Revisions to Schwemmer et al. Menidia DEB manuscript

1. Outline of most important points to discuss
   1. Embryo to larval transition
      1. Reviewer 1 is concerned about our statement that hatching occurs when yolk is fully depleted. This could be addressed in one or more of the following ways:
         1. Rephrase it to say that birth occurs when yolk is fully depleted (mass=0) and that we are assuming in our model that hatching = birth, because we do not have data on time of yolk depletion but we do have data on hatch timing.
         2. Highlight that *Menidia menidia* tend to hatch with little to no yolk (with references) and start feeding the same day (I’m still looking for a reference but have observed it in the lab).
            1. Our data on time to hatching is recorded in days, not hours or fractions of days, so even if feeding starts several hours after hatching the data would not reflect the difference.
         3. Address the implications of our assumption. How could it affect the energy budget and parameters if we are assuming birth happens earlier than it really does? Do we expect the difference between hatching and yolk depletion to be the same or different across oxygen treatments?
   2. Reviewer 1: the paper is too long and some of it is more like a review paper. They suggest shortening it and/or moving some to an SI section: “focussing on the essentials of the modelling exercise, reducing the wider context and some of the speculations”
   3. Reviewer 2: “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.”
      1. This seems like a really good point and these values need to be revisited.
      2. If yVA = 0.365 then should the newly hatched larva only be about 1/3 the dry weight of a fresh egg?
      3. They also think the dry weight density of 0.4 seems high
   4. Reviewer 2: “Lines 258-259. The 3 reasons for using DEBkiss instead of a “standard” DEB model stated in this sentence do not fully support this choice.”
      1. Need to strengthen this paragraph of Section 2.2 either by adding more justification or making it more closely match what we said in the introduction.
      2. Not sure if it is significant that she commented on this section but not Lines 165-173, or if the comments apply to both sections.
   5. Reviewer 2: “Line 238: 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.”
      1. Delta\_M is not a model parameter that can be estimated in DEBkiss/BYOM. It is a global parameter.
      2. I’m going to take a closer look but it looks like it is only used when converting length at puberty to weight at puberty.
   6. Reviewer 2: “Line 303. 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.
      1. Would the appropriate response to be to remove the mention of the literature value and simply say that we had sufficient data to estimate it?
      2. It may also be useful to note here that the estimated yVA of 0.365 is close to the growth efficiency measured for *M. beryllina* of 0.375.
   7. Reviewer 2: “Line 567... As it is discussed in lines 628-641, 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.”
      1. Reproductive data?
      2. Direct measurement of food consumption and growth to measure y\_VA at different hypoxia levels
2. Detailed Notes of Revisions

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 suggest that the organism undergoes 3 life stages embryo, larval, and adult. Is larva modeled different from juvenile? In the text (line 197) 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”.

* *This should be simple to fix but may depend on how we respond to Reviewer 1 – revisit after.*
* *Add juvenile to Figure 1, as post-hatching survival does include juveniles as well.*
* *Edit line 197 to state that larvae and juveniles feed.*
* *Move the red box in Figure 1 to “J\_M”.*

Lines 197-198. 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.

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.

* *I think it should be changed to “yield of assimilates on structure”*

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.

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.

Line 229. 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).

Line 238: 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.

* *May need to rerun with estimation turned on for delta\_M*
* *Is this going to cause problems with getting NLL=Inf*

Lines 258-259. 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.

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

Line 303. 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.

* *Would the appropriate response to be to remove the mention of the literature value and simply say that we had sufficient data to estimate it?*
* *It may also be useful to note here that the estimated yVA of 0.365 is close to the growth efficiency measured for* M. beryllina *of 0.375.*

Lines 339-341. Include the symbols and the names of parameters as introduced in Table 1 for clarity.

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.

Line 567. 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, 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.

* *Reproductive data?*
* *Direct measurement of food consumption and growth to measure y\_VA at different hypoxia levels*

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). 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: "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).

* *Change it to “Birth occurs when the egg buffer is fully depleted” and say that we are assuming hatching occurs at this time by using time to hatch to get our state variable for time to birth. The studies we are using data from*

Related to the previous point, also the calculations in Line 226-233 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).  
  
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).  
- 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?  
- 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.  
- In Table 1, the mortality rates for embryos and larvae need a unit (1/d).  
- 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.  
- In Line 216-217, you could add for clarification that the non-somatic fraction is dissipated and therefore does not contribute to biomass.  
- Line 236-241: 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?

* *Is there a way to have delta\_M change with life stage?*

- Line 254: 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).  
- Line 312-314: this could use a bit more explanation, perhaps in the SI (with a figure), as it is not a trivial calculation.  
- Line 389: fluxes in DEBkiss are not in carbon units but in biomass units (mg of structure or assimilates).  
- Line 466: "exponential" does not seem to be a correct term for Z.

* *This is correct, that word was left over from the previous version where we used a different function for the correction factor and I will remove it.*

- Line 492-495 and Line 632: 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).  
- Line 537: should "increasing" be "decreasing" here?

* *Yes, I will fix it*

- Line 677: 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).

* *This is a valuable insight, I will see if I can find a reference for it and adjust how we discuss it in the text*

- Line 746-749: 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.