dry mass over time”, omit “over time” all state variables are functions of time. The units refer to the rate of change of the state variable. In this case the survival equation is not unitless. I suggest you refer to the units of the state variable.

* *We have removed “over time” from Table 2 and corrected the units of survival.*

Line 229 (*now Line 269*). Equation 1 is written in a complicated form while it can be written as W\_V = a LM^3 (and estimate only a). This will then be consistent with equations 2 and 3 which state, respectively, that W\_V is proportional to the structural volume and total length proportional to volumetric length (i.e, structural volume to the power 1/3).

* *We did not estimate the parameters of the total length to dry weight conversion, but rather they were estimated empirically in previous work. We have replaced it with the simplified version as you suggested, clarified the text explaining where the conversion came from, and updated the reference to the study where this function is now published, rather than citing the personal communication by which we previously received it (Lines 265-268).*

Line 238 (*now Line 286*): Why delta\_M is manually adjusted to fit the length-at-time data (Figure 3A)? Why not include with the estimation of the remaining parameters? The best practice is to estimate all parameters simultaneously.

* *We did not fit delta\_M simultaneously with the other parameters to avoid risking overparameterization. In DEBkiss delta\_M is defined as a conversion or auxiliary parameter rather than a primary parameter, and we had data to calculate a reasonable starting value for it, which we then adjusted slightly. The original calculated value was slightly too high, resulting in quickly depleted yolk or too low growth rate and ultimate size, even with estimating new values of J\_Am^a and y\_VA to try and correct this. Length, which is controlled by delta\_M, is multiplied by J\_Am^a to get JA so estimating both simultaneously using the growth data would be problematic.*
* *We have added additional explanation as to why we believe the higher delta\_M did not work (Lines 283-286). We only had data to estimate volume of embryos the day before hatching, when they can be approximated as a sphere but assumed to be similar in length to those measured immediately after hatching. As Reviewer 1 pointed out, it may change over time as the fish grows and body shape changes, a point which we reference at Lines 263-265 and 283-286. Furthermore, we tried applying the hypoxia-based correction factor according to Section 2.5 using the two different values of delta\_M and did not find a difference in the results. In both cases, the best parameters to which to apply the correction factor to obtain the best fit to the different oxygen treatment data were y\_VA, mu\_emb, and mu\_lar. We therefore chose to use the delta\_M of 0.107 that allowed a closer fit to the full-life data.*

Lines 258-259 (*now Lines 303-307*). The 3 reasons for using DEBkiss instead of a “standard” DEB model stated in this sentence do not fully support this choice. Data from different studies could be used to estimate DEB parameters as one can see in the AmP database. In any model, one could hypothesize plausible values for parameters, but these values must be supported by some degree of evidence, or the model’s sensitivity to those parameters should be checked.

* *Early in our work we (TGS and RMN) spent a large amount of time and effort attempting this and other ways of using AmP. We never achieved any set of interpretable parameters. Whether the treatment of embryos in standard DEB versus DEBkiss is preferable is debatable but elaborating on this is beyond the scope of the paper. However, to highlight the key difference between the models without distracting from the paper’s main theme, we have added an explanatory sentence (Line 214-216). Whether or not DEBkiss or standard DEB (possibly modified) should be the default starting option for any specific application involves many subtleties lucidly discussed in a paper by Romoli et al. (2024, cited in our manuscript at Lines 163-170 and Lines 303-307).*

Section 2.3 should be reduced. Details on the procedure of parameter estimation should be moved to an online SI.

* *Thank you for this suggestion. We have moved the details on parameter estimation from Lines 339-353 to the SI (Line 5-19) so that the main text focuses on explaining whether each parameter was estimated by fitting to data, calculated, or fixed at a suggested value.*

Line 303 (*now Line 355*). It should not come as a surprise that the yield of structure on assimilates does not have the same value of that suggested for the DEB model since the structure of the models differ and the interpretation of the parameters differ.

* *We agree that this remark was unnecessary and have removed the mention of the suggested value, instead simply saying that we had sufficient data to estimate it.*

Lines 339-341 (*now Lines 395-397*). Include the symbols and the names of parameters as introduced in Table 1 for clarity.

* *We have added the symbols and edited Table 1 so that the names reflect those used in the text.*

Give units to the parameters involved in equations 2-5 and use another symbol to combine parameters k\_i and Z. The new compound parameter will have different units than Z.

* *Thank you for bringing our attention to these equations. We have added the units, and renumbered the equations as number 2 and 3 were repeated. These are now equations 4-7 (Lines 502-517).*
* *We have replaced the first Z with B, so that Z is the product of k\_i and B.*

Line 567 (*now Line 555-558*). Assimilation rate J\_Am^a and the yield coefficient y\_VA both affect the growth flux, while J\_Am^a affects explicitly also the reproduction flux as well as the maximum length. As it is discussed in lines 628-641 (*now Lines 694-701*), because of the correlation of the two parameters, it is difficult to identify the contribution of those parameters on the hypoxia effects. Can you suggest what type of data are needed to disentangle their contribution.

* *We have added a section to this paragraph (Line 707-711) stating the data that would be needed to more directly estimate the effect of hypoxia on y\_VA and J\_Am^a. Thank you for this suggestion which enhances the discussion of these parameters. “Future work examining the effects of hypoxia on ingestion, defecation, respiration, and growth could help tease apart the relative contributions of yVA and JaAm by allowing direct calculation of yVA. Data on fecundity at different DO levels would provide information on the contribution of JaAm, although constant hypoxia through adulthood is unrealistic and this would assume the energy budget is impacted similarly across life stages.”*