

Ecological Modelling

Attributing hypoxia responses of early life Menidia menidia to energetic mechanisms with Dynamic Energy Budget theory

--Manuscript Draft--

Manuscript Number:	ECOMOD-24-322R1
Article Type:	VSI:DEB2023
Keywords:	Dynamic Energy Budget; DEBkiss; early life stages; Atlantic silverside; hypoxia; stressors
Corresponding Author:	Teresa Grace Schwemmer, Ph.D. Stony Brook University School of Marine and Atmospheric Sciences South Kingstown, RI UNITED STATES
First Author:	Teresa G. Schwemmer, Ph.D.
Order of Authors:	Teresa G. Schwemmer, Ph.D. Roger M. Nisbet, Ph.D. Janet A. Nye, Ph.D.
Abstract:	<p>Highlights</p> <ul style="list-style-type: none"> • Bioenergetic mechanisms of Atlantic silverside hypoxia responses were investigated. • Hypoxia effects were modeled with a simplified Dynamic Energy Budget model. • We connected physiology with energetic processes to identify potential mechanisms. • Conversion efficiency and mortality parameters best explained hypoxia effects. • This mechanism could impact energy flow across generations and trophic levels.
Suggested Reviewers:	<p>Benjamin Martin University of Amsterdam Department of Theoretical and Computation Ecology b.t.martin@uva.nl Expertise in DEB models of fish, hypoxia and metabolism research, has used a DEBkiss model.</p> <p>Tjalling Jager DEBtox Research tjalling@debtotox.nl Extensive expertise and experience with DEB modeling, developed the DEBkiss model, and has used it to model environmental stressor effects.</p> <p>Laure Pecquerie Université de Brest laure.pecquerie@ird.fr Expertise in DEB models of fish, fish metabolism and growth.</p> <p>Nina Marn Ruđer Bošković Institute nina.marn@irb.hr Expertise in DEB theory, physiology and energetics.</p>
Response to Reviewers:	<p>Our responses to reviewers are bulleted below. We have also provided this in the uploaded Revision Notes document with <i>italics</i> to differentiate our responses from the reviewer comments.</p> <p>Reviewer #1</p> <p>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).</p>

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 $W_B0 \times y_{VA} \times \kappa$ (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 $W_B0 \times y_{VA} \times \kappa$.

• 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} , μ_{emb} , and μ_{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 d_V 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 δ_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 δ_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 δ_M is used to calculate length, which is then used in JA and JV, too great of a δ_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 $J_M \cdot v$. 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 k value rather than being estimated: $J_J^v = (1-k)/k * J_M^v$. 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 δ_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 δ_M simultaneously with the other parameters to avoid risking overparameterization. In DEBkiss δ_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 yVA to try and correct this. Length, which is controlled by δ_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 δ_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 δ_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 yVA , μ_{emb} , and μ_{lar} . We therefore chose to use the δ_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 y_{VA} and J_{Am^a} by allowing direct calculation of y_{VA} . Data on fecundity at different DO levels would provide information on the contribution of J_{Am^a} , although constant hypoxia through adulthood is unrealistic and this would assume the energy budget is impacted similarly across life stages."

Dr. Teresa G. Schwemmer, Ph.D.
Stony Brook University
Stony Brook, NY 11794-5000
teresa.schwemmer@gmail.com

July 19, 2024

Dr. Dina Lika, Ph.D.
Guest Editor, Special Issue
Ecological Modelling

Dear Dr. Lika:

I am writing to resubmit our manuscript entitled “Attributing hypoxia responses of early life *Menidia menidia* to energetic mechanisms with Dynamic Energy Budget theory” (ECOMOD-24-322) after completing revisions. Thank you for taking the time to personally review the manuscript to provide timely reviews in light of the delayed reviewer. We are grateful for the constructive and insightful feedback provided by you and the first reviewer, and for the opportunity to resubmit our manuscript. After taking the time to carefully implement and respond to the suggestions and concerns, we believe the manuscript is substantially improved.

Specifically, we have created a Supplemental Methods section and moved the details about the parameter estimation procedure from Section 2.3 there, along with the bulk of Section 2.4 explaining the physiological connections between hypoxia responses in fishes, synthesizing units, and DEB parameters. We also added a section to the Supplemental Methods explaining how we calculated the specific maintenance cost using data on weight loss during starvation.

We also addressed concerns about our assumption that birth occurs at hatching by adding information and references about how *M. menidia* have little to no yolk sac larval period and begin hatching immediately, as well as reexamining and responding to questions about our values for δ_M and d_V . The detailed responses to all comments with line numbers are listed below.

Thank you for your time and consideration in reviewing our revised manuscript. We look forward to hearing from you.

Sincerely,

Teresa G. Schwemmer, Ph.D.
Corresponding Author

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_AV 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 y_{AV} 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 d_V and δ_M .*

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: $J_J^v = (1-\kappa)/\kappa * J_M^v$. According to Jager (2018) this allows the investment in maturity to be independent of food availability. Given a calculated J_M^v of $0.0214 \text{ mg mm}^{-3} \text{ d}^{-1}$, $J_J^v = 0.00535 \text{ mg mm}^{-3} \text{ d}^{-1}$. 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 δ_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 δ_M simultaneously with the other parameters to avoid risking overparameterization. In DEBkiss δ_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 $J_A m^a$ and y_{VA} to try and correct this. Length, which is controlled by δ_M , is multiplied by $J_A m^a$ to get J_A so estimating both simultaneously using the growth data would be problematic.*

- We have added additional explanation as to why we believe the higher δ_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 δ_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} , μ_{emb} , and μ_{lar} . We therefore chose to use the δ_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 y_{VA} and J_{Am}^a by allowing direct calculation of y_{VA} . Data on fecundity at different DO levels would provide information on the contribution of J_{Am}^a , although constant hypoxia through adulthood is unrealistic and this would assume the energy budget is impacted similarly across life stages.”*

Highlights for Schwemmer et al.

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

Highlights

- Bioenergetic mechanisms of Atlantic silverside hypoxia responses were investigated.
- Hypoxia effects were modeled with a simplified Dynamic Energy Budget model.
- We connected physiology with energetic processes to identify potential mechanisms.
- Conversion efficiency and mortality parameters best explained hypoxia effects.
- This mechanism could impact energy flow across generations and trophic levels.

1 **Attributing hypoxia responses of early life *Menidia menidia* to energetic mechanisms with**
2 **Dynamic Energy Budget theory**

3

4 Teresa G. Schwemmer^{a,1}, Roger M. Nisbet^b, and Janet A. Nye^c

5

6 ^aSchool of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794,
7 U.S.A., teresa.schwemmer@gmail.com

8 ^bDepartment of Ecology, Evolution and Marine Biology, University of California Santa Barbara,
9 Santa Barbara, CA 93106, U.S.A., rogerenisbet@ucsb.edu

10 ^cDepartment of Earth, Marine and Environmental Sciences, University of North Carolina at
11 Chapel Hill, Institute of Marine Sciences, Morehead City, NC 28557, U.S.A., jnye@nyelab.org

12

13 ¹Corresponding author, present affiliation: Teresa G. Schwemmer, Mid-Atlantic Coastal
14 Acidification Network, Newark, DE 19716, U.S.A., teresa.schwemmer@gmail.com

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38 To be submitted to: *Ecological Modelling*, Special issue: Metabolic organization across scales of
39 space and time

- 40 **Highlights**
- 41 • Bioenergetic mechanisms of Atlantic silverside hypoxia responses were investigated.
- 42 • Hypoxia effects were modeled with a simplified Dynamic Energy Budget model.
- 43 • We connected physiology with energetic processes to identify potential mechanisms.
- 44 • Conversion efficiency and mortality parameters best explained hypoxia effects.
- 45 • This mechanism could impact energy flow across generations and trophic levels.

46
47

48 **Abstract**

49 Ocean deoxygenation is intensifying worldwide due to warming and eutrophication,
50 particularly in estuaries and coastal waters. Although the Atlantic silverside (*Menidia menidia*) is
51 tolerant of the fluctuating environmental conditions in its estuarine habitat, chronic hypoxia
52 impairs hatching, growth, and survival in the early life stages. We used a simplified version of a
53 Dynamic Energy Budget model (DEBkiss) to test the hypothesis that experimentally observed
54 changes in animal performance can be explained by one or more of the rate processes in the
55 model. We sought to identify the DEBkiss parameters that, when adjusted with a correction
56 factor based on inhibition of Synthesizing Units, provided the best fit to hypoxia effects in the
57 three state variables of total length, egg buffer mass, and survival over time. Because hypoxia
58 reduces survival in embryos and newly hatched larvae, we added a survival state variable
59 controlled by pre- and post-hatching mortality parameters. Applying the hypoxia effects to
60 reduce the conversion efficiency of assimilates to structure accounted for some of the hypoxia-
61 related changes in all three state variables. However, the best fit was achieved by simultaneously
62 reducing the conversion efficiency and increasing both mortality parameters. In contrast,
63 changing the parameter for maintenance rate with hypoxia provided little to no improvement of
64 fit to the data. Reduced conversion efficiency under hypoxia would suggest that less of the
65 energy invested by parents and consumed through predation is converted into biomass in *M.*
66 *menidia* offspring, with implications for size at age that could threaten recruitment and alter the
67 flow of energy through the food web.

68

69 **Keywords**

70 Dynamic Energy Budget; DEBkiss; early life stages; Atlantic silverside; hypoxia; stressors

71

72 **1. Introduction**

73 Hypoxia is common in coastal and estuarine waters and is expected to intensify with
74 global warming (Diaz and Rosenberg, 2008; Breitburg et al., 2018). Between anthropogenic
75 influence on nearshore waters and the natural dynamics of shallow, partially enclosed water
76 bodies, hypoxia often co-occurs with other stressors such as high temperature, ocean
77 acidification, and pollutants (Gruber, 2011). In temperate estuaries, stratification and
78 productivity associated with high temperatures in spring and summer cause hypoxic and
79 eutrophic zones to form with great fluctuations in dissolved oxygen (DO) on diel to monthly
80 time scales (O'Donnell et al., 2008; Baumann and Smith, 2018; Testa et al., 2018). While fish
81 species that currently live in such areas tend to have mechanisms to cope with episodic hypoxia
82 (Farrell and Brauner, 2009; Zhu et al., 2013; Baumann, 2019), these are not necessarily adequate
83 for tolerance of longer duration events. Fishes that spawn in the spring and summer may be
84 particularly vulnerable because they are exposed to hypoxia during the sensitive early life stages.
85 Embryos and young larvae rely largely on diffusion for oxygen uptake and lack well-developed
86 mechanisms, such as high surface area gills, to meet oxygen demands in low DO water
87 (Rombough, 1988). While later stage fishes and even some early larvae can swim to avoid
88 hypoxic habitats (Niklitschek and Secor, 2005; Chapman and McKenzie, 2009), embryos cannot
89 utilize this response. Mortality can result directly from severe hypoxia or indirectly from reduced
90 growth increasing susceptibility to predation. Even fish that survive may incur sublethal effects
91 with lasting, lifelong consequences for growth, development, and reproduction (Stierhoff et al.,
92 2006; Vanderplancke et al., 2015; Zambonino-Infante et al., 2017). Modeling the energetic
93 mechanisms of responses to hypoxia using unified principles on model species can help connect
94 physiology and life history to population-level changes and serve as a valuable alternative and/or
95 supplement to time- and labor-intensive laboratory experiments on other species, particularly
96 with very small embryos and larvae.

97 Hypoxia is known to inhibit growth and survival in early life fishes (Rombough, 1988;
98 Cross et al., 2019; Del Rio et al., 2019), as oxygen is required for the processes that maintain
99 homeostasis and convert food for growth and activity. Anaerobic energy production fuels these
100 processes with only about 1/15th the ATP yield of aerobic respiration. Hypoxic exposure may
101 lead to physiological responses such as depressed metabolism (Richards, 2009; Schwemmer,

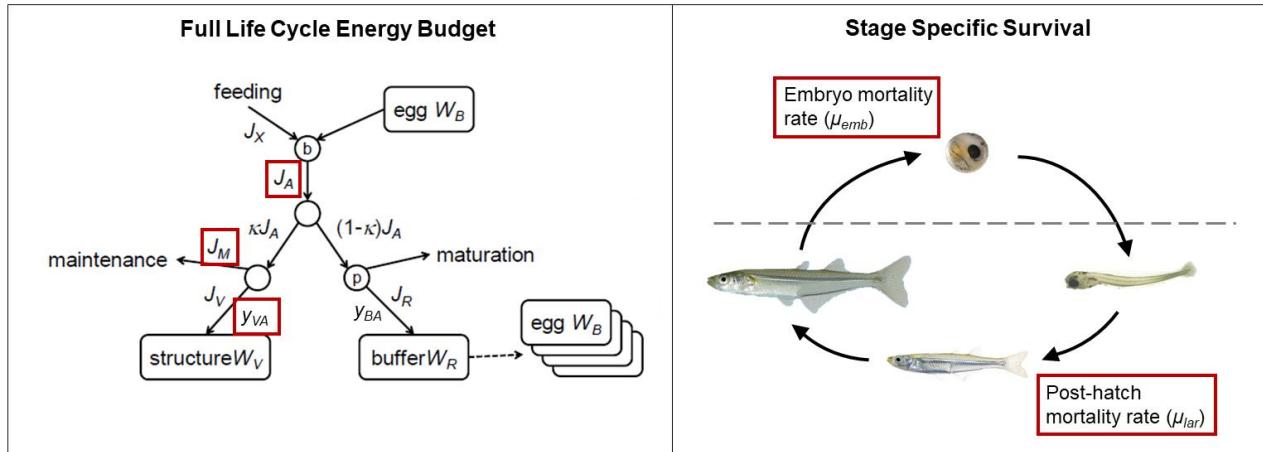
102 2023), limited growth, increased ventilation, and changes to hematocrit, hemoglobin, and
103 erythrocyte quantities and characteristics (Taylor and Miller, 2001; Stierhoff et al., 2009;
104 Bianchini and Wright, 2013). Metabolism has also been shown to increase after temporary
105 hypoxia as fish remove lactate accumulated from anaerobic respiration (Heath and Pritchard,
106 1965). While the growth and survival effects of hypoxia have been demonstrated in many
107 species, the mechanisms are poorly understood. The Atlantic silverside (*Menidia menidia*) is an
108 estuarine forage fish that has frequently been used as a model species to understand effects of
109 stressors, including hypoxia, on fish growth and physiology (DePasquale et al., 2015; Miller et
110 al., 2016; Schwemmer et al., 2020). Hypoxia significantly delays *M. menidia* hatching and
111 reduces embryo and larval growth (Cross et al., 2019).

112 Dynamic Energy Budget (DEB) theory is a bioenergetic framework designed to bridge
113 multiple levels of biological organization in assessing stressor effects and their mechanisms in a
114 vast variety of species (Kooijman, 2010a; AmP, 2023). This approach follows energy allocation,
115 represented in suborganismal metabolic fluxes, and how it leads to life history outcomes such as
116 growth rate, reproductive output, and survival, using physical and biological concepts that are
117 generalizable to most species (Jusup et al., 2017). It accounts for differences in the energy budget
118 at each stage to allow modeling of life stage transition timing and stage-specific responses to
119 stressors (Kooijman, 2010a). DEB theory is often used to connect experimental observations of
120 multiple stressor effects to both the underlying energetic mechanisms (Kooijman, 2018) and life
121 history outcomes that feed into population dynamics (Nisbet et al., 2000; Martin et al., 2013;
122 Smallegange et al., 2017). It is important to connect suborganismal and organismal responses to
123 population implications because targeted conservation actions typically operate at this level, but
124 this scaling requires additional steps and remains a challenge (but see Nisbet et al., 1989; Martin
125 et al., 2013; Grear et al., 2020; Tai et al., 2021). The ability to bridge levels of biological
126 organization from the molecular to population level makes DEB theory an excellent tool for
127 enhancing the utility of experimental hypoxia data for conservation and management (Lavaud et
128 al., 2021). However, there is a conceptual disconnect between the abstract variables and fluxes in
129 DEB models and the chemically defined quantities reported in most molecular-level studies (e.g.
130 Murphy et al., 2018). Some of the interpretation of our results in this paper rests on hypothesized
131 connections between these different levels of description, and for this reason we discuss some

132 suborganismal literature in detail in the Supplementary Materials, section *Relating DEB*
133 *processes to physiology.*

134 Depending on the application and types of data available, simplified versions of the
135 standard DEB model can be used (e.g. Kooijman and Metz, 1984; Jager, 2018; Martin et al.,
136 2017). Although complexity can sometimes be beneficial (Evans et al., 2013), simple parameter-
137 sparse models are often preferable for their predictive power and ability to be applied, tested, and
138 interpreted widely (Holling, 1966; Jusup et al., 2017). The DEBkiss framework (Figure 1) is a
139 moderately simplified variation on the standard DEB model for animals that eliminates explicit
140 representation of reserve and assumes that assimilates are immediately allocated to structure,
141 maintenance, and reproduction (Jager et al., 2013). This reduces the data requirements and,
142 depending on the data, the total number of parameters to be estimated (Jager et al., 2013). The
143 simplicity of DEBkiss makes it ideal for adaptation to many species of ecological or commercial
144 value, even when the existing studies were not originally intended for this use. Romoli et al.
145 (2024) present a detailed comparison of the advantages and limitations for ecological risk
146 assessment of a model based on DEBkiss versus a model based on Kooijman’s “standard” DEB
147 model. They highlight several “modeling choices” that should influence the choice of approach,
148 including: (i) insufficient information in data sets; (ii) capturing differences between data sets for
149 the same species; and (iii) auxiliary hypotheses. Consideration of each of these led us to choose
150 DEBkiss for our work after much unsuccessful effort attempting to interpret parameter estimates
151 with the standard model (using the Add-my-Pet software; AmPtool, 2022; Marques et al., 2018).

152
153
154



155
156

157 **Figure 1. Conceptual diagram of the DEBkiss model highlighting parameters of interest for**
158 **hypoxia effects.** The DEBkiss model (diagram adapted from Jager et al., 2013) used in this study
159 includes stage-specific survival parameters. The hypothesized parameters for hypoxia stress
160 mechanisms are highlighted in red boxes. The left panel shows the energy budget for the full life
161 cycle and the right panel shows how the stage-specific survival modification is applied to
162 embryos, larvae, juveniles, and adults of *M. menidia*.

163
164

165 We used DEBkiss to test the hypothesis that changes in animal performance under
166 hypoxia can be explained by changes in one or more of the rate processes in the model, and to
167 identify the bioenergetic mechanisms underlying experimental hatching, growth, and survival
168 effects of hypoxia in early life stages of *M. menidia* observed in Cross et al. (2019). First, we fit
169 the DEBkiss model to full-life data on total length, reproductive output, hatch timing, and
170 survival and estimated or calculated parameters under fully oxygenated conditions. Second, we
171 used the concept of Synthesizing Units (SU) that are inhibited or damaged by hypoxia to directly
172 or indirectly change key parameters in the DEBkiss model (Muller et al 2019). SUs are
173 generalized enzymes that produce products such as body structure or support maintenance
174 requirements from incoming fluxes of substrate, i.e. food or egg buffer (Kooijman, 1998;
175 Kooijman, 2010a). With single substrates for each life stage the SU formalism is equivalent to
176 standard Michaelis-Menten kinetics, but the SU interpretation allowed us to exploit the subtleties
177 in describing inhibition set out by Muller et al. (2019). We used a correction factor based on
178 inhibition or damage to the SU to fit the model to early-life data for four DO treatments. We

evaluated which parameter or combination of parameters, when adjusted with the correction factor, was able to best account for the full set of hypoxia responses observed in experiments and thus allow inference of mechanism.

182

183 2. Methods

184 2.1. DEBkiss model description

185 The material flows are shown in Figure 1. The yolk in an egg is treated as a buffer of
186 “food” for the developing embryo that initially has an infinitesimally small structural biomass. In
187 this regard, DEBkiss differs from standard DEB where the yolk is considered to be “reserve”
188 whose dynamics are treated similarly to that for feeding life stages. There is no reserve
189 compartment between food assimilation and its utilization. Birth occurs when the egg buffer is
190 fully depleted. After birth, larvae and juveniles, which are treated identically in this model, feed
191 and assimilated food is allocated to growth, maturation, and both somatic and maturity
192 maintenance in accordance with the κ -rule. After reaching puberty and entering the adult stage,
193 individuals feed and reproduce while maturation ceases. Somatic and maturity maintenance
194 continue in adults.

195 The DEBkiss assumptions and equations are from Jager (2018). The parameters are
196 defined in Table 1 and the variables, differential equations, and conversions are defined in Table
197 2. The flux of food or, for embryos, from the egg buffer (W_B) is immediately converted to
198 assimilates which are allocated to a somatic fraction (κ) and a reproductive fraction ($1-\kappa$; Figure
199 1). The assimilation flux (J_A) is the product of the scaled measure of resource availability (f), the
200 volumetric surface area (L^2), and the parameter maximum area-specific assimilation rate (J_{Am}^a)
201 where $f = 1$ for embryos and for post-hatching fish fed *ad libitum*. Within the somatic branch, a
202 flux to maintenance (J_M) is prioritized while the remainder goes to structural mass (J_V) with a
203 conversion efficiency y_{VA} . The maintenance flux is proportional to structure.

204

Parameter	Symbol	Fixed or estimated	Value
Max. area-specific assimilation rate	J_{Am}^a	Estimated	0.333 mg mm ⁻² d ⁻¹
Max. volume-specific maintenance rate	J_M^v	Fixed	0.0214 mg mm ⁻³ d ⁻¹
Initial egg buffer mass	W_{B0}	Fixed	0.15 mg
Total physical length at puberty	L_{Vp}	Fixed	102 mm
Yield of assimilates on structure	y_{AV}	Fixed	0.8

Yield of egg buffer on assimilates	y_{BA}	Fixed	0.95
Conversion efficiency of assimilates to structure	y_{VA}	Estimated	0.365
Fraction of assimilates allocated to soma	κ	Fixed	0.8
Scaled food level	f	Fixed	1
Scaled food level for embryo	f_B	Fixed	1
Embryo mortality rate	μ_{emb}	Estimated	0.0639 d ⁻¹
Post-hatch mortality rate	μ_{lar}	Estimated	0.0294 d ⁻¹

205 **Table 1. DEBkiss parameters, their abbreviations, and their fixed or estimated values from**
 206 **fitting to full life data.** Units are given with the value unless the parameter is a unitless ratio. All
 207 masses are in mg of dry weight.

208

209

Flux	Symbol	Equation	Units
Assimilation flux	J_A	$J_A = f J_{Am}^a L^2$	mg day ⁻¹
Maintenance flux	J_M	$J_M = J_M^v L^3$	mg day ⁻¹
Flux to structural growth	J_V	$J_V = y_{VA} (\kappa J_A - J_M)$	mg day ⁻¹
Flux to reproduction buffer	J_R	$J_R = (1 - \kappa) J_A - J_J \text{ when } W_V \geq W_{Vp}$ $J_R = 0 \text{ when } W_V < W_{Vp}$	mg day ⁻¹
Flux to maturity maintenance	J_J	$J_J = J_J^v L^3 \text{ when } W_V < W_{Vp}$ $J_J = J_J^v \frac{W_{Vp}}{d_V} \text{ when } W_V \geq W_{Vp}$	mg day ⁻¹
State Variable	Symbol	Equation	Units
Structural dry mass	W_V	$\frac{dW_V}{dt} = J_V$	mg day ⁻¹
Continuous reproduction rate	R	$\frac{dR}{dt} = \frac{y_{BA} J_R}{W_{B0}}$	eggs day ⁻¹
Egg buffer (yolk) mass	W_B	$\frac{dW_B}{dt} = -J_A$	mg day ⁻¹
Survival	S	$\frac{dS}{dt} = -\mu_{emb} S \text{ when } W_B > 0$ $\frac{dS}{dt} = -\mu_{lar} S \text{ when } W_B = 0$	day ⁻¹
Other variables and conversions	Symbol	Equation	Units
Total physical length	L^M	$L^M = \frac{L}{\delta_M}$	mm

Volumetric length	L	$L = \delta_M L^M = \sqrt[3]{\frac{W_V}{d_V}}$	mm
Shape coefficient	δ_M	$\delta_M = \frac{L}{L^M}$	unitless
Dry weight density of structure	d_V	$d_V = \frac{W_V}{L^3}$	mg mm^{-3}
Dry mass at puberty	W_{Vp}	$W_{Vp} = d_V * (L_{Vp} * \delta_M)^3$	mg
Volume-specific maturity maintenance costs	J_J^v	$J_J^v = \frac{1 - \kappa}{\kappa} * J_M^v$	$\text{mg mm}^{-3} \text{ day}^{-1}$
Scaled measure of resource availability	f	-	unitless (range 0-1)

210 **Table 2. Model definition.** Fluxes, state variables, and differential equations in the DEBkiss
211 model.

212

213 For larvae and juveniles, the non-somatic fraction of assimilates is spent on maturation,
214 or increasing complexity, through which it is dissipated and does not contribute to biomass.
215 While the standard DEB formulation uses a state variable for maturity that triggers changes
216 between life stages, DEBkiss instead uses a constant size at puberty to specify when
217 reproduction is initiated (Kooijman, 2010b; Jager et al., 2013), so the maturity variable plays no
218 role in the current work. Once the mass at puberty is reached (W_{Vp}), reproductive flux (J_R)
219 toward egg production begins in adults with a conversion efficiency y_{BA} . The flux to maturity
220 maintenance (J_J) is the product of the volume-specific maintenance costs (J_J^v) and structural
221 volume, or the volume at puberty for adults. J_J^v is calculated from κ and J_M^v (Table 2), rather
222 than estimated, as connecting the two maintenance costs allows cumulative investment in
223 maturity at puberty to be independent of food level (Jager, 2018).

224 Starvation is defined in two stages, with the first stage being insufficient flux of
225 assimilates to the somatic fraction to meet maintenance requirements so that energy is diverted
226 from the flux to maturation or the reproduction buffer. In the second stage, when the flux to both
227 the somatic and reproductive branches is insufficient and the reproduction buffer is empty or
228 puberty has not been reached, structure is converted to assimilates with conversion efficiency y_{AV}
229 to go towards maintenance costs (Jager, 2018).

230 Because our growth data are in total length, we used a shape correction coefficient (δ_M)
231 and dry weight density (d_V) to connect length with the model state variables (Table 2). δ_M

232 connects the total length (L^M) to the volumetric length (L) which is the cubic root of volume, and
233 d_V connects volume to structural dry mass. δ_M could plausibly have different values in different
234 life stages (section 7.8 of Kooijman, 2010a), but lacking relevant data we here assume a single
235 stage-independent value. We calculated these constants using data on *M. menidia* length (Klahre,
236 1997) and embryo volume (Schwemmer, unpublished data) and a total length (L^M) to dry weight
237 (W_V) conversion empirically derived from data on larval to adult stages (Concannon et al., 2021):

$$238 \quad W_V = 0.0012 * L^{M \cdot 2.997} \quad (1)$$

239 After calculating W_V from $L^M = 5.3$ mm at hatching (Cross et al., 2019), we obtained a dry
240 weight at hatching of 0.18 mg. Assuming there is negligible change in weight or volume during
241 hatching, we used the volume of an embryo immediately before hatching, $L^3 = 0.45$ mm³, to
242 calculate d_V using:

$$243 \quad d_V = \frac{W_V}{L^3} \quad (2)$$

244 This gave us $d_V = 0.4$ mg mm⁻³. This is slightly higher than the d_V values used for other fish
245 species (e.g. Jager et al., 2022), but the overall results were not sensitive to this parameter and it
246 allowed for a good fit to growth data across all life stages. More details on this calculation can be
247 found in the Supplemental Materials. We similarly used the embryo volume to calculate
248 volumetric length of an embryo as $L = 0.77$ mm, which gives us a δ_M of 0.145 using the
249 following equation:

$$250 \quad \delta_M = \frac{L}{L^M} \quad (3)$$

251 However, this value led the model to underestimate total length later in the life span, suggesting
252 the δ_M value was too high for this long and slender fish. This underestimation indicates that the
253 shape of a newly hatched larva is not representative of the shape throughout life and after feeding
254 begins, and this conversion could be refined for future work by making volume and length
255 measurements at multiple life stages to implement stage-specific δ_M values. We manually
256 adjusted δ_M to a final value of 0.107 which provided a reasonable fit to length data and a better
257 starting point for parameter estimation.

258 We added a survival state variable (S) which, in addition to allowing an alternative
259 outcome to hatching, enabled us to model survival as a consequence of hypoxia effects on the
260 energy budget. We fit mortality parameters for embryos and post-hatch fish (μ_{emb} and μ_{lar}) to data
261 for survival to hatching and larval/juvenile survival (Figure 1; Table 2). In our implementation of

survival, the only DEB process influencing survival is egg buffer depletion, which determines the time to hatch and thus when the embryo mortality rate switches to the post-hatch mortality rate. This means survival is indirectly affected by the assimilation rate and conversion efficiency of assimilates into structure.

266

267 2.2. Data

268 We calculated and estimated DEBkiss parameters in normoxic conditions (Section 2.3)
269 and modeled hypoxia effects (Section 2.5) based on four types of data: total length over time,
270 egg buffer mass over time (initial egg mass and age at hatching when egg buffer mass is assumed
271 to be zero), cumulative egg production over time, and proportion surviving since fertilization
272 over time. As described in the introduction, the data available for this model led us to use
273 DEBkiss over the “standard” DEB model based on the factors highlighted by Romoli et al.
274 (2024) and unsuccessful attempts to use standard DEB. We had insufficient data, had to integrate
275 information from multiple studies of the same (and similar) species, and had to hypothesize
276 plausible values for a few parameters.

277 Data for total length were sourced from four studies. Length at hatching and 15 days
278 post-hatching (dph) came from a study that reared *M. menidia* offspring in different static
279 oxygen levels across two experiments (Cross et al., 2019). This provided data for parameter
280 estimation at control oxygen levels described in Section 2.3 and modeling three reduced oxygen
281 treatments (Section 2.5 and Table 2). We sourced additional length data for the full life span
282 from control levels of experiments that exposed *M. menidia* offspring to ambient and elevated
283 CO₂ levels (Murray and Baumann, 2018; Murray and Baumann, 2020; Concannon et al., 2021).
284 All total length data were obtained from fish maintained in static laboratory conditions at 24°C.

285 Data for the state variables of egg buffer mass (via time to hatching, when egg buffer
286 mass is zero), as well as survival at hatching and at 15 dph, were obtained from Cross et al.
287 (2019). Because *M. menidia* hatch with little to no yolk sac (Bayliff, 1950; Bigelow and
288 Schroeder, 1953) and begin feeding the day of hatching (Middaugh and Lempesis, 1976), we
289 equate hatching with birth and assume the egg buffer mass reaches zero at hatching. The hatch
290 timing data use time steps of 1 day, so any very short delay between hatching and the start of
291 feeding would not be reflected in the model. The control data from these experiments were used
292 to estimate parameters under normoxia (Section 2.3). We also obtained normoxic survival data

293 from a study on the effects of temperature and CO₂ on *M. menidia* early life survival, using only
294 the 24°C and control CO₂ data (Murray and Baumann, 2018). Four additional data points for
295 long-term survival in laboratory conditions at 17°C were obtained from a study that exposed *M.*
296 *menidia* offspring until 122 dph to two CO₂ levels, of which we only used data from the control
297 level (Murray et al., 2017). Lastly, the data for cumulative egg production over time, used to
298 estimate parameters under normoxia (Section 2.3), were also obtained from control groups in
299 Concannon et al. (2021), a study in which wild-caught juveniles were held in the laboratory at
300 20°C in different CO₂ treatments and strip-spawned once they reached reproductive maturity.
301

302 2.3. Parameter estimation under normoxia

303 We estimated four parameters by fitting them to full-lifespan data listed in Section 2.2
304 (J^a_{Am} , y_{VA} , μ_{emb} , and μ_{lar}), calculated four parameters from data (J^r_M , W_{B0} , L_{Vp} , and f), and fixed at
305 suggested values for which we had insufficient data to calculate or estimate (y_{AV} , y_{BA} ,
306 κ , and f_B ; Jager, 2018). The primary parameters and their calculated or estimated values are
307 found in Table 1. Fitting was done in Matlab with the platform BYOM v.6.4 and the package
308 DEBkiss v.2.3a (<https://www.debtox.info/byom.html>). Details on parameter estimation in
309 BYOM can be found in the Supplementary Materials.

310 We were able to obtain a reasonable fit using suggested values for y_{AV} , y_{BA} , and κ for
311 unstressed fish that are thought to be widely applicable across species (Lika et al., 2011; Jager,
312 2018). We used length, reproduction, and egg buffer depletion data to estimate y_{VA} with the
313 BYOM optimization. Ultimate length was used to fit J^a_{Am} to a reasonable value while fixing all
314 other parameters before estimating y_{VA} , because both parameters affect growth and egg buffer
315 depletion in the model and therefore cannot be estimated simultaneously. Finally, we used the
316 BYOM optimization to estimate μ_{emb} and μ_{lar} .

317 The length and reproductive data allowed us to calculate “length at puberty” (L_{Vp}),
318 defined as the length at which egg production begins. We obtained W_{B0} from *M. menidia* egg dry
319 weight data (Klahre, 1997) and calculated δ_M and d_V from total length, egg diameter, and egg
320 mass data (Cross et al., 2019; Klahre, 1997; Concannon et al., 2021). To calculate volume-
321 specific maintenance costs (J^r_M), we used data on the rate of decrease in larval dry weight over a
322 period of starvation in the congeneric species *M. beryllina* (Letcher and Bengtson, 1993). More
323 detail on this calculation can be found in the Supplemental Materials. Borrowing from closely

related species is a common practice in bioenergetic modeling when the species has similar habitat, life history, and physiology (Sibly et al., 2013). *M. menidia* and *M. beryllina* have overlapping habitats and similar life history, egg sizes, and body sizes, although *M. beryllina* reaches a smaller ultimate length (Middaugh, 1981; Bengtson, 1984; Middaugh and Hemmer, 1992). All *M. menidia* experiments used in this study fed fish *ad libitum* in all treatment levels, so f was set to 1. For studies that exposed fish to different CO₂ levels, we only used data from control groups to avoid potential CO₂ effects in the data.

331

332 2.4. Relating DEB processes to physiology

333 We aimed to identify the DEBkiss parameters responsible for observed whole-organism
334 effects of rearing *M. menidia* in hypoxia by applying a correction factor to modify one or more
335 parameters with decreasing oxygen based on inhibition of or damage to a SU. The SU controls
336 assimilation, the transformation of food (or yolk) and oxygen into compounds that will go to
337 structure, maintenance, or reproduction (Kooijman, 2010a; Jager, 2018). Although oxygen can
338 be a limiting substrate in SUs, previous work suggests that *M. menidia* embryos only become
339 metabolically oxygen-limited below a critical level of 2.04 mg L⁻¹ (Schwemmer, 2023), while it
340 remains oxygen-independent at the treatments for which we have data (2.7, 3.1, 4.2, and 7.7 mg
341 L⁻¹; Schwemmer et al., 2020). We therefore considered a single-substrate growth SU in which
342 food or egg buffer was the substrate. The mathematical characterization of inhibition and damage
343 is in Section 2.5.

344 Inhibiting agents reversibly bind to SUs, preventing them from accepting substrates to
345 proceed with their reaction. Damage, in contrast, induces dysfunction that is irreversible upon
346 removal of the damaging agent; however, damaged SUs can be repaired or replaced (Muller et
347 al., 2019). The idea is that hypoxia induces the production of compounds that in turn bind to
348 SUs. We used existing information on the physiological responses of fish early life stages to
349 hypoxia to identify the following candidate DEBkiss parameters to which to apply the hypoxia-
350 based correction factor: maximum assimilation rate (J^a_{Am}), conversion efficiency of assimilates
351 into structure (growth, y_{VA}), maximum somatic maintenance rate (J^r_M , mg mm⁻³ d⁻¹), embryo
352 mortality rate (μ_{emb}), and post-hatch mortality rate (μ_{lar}). Hypoxia effects on growth and hatching
353 time can occur either through inhibition of assimilation or through damage that reduces the
354 conversion efficiency of assimilates to growth. Hypoxia may impact survival directly through

355 damage or by inhibition of damage repair processes. Hypoxia's impact on somatic maintenance
356 rate may be most plausibly represented as damage. Inhibition of or damage to SUs could affect
357 these parameters as a direct or indirect result of several hypoxia responses in fish, such as
358 anaerobic respiration, behavior, and action of hypoxia-inducible factors (Farrell and Brauner,
359 2009). A detailed review of how these mechanisms relate to the DEB parameters and SUs can be
360 found in the Supplementary Materials.

361

362

363 2.5. Hypoxia effects

364 We tested the hypothesis that changes in *M. menidia* early life growth, hatch timing, and
365 survival under reduced oxygen (Cross et al., 2019) can be explained by inhibition or damage
366 linked to one or more DEBkiss processes (Figure 1). To summarize the experimental data on
367 static hypoxia effects we are attempting to explain by altering these parameters, the mean values
368 of data for each oxygen treatment are listed in Table 3. We used the parameter values from the
369 model fit to full life data and altered one or more parameters at a time with oxygen-dependent
370 correction factors, then fit the model to data for only the first 136 days by estimating a parameter
371 that controls the correction factor's relationship with DO. We only used early life data to fit the
372 hypoxia-altered parameters because we did not have late-life or reproductive data for multiple
373 oxygen treatments against which to validate observed changes. It did not make sense to include
374 later life data in the calculations of NLL that influence the parameter estimates or to speculate
375 about how well the predicted data match what we might expect to happen later in life if we not
376 only lack late-life hypoxia data but also do not expect full-life hypoxia to occur in nature.

377

Variable	7.7 mg L ⁻¹	4.2 mg L ⁻¹	3.1 mg L ⁻¹	2.7 mg L ⁻¹
Survival to hatching	74.3%	70.6%	85.8%	30.2%
Hatch time (egg buffer mass = 0)	6 days	7 days	8 days	9 days
Length at hatching	5.3 mm	4.6 mm	4.4 mm	4.1 mm
Larval length at 15 dph	15.8 mm	12.2 mm	9.2 mm	-
Larval survival to 15 dph	44.0%	22.2%	20.9%	0%

378 **Table 3. Summary of experimental data for each DO level.** The mean survival to hatching,
379 hatch time (at which egg buffer is zero), length at hatching, length at 15 dph, and survival to 15
380 dph from the different DO treatments in Cross et al. (2019). The control DO level means (7.7 mg
381 L⁻¹) also include data from Murray and Baumann (2018).

382

We derived a correction factor for *inhibition* using the framework developed by Muller et al. (2019), in which inhibitors can act on SU dynamics in five different ways. Out of these, *noncompetitive inhibition* is well-suited to this study because of the limitations of data availability for *M. menidia*. In noncompetitive inhibition the arrival rate of substrate does not affect the binding and dissociation of inhibitors and therefore requires little information about the rate of food uptake (Muller et al., 2019). In this form of inhibition, the rate of assimilation by the SU is:

$$J_A = f J_{Am}^a L^2 \left(\frac{1}{1 + \frac{j_i}{k_i}} \right) \quad (4)$$

where j_i (mg d^{-1}) is the arrival flux of the inhibitor and k_i (mg d^{-1}) is the dissociation parameter. The effect of this relationship in our model is that assimilation declines as the arrival rate of hypoxia-related inhibitors increases. We set j_i to depend on DO treatment above a DO threshold, DO_c (mg L^{-1}), below which j_i is infinitely large, which would bring the rate of the process it is inhibiting to zero:

$$j_i = \begin{cases} \infty & \text{if } \text{DO} \leq \text{DO}_c \\ \frac{1}{B(\text{DO} - \text{DO}_c)} & \text{if } \text{DO} > \text{DO}_c \end{cases} \quad (5)$$

B ($\text{L} \cdot \text{d} \cdot \text{mg inhibitor}^{-1} \cdot \text{mg O}_2^{-1}$) is a parameter that influences the shape of the relationship between j_i and DO. We defined the correction factor c as the inhibition term (in parentheses in Equation 4) and replace j_i with the function from Equation 5 for $\text{DO} > \text{DO}_c$ to derive the correction factor c :

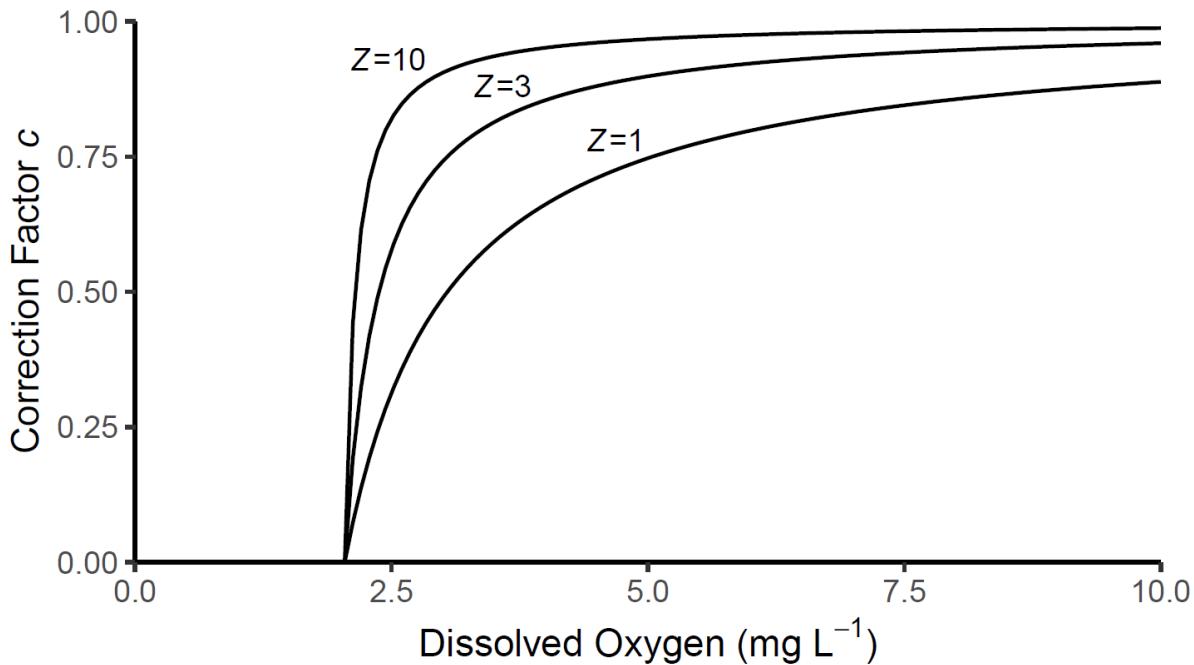
$$c = \frac{1}{1 + \frac{1}{k_i B (\text{DO} - \text{DO}_c)}} \quad (6)$$

As only the product of the parameters k_i and B appear in the formula and we have no need to estimate them separately, they can be combined into one parameter as Z (L mg O_2^{-1}). Simplifying Equation 6 and adding in the case for which $\text{DO} \leq \text{DO}_c$ gives us the following correction factor:

$$c = \begin{cases} 0 & \text{if } \text{DO} \leq \text{DO}_c \\ \frac{Z(\text{DO} - \text{DO}_c)}{1 + Z(\text{DO} - \text{DO}_c)} & \text{if } \text{DO} > \text{DO}_c \end{cases} \quad (7)$$

The relationship between c and DO for three different sample values of Z , the parameter to be estimated, is shown in Figure 2. A larger Z value keeps c higher as oxygen decreases before a more abrupt drop, while a smaller Z gives a more constant decline in c with hypoxia. The value

409 of c cannot exceed 1. DO_c was fixed at a biologically relevant level of 2.04 mg L^{-1} , which is the
410 critical oxygen level below which embryonic routine metabolism becomes highly oxygen-
411 dependent (Schwemmer, 2023). Attempts to estimate DO_c and Z simultaneously showed that
412 leaving DO_c free did not improve the ability of the correction factor to fit the hypoxia data.
413



414

415 **Figure 2. The correction factor c used to apply hypoxia effects to DEBkiss parameters.** The
416 effect of DO on correction factor c is shown at three different values of the parameter Z . Actual
417 estimated Z values are listed in Table 4, and the three Z values used in this figure are sample
418 values to show how Z affects the relationship between DO and c .

419

420 Similar simplification of the reasoning by Muller et al. (2019) can be used to derive an
421 analogous correction factor for *damage*. Assuming a proportional change in the rate of damage
422 production to the SU (e.g. via “damage inducing compounds”; Kooijman 2010a), j_d has the same
423 form as Equation (5). If damage production is quickly balanced by repair or mitigation, then
424 fluxes that decrease through hypoxia will again be reduced by the factor given by Equation (7).
425 This was recognized by Muller et al. (2019) who noted that if damage production is much slower
426 than the maximum production rate of an SU, the formalism for noncompetitive damage is

equivalent to that of noncompetitive inhibition (Muller et al., 2019). Further submodels relating damage to rates that may increase in response to hypoxia (e.g. maintenance and mortality) are needed to derive functional forms for appropriate conversion factors here. Absent information to support such submodels, we hypothesize that the increase was inversely proportional to c defined by Equation (7).

The correction factor c was multiplied by J^a_{Am} and y_{VA} because these parameters were hypothesized to decrease under hypoxia irrespective of the underlying cause (inhibition or damage). Reductions in the parameter y_{VA} through hypoxia are most plausibly interpreted as damage, the irreversible destruction of functionality of an SU. However, the parameters for maintenance and mortality were divided by c because they were hypothesized to increase, rather than decrease, with damage production and inhibition.

To find the best value of Z for each DEBkiss parameter or combination of parameters, we added Z as a model parameter and estimated it using the BYOM optimization to minimize NLL. We weighted the data equally across treatments to correct for differences in sample size across treatments and prevent one treatment group from disproportionately affecting the estimation of Z , so that all weights within each treatment added up to the same number. We did not apply the correction factor to J^a_{Am} and y_{VA} simultaneously because they both contribute to J_V and their individual contributions to the growth and egg buffer depletion are difficult to disentangle, particularly when J_M is very small as in the early life stages. We only compared the fit of models in which c was applied to parameter(s) that resulted in all three early life datasets – total length, egg buffer mass, and survival – being affected by hypoxia. As a result, either J^a_{Am} or y_{VA} is in each candidate model, because J'_M , μ_{emb} , and μ_{lar} do not affect egg buffer depletion.

To identify the most likely version of the model (which parameter or combination of parameters best explains the hypoxia effects on the state variables), we estimated Z for each of these scenarios and calculated Akaike's Information Criterion for small sample sizes (AICc). We compared the AICc between each model using the difference between AICc values (ΔAICc) and the relative likelihood of each model using Akaike weights:

$$w_i(\text{AICc}) = e^{-0.5 \cdot \Delta_i \text{AICc}} / \sum_{k=1}^K e^{-0.5 \cdot \Delta_k \text{AICc}}, \quad (8)$$

where $w_i(\text{AICc})$ is the Akaike weight of each model i , $\Delta_i \text{AICc}$ is the difference between each model i and the model with the lowest AICc (AICc_{\min}), and the denominator calculates the sum of relative likelihoods for every model starting at the first model k (Wagenmakers and Farrell,

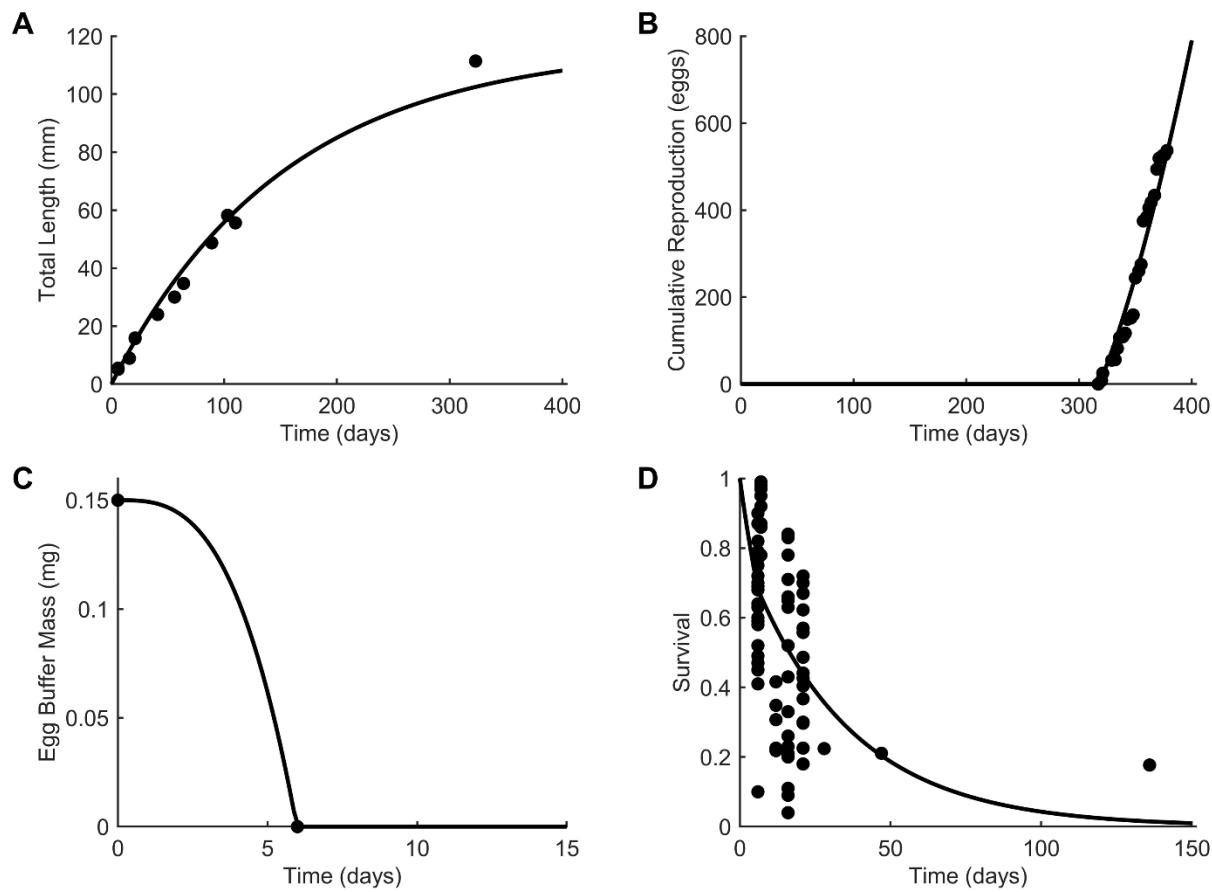
458 2004). We used $\Delta AICc$ and ratios of Akaike weights to determine which combination of
459 parameters best fit the data when inhibition or damage was applied and, therefore, which DEB
460 processes best explain the hypoxia effects observed in experiments (Table 4).

461

462 3. Results

463 3.1. DEBkiss model

464 We obtained realistic fits to the full life cycle data (Figure 3). The only exception is late-
465 life survival, for which the mortality was too high beyond the larval stage but could not be better
466 fit due to lack of full-life survival data (Figure 3D). Silversides are an annual species so survival
467 should be greater than 0% after 150 days. However, this did not impair our ability to model the
468 effects of hypoxia on early life survival, which is most important given that the present study
469 focuses on hypoxia in the early life stages. Estimating y_{VA} returned a value much lower than 0.8,
470 which is the value suggested by Jager (2018) and has been applied in DEBkiss models of other
471 species (e.g. Jager et al., 2018; Hamda et al., 2019). However, our value of $y_{VA} = 0.365$ is close to
472 the maximum growth efficiency of 0.375 measured in the closely related *M. beryllina* (Letcher
473 and Bengtson, 1993). This gave a realistic fit to the length data and allowed a detailed and very
474 close fit to egg buffer mass over time (hatch timing). The observed and predicted data for full life
475 span are plotted in Figure 3.



476

477

478 **Figure 3. Full life model fits to data for four state variables.** Predicted (lines) and observed
 479 data (dots) for the DEBkiss model of *M. menidia* are shown. The state variables are (A) total
 480 length (mm) over time (days), (B) cumulative reproduction (eggs) over time (days), (C) egg
 481 buffer mass (mg) over time (days), and (D) survival over time (days). Predicted data lines were
 482 calculated with the parameter values listed in Table 1.

483

484

485 3.2. Hypoxia effects

486 Applying the oxygen-dependent correction factor to the parameter combinations listed in
 487 Table 4 reproduced the direction of experimentally observed hypoxia effects, e.g. decreasing
 488 J^a_{Am} reduced total length, increased time until egg buffer mass reaches 0, and reduced survival.
 489 The best model of experimental hypoxia effects on *M. menidia* early life stages simultaneously
 490 had y_{VA} multiplied by c , and μ_{emb} and μ_{lar} divided by c (Figure 4, Table 4, Figure S1). Although

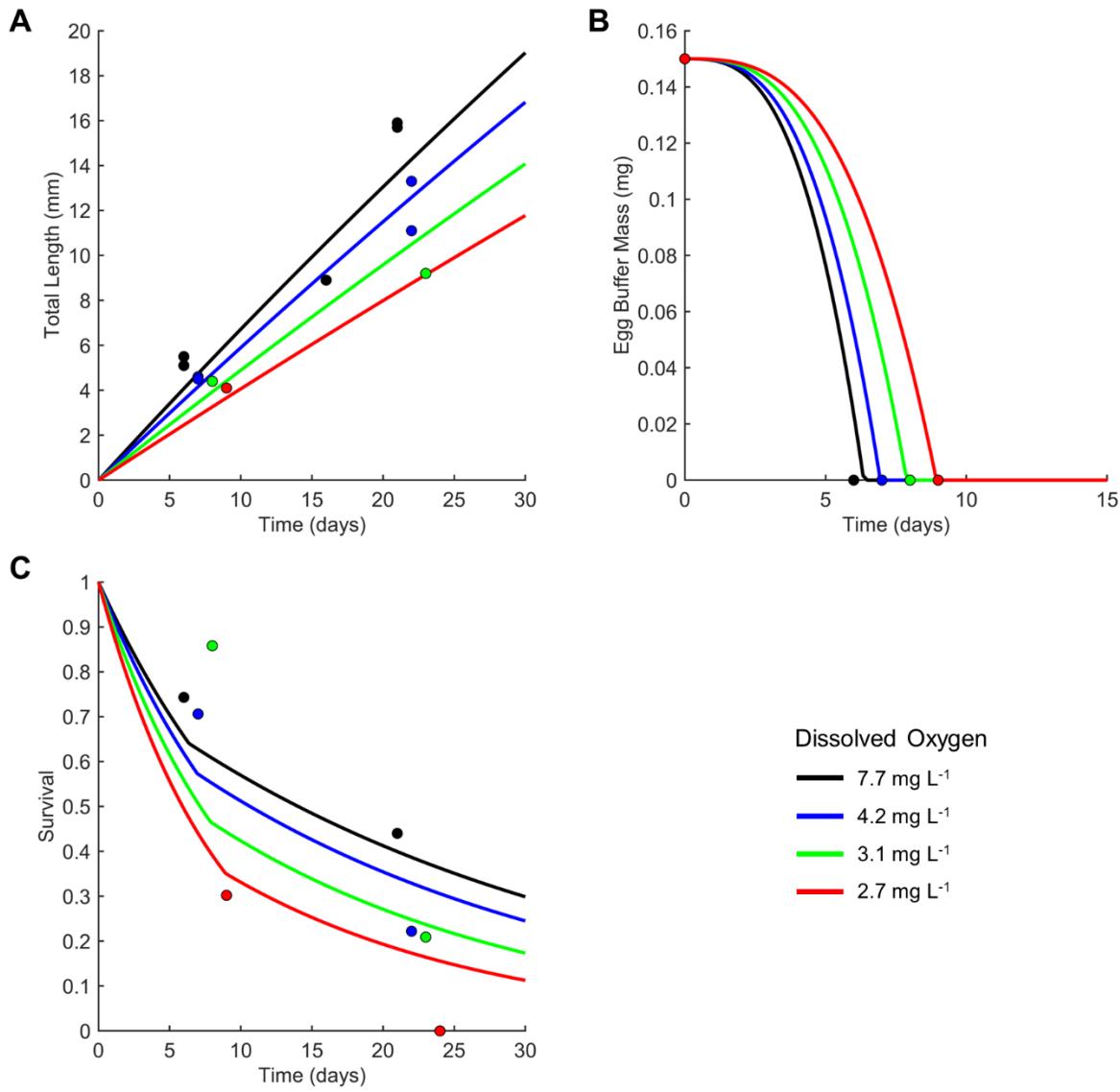
491 applying damage to y_{VA} alone affected all three state variables, concurrently increasing both
 492 mortality parameters improved the fit to the data (Table 4). The model in which the correction
 493 factor was applied to y_{VA} , μ_{emb} , and μ_{lar} also had the lowest AICc of all candidate models, with an
 494 AICc of 794.03 (AICc_{min}). Adding a correction factor to J^v_M in simultaneously with these three
 495 parameters yielded a slightly higher AICc of 795.97 (Table 4). The ratio of Akaike weights
 496 shows that the model with c applied to y_{VA} , μ_{emb} , and μ_{lar} , is 2.67 times as likely as the one with c
 497 concurrently applied to J^v_M (Table 4). Applying a damage effect to maintenance was therefore
 498 not considered to have improved the fit. After estimating Z , we calculated the values of y_{VA} , μ_{emb} ,
 499 and μ_{lar} when their respective correction factors are applied for each DO level (Table 5).

500
 501

Parameter(s) affected by hypoxia correction factor	Estimated Z [95% CI]	AICc	ΔAICc	Akaike weight
J^a_{Am}	3.019 [2.512-3.612]	856.06	62.03	2.5e-14
y_{VA}	1.818 [1.601-2.342]	848.65	54.62	1.0e-12
$J^a_{Am} + J^v_M$	3.105 [2.651-3.726]	855.00	60.97	4.2e-14
$y_{VA} + J^v_M$	1.985 [1.688-2.774]	850.64	56.61	3.7e-13
$J^a_{Am} + \mu_{emb}$	2.804 [1.605-3.287]	823.24	29.21	3.3e-7
$y_{VA} + \mu_{emb}$	1.801 [1.570-2.167]	808.12	14.09	6.3e-4
$J^a_{Am} + \mu_{lar}$	2.930 [2.165-3.428]	838.17	44.14	1.9e-10
$y_{VA} + \mu_{lar}$	1.767 [1.536-2.111]	821.30	27.27	8.7e-7
$J^a_{Am} + \mu_{emb} + \mu_{lar}$	2.819 [1.920-3.286]	810.21	16.18	2.2e-4
$y_{VA} + \mu_{emb} + \mu_{lar}$	1.827 [1.620-2.269]	794.03	0	0.72
$J^a_{Am} + J^v_M + \mu_{emb} + \mu_{lar}$	2.913 [2.288, 3.387]	809.96	15.93	2.5e-4
$y_{VA} + J^v_M + \mu_{emb} + \mu_{lar}$	1.981 [1.700, 2.456]	795.97	1.94	0.27

502 **Table 4. Parameter Z estimates and model selection results.** The estimated Z value, AICc,
 503 ΔAICc, and Akaike weights when the correction factors were applied to each parameter or
 504 combination of parameters. ΔAICc and Akaike weights were calculated with AICc_{min} = 794.03
 505 for the $y_{VA} + \mu_{emb} + \mu_{lar}$ model (bold).

506
 507



508
509

510 **Figure 4. Best fit of DEBkiss model to experimental data from four DO levels.** The best fit of
511 the predicted data (lines) to the observed data (dots) for four DO levels is shown, for early life
512 data only. The best fitting model was selected based on lowest AICc. (A) is total length (mm)
513 over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time
514 (days), with means rather than all data plotted for survival for ease of viewing. Full datasets used
515 to estimate the correction factor parameter Z are plotted in Figure S1.

516

517 Interestingly, although J^a_{Am} affects the variables similarly to y_{VA} , the ratio of Akaike
518 weights showed that the best fitting model is about 3000 times as likely as the version applying

519 inhibition to J^a_{Am} , μ_{emb} , and μ_{lar} (Table 4). Reducing J^a_{Am} with hypoxia using the correction factor
520 resulted in a visually good fit to the data across oxygen levels and variables. Simultaneously
521 applying c to J^a_{Am} and both mortality parameters improved the fit compared to only applying it to
522 J^a_{Am} , but this model fit less well than the version that applied c to y_{VA} , μ_{emb} , and μ_{lar} , with an AIC
523 value of 810.21 in the former model compared to 794.03 in the latter.

524 The estimated best value of Z , the exponential coefficient in the correction factor c ,
525 enables us to calculate that y_{VA} at the lowest oxygen level is 55% of its value with no hypoxia
526 stress. Reducing conversion efficiency alone produced small differences in survival at hatching
527 because it prolongs the time spent in the embryo stage, which has a greater mortality rate than
528 post-hatching in our model. Dividing both the pre- and post-hatching mortality rates by c more
529 closely predicted the reduced survival rates in the low DO treatments, resulting in a best fitting
530 model that explained observed hypoxia effects well by altering conversion efficiency, embryo
531 mortality, and post-hatch mortality.

532

	Product of correction factor and initial parameter value			
	7.7 mg L ⁻¹	4.2 mg L ⁻¹	3.1 mg L ⁻¹	2.7 mg L ⁻¹
y_{VA}	0.333 [0.329, 0.339]	0.291 [0.284, 0.303]	0.240 [0.230, 0.257]	0.199 [0.188, 0.218]
μ_{emb}	0.0701 [0.0689, 0.0709]	0.0801 [0.0770, 0.0822]	0.0970 [0.0906, 0.101]	0.117 [0.107, 0.124]
μ_{lar}	0.0322 [0.0317, 0.0326]	0.0369 [0.0354, 0.0378]	0.0446 [0.0417, 0.0466]	0.0539 [0.0492, 0.0571]

533 **Table 5. Effects of correction factor c on parameters.** The value of the DEBkiss parameters
534 that best reproduce the hypoxia effects observed experimentally, calculated (with 95%
535 confidence intervals in brackets) for each DO treatment level using the correction factor c and
536 the estimated value of $Z = 1.827$.

537

538

539 4. Discussion

540 By combining experimental data with unified principles for energetic allocation that are
541 broadly applicable across species, we identified the conversion efficiency of assimilates into
542 structure as the most likely process by which low oxygen levels affect early life stages of *M.*
543 *menidia*. In comparing combinations of DEBkiss parameters that influence the ecological
544 endpoints (total length, hatch timing, and survival), we discovered that applying correction

545 factors based on damage production to the growth SU to reduce conversion efficiency (y_{VA}) and
546 increase pre- and post-hatching mortality rates (μ_{emb} and μ_{lar}) best predicted the experimental
547 effects of hypoxia on larval length, time to hatching, and early life survival. Through this model
548 we have found evidence that the mechanism largely responsible for the observed hypoxia
549 impacts on growth, hatch timing, and survival is the efficiency with which assimilated food or
550 egg yolk is converted into structure. The limitations of this inference are discussed later.

551 Changes to assimilation in response to hypoxia have been recorded in other species, but
552 the direction of that effect is species-dependent (reviewed in Thomas et al., 2019). In *M.*
553 *menidia*, however, reducing assimilation with hypoxia rather than conversion efficiency yielded
554 a worse fit despite the two parameters' similar contributions to the DEBkiss model in that both
555 parameters are used to calculate predicted growth and egg buffer depletion. Reducing either
556 assimilation or conversion efficiency would extend developmental time, which is consistent with
557 previous work showing yolk absorption slows under hypoxia (Polymeropoulos et al., 2017). As
558 maintenance costs must continue to be paid, this would increase the energy expended to produce
559 each unit of structure (Kamler, 2008). Unlike assimilation, the mechanism for reduced
560 conversion efficiency is most plausibly interpreted as damage to the synthesizing unit, perhaps
561 from buildup of anaerobic byproducts, along with far less efficient ATP production through
562 anaerobic respiration and slower rates of tissue differentiation (Bouma et al., 1994; Kooijman,
563 2010a; Muller et al., 2019). The experimental DO levels are greater than the critical oxygen levels
564 for oxygen-independent routine metabolism (P_{crit}) of 2.04 mg L^{-1} and 1.56 mg L^{-1} for embryos
565 and 5dph larvae, respectively (Schwemmer, 2023). P_{crit} has been assumed by some to be the
566 oxygen level at which anaerobic metabolism is triggered, but there is abundant evidence that
567 some level of anaerobic metabolism can occur well above P_{crit} (Nonnotte et al., 1993; Maxime et
568 al., 2000; Wood et al., 2018). Additional activity such as swimming bursts can drive up the need
569 for anaerobiosis (Di Santo et al., 2017). Our evidence that conversion efficiency is reduced by
570 hypoxia-induced damage suggests that anaerobic metabolism may be a mechanism of hypoxia
571 effects in *M. menidia* early life stages even at oxygen levels above P_{crit} .

572 While y_{VA} is the best parameter to explain the hypoxia effects according to our model and
573 AICc, it is nonetheless possible that J^a_{Am} is responsible for an unknown portion of the hypoxia
574 effects. Because of near collinearity between J^a_{Am} and y_{VA} , our model does not allow us to test for
575 the possibility that both parameters are simultaneously contributing to the observed hypoxia

576 effects. It is not possible to simultaneously estimate both parameters, particularly when J_M is
577 negligible as in the early life stages and J^a_{Am} and y_{VA} are directly multiplied to calculate growth
578 in the model; we can adjust one or the other with the correction factor and get similar effects on
579 the flux for growth with no way of determining which is correct. We therefore cannot test for
580 partial contribution of the two parameters to hypoxia effects or quantify their relative
581 contributions. If conversion efficiency were the only parameter varying across hypoxia
582 treatments, one might expect all offspring to fully deplete the egg buffer and hatch at the same
583 time, but with hatch size increasing with DO level. However, adjusting conversion efficiency
584 with hypoxia does account for the observed significant differences in hatch timing between DO
585 treatments in *M. menidia* larvae (Cross et al., 2019) because y_{VA} reduces the body size at a given
586 time, indirectly reducing the assimilation flux due to smaller body volume. Future work
587 examining the effects of hypoxia on ingestion, defecation, respiration, and growth could help
588 tease apart the relative contributions of y_{VA} and J^a_{Am} by allowing direct calculation of y_{VA} . Data
589 on fecundity at different DO levels would provide information on the contribution of J^a_{Am} ,
590 although constant hypoxia through adulthood is unrealistic and this would assume the energy
591 budget is impacted similarly across life stages.

592 Although both conversion efficiency and assimilation can explain hypoxia effects on total
593 length and egg buffer mass over time, reducing them only produced a small decrease in survival
594 relative to the data. Simultaneously applying c to both mortality rates better predicted the great
595 reductions in survival at both hatching and 15 dph with hypoxia and improved the fit based on
596 ΔAICc (Table 4). In the experiments, the lowest oxygen level (2.7 mg L^{-1}) had a mean hatch
597 survival of 30.2% while the mean survival in the other three treatments was over 70% (Cross et
598 al., 2019). By 15 dph fish from all three low oxygen treatments had lower survival than those
599 from the normoxic treatment (Cross et al., 2019; Table 3). The additional mortality that was not
600 accounted for by y_{VA} may have been related to unrepaired damage from buildup of toxic
601 compounds during anaerobic metabolism (Richards, 2011). The mortality could also have
602 resulted from failing to meet energetic demands with either aerobic or anaerobic metabolism
603 (Richards, 2009) and, specifically in embryos, failure to reach a viable level of complexity
604 before the yolk is depleted (Jager et al., 2013). Measurement of anaerobic byproducts such as
605 lactate and morphometric assessment of dead embryos and larvae could help to identify the
606 mechanisms underlying the mortality rates in future work. Although survival does not approach

607 0% during the larval stage in our best fitting model (Figure 4), all experimental replicates of the
608 2.7 mg L⁻¹ DO treatment had 0% survival by 15 dph, making larvae apparently more sensitive
609 than embryos (Cross et al., 2019). The authors of the study attribute this to a possibly lower
610 ability to suppress metabolism in larvae compared to embryos. While the increased mobility of
611 larvae may allow aquatic surface respiration (Miller et al., 2016; Cross et al., 2019) and escape
612 from hypoxia in a patchy and stratified estuarine environment, activity comes with elevated
613 maintenance costs in addition to those required to begin feeding almost immediately after
614 hatching (Middaugh and Lempesis, 1976). This may also be a crucial time to repair damage to
615 the SU (Muller et al., 2019), and the combination of these additional maintenance demands may
616 be too great to meet without restoration of normoxia. Though beyond the scope of this work, a
617 model that captures stage-specific differences in maintenance costs and links them explicitly to
618 survival may better capture the mechanism of high mortality in larvae.

619 Adding a correction factor to the volume-specific maintenance rate in addition to y_{VA} ,
620 μ_{emb} , and μ_{lar} did not substantially improve the fit according to AICc, suggesting that increasing
621 maintenance costs is not an important bioenergetic mechanism underlying hypoxia response in
622 early life stages. This is consistent with laboratory measurements showing no effect of these
623 hypoxia levels on embryonic or larval metabolic rates (Schwemmer et al., 2020), but as noted
624 earlier interpretation of respiration data is challenging and there was high individual variability
625 in the data. In our model, egg buffer depletion is insensitive to changes in volume-specific
626 maintenance costs, requiring a quadrupling to see a noticeable delay in hatching. Changing
627 maintenance has much greater effects on length later in life while failing to explain differences in
628 length at the time of hatching. Because maintenance is dependent on volume, it is a relatively
629 small portion of the energy budget in the very small early life stages but increases substantially
630 relative to the surface area-specific assimilation when larger sizes are reached, increasing its
631 relative role in determining growth rate and, indirectly, all size-specific fluxes. Repairing
632 damage and increasing ventilation and swimming activity could both increase maintenance costs
633 (Thomas et al., 2019), but at the embryo stage very little activity is possible. Some studies on
634 fish responses to hypoxia suggest maintenance may drop temporarily due to the reduced capacity
635 for aerobic metabolism at low DO levels, then subsequently be temporarily elevated after oxygen
636 is restored because of recovery demands such as paying oxygen debt and removing or repairing
637 damage from anaerobic byproducts (Heath and Pritchard, 1965; Claireaux and Chabot, 2016;

638 Thomas et al., 2019). Such fluctuations in maintenance were not discernible in the time scale of
639 our model, but future work should attempt to model the *M. menidia* early life energy budget
640 during recovery from hypoxia.

641 Understanding the mechanisms of reduced growth and survival under hypoxia through
642 DEB theory is useful for predicting life history effects, and although modeling population growth
643 rates was not within the scope of this study, our results have implications for processes that
644 influence fish population dynamics. The best fitting model predicts hypoxia-related reductions in
645 long-term growth and survival that would certainly be detrimental to population growth under
646 extended periods of low oxygen. Under this model, even restoring normoxia after 15 days would
647 result in smaller size at age and survival rates than the control group, and damage to the SU is
648 not reversed upon return to normoxia, but rather requires energy to repair (Muller et al., 2019).
649 Delayed hatching and slower growth can lead to enhanced vulnerability to predation (Chambers
650 and Leggett, 1987; Takasuka et al., 2007), further reducing fish survival rates beyond those
651 observed in controlled laboratory conditions, although this is not always the case (Lankford et
652 al., 2001; Robert et al., 2023). However, compensation of growth may be possible in aquatic
653 ectotherms after exposure to hypoxia (Wei et al., 2008). An important assumption of our model is
654 that several of the parameters have the same value across life stages (e.g. J^a_{Am} , J^r_M , y_{VA}) and
655 similarly that values of the hypoxia correction factors are the same regardless of life stage. We
656 lacked data on the effects of hypoxia on the proportion of total energy allocated to reproduction
657 ($1-\kappa$), which is an additional component of DEB useful in connecting organismal effects to
658 populations. Future experimentation could provide the adult-stage information that is needed to
659 extend this DEB model to predict population growth, which would be useful for resource
660 management applications (Kooijman et al., 2020; Lavaud et al., 2021), given the ecological
661 importance of forage fishes and the value of model species like *M. menidia*.

662 The oxygen levels in the estuaries inhabited by *M. menidia* undergo great diel and
663 seasonal fluctuations (Baumann et al., 2015). The effects of fluctuating DO cannot be resolved in
664 the time scales used by our DEBkiss model, so we assumed constant DO levels. As a result, the
665 model is more useful in identifying mechanisms than in quantitatively predicting how *M.*
666 *menidia* will respond to realistic hypoxia scenarios, as lifelong constant hypoxia is unrealistic
667 and this assumption may lead to overestimation of hypoxia effects. Studies comparing fish
668 responses to static and fluctuating hypoxia treatments have suggested that fluctuations provided

temporary relief and reduced sensitivity (Cross et al., 2019; Williams et al., 2019; Wang et al., 2021), although conflicting results also exist (Morrell and Gobler, 2020). It is also unrealistic for only a single environmental factor, in this case hypoxia, to influence the energy budget. Other studies have applied correction factors to DEB parameters to model other species' responses to hypoxia (Lavaud et al., 2019; Aguirre-Velarde et al., 2019), seawater acidification (Jager et al., 2016; Moreira et al., 2022; Pousse et al., 2022) and pollutants (Muller et al., 2010; Desforges et al., 2017). The success of this approach with a wide variety of stressors makes it an ideal supplement to multistressor experiments, which are limited by logistical constraints. Modeling stressor effects with DEBkiss parameters can yield additional information about energetic mechanisms of responses and, with careful attention to the assumptions being made, may be useful in extrapolating stressor effects to additional magnitudes or combinations of stressors that would have been impractical to test experimentally, or to species with certain shared physiology or life history traits (Goussen et al., 2020; Boult and Evans, 2021). In the case of *M. menidia*, previous work showed that high CO₂ increases oxygen dependence of metabolism under both chronic (Schwemmer et al., 2020) and acute hypoxia (Schwemmer, 2023). Adding oxygen as a second substrate in the SU would allow a DEB model to incorporate the oxygen limitation that is evidently induced by acidification.

Our best fitting model overestimated time to hatching at 7.7 mg L⁻¹ DO and overestimated survival at age for the 2.7 and 4.2 mg L⁻¹ treatments, which suggests there either may be a different nonlinear correction factor function that better fits the relationship between DO and the DEBkiss parameters or that there were additional factors contributing to these differences that the model does not account for. 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. Despite the potential for improvements with more data, the model was able to replicate the direction of effects and even account for some hypoxia effects in all three state variables simultaneously by changing only one parameter, either conversion efficiency or assimilation. Further, it provided these reasonable fits using an SU model based in well-studied and widely applicable Michaelis-Menten-Briggs-Haldane enzyme kinetics (Muller et al., 2019) rather than a more specialized or complex correction factor. While the generalized framework allows this model to be applied to

700 other species, one species-specific assumption is that birth occurs at hatching. This is a fitting
701 assumption for *M. menidia*, which are known to hatch with no detectable yolk (Bayliff, 1950)
702 and begin feeding the day of hatching (Middaugh and Lempesis, 1976). However, investigators
703 would need to alter the model or use different types of data before applying this approach to
704 species that have an extended yolk-sac larval stage before feeding begins.

705 We end with a comment on the limitation of the “DEBtox” approach (Kooijman et al.,
706 2009), a toxicology application of DEB from which DEBkiss stems, to identifying physiological
707 modes of action in response to environmental stress. In Section 1 we cite the paper by Romoli et
708 al. (2024) that highlighted the difficult modeling choices that are required. Here we chose to use
709 DEBkiss coupled with several hypothesized responses to hypoxia. We selected the combination
710 of DEB model and response hypothesis that best described given data (in an information
711 theoretic sense using AICc), *conditional on the “correctness” of the model and of assumed*
712 *values for some parameters*. Yet, for a case study in ecotoxicology, Romoli et al. showed that a
713 different dominant physiological model of action was selected when using two different
714 underlying DEB models that both give visually good fits to control data. Muller et al. (2010)
715 demonstrated a related issue for a study of early life stage growth by identifying best fit
716 submodel for larval growth of two closely related bivalve species exposed to mercury.
717 Implausibly, the selected submodels were different to the extent that the best fit for one species
718 was the worst for the other.

719 In the preceding discussion, we have offered a few suggestions for empirical work on
720 whole organisms that would significantly help narrow down the DEB processes responsible for
721 responses to hypoxia. However, it is likely that an additional, very promising way forward is to
722 determine *suborganismal* processes co-occurring with the observed whole-organism responses.
723 Transcriptomic data represent a particularly promising candidate (Murphy et al 2018). We
724 recognized this qualitatively in Section 2.4 when invoking genes controlling cell division and
725 protein synthesis that are regulated by hypoxia-inducible factors. Stevenson et al. (2023)
726 demonstrated the power of transcriptomic data in a study of killifish embryos exposed to a
727 toxicant. The molecular data helped to identify damage mechanisms that in turn led to changes in
728 DEB parameters. There are many further exciting possibilities for integrating suborganismal
729 (molecular) data with DEBtox modeling.

730

731 **5. Conclusions**

732 With this simple and widely applicable DEBkiss model we were able to attribute
733 hypoxia-related variability in *M. menidia* growth, hatch timing, and survival to damage-induced
734 reductions in conversion efficiency of assimilates into structure. Applying hypoxia corrections
735 simultaneously to conversion efficiency and the mortality parameters for embryos and larvae
736 provided the best fit, suggesting that hypoxia leads to both wasted energy and damage that
737 cannot be sufficiently repaired in the early life stages. As lifelong, constant oxygen conditions
738 are unrealistic in nature, the patterns modeled in this study should not be interpreted as a
739 standalone prediction of what will happen to wild *M. menidia* populations as coastal hypoxia
740 intensifies. Instead, this approach demonstrates the value of identifying energetic processes
741 responsible for whole-organism effects of hypoxia to understand underlying energetic processes
742 that are often time, labor, and cost-intensive to measure empirically, particularly in the early life
743 stages, when biomass available for sampling is small and developmental changes are rapid.
744 Through doing so we were able to support the utility of modeling inhibition and damage to
745 synthesizing units and highlight conversion efficiency of food into growth as a primary
746 mechanism by which hypoxia impacts an ecologically important forage fish and model species.
747 Measuring suborganismal processes to identify physiological modes of action can refine this
748 model so that it can better model this species' response to realistic hypoxia scenarios and,
749 ultimately, how reductions in conversion efficiency could affect energy flow through food webs.

750

751 **Declaration of Competing Interest**

752 The authors do not declare any competing interests.

753

754 **Acknowledgements**

755 The authors would like to acknowledge the researchers who collected the data used in this
756 model, and without whose hard work this study could not exist: Hannes Baumann, Christopher
757 S. Murray, Emma L. Cross, Callie Concannon, Lucas F. Jones, Catherine M. Matassa, and
758 Richard S. McBride. We would also like to thank Robert Cerrato, Michael Frisk, Amy Maas and
759 Louise Stevenson for valuable feedback on this research and manuscript. Finally, we would like
760 to express our gratitude to the guest editor Dina Lika and an anonymous reviewer for their
761 constructive and insightful feedback on this manuscript.

762

763 **Author Contributions**

764 Conceptualization – T.G.S., R.M.N., J.A.N.; Data curation – T.G.S.; Methodology – R.M.N.,
765 T.G.S.; Formal analysis – T.G.S.; Funding acquisition – T.G.S., J.A.N.; Visualization – T.G.S.,
766 Writing, original draft – T.G.S., R.M.N.; Writing, reviewing and editing – T.G.S., R.M.N.,
767 J.A.N.

768

769 **Funding Sources**

770 This research and the preparation of this article were supported by NOAA Sea Grant; NOAA
771 Ocean Acidification Program [NA19OAR170349]; the New York State Department of
772 Environmental Conservation [AM10560].

773

774 **Data and Code**

775 The datasets used for modeling can be found on BCO-DMO: early life total length, survival, and
776 hatching: doi: 10.1575/1912/bco-dmo.742200; early life total length with oxygen treatments: doi:
777 10.1575/1912/bco-dmo.777130.1; hatching and survival with oxygen treatments: doi:
778 10.1575/1912/bco-dmo.777117.1; total length of adults: doi: 10.26008/1912/bco-dmo.845906.1;
779 total length of larvae and juveniles: doi: 10.1575/1912/bco-dmo.652124; egg production: doi:
780 10.26008/1912/bco-dmo.845633.1. The BYOM and DEBkiss packages can be found at
781 <https://www.debtox.info/byom.html>. The code for inputting data, parameter estimation, and
782 plotting, for both the normoxic model and the model with hypoxia effects, can be found at
783 github.com/tschwemmer/MenidiaDEB.

784

785 **References**
786

- 787 Aguirre-Velarde, A., Pecquerie, L., Frederic, J., Gerard, T., and Flye-Sainte-Marie, J. 2019.
788 Predicting the energy budget of the scallop *Argopecten purpuratus* in an oxygen-limiting
789 environment. *J. Sea Res.*, 143: 254-261. <https://doi.org/10.1016/j.seares.2018.09.011>
- 790
- 791 AmP. 2021. Online database of DEB parameters, implied properties and referenced underlying
792 data. www.bio.vu.nl/thb/deb/deblab/add_my_pet/ (data accessed: March 3, 2023).
- 793
- 794 AmPtool, 2022. Software package, <https://github.com/add-my-pet/AmPtool/>
- 795
- 796 Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean
797 acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97: 399-408.
798 [dx.doi.org/10.1139/cjz-2018-0198](https://doi.org/10.1139/cjz-2018-0198)
- 799
- 800 Baumann, H. and Smith, E. M. 2018. Quantifying Metabolically Driven pH and Oxygen
801 Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuaries and*
802 *Coasts*, 41: 1102-1117. <https://doi.org/10.1007/s12237-017-0321-3>
- 803
- 804 [dataset] Baumann, H., Nye, J. (2016) Laboratory study of long-term growth in juvenile *Menidia*
805 *menidia* (Atlantic silverside) at contrasting CO₂ levels for 16 to 122 days in 2015.
806 Biological and Chemical Oceanography Data Management Office (BCO-DMO).
807 (Version final) Version Date 2016-07-07. doi:10.1575/1912/bco-dmo.652124 [accessed
808 2022-03-30]
- 809
- 810 [dataset] Baumann, H., Cross, E. (2019) Growth data from static and fluctuating pCO₂ x
811 dissolved oxygen (DO) experiments on *Menidia menidia*. Biological and Chemical
812 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2019-
813 09-20. doi:10.1575/1912/bco-dmo.777130.1 [accessed 2022-03-30]
- 814
- 815 [dataset] Baumann, H., Cross, E. (2019) Survival data from static and fluctuating pCO₂ x
816 dissolved oxygen (DO) experiments on *Menidia menidia*. Biological and Chemical
817 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2019-
818 09-20. doi:10.1575/1912/bco-dmo.777117.1 [accessed 2022-03-30]
- 819
- 820 [dataset] Baumann, H., Nye, J. (2021) Data from the spawning trial in a study of CO₂ and
821 temperature-specific reproductive traits in *Menidia menidia*. Biological and Chemical
822 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-
823 04-23. doi:10.26008/1912/bco-dmo.845633.1 [accessed 2022-03-30]
- 824
- 825 [dataset] Baumann, H., Nye, J. (2021) Data from the fecundity trial in a study of CO₂ and
826 temperature-specific reproductive traits in *Menidia menidia*. Biological and Chemical
827 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-
828 03-18. doi:10.26008/1912/bco-dmo.845906.1 [accessed 2022-03-30]
- 829

- 830 Baumann, H., Wallace, R. B., Tagliaferri, T., and Gobler, C. J. 2015. Large Natural pH, CO₂ and
831 O₂ Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time
832 Scales. *Estuaries Coasts*, 38: 220-231. doi: 10.1007/s12237-014-9800-y
- 833
- 834 Bayliff, W. H. (1950). *The life history of the silverside Menidia menidia (Linnaeus)*. Chesapeake
835 Bay Laboratory, Solomons Island, Maryland: State of Maryland Board of Natural
836 Resources, Department of Research and Education.
- 837
- 838 Bengtson, D. A. 1984. Resource partitioning by *Menidia menidia* and *Menidia beryllina*
839 (Osteichthyes: Atherinidae). *Mar. Ecol. Prog. Ser.*, 18: 21-30.
- 840
- 841 Bianchini, K. and Wright, P. A. 2013. Hypoxia delays hematopoiesis: retention of embryonic
842 hemoglobin and erythrocytes in larval rainbow trout, *Oncorhynchus mykiss*, during
843 chronic hypoxia exposure. *J. Exp. Biol.*, 216(23): 4415-4425.
844 <https://doi.org/10.1242/jeb.083337>
- 845
- 846 Bigelow, H. B. and Schroeder, W. C. (1953). *Fishes of the Gulf of Maine*. U.S. Fish and Wildlife
847 Service, *Fish. Bull.*, 53(74). 577 pp.
- 848
- 849 Boult, V. L. and Evans, L. C. 2021. Mechanisms matter: Predicting the ecological impacts of
850 global change. *Glob. Change Biol.*, 27(9): 1689-1691. <https://doi.org/10.1111/gcb.15527>
- 851
- 852 Bouma, T. J., De Visser, R., Janssen, J. H. J. A., De Kock, M. J., Van Leeuwen, P. H., and
853 Lambers, H. 1994. Respiratory energy requirements and rate of protein turnover in vivo
854 determined by the use of an inhibitor of protein synthesis and a probe to assess its effect.
855 *Physiol. Plant.*, 92: 585-594. <https://doi.org/10.1111/j.1399-3054.1994.tb03027.x>
- 856
- 857 Breitburg, D., Levin, L. A., Oschlies, A., et al. 2018. Declining oxygen in the global ocean and
858 coastal waters. *Science*, 359(6371): eaam7240. doi: 10.1126/science.aam7240
- 859
- 860 Chambers, R. C. and Leggett, W. C. 1987. Size and age at metamorphosis in marine fishes – an
861 analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a
862 review of variation in other species. *Can. J. Fish. Aquat. Sci.*, 44(11): 1936-1947.
863 <https://doi.org/10.1139/f87-238>
- 864
- 865 Chapman, L. J. and McKenzie, D. J. 2009. Behavioral responses and ecological consequences.
866 In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. Jeffrey G. Richards, Anthony P. Farrell, and
867 Colin J. Brauner), pp. 25-77. San Diego: Academic Press.
- 868
- 869 Claireaux, G. and Chabot, D. 2016. Responses by fishes to environmental hypoxia: integration
870 through Fry's concept of aerobic metabolic scope. *J. Fish Biol.*, 88: 232-251.
871 <https://doi.org/10.1111/jfb.12833>
- 872
- 873 Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., and
874 Baumann, H. 2021. Temperature-dependent effects on fecundity in a serial broadcast

- 875 spawning fish after whole-life high CO₂ exposure. *ICES J. Mar. Sci.*, 78(10): 3724-3734.
876 <https://doi.org/10.1093/icesjms/fsab217>
- 877
- 878 Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal pCO₂ x O₂ fluctuations
879 provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9:
880 18146. <https://doi.org/10.1038/s41598-019-53930-8>
- 881
- 882 Del Rio, A. M., Davis, B. E., Fangue, N. A., and Todgham, A. E. 2019. Combined effects of
883 warming and hypoxia on early life stage Chinook salmon physiology and development.
884 *Conserv. Physiol.*, 7(1): coy078. doi: 10.1093/conphys/coy078
- 885
- 886 DePasquale, E., Baumann, H., and Gobler, C. J. 2015. Vulnerability of early life stage Northwest
887 Atlantic forage fish to ocean acidification and low oxygen. *Mar. Ecol. Prog. Ser.*, 523:
888 145-156. doi: 10.3354/meps11142
- 889
- 890 Desforges, J.-P. W., Sonne, C., and Dietz, R. 2017. Using energy budgets to combine ecology
891 and toxicology in a mammalian sentinel species. *Sci. Rep.*, 7: 46267. doi:
892 10.1038/srep46267
- 893
- 894 Di Santo, V., Kenaley, C. P., and Lauder, G. V. 2017. High postural costs and anaerobic
895 metabolism during swimming support the hypothesis of a U-shaped metabolism-speed
896 curve in fishes. *Proc. Nat. Acad. Sci.*, 114(49): 13048-13053.
897 <https://doi.org/10.1073/pnas.1715141114>
- 898
- 899 Diaz, R. J. and Rosenberg, R. 2008. Spreading Dead Zones and Consequences for Marine
900 Ecosystems. *Science*, 321: 926-929. doi: 10.1126/science.1156401
- 901
- 902 Evans, M. R., Grimm, V., Johst, K., et al. 2013. Do simple models lead to generality in ecology?
903 *Trends in Ecology & Evolution*, 28(10): 578-583.
904 <https://doi.org/10.1016/j.tree.2013.05.022>
- 905
- 906 Farrell, A. P. and Brauner, C. J. 2009. Fish Physiology, Vol. 27: Hypoxia. Academic Press,
907 London.
- 908
- 909 Goussen, B., Rendal, C., Sheffield, D., Butler, E., Price, O. R., and Ashauer, R. 2020.
910 Bioenergetics modelling to analyze and predict the joint effects of multiple stressors:
911 Meta-analysis and model corroboration. *Sci. Total. Environ.*, 749: 141509.
912 <https://doi.org/10.1016/j.scitotenv.2020.141509>
- 913
- 914 Gear, J. S., O'Leary, C. A., Nye, J. A., Tettelbach, S. T., and Gobler, C. J. 2020. Effects of
915 coastal acidification on North Atlantic bivalves: interpreting laboratory responses in the
916 context of *in situ* populations. *Mar. Ecol. Prog. Ser.*, 633: 89-104.
917 <https://doi.org/10.3354/meps13140>
- 918
- 919 Gruber, J. 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global
920 change. *Phil. Trans. R. Soc. A*, 369: 1980-1996. <https://doi.org/10.1098/rsta.2011.0003>

- 921
922 Hamda, N. T., Martin, B., Poletto, J. B., Cocherell, D. E., Fangue, N. A., Van Eenennaam, J.,
923 Mora, E. A., and Danner, E. 2019. Applying a simplified energy-budget model to explore
924 the effects of temperature and food availability on the life history of green sturgeon
925 (*Acipenser medirostris*). *Ecol. Modell.*, 395: 1-10.
926 <https://doi.org/10.1016/j.ecolmodel.2019.01.005>
927
928 Heath, A. G. and Pritchard, A. W. 1965. Effects of severe hypoxia on carbohydrate energy stores
929 and metabolism in two species of fresh-water fish. *Physiol. Zool.*, 38(4): 325-334.
930 <https://doi.org/10.1086/physzool.38.4.30152409>
931
932 Holling, C. S. 1966. The strategy of building models of complex ecological systems. In: Systems
933 Analysis in Ecology. (K. E. F. Watt, Ed.) Academic Press. Pp. 195-214.
934
935 Jager, T. 2018. DEBkiss: A Simple Framework for Animal Energy Budgets. Version 2.0.
936 Leanpub: https://leanpub.com/debkiss_book.
937
938 Jager, T., Martin, B. T., and Zimmer, E. I. 2013. DEBkiss or the quest for the simplest generic
939 model of animal life history. *J. Theor. Biol.*, 328: 9-18.
940 <https://doi.org/10.1016/j.jtbi.2013.03.011>
941
942 Jager, T., Ravagnan, E., and Dupont, S. 2016. Near-future ocean acidification impacts
943 maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points
944 using a DEB modelling approach. *J. Exp. Mar. Biol. Ecol.*, 474: 11-17.
945 <https://doi.org/10.1016/j.jembe.2015.09.016>
946
947 Jager, T., Nepstad, R., Hansen, B. H., and Farkas, J. 2018. Simple energy-budget model for yolk-
948 feeding stages of Atlantic cod (*Gadus morhua*). *Ecol. Modell.*, 385: 213-219.
949 <https://doi.org/10.1016/j.ecolmodel.2018.08.003>
950
951 Jager, T., Malzahn, A. M., Hagemann, A., and Hansen, B. H. 2022. Testing a simple energy-
952 budget model for yolk-feeding stages of cleaner fish. *Ecol. Modell.*, 469: 110005.
953 <https://doi.org/10.1016/j.ecolmodel.2022.110005>
954
955 Jusup, M., Sousa, T., Domingos, T., Labinac, V., Marn, N., Wang, Z., and Klanjšček, T. 2017.
956 Physics of metabolic organization. *Physics of Life Reviews*, 20: 1-39.
957 <https://doi.org/10.1016/j.plrev.2016.09.001>
958
959 Kamler, E. 2008. Resource allocation in yolk-feeding fish. *Rev. Fish. Biol. Fisheries*, 18: 143-
960 200. <https://doi.org/10.1007/s11160-007-9070-x>
961
962 Klahre, L. E. 1997. Countergradient Variation in Egg Production Rate of the Atlantic Silverside
963 *Menidia menidia*. [Master's thesis]. Stony Brook University.
964

- 965 Kooijman, S. A. L. M. 1998. The Synthesizing Unit as model for the stoichiometric fusion and
966 branching of metabolic fluxes. *Biophys. Chem.*, 73: 179-188.
967 [https://doi.org/10.1016/S0301-4622\(98\)00162-8](https://doi.org/10.1016/S0301-4622(98)00162-8)
- 968
- 969 Kooijman, S. A. L. M. 2010a. Dynamic Energy Budget Theory for Metabolic Organisation.
970 Cambridge University Press, Cambridge.
- 971
- 972 Kooijman, S. A. L. M. 2010b. Comments on Dynamic Energy Budget Theory for Metabolic
973 Organisation. Cambridge University Press, Cambridge.
- 974
- 975 Kooijman, S. A. L. M. 2018. Models in stress research. *Ecol. Complex.*, 34: 161-177.
976 <https://doi.org/10.1016/j.ecocom.2017.07.006>
- 977
- 978 Kooijman, S. A. L. M., and Metz, J. A. J. 1984. On the dynamics of chemically stressed
979 populations: The deduction of population consequences from effects on individuals.
980 *Ecotoxicology and Environmental Safety*, 8(3): 254-274. [https://doi.org/10.1016/0147-6513\(84\)90029-0](https://doi.org/10.1016/0147-6513(84)90029-0)
- 981
- 982
- 983 Kooijman, S. A. L. M., Baas, J., Bontje, D., Broerse, M., van Gestel, C. A. M., and Jager, T.
984 2009. Ecotoxicological Applications of Dynamic Energy Budget Theory. In: *Emerging
985 Topics in Ecotoxicology, Vol. 2: Ecotoxicology Modeling* (Ed. Devillers, J.), pp. 237-259.
986 Boston, MA: Springer. https://doi.org/10.1007/978-1-4419-0197-2_9
- 987
- 988 Kooijman, S. A. L. M., Lika, K., Augustine, S., Marn, N., and Kooi, B. W. 2020. The energetic
989 basis of population growth in animal kingdom. *Ecol. Modell.*, 428: 109055.
990 <https://doi.org/10.1016/j.ecolmodel.2020.109055>
- 991
- 992 Landry, C. A., Steele, S. L., Manning, S., and Cheek, A. O. 2007. Long term hypoxia suppresses
993 reproductive capacity in the estuarine fish, *Fundulus grandis*. *Comp. Biochem. Physiol.
994 Part A: Mol. Integr. Physiol.*, 148(2): 317-323.
995 <https://doi.org/10.1016/j.cbpa.2007.04.023>
- 996
- 997 Lankford, T. E., Billerbeck, J. M., and Conover, D. O. 2001. Evolution of intrinsic growth and
998 energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia
999 menidia*. *Evolution*, 55(9): 1873-1881. <https://doi.org/10.1111/j.0014-3820.2001.tb00836.x>
- 1000
- 1001
- 1002 Lavaud, R., Filgueira, R., and Augustine, S. 2019. The role of Dynamic Energy Budgets in
1003 conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi:
1004 [10.1093/conphys/coab083](https://doi.org/10.1093/conphys/coab083)
- 1005
- 1006 Lavaud, R., Filgueira, R., and Augustine, S. 2021. The role of Dynamic Energy Budgets in
1007 conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi:
1008 [10.1093/conphys/coab083](https://doi.org/10.1093/conphys/coab083).
- 1009

- 1010 Letcher, B. H. and Bengtson, D. A. 1993. Effects of food density and temperature on feeding and
1011 growth of young inland silversides (*Menidia beryllina*). *J. Fish Biol.*, 43: 671-686.
1012 <https://doi.org/10.1111/j.1095-8649.1993.tb01145.x>
1013
- 1014 Lika, K., Kearney, M. R., Freitas, V., van der Veer, H. W., van der Meer, J., Mijsman, J. W. M.,
1015 Pecqueries, L., and Kooijman, S. A. L. M. 2011. The “covariation method” for estimating
1016 the parameters of the standard Dynamic Energy Budget model I: Philosophy and
1017 approach. *J. Sea Res.*, 66(4): 270-277. <https://doi.org/10.1016/j.seares.2011.07.010>
1018
- 1019 Marques, G. M., Augustine, S., Lika, K., Pecquerie, L., Domingos, T., and Kooijman, S. A. L.
1020 M. 2018. The AmP project: Comparing species on the basis of dynamic energy budget
1021 parameters. *PLoS Comput. Biol.*, 14(5): e1006100.
1022 <https://doi.org/10.1371/journal.pcbi.1006100>
1023
- 1024 Martin, B. T., Jager, T., Nisbet, R. M., Preuss, T. G., and Grimm, V. 2013. Predicting Population
1025 Dynamics from the Properties of Individuals: A Cross-Level Test of Dynamic Energy
1026 Budget Theory. *The American Naturalist*, 181(4): 506-519.
1027 <https://doi.org/10.1086/669904>
1028
- 1029 Martin, B. T., Heintz, R., Danner, E. M., and Nisbet, R. M. 2017. Integrating lipid storage into
1030 general representations of fish energetics. *Journal of Animal Ecology*, 86: 812-825.
1031 <https://doi.org/10.1111/1365-2656.12667>
1032
- 1033 Maxime, V., Pichavant, K., Boeuf, G., and Nonnotte, G. 2000. Effects of hypoxia on respiratory
1034 physiology of turbot, *Scophthalmus maximus*. *Fish Physiology and Biochemistry*, 22: 51-
1035 59. <https://doi.org/10.1023/A:1007829214826>
1036
- 1037 Middaugh, D. P. 1981. Reproductive Ecology and Spawning Periodicity of the Atlantic
1038 Silverside, *Menidia menidia* (Pisces: Atherinidae). *Copeia*, 1981(4): 766-776.
1039 <https://doi.org/10.2307/1444176>
1040
- 1041 Middaugh, D. P. and Lempesis, P. W. 1976. Laboratory spawning and rearing of a marine fish,
1042 the silverside *Menidia menidia menidia*. *Mar. Biol.*, 35: 295-300.
1043 <https://doi.org/10.1007/BF00386640>
1044
- 1045 Middaugh, D. P. and Hemmer, M. J. 1992. Reproductive Ecology of the Inland Silverside,
1046 *Menidia beryllina*, (Pisces: Atherinidae) from Blackwater Bay, Florida. *Copeia*, 1992(1):
1047 53-61. <https://doi.org/10.2307/1446535>
1048
- 1049 Miller, S. H., Breitburg, D. L., Burrell, R. B., Keppel, A. G. 2016. Acidification increases
1050 sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.*, 549: 1-8.
1051 <https://doi.org/10.3354/meps11695>
1052
- 1053 Moreira, J. M., Candeias Mendes, A., Maulvault, A. L., Marques, A., Rosa, R., Pousão-Ferreira,
1054 P., Sousa, T., Anacleto, P., and Marques, G. M. 2022. Impacts of ocean warming and

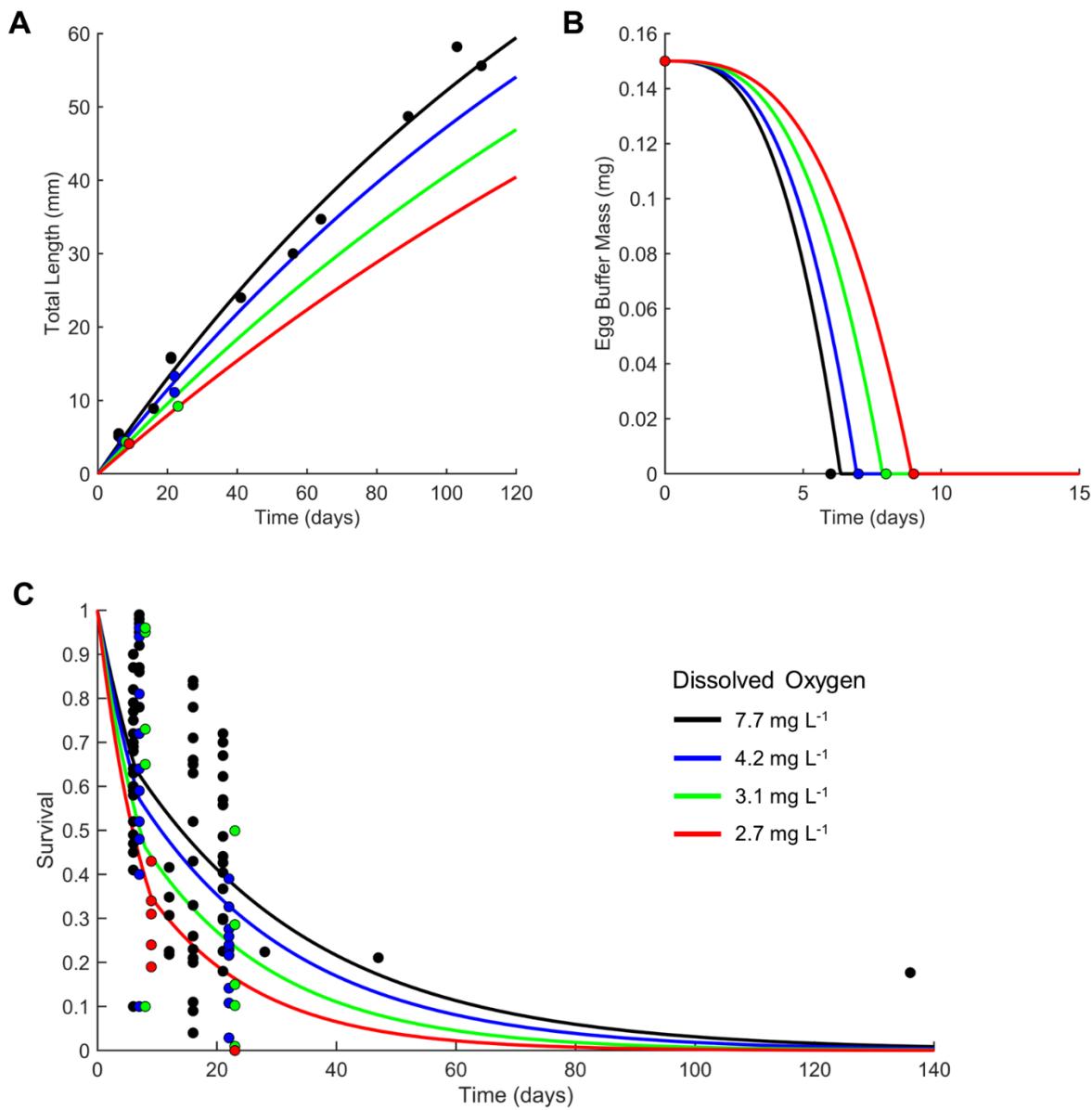
- 1055 acidification on the energy budget of three commercially important fish species. *Conserv.*
1056 *Physiol.*, 10(1): coac048. <https://doi.org/10.1093/conphys/coac048>
- 1057
- 1058 Morrell, B. K. and Gobler, C. J. 2020. Negative Effects of Diurnal Changes in Acidification and
1059 Hypoxia on Early-Life Stage Estuarine Fishes. *Diversity*, 12: 25. doi: 10.3390/d12010025
- 1060
- 1061 Muller, E. B., Nisbet, R. M., and Berkley, H. A. 2010. Sublethal toxicant effects with dynamic
1062 energy budget theory: model formulation. *Ecotoxicology*, 19: 48-60.
1063 <https://doi.org/10.1007/s10646-009-0385-3>
- 1064
- 1065 Muller, E. B., Klanjšček, T., and Nisbet, R. M. 2019. Inhibition and damage schemes within the
1066 synthesizing unit concept of dynamic energy budget theory. *J. Sea Res.*, 143: 165-172.
1067 <https://doi.org/10.1016/j.seares.2018.05.006>
- 1068
- 1069 Murphy, C. A., Nisbet, R. M., Antczak, P., Garcia-Reyero, N., Gergs, A., Lika, K., Mathews, T.,
1070 Muller, E. B., Nacci, D., Peace, A., Remien, C. H., Schultz, I. R., Stevenson, L. M., and
1071 Watanabe, K. H. 2018. Incorporating Suborganismal Processes into Dynamic Energy
1072 Budget Models for Ecological Risk Assessment. *Integr. Environ. Assess. Manag.*, 14(5):
1073 615-624. doi: 10.1002/ieam.4063
- 1074
- 1075 Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ × Temperature
1076 Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*,
1077 10: 69. doi: 10.3390/d10030069
- 1078
- 1079 [dataset] Murray, C., Baumann, H. (2018) CO₂ × temperature specific early life survival and
1080 growth of *Menidia menidia* assessed by 5 factorial experiments. Biological and Chemical
1081 Oceanography Data Management Office (BCO-DMO). (Version 05 April 2018) Version
1082 Date 2018-04-05. doi:10.1575/1912/bco-dmo.742200 [accessed 2022-03-30]
- 1083
- 1084 Murray, C. S. and Baumann, H. 2020. Are long-term growth responses to elevated pCO₂ sex-
1085 specific in fish? *PLoS ONE*, 15(7): e0235817.
1086 <https://doi.org/10.1371/journal.pone.0235817>
- 1087
- 1088 Murray, C. S., Fuiman, L. A., and Baumann, H. 2017. Consequences of elevated CO₂ exposure
1089 across multiple life stages in a coastal forage fish. *ICES J. Mar. Sci.*, 74(4): 1051-1061.
1090 doi: 10.1093/icesjms/fsw179
- 1091
- 1092 Niklitschek, E. J. and Secor, D. H. 2005. Modeling spatial and temporal variation of suitable
1093 nursery habitats for Atlantic sturgeon in the Chesapeake Bay. *Estuar. Coast. Shelf Sci.*,
1094 64: 135-148. <https://doi.org/10.1016/j.ecss.2005.02.012>
- 1095
- 1096 Nisbet, R. M., Gurney, W. S. C., Murdoch, W. W., and McCauley, E. 1989. Structured
1097 population models: a tool for linking effects at individual and population level. *Biol. J.*
1098 *Linn. Soc.*, 37: 79-99. <https://doi.org/10.1111/j.1095-8312.1989.tb02006.x>
- 1099

- 1100 Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to
1101 ecosystems through dynamic energy budget models. *Journal of Animal Ecology*, 69: 913-
1102 926.
- 1103
- 1104 Nonnotte, G., Maxime, V., Truchot, J. P., Williot, P., and Peyraud, C. 1993. Respiratory
1105 responses to progressive ambient hypoxia in the sturgeon, *Acipenser baeri*. *Respir.*
1106 *Physiol.*, 91: 71-82. [https://doi.org/10.1016/0034-5687\(93\)90090-W](https://doi.org/10.1016/0034-5687(93)90090-W)
- 1107
- 1108 O'Donnell, J., Dam, H. G., Bohlen, W. F., Fitzgerald, W., Gay, P. S., Houk, A. E., Cohen, D. C.,
1109 and Howard-Strobel, M. M. 2008. Intermittent ventilation in the hypoxic zone of western
1110 Long Island Sound during the summer of 2004. *J. Geophys. Res.*, 113: C09025.
1111 <https://doi.org/10.1029/2007JC004716>
- 1112
- 1113 Polymeropoulos, E. T., Elliott, N. G., and Frappell, P. B. 2017. Hypoxic acclimation leads to
1114 metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comp.*
1115 *Biochem. Physiol. A*, 213: 28-35. <https://doi.org/10.1016/j.cbpa.2017.08.011>
- 1116
- 1117 Pousse, É., Munroe, D., Hart, D., Hennen, D., Cameron, L. P., Rheuban, J. E., Wang, Z. A.,
1118 Wikfors, G. H., and Meseck, S. L. 2022. Dynamic energy budget modeling of Atlantic
1119 surfclam, *Spisula solidissima*, under future ocean acidification and warming. *Mar.*
1120 *Environ. Res.*, 177: 105602. <https://doi.org/10.1016/j.marenvres.2022.105602>
- 1121
- 1122 Richards, J. G. 2009. Metabolic and Molecular Responses of Fish to Hypoxia. In: *Fish*
1123 *Physiology, Vol. 27: Hypoxia*. (Ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp.
1124 443-485. San Diego: Academic Press.
- 1125
- 1126 Richards, J. G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes
1127 to hypoxia. *J. Exp. Biol.*, 214: 191-199. <https://doi.org/10.1242/jeb.047951>
- 1128
- 1129 Robert, D., Shoji, J., Sirois, P., Takasuka, A., Catalán, I. A., et al. 2023. Life in the fast lane:
1130 Revisiting the fast growth—High survival paradigm during the early life stages of fish.
1131 *Fish and Fisheries*, 24: 863-888. <https://doi.org/10.1111/faf.12774>
- 1132
- 1133 Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia
1134 during early life. In: *Fish Physiology, Vol. 11: The Physiology of Developing Fish, Part*
1135 *A: Eggs and Larvae*. (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego:
1136 Academic Press.
- 1137
- 1138 Romoli, C., Jager, T., Trijau, M., Goussen, B., and Gergs, A. 2024. Environmental Risk
1139 Assessment with Energy Budget Models: A Comparison Between Two Models of
1140 Different Complexity. *Environ. Toxicol. Chem.*, 43(2): 440-449. doi: 10.1002/etc.5795
- 1141
- 1142 Schwemmer, T. G. 2023. Early Life Physiological and Energetic Responses of Atlantic
1143 Silversides (*Menidia menidia*) to Ocean Acidification, Warming, and Hypoxia. Doctoral
1144 dissertation. ProQuest Dissertations Publishing. State University of New York at Stony
1145 Brook, Stony Brook, NY.

- 1146
1147 Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., and Nye, J. A. 2020.
1148 Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of
1149 the coastal forage fish *Menidia menidia*. *J. Exp. Biol.*, 223: jeb228015. doi:
1150 10.1242/jeb.228015
1151
1152 Sibly, R. M., Grimm, V., Martin, B. T., Johnston, A. S. A., et al. 2013. Representing the
1153 acquisition and use of energy by individuals in agent-based models of animal
1154 populations. *Methods in Ecology and Evolution*, 4: 151-161.
1155 <https://doi.org/10.1111/2041-210x.12002>
1156
1157 Smallegange, I. M., Caswell, H., Toorians, M. E. M., and de Roos, A. M. 2017. Mechanistic
1158 description of population dynamics using dynamic energy budget theory incorporated
1159 into integral projection models. *Methods in Ecology and Evolution*, 8: 146-154.
1160 <https://doi.org/10.1111/2041-210X.12675>
1161
1162 Stevenson, L. M., Muller, E. B., Nacci, D., Clark, B. W., Whitehead, A., and Nisbet, R. M. 2023.
1163 Connecting Suborganismal Data to Bioenergetic Processes: Killifish Embryos Exposed to
1164 a Dioxin-Like Compound. *Environ. Toxicol. Chem.*, 42(9): 2040-2053. doi:
1165 10.1002/etc.5680
1166
1167 Stierhoff, K. L., Targett, T. E., and Miller, K. 2006. Ecophysiological responses of juvenile
1168 summer and winter flounder to hypoxia: experimental and modeling analyses of effects
1169 on estuarine nursery quality. *Mar. Ecol. Prog. Ser.*, 325: 255-266.
1170 doi:10.3354/meps325255
1171
1172 Stierhoff, K. L., Targett, T. E., and Power, J. H. 2009. Hypoxia-induced growth limitation of
1173 juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics
1174 using RNA:DNA. *Can. J. Fish. Aquat. Sci.*, 66(7): 1033-1047.
1175 <https://doi.org/10.1139/F09-066>
1176
1177 Tai, T. C., Sumaila, U. R., and Cheung, W. W. L. 2021. Ocean Acidification Amplifies Multi-
1178 Stressor Impacts on Global Marine Invertebrate Fisheries. *Front. Mar. Sci.*, 8: 596644.
1179 doi: 10.3389/fmars.2021.596644
1180
1181 Takasuka, A., Aoki, I., and Oozeki, Y. 2007. Predator-specific growth-selective predation on
1182 larval Japanese anchovy *Engraulis japonicus*. *Mar. Ecol. Prog. Ser.*, 350: 99-107.
1183 <https://doi.org/10.3354/meps07158>
1184
1185 Taylor, J. C. and Miller, J. M. 2001. Physiological performance of juvenile southern flounder,
1186 *Paralichthys lethostigma* (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. *J.*
1187 *Exp. Mar. Biol. Ecol.*, 258: 195-214. [https://doi.org/10.1016/S0022-0981\(01\)00215-5](https://doi.org/10.1016/S0022-0981(01)00215-5)
1188
1189 Testa, J. M., Murphy, R. R., Brady, D. C., and Kemp, W. M. 2018. Nutrient- and Climate-
1190 Induced Shifts in the Phenology of Linked Biogeochemical Cycles in a Temperate
1191 Estuary. *Front. Mar. Sci.*, 5: 114. <https://doi.org/10.3389/fmars.2018.00114>

- 1192
1193 Thomas, P., Rahman, M. S., Kummer, J. A., and Lawson, S. 2006. Reproductive endocrine
1194 dysfunction in Atlantic croaker exposed to hypoxia. *Mar. Environ. Res.*, 62: S249-S252.
1195 <https://doi.org/10.1016/j.marenvres.2006.04.031>
1196
1197 Thomas, Y., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G. M., and
1198 Pecquerie, Laure. 2019. Effects of hypoxia on metabolic functions in marine organisms:
1199 Observed patterns and modelling assumptions within the context of Dynamic Energy
1200 Budget (DEB) theory. *J. Sea Res.*, 143: 231-242.
1201 <https://doi.org/10.1016/j.seares.2018.05.001>
1202
1203 Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferrarezzo, S., Sévère, A.,
1204 Zambonino-Infante, J.-L., and Mazurais, D. 2015. Hypoxic episode during the larval
1205 period has long-term effects on European sea bass juveniles (*Dicentrarchus labrax*). *Mar.*
1206 *Biol.*, 162: 367-376. <https://doi.org/10.1007/s00227-014-2601-9>
1207
1208 Wagenmakers, E.-J. and Farrell, S. 2004. AIC model selection using Akaike weights. *Psychon.*
1209 *Bull. Rev.*, 11(1): 192-196. <https://doi.org/10.3758/BF03206482>
1210
1211 Wang, J., Yang, Y., Wang, Z., Xu, K., Xiao, X., and Mu, W. 2021. Comparison of effects in
1212 sustained and diel-cycling hypoxia on hypoxia tolerance, histology, physiology, and
1213 expression of clock genes in high latitude fish *Phoxinus lagowskii*. *Comp. Biochem.*
1214 *Physiol. A Mol. Integr. Physiol.*, 260: 111020.
1215 <https://doi.org/10.1016/j.cbpa.2021.111020>
1216
1217 Wei, L.-Z., Zhang, X.-M., Li, J., and Huang, G.-Q. 2008. Compensatory growth of Chinese
1218 shrimp, *Fenneropenaeus chinensis* following hypoxic exposure. *Aquacult. Int.*, 16: 455-
1219 470. <https://doi.org/10.1007/s10499-007-9158-2>
1220
1221 Williams, K. J., Cassidy, A. A., Verhille, C. E., Lamarre, S. G., and MacCormack, T. J. 2019.
1222 Diel cycling hypoxia enhances hypoxia tolerance in rainbow trout (*Oncorhynchus*
1223 *mykiss*): evidence of physiological and metabolic plasticity. *J. Exp. Biol.*, 222(14):
1224 jeb206045. <https://doi.org/10.1242/jeb.206045>
1225
1226 Wood, C. M. 2018. The fallacy of the P_{crit} – are there more useful alternatives? *J. Exp. Biol.*,
1227 221: jeb163717. doi: 10.1242/jeb.163717
1228
1229 Wu, R. S. S., Zhou, B. S., Randall, D. J., Woo, N. Y. S., and Lam, P. K. S. 2003. Aquatic
1230 Hypoxia Is an Endocrine Disruptor and Impairs Fish Reproduction. *Environ. Sci.*
1231 *Technol.*, 37(6): 1137-1141. <https://doi.org/10.1021/es0258327>
1232
1233 Zambonino-Infante, J. L., Mazurais, D., Dubuc, A., Quéau, P., Vanderplancke, G., Servili, A.,
1234 Cahu, C., Le Bayon, N., Huelvan, C., and Claireaux, G. 2017. An early life hypoxia event
1235 has a long-term impact on protein digestion and growth in juvenile European sea bass. *J.*
1236 *Exp. Biol.*, 220(10): 1846-1851. <https://doi.org/10.1242/jeb.154922>
1237

1238 Zhu, C.-D., Wang, Z.-H., and Yan, B. 2013. Strategies for hypoxia adaptation in fish species: a
1239 review. *J. Comp. Physiol. B*, 183: 1005-1013. <https://doi.org/10.1007/s00360-013-0762-3>
1240

1241 **Supplementary Figure**

1242

1243 **Figure S1. Best fit of DEBkiss model to all experimental data from four DO levels.** The
 1244 model was fitted to early life data (embryos, larvae, and juveniles) and the best fitting model was
 1245 selected based on lowest AICc. (A) is total length (mm) over time (days), (B) is egg buffer mass
 1246 (mg) over time (days), and (C) is survival over time (days).

1247

1 **Attributing hypoxia responses of early life *Menidia menidia* to energetic mechanisms with**
2 **Dynamic Energy Budget theory**

3

4 Teresa G. Schwemmer^{a,1}, Roger M. Nisbet^b, and Janet A. Nye^c

5

6 ^aSchool of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794,
7 U.S.A., teresa.schwemmer@gmail.com

8 ^bDepartment of Ecology, Evolution and Marine Biology, University of California Santa Barbara,
9 Santa Barbara, CA 93106, U.S.A., rogerenisbet@ucsb.edu

10 ^c[Institute of Marine Sciences](#), [Department of Earth, Marine and Environmental Sciences](#),
11 University of North Carolina at Chapel Hill, [Institute of Marine Sciences](#), Morehead City, NC
12 28557, U.S.A., jnye@nyelab.org

13

14 ¹Corresponding author, present affiliation: Teresa G. Schwemmer, Mid-Atlantic Coastal
15 Acidification Network, Newark, DE 19716, U.S.A., teresa.schwemmer@gmail.com

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39 To be submitted to: *Ecological Modelling*, Special issue: Metabolic organization across scales of

40 space and time

41 **Highlights**

- 42 • Bioenergetic mechanisms of Atlantic silverside hypoxia responses were investigated.
43 • Hypoxia effects were modeled with a simplified Dynamic Energy Budget model.
44 • We connected physiology with energetic processes to identify potential mechanisms.
45 • Conversion efficiency and mortality parameters best explained hypoxia effects.
46 • This mechanism could impact energy flow across generations and trophic levels.

47

48

49 **Abstract**

50 Ocean deoxygenation is intensifying worldwide due to warming and eutrophication,
51 particularly in estuaries and coastal waters. Although the Atlantic silverside (*Menidia menidia*) is
52 tolerant of the fluctuating environmental conditions in its estuarine habitat, chronic hypoxia
53 impairs hatching, growth, and survival in the early life stages. We used a simplified version of a
54 Dynamic Energy Budget model (DEBkiss) to test the hypothesis that experimentally observed
55 changes in animal performance can be explained by one or more of the rate processes in the
56 model. We sought to identify the DEBkiss parameters that, when adjusted with a correction
57 factor based on inhibition of Synthesizing Units, provided the best fit to hypoxia effects in the
58 three state variables of total length, egg buffer mass, and survival over time. Because hypoxia
59 reduces survival in embryos and newly hatched larvae, we added a survival state variable
60 controlled by pre- and post-hatching mortality parameters. Applying the hypoxia effects to
61 reduce the conversion efficiency of assimilates to structure accounted for some of the hypoxia-
62 related changes in all three state variables. However, the best fit was achieved by simultaneously
63 reducing the conversion efficiency and increasing both mortality parameters. In contrast,
64 changing the parameter for maintenance rate with hypoxia provided little to no improvement of
65 fit to the data. Reduced conversion efficiency under hypoxia would suggest that less of the
66 energy invested by parents and consumed through predation is converted into biomass in *M.*
67 *menidia* offspring, with implications for size at age that could threaten recruitment and alter the
68 flow of energy through the food web.

69

70 **Keywords**

71 Dynamic Energy Budget; DEBkiss; early life stages; Atlantic silverside; hypoxia; stressors

72

73 **1. Introduction**

74 Hypoxia is common in coastal and estuarine waters and is expected to intensify with
75 global warming (Diaz and Rosenberg, 2008; Breitburg et al., 2018). Between anthropogenic
76 influence on nearshore waters and the natural dynamics of shallow, partially enclosed water
77 bodies, hypoxia often co-occurs with other stressors such as high temperature, ocean
78 acidification, and pollutants (Gruber, 2011). In temperate estuaries, stratification and
79 productivity associated with high temperatures in spring and summer cause hypoxic and
80 eutrophic zones to form with and great fluctuations in dissolved oxygen (DO) on diel to monthly
81 time scales (O'Donnell et al., 2008⁸⁴; Baumann and Smith, 2018; Testa et al., 2018). While fish
82 species that currently live in such areas tend to have mechanisms to cope with episodic hypoxia
83 (Farrell and Brauner, 2009; Zhu et al., 2013; Baumann, 2019), these are not necessarily adequate
84 for tolerance of longer duration events. Fishes that spawn in the spring and summer may be
85 particularly vulnerable because they are exposed to hypoxia during the sensitive early life stages.
86 Embryos and young larvae rely largely on diffusion for oxygen uptake and lack well-developed
87 mechanisms, such as high surface area gills, to meet oxygen demands in low DO water
88 (Rombough, 1988). While later stage fishes and even some early larvae can swim to avoid
89 hypoxic habitats (Niklitschek and Secor, 2005; Chapman and McKenzie, 2009), embryos cannot
90 utilize this response. Mortality can result directly from severe hypoxia or indirectly from reduced
91 growth increasing susceptibility to predation. Even fish that survive may incur sublethal effects
92 with lasting, lifelong consequences for growth, development, and reproduction (Stierhoff et al.,
93 2006; Vanderplancke et al., 2015; Zambonino-Infante et al., 2017). Modeling the energetic
94 mechanisms of responses to hypoxia using unified principles on model species can help connect
95 physiology and life history to population-level changes and serve as a valuable alternative and/or
96 supplement to time- and labor-intensive laboratory experiments on other species, particularly
97 with very small embryos and larvae.

98 Hypoxia is known to inhibit growth and survival in early life fishes (Rombough, 1988;
99 Cross et al., 2019; Del Rio et al., 2019), as oxygen is required for the processes that maintain
100 homeostasis and convert food for growth and activity. Anaerobic energy production fuels these
101 processes with only about 1/15th the ATP yield of aerobic respiration. Hypoxic exposure may
102 lead to physiological responses such as depressed metabolism (Richards, 2009; Schwemmer,

103 2023), limited growth, increased ventilation, and changes to hematocrit, hemoglobin, and
104 erythrocyte quantities and characteristics (Taylor and Miller, 2001; Stierhoff et al., 2009;
105 Bianchini and Wright, 2013). Metabolism has also been shown to increase after temporary
106 hypoxia as fish remove lactate accumulated from anaerobic respiration (Heath and Pritchard,
107 1965).

108 Hypoxia often has interactive effects with other stressors such as temperature (Brandt et
109 al., 2009; McBryan et al., 2013; Earhart et al., 2022) and high carbon dioxide (CO₂; Hancock and
110 Place, 2016; Miller et al., 2016; Morrell and Gobler, 2020). While the growth and survival
111 effects of hypoxia have been demonstrated in many species, the mechanisms are poorly
112 understood. The Atlantic silverside (*Menidia menidia*) is an estuarine forage fish that has
113 frequently been used as a model species to understand interactive effects of high CO₂ and effects
114 of stressors, including hypoxia, on fish growth and physiology (DePasquale et al., 2015; Miller et
115 al., 2016; Murray and Baumann, 2018; Schwemmer et al., 2020). Rearing *M. menidia* offspring
116 in static low DO Hypoxia significantly delays *M. menidia* hatching, reduced survival to
117 hatching and larval survival, and reduced embryo and larval growth (Cross et al., 2019).
118 However, the negative effects of both hypoxia and high CO₂ were mitigated when offspring were
119 exposed to diel fluctuating treatments rather than static (Cross et al., 2019). While diel
120 fluctuations are a realistic representation of current changes in community photosynthesis and
121 respiration between day and night, environmental change in coming years could extend hypoxic
122 duration to reduce periods of relief. Warming reduces oxygen solubility while increasing
123 metabolic rates of organisms that draw down oxygen when densely aggregated. At the same
124 time, higher summer temperatures and freshwater input in some regions will intensify
125 stratification that separates low oxygen water from surface oxygen diffusion (Rabalais et al.,
126 2009; Howarth et al., 2011). Currently *M. menidia* is tolerant enough that population declines are
127 not a concern, but without knowledge of the mechanisms of early life impacts it is hard to
128 anticipate whether this will change under intensifying deoxygenation or with additional stressors
129 (Baumann, 2019).

130 Dynamic Energy Budget (DEB) theory is a bioenergetic framework designed to bridge
131 multiple levels of biological organization in assessing stressor effects and their mechanisms in a
132 vast variety of species (Kooijman, 2010a; AmP, 2023). This approach follows energy allocation,
133 represented in suborganismal metabolic fluxes, and how it leads to life history outcomes such as

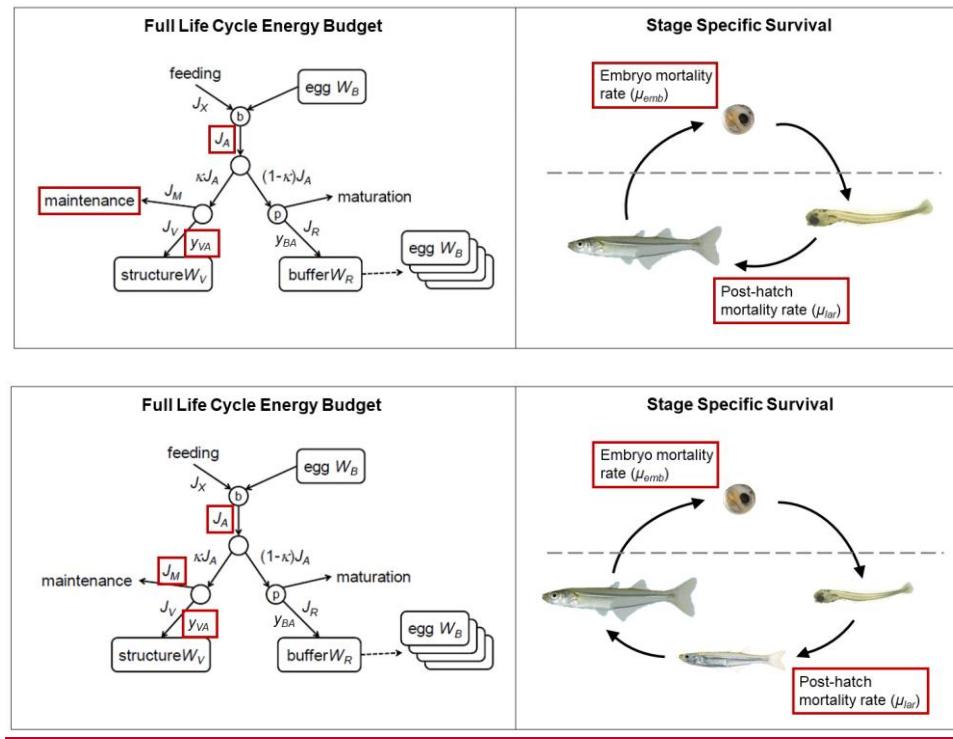
134 growth rate, reproductive output, and survival, using physical and biological concepts that are
135 generalizable to most species (Jusup et al., 2017). It accounts for differences in the energy budget
136 at each stage to allow modeling of life stage transition timing and stage-specific responses to
137 stressors (Kooijman, 2010a). DEB theory is often used to connect experimental observations of
138 multiple stressor effects to both the underlying energetic mechanisms (Kooijman, 2018) and life
139 history outcomes that feed into population dynamics (Nisbet et al., 2000; Martin et al., 2013;
140 Smallegange et al., 2017). It is important to connect suborganismal and organismal responses to
141 population implications because targeted conservation actions typically operate at this level, but
142 this scaling requires additional steps and remains a challenge (but see Nisbet et al., 1989; Martin
143 et al., 2013; Grear et al., 2020; Tai et al., 2021). The ability to bridge levels of biological
144 organization from the molecular to population level makes DEB theory an excellent tool for
145 enhancing the utility of experimental hypoxia data for conservation and management (Lavaud et
146 al., 2021). However, there is a conceptual disconnect between the abstract variables and fluxes in
147 DEB models and the chemically defined quantities reported in most molecular-level studies (e.g.
148 Murphy et al., 2018). Some of the interpretation of our results in this paper rests on hypothesized
149 connections between these different levels of description, and for this reason we discuss some
150 suborganismal literature in detail in the Supplementary Materials, section *Relating DEB*
151 *processes to physiology.*

152 Depending on the application and types of data available, simplified versions of the
153 standard DEB model can be used (e.g. Kooijman and Metz, 1984; Jager, 2018; Martin et al.,
154 2017). Although complexity can sometimes be beneficial (Evans et al., 2013), simple parameter-
155 sparse models are often preferable for their predictive power and ability to be applied, tested, and
156 interpreted widely (Holling, 1966; May, 2001; Jusup et al., 2017). The DEBkiss framework
157 (Figure 1) is a moderately simplified variation on the standard DEB model for animals that
158 eliminates explicit representation of reserve and assumes that assimilates are immediately
159 allocated to structure, maintenance, and reproduction (Jager et al., 2013). This reduces the data
160 requirements, the role of compound parameters, and, depending on the data, the total number of
161 parameters to be estimated (Jager et al., 2013). The simplicity of DEBkiss makes it ideal for
162 adaptation to many species of ecological or commercial value, even when the existing studies
163 were not originally intended for this use. Romoli et al. (2024) present a detailed comparison of
164 the advantages and limitations for ecological risk assessment of a model based on DEBkiss

165 versus a model based on Kooijman's "standard" DEB model. They highlight several "modeling
 166 choices" that should influence the choice of approach, including: (i) insufficient information in
 167 data sets; (ii) capturing differences between data sets for the same species; and (iii) auxiliary
 168 hypotheses. Consideration of each of these led us to choose DEBkiss for our work after much
 169 unsuccessful effort attempting to interpret parameter estimates with the standard model (using
 170 the Add-my-Pet software; AmPtool, 2022; Marques et al., 2018).

171

172



173

174

175 **Figure 1. Conceptual diagram of the DEBkiss model highlighting parameters of interest for**
 176 **hypoxia effects.** The DEBkiss model (diagram adapted from Jager et al., 2013) used in this study
 177 includes stage-specific survival parameters. The hypothesized parameters for hypoxia stress
 178 mechanisms are highlighted in red boxes. The left panel shows the energy budget for the full life
 179 cycle.

180 cycle and the right panel shows how the stage-specific survival modification is applied to
181 embryos, larvae, juveniles, and adults of *M. menidia*.

182
183

184 ~~Romoli et al. (2024) present a detailed comparison of the advantages and limitations for~~
185 ~~ecological risk assessment of a model based on DEBkiss versus a model based on Kooijman's~~
186 ~~"standard" DEB model. The comparison focuses on application in ecotoxicology but the~~
187 ~~reasoning carries over to other forms of environmental stress, including this study on hypoxia.~~
188 ~~They highlight several "modeling choices" that should influence the choice of approach,~~
189 ~~including: (i) insufficient information in data sets; (ii) capturing differences between data sets for~~
190 ~~the same species; and (iii) auxiliary hypotheses. Consideration of each of these led us to choose~~
191 ~~DEBkiss for our work after much unsuccessful effort attempting to interpret parameter estimates~~
192 ~~with the standard model (using the Add my Pet software; AmPtool, 2022; Marques et al., 2018).~~

193 We used DEBkiss to test the hypothesis that changes in animal performance under
194 hypoxia can be explained by changes in one or more of the rate processes in the model, and to
195 identify the bioenergetic mechanisms underlying experimental hatching, growth, and survival
196 effects of hypoxia in early life stages of *M. menidia* observed in Cross et al. (2019). First, we fit
197 the DEBkiss model to full-life data on total length, reproductive output, hatch timing, and
198 survival and estimated or calculated parameters under fully oxygenated conditions. Second, we
199 used the concept of Synthesizing Units (SU) that are inhibited or damaged by hypoxia to directly
200 or indirectly change key parameters in the DEBkiss model (Muller et al 2019). SUs are
201 generalized enzymes that produce products such as body structure or support maintenance
202 requirements from incoming fluxes of substrate, i.e. food or egg buffer (Kooijman, 1998;
203 Kooijman, 2010a). With single substrates for each life stage the SU formalism is equivalent to
204 standard Michaelis-Menten kinetics, but the SU interpretation allowed us to exploit the subtleties
205 in describing inhibition set out by Muller et al. (2019). We used a correction factor based on
206 inhibition or damage to the SU to fit the model to early-life data for four DO treatments. We
207 evaluated which parameter or combination of parameters, when adjusted with the correction
208 factor, was able to best account for the full set of hypoxia responses observed in experiments and
209 thus allow inference of mechanism.

210

211 **2. Methods**212 **2.1. DEBkiss *m*Model *d*Description**

213 The material flows are shown in Figure- 1. The yolk in an egg is treated as a buffer of
 214 “food” for the developing embryo that initially has an infinitesimally small structural biomass. In
 215 this regard, DEBkiss differs from standard DEB where the yolk is considered to be “reserve”
 216 whose dynamics are treated similarly to that for feeding life stages. There is no reserve
 217 compartment between food assimilation and its utilization. Hatching Birth occurs when the egg
 218 buffer is fully depleted. After hatchingbirth, larvae and juveniles, which are treated identically in
 219 this model, feed and assimilated food is allocated to growth, maturity, -and both somatic and
 220 maturity maintenance versus maturity or reproduction in accordance with the κ -rule. After
 221 reaching puberty and entering the adult stage, individuals feed and reproduce while maturation
 222 ceases. Somatic and maturity maintenance continue in adults.

223 The DEBkiss assumptions and equations are from Jager (2018). The parameters are
 224 defined in Table 1 and the variables, differential equations, and conversions are defined in Table
 225 2. The flux of food or, for embryos, from the egg buffer (W_B) is immediately converted to
 226 assimilates which are allocated to a somatic fraction (κ) and a reproductive fraction ($1-\kappa$; Figure
 227 1). The assimilation flux (J_A) is the product of the scaled measure of resource availability (f), the
 228 volumetric surface area (L^2), and the parameter maximum area-specific assimilation rate (J_{Am}^a)
 229 where $f = 1$ for embryos and for post-hatching fish fed *ad libitum*. Within the somatic branch, a
 230 flux to maintenance (J_M) is prioritized while the remainder goes to structural mass (J_V) with a
 231 conversion efficiency y_{VA} . The maintenance flux is proportional to structure.
 232

Parameter	Symbol	Fixed or estimated	Value
Max. area-specific assimilation rate	J_{Am}^a	Estimated	0.333 mg mm ⁻² d ⁻¹
Max. volume-specific maintenance rate	J_M^v	Fixed	0.0214 mg mm ⁻³ d ⁻¹
Initial egg buffer mass	W_{B0}	Fixed	0.15 mg
Total <u>physical</u> length at puberty	L_{Vp}	Fixed	102 mm
Yield of assimilates on <u>structurevolume</u>	y_{AV}	Fixed	0.8
Yield of egg buffer on assimilates	y_{BA}	Fixed	0.95
<u>Yield of structure on assimilatesConversion</u> <u>efficiency of assimilates to structure</u>	y_{VA}	Estimated	0.365
Fraction of assimilates allocated to soma	κ	Fixed	0.8
Scaled food level	f	Fixed	1
Scaled food level for embryo	f_B	Fixed	1

<u>Embryo mortality rate for embryos</u>	μ_{emb}	Estimated	$0.0639 \frac{d^{-1}}{d^{-1}}$
<u>Post-hatch mortality rate for larvae</u>	μ_{lar}	Estimated	$0.0294 \frac{d^{-1}}{d^{-1}}$

Formatted: Superscript

Formatted: Superscript

Table 1. DEBkiss parameters, their abbreviations, and their fixed or estimated values from

fitting to full life data. Units are given with the value unless the parameter is a unitless ratio. All
masses are in mg of dry weight.

Flux	Symbol	Equation	Units
Assimilation flux	J_A	$J_A = f J_{Am}^a L^2$	$mg \text{ day}^{-1}$
Maintenance flux	J_M	$J_M = J_M^v L^3$	$mg \text{ day}^{-1}$
Flux to structural growth	J_V	$J_V = y_{VA} (\kappa J_A - J_M)$	$mg \text{ day}^{-1}$
Flux to reproduction buffer	J_R	$J_R = (1 - \kappa) J_A - J_J \text{ when } W_V \geq W_{Vp}$ $J_R = 0 \text{ when } W_V < W_{Vp}$	$mg \text{ day}^{-1}$
Flux to maturity maintenance	J_J	$J_J = J_J^v L^3 \text{ when } W_V < W_{Vp}$ $J_J = J_J^v \frac{W_{Vp} L^3}{d_V} \text{ when } W_V \geq W_{Vp}$	$mg \text{ day}^{-1}$

State Variable	Symbol	Equation	Units
Structural dry mass over time	W_V	$\frac{dW_V}{dt} = J_V$	$mg \text{ day}^{-1}$
Continuous reproduction rate	R	$\frac{dR}{dt} = \frac{y_{BA} J_R}{W_{B0}}$	eggs day^{-1}
Egg buffer (yolk) mass	W_B	$\frac{dW_B}{dt} = -J_A$	$mg \text{ day}^{-1}$
Survival	S	$\frac{dS}{dt} = -\mu_{emb} S \text{ when } W_B > 0$ $\frac{dS}{dt} = -\mu_{lar} S \text{ when } W_B = 0$	unitless (range 0-1) $\frac{1}{day}$

Formatted: Superscript

Other variables and conversions	Symbol	Equation	Units
Total physical length	L^M	$L^M = \frac{L}{\delta_M}$	mm
Volumetric length	L	$L = \delta_M L^M = \sqrt[3]{\frac{W_V}{d_V} \cdot \frac{L^3}{d_V}}$	mm
Shape coefficient	δ_M	$\delta_M = \frac{L}{L^M}$	unitless
Dry weight density of structure	d_V	$d_V = \frac{W_V}{L^3}$	$mg \text{ mm}^{-3}$

Dry mass at puberty	W_{Vp}	$W_{Vp} = d_V * (L_{Vp} * \delta_M)^3$	mg
Volume-specific maturity maintenance costs	J'_J	$J'_J = \frac{1-\kappa}{\kappa} * J'_M$	mg mm ⁻³ day ⁻¹
Structural volume at puberty	L_{Vp}^3	-	mm ⁻³
Scaled measure of resource availability	f	-	unitless (range 0-1)

238 **Table 2. Model definition.** Fluxes, state variables, and differential equations in the DEBkiss

239 model.

240

241 For larvae and juveniles, the non-somatic fraction of assimilates is spent on maturation,
 242 or increasing complexity, through which it is dissipated and does not contribute to biomass.
 243 While the standard DEB formulation uses a state variable for maturity that triggers changes
 244 between life stages, DEBkiss instead uses a constant size at puberty to specify when
 245 reproduction is initiated (Kooijman, 2010b; Jager et al., 2013), so the maturity variable plays no
 246 role in the current work. Once the mass at puberty is reached (W_{Vp}), reproductive flux (J_R)
 247 toward egg production begins in adults with a conversion efficiency y_{BA} . The flux to maturity
 248 maintenance (J_J) is the product of the volume-specific maintenance costs (J'_J) and structural
 249 volume, or the volume at puberty for adults. J'_J is calculated from κ and J'_M (Table 2), rather
 250 than estimated, as connecting the two maintenance costs allows cumulative investment in
 251 maturity at puberty to be independent of food level (Jager, 2018). Although *M. menidia* have a
 252 distinct larval and juvenile stage, we treated both as the juvenile stage because the relevant
 253 aspects of their energy budget for DEBkiss are assumed to be identical.

254 Starvation is defined in two stages, with the first stage being insufficient flux of
 255 assimilates to the somatic fraction to meet maintenance requirements so that energy is diverted
 256 from the flux to maturation or the reproduction buffer. In the second stage, when the flux to both
 257 the somatic and reproductive branches is insufficient and the reproduction buffer is empty or
 258 puberty has not been reached, structure is converted to assimilates with conversion efficiency y_{AV}
 259 to go towards maintenance costs (Jager, 2018).

260 Because our growth data are in total length, we used a shape correction coefficient (δ_M)
 261 and dry weight density (d_V) to connect length with the model state variables (Table 2). δ_M
 262 connects the total length (L^M) to the volumetric length (L) which is the cubic root of volume, and

263 d_V connects volume to structural dry mass. δ_M could plausibly have different values in different
264 life stages (section 7.8 of Kooijman, 2010a), but lacking relevant data we here assume a single
265 stage-independent value. We calculated these constants using data on *M. menidia* length (Klahre,
266 1997) and embryo volume (Klahre, 1997) (Schwemmer, unpublished data) and a total length
267 (L^M) to dry weight (W_V) conversion empirically derived from data on larval to adult stages (H.
268 Baumann, personal communicationConcannon et al., 2021):

$$W_V = 0.0012 * L^{M \cdot 2.997} e^{(-2.997 \cdot \ln(L^M) - 6.7)} \quad (1)$$

270 After calculating W_V from $L^M = 5.3$ mm at hatching (Cross et al., 2019), we obtained a dry
271 weight at hatching of 0.18 mg. Assuming there is negligible change in weight or volume during
272 hatching, we used the volume of an embryo immediately before hatching, $L^3 = 0.45$ mm³, to
273 calculate d_V using:

$$d_V = \frac{W_V}{L^3} \quad (2)$$

274 This gave us $d_V = 0.4$ mg mm⁻³. This is slightly higher than the d_V values used for other fish
275 species (e.g. Jager et al., 2022), but the overall results were not sensitive to this parameter and it
276 allowed for a good fit to growth data across all life stages. More details on this calculation can be
277 found in the Supplemental Materials. We similarly used the embryo volume to calculate
278 volumetric length of an embryo as $L = 0.77$ mm, which gives us a δ_M of 0.145 using the
279 following equation:

$$\delta_M = \frac{L}{L^M} \quad (3)$$

280 However, this value led the model to underestimate total length later in the life span, suggesting
281 the δ_M value was too high for this long and slender fish. This underestimation indicates that the
282 shape of a newly hatched larva is not representative of the shape throughout life and after feeding
283 begins, and this conversion could be refined for future work by making volume and length
284 measurements at multiple life stages to implement stage-specific δ_M values. We manually
285 adjusted δ_M to a final value of 0.107 which provided a reasonable fit to length data and a better
286 starting point for parameter estimation.

287 We added a survival state variable (S) which, in addition to allowing an alternative
288 outcome to hatching, enabled us to model survival as a consequence of hypoxia effects on the
289 energy budget. We fit mortality parameters for embryos and post-hatch fish (μ_{emb} and μ_{lar}) to data
290 for survival to hatching and larval/juvenile survival (Figure 1; Table 2). In our implementation of

293 survival, the only DEB process influencing survival is egg buffer depletion, which determines
294 the time to hatch and thus when the embryo mortality rate switches to the post-hatch mortality
295 rate. This means survival is indirectly affected by the assimilation rate and conversion efficiency
296 of assimilates into structure.

297

298 2.2. Data

299 We calculated and estimated DEBkiss parameters in normoxic conditions (Section 2.3)
300 and modeled hypoxia effects (Section 2.5) based on four types of data: total length over time,
301 egg buffer mass over time (~~extrapolated from knowledge of initial~~ egg mass and age at hatching
302 ~~when egg buffer mass is assumed to be zero~~, cumulative egg production over time, and
303 proportion surviving since fertilization over time. As described in the introduction, the data
304 available for this model led us to use DEBkiss over the “standard” DEB model based on the
305 factors highlighted by Romoli et al. (2024) ~~and unsuccessful attempts to use standard DEB~~. We
306 had insufficient data, had to integrate information from multiple studies of the same (and similar)
307 species, and had to hypothesize plausible values for a few parameters.

308 Data for total length were sourced from four studies. Length at hatching and 15 days
309 post-hatching (dph) came from a study that reared *M. menidia* offspring in different static
310 oxygen levels across two experiments (Cross et al., 2019). This provided data for parameter
311 estimation at control oxygen levels described in Section 2.3 and modeling three reduced oxygen
312 treatments (Section 2.5 ~~-and~~ Table 2). We sourced additional length data for the full life span
313 from control levels of experiments that exposed *M. menidia* offspring to ambient and elevated
314 CO₂ levels (Murray and Baumann, 2018; Murray and Baumann, 2020; Concannon et al., 2021).
315 All total length data were obtained from fish maintained in static laboratory conditions at 24°C.

316 Data for the state variables of egg buffer mass (via time to hatching, when egg buffer
317 mass is zero), as well as survival at hatching and at 15 dph, were obtained from Cross et al.
318 (2019). ~~Because *M. menidia* hatch with little to no yolk sac (Bayliff, 1950; Bigelow and
319 Schroeder, 1953) and begin feeding the day of hatching (Middaugh and Lempesis, 1976), we
320 equate hatching with birth and assume the egg buffer mass reaches zero at hatching. The hatch
321 timing data use time steps of 1 day, so any very short delay between hatching and the start of
322 feeding would not be reflected in the model.~~ The control data from these experiments were used
323 to estimate parameters under normoxia (Section 2.3). We also obtained normoxic survival data

324 from a study on the effects of temperature and CO₂ on *M. menidia* early life survival, using only
325 the 24°C and control CO₂ data (Murray and Baumann, 2018). Four additional data points for
326 long-term survival in laboratory conditions at 17°C were obtained from a study that exposed *M.*
327 *menidia* offspring until 122 dph to two CO₂ levels, of which we only used data from the control
328 level (Murray et al., 2017). Lastly, the data for cumulative egg production over time, used to
329 estimate parameters under normoxia (Section 2.3), were also obtained from control groups in
330 Concannon et al. (2021), a study in which wild-caught juveniles were held in the laboratory at
331 20°C in different CO₂ treatments and strip-spawned once they reached reproductive maturity.
332

333 2.3. Parameter estimation under normoxia

334 We estimated four parameters by fitting them to full-lifespan data listed in Section 2.2
335 (J^a_{Am} , y_{VA} , μ_{emb} , and μ_{lar}), calculated four parameters from data (J^v_M , W_{Bo} , L_{Vp} , and f), and fixed at
336 suggested values parameters for which we had insufficient data to calculate or estimate (y_{AV} , y_{BA} ,
337 κ , and f_B ; Jager, 2018). The primary parameters and their calculated or estimated values are
338 found in Table 1. Fitting was done in Matlab with the platform BYOM v.6.4 and the package
339 DEBkiss v.2.3a (<https://www.debtox.info/byom.html>). [Details on parameter estimation in](#)
340 [BYOM can be found in the Supplementary Materials](#). BYOM uses a Nelder Mead simplex
341 search to optimize the parameters for a set of ordinary differential equations by minimizing
342 negative log likelihood (NLL). The DEBkiss package works under BYOM to estimate model
343 parameters based on their effect on the DEBkiss equations and the auxiliary equations. The
344 differential equations predict length, egg production, egg buffer mass, and survival over time
345 with the differences from observations used to calculate NLL.

346 Before estimating any parameters with the optimization described above, we ran
347 simulations with fitting turned off using a set of recommended parameters (Jager, 2018) and
348 parameters we calculated from data on *M. menidia*, as described below. We visually assessed fit
349 and noted the NLL calculated from each simulation as we adjusted parameters to obtain a
350 reasonable set of initial parameters before estimating any. Testing a range of parameters and
351 obtaining realistic initial parameters helps avoid detecting local minima with the optimization.
352 This also helped us reduce the number of parameters being estimated to avoid overfitting and so
353 that there were not multiple correlated parameters free at once. Furthermore, we were able to
354 obtain a reasonable fit using suggested values for y_{AV} , y_{BA} , and κ for unstressed fish that are

355 thought to be widely applicable across species (Lika et al., 2011; Jager, 2018). ~~The suggested~~
356 ~~value for y_{VA} of 0.8 from the literature (Lika et al., 2011; Jager, 2018) did not allow a realistic fit~~
357 ~~to the length data, but the~~ We used length, reproduction, and egg buffer depletion data allowed it
358 to be re-estimated ~~with~~ with the BYOM optimization. Ultimate length was used to fit J^a_{Am} to a
359 reasonable value while fixing all other parameters before estimating y_{VA} , because both
360 parameters affect growth and egg buffer depletion in the model and therefore cannot be
361 estimated simultaneously. Finally, we used the BYOM optimization to estimate μ_{emb} and μ_{lar} .

362 The length and reproductive data allowed us to calculate “length at puberty” (L_{Vp}),
363 defined as the length at which egg production begins. We obtained W_{B0} from *M. menidia* egg dry
364 weight data (Klahre, 1997) and calculated δ_M and dv from total length, egg diameter, and egg
365 mass data (Cross et al., 2019; Klahre, 1997; Concannon et al., 2021). To calculate volume-
366 specific maintenance costs (J^v_M), we used data on the rate of decrease in larval dry weight over a
367 period of starvation in the congeneric species *M. beryllina* (Letcher and Bengtson, 1993). [More](#)
368 [detail on this calculation can be found in the Supplemental Materials.](#) Borrowing from closely
369 related species is a common practice in bioenergetic modeling when the species has similar
370 habitat, life history, and physiology (Sibly et al., 2013). *M. menidia* and *M. beryllina* have
371 overlapping habitats and similar life history, egg sizes, and body sizes, although *M. beryllina*
372 reaches a smaller ultimate length (Middaugh, 1981; Bengtson, 1984; Middaugh and Hemmer,
373 1992). All *M. menidia* experiments used in this study fed fish *ad libitum* in all treatment levels,
374 so f was set to 1. For studies that exposed fish to different CO₂ levels, we only used data from
375 control groups to avoid potential CO₂ effects in the data.
376

377 2.4. Relating DEB processes to physiology

378 We aimed to identify the DEBkiss parameters responsible for observed whole-organism
379 effects of rearing *M. menidia* in hypoxia by applying a correction factor to modify one or more
380 parameters with decreasing oxygen based on inhibition of or damage to a SU. [The SU controls](#)
381 [assimilation, the transformation of food \(or yolk\) and oxygen into compounds that will go to](#)
382 [structure, maintenance, or reproduction \(Kooijman, 2010a; Jager, 2018\).](#) Although oxygen can
383 be a limiting substrate in SUs, previous work suggests that *M. menidia* embryos only become
384 metabolically oxygen-limited below a critical level of 2.04 mg L⁻¹ (Schwemmer, 2023), while it
385 remains oxygen-independent at the treatments for which we have data (2.7, 3.1, 4.2, and 7.7 mg

386 L⁻¹; Schwemmer et al., 2020). We therefore considered a single-substrate growth SU in which
387 food or egg buffer was the substrate. The mathematical characterization of inhibition and damage
388 is in Section 2.5.

389 Inhibiting agents reversibly bind to SUs, preventing them from accepting substrates to
390 proceed with their reaction. Damage, in contrast, induces dysfunction that is irreversible upon
391 removal of the damaging agent; however, damaged SUs can be repaired or replaced (Muller et
392 al., 2019). The idea is that hypoxia induces the production of compounds that in turn bind to
393 SUs. We used existing information on the physiological responses of fish early life stages to
394 hypoxia to identify the following candidate DEBkiss parameters to which to apply the hypoxia-
395 based correction factor: maximum assimilation rate (J^A_{Am}), conversion efficiency of assimilates
396 into structure (growth, ν_{VA}), maximum somatic maintenance rate (J^M_M , mg mm⁻³ d⁻¹), embryo
397 mortality rate (μ_{emb}), and post-hatch mortality rate (μ_{lar}). Hypoxia effects on growth and hatching
398 time can occur either through inhibition of assimilation or through damage that reduces the
399 conversion efficiency of assimilates to growth. Hypoxia ~~impact on survival may be caused~~ may
400 ~~impact survival~~ directly through damage or by inhibition of damage repair processes. Hypoxia's
401 impact on somatic maintenance rate may be most plausibly represented as damage. Inhibition of
402 or damage to SUs could affect these parameters as a direct or indirect result of several hypoxia
403 responses in fish, such as anaerobic respiration, behavior, and action of hypoxia-inducible factors
404 (Farrell and Brauner, 2009). A detailed review of how these mechanisms relate to the DEB
405 parameters and SUs can be found in the Supplementary Materials. We now discuss how these
406 abstract concepts, damage and inhibition, may relate to observations on hypoxia.

407 Assimilation is the transformation of food (or yolk) and oxygen into compounds that will
408 go to structure, maintenance, or reproduction (Kooijman, 2010a; Jager, 2018). Reduced food
409 consumption or reduced conversion of food into utilizable compounds, and thus limitation of
410 input of substrate to the SU, is a primary mechanism by which the fish energy budget is thought
411 to be impacted by hypoxia (Chabot and Dutil, 1999; Thomas et al., 2019 with findings
412 reinterpreted for DEBkiss).

413 Several genes controlling cell division and protein synthesis are regulated by hypoxia
414 (Ton et al., 2003), such as insulin-like growth factor binding protein 1 (IGFBP 1), a protein
415 controlled by hypoxia-inducible factor 1 (Hif 1) that has been shown to reduce growth and delay
416 development in fish embryos exposed to hypoxia (Kajimura et al., 2005; Kajimura et al., 2006;

417 Sun et al., 2011; Tian et al., 2014). This factor is thought to trade off growth for other oxygen-
418 demanding processes and help fish tolerate hypoxia. By preventing insulin-like growth factors
419 from binding to their receptors, IGFBP-1 inhibits signaling for cell division and differentiation
420 and energy can be diverted to processes necessary for survival (Kajimura et al., 2005). In the
421 DEBkiss model such forms of inhibition to the SU under hypoxia would be represented by
422 reduced assimilation rates, though the link to survival is not represented explicitly.

423 The conversion efficiency of assimilates to structure determines growth and hatch timing
424 because it represents the fraction of assimilates that are converted into structure rather than
425 burned on overhead costs of growth (Jager, 2018). When oxygen is low enough that anaerobic
426 metabolism must be used, this reduces conversion efficiency so that less growth results from the
427 same amount of yolk or food (Thomas et al., 2019). Damage to the SU may also be responsible
428 for reductions in conversion efficiency through lactate accumulation and consequential declines
429 in internal pH. Even at oxygen levels above the critical level at which oxygen consumption
430 declines, anaerobic glycolysis may increase (Nonnotte et al., 1993; Maxime et al., 2000; Wood et
431 al., 2018). Although capability for anaerobic glycolysis in embryos and yolk sac larvae appears
432 to vary widely across species (Wieser, 1995; Finn, 1995; Rombough, 1988), smaller fishes reach
433 harmful levels of anaerobic end products much faster than larger fishes due to their higher mass-
434 specific metabolic rates (Nilsson and Östlund-Nilsson, 2008). We hypothesized that this
435 contributed to a smaller hatch size and slower growth post-hatch.

436 Maintenance in DEBkiss is the energy allocated to any processes that support the
437 integrity and functioning of the structural body (Jager, 2018), including homeostasis, damage
438 repair, and activity. Demand for more protein turnover and cell repair can increase the volume-
439 specific maintenance rate following damage (Bouma et al., 1994; Kooijman, 2010a) and indeed
440 maintenance has been shown to increase with damage to structural proteins (Maury et al., 2019).
441 In addition to damage repair, maintenance rate could be elevated by the activity required for
442 some of the behavioral responses fish exhibit under hypoxia (Thomas et al., 2019). *M. menidia*
443 exposed to hypoxia swim to the surface to use aquatic surface respiration, taking advantage of
444 the diffusion of oxygen from the air (Miller et al., 2016). This behavior is impossible in embryos
445 but has been observed in larvae (Cross et al., 2019). Fishes also expend energy on faster
446 ventilation and heartbeat when ambient DO is low to increase oxygen uptake (Kramer, 1987;

447 Maxime et al., 2000) and remove accumulated CO₂ and lactate (Perry et al., 2009; Heath and
448 Pritchard, 1965), but these capabilities may be limited until development has progressed further.

449 The maintenance flux in DEBkiss is represented in units of assimilated carbon required to
450 meet the energy demand from maintenance. It therefore is only indirectly related to respiration
451 rates measured as oxygen consumption. For example, an increase in the abstract maintenance
452 parameter in the model could be caused by an increase in anaerobic processes without impacting
453 oxygen consumption. The *measured* oxygen consumption rates of *M. menidia* early life stages
454 did not significantly increase under experimental chronic hypoxia, but great variability in
455 metabolic rates among individuals combined with the short respirometry periods used (<1 hour)
456 may make small increases related to damage repair or activity difficult to detect (Schwenmmer et
457 al., 2020).

458 Although mortality is not a process directly represented by an SU, it could indirectly be
459 impacted by hypoxia effects on SUs through failure to meet developmental milestones—
460 particularly for hatching—or directly through increase in damage production or inhibition of
461 damage repair rates. In the parameter estimation using data from normoxic conditions (Section
462 2.3), our estimated survival parameter for embryo mortality was greater than that of larvae
463 (Table 1). If assimilation rate or conversion efficiency of *M. menidia* decreases under hypoxia,
464 the resulting slower egg buffer depletion would delay hatching, extending individuals' time in
465 the stage with greater mortality and thus accounting for reduced hatch survival under hypoxia.
466 We therefore hypothesized that if either assimilation rate or conversion efficiency is modified by
467 the hypoxia-based correction factor, additionally modifying the embryo mortality parameter
468 would consequently not be necessary to account for hypoxia effects on all four state variables.
469 However, this would not be the case for the post-hatch mortality parameter because none of the
470 processes in the DEBkiss model indirectly affect mortality after hatching, so changing either the
471 assimilation or conversion efficiency parameter in combination with the post-hatch mortality
472 parameter may be necessary to fully replicate the observed changes to growth, hatch timing, and
473 survival under hypoxia.

474

475 2.5. Hypoxia *eF*fects

476 We tested the hypothesis that changes in *M. menidia* early life growth, hatch timing, and
477 survival under reduced oxygen (Cross et al., 2019) can be explained by inhibition or damage

478 linked to one or more DEBkiss processes (Figure 1). To summarize the experimental data on
 479 static hypoxia effects we are attempting to explain by altering these parameters, the mean values
 480 of data for each oxygen treatment are listed in Table 3. We used the parameter values from the
 481 model fit to full life data and altered one or more parameters at a time with oxygen-dependent
 482 correction factors, then fit the model to data for only the first 136 days by estimating a parameter
 483 that controls the correction factor's relationship with DO. We only used early life data to fit the
 484 hypoxia-altered parameters because we did not have late-life or reproductive data for multiple
 485 oxygen treatments against which to validate observed changes. It did not make sense to include
 486 later life data in the calculations of NLL that influence the parameter estimates or to speculate
 487 about how well the predicted data match what we might expect to happen later in life if we not
 488 only lack late-life hypoxia data but also do not expect full-life hypoxia to occur in nature.
 489

Variable	7.7 mg L ⁻¹	4.2 mg L ⁻¹	3.1 mg L ⁻¹	2.7 mg L ⁻¹
Survival to hatching	74.3%	70.6%	85.8%	30.2%
Hatch time (egg buffer mass = 0)	6 days	7 days	8 days	9 days
Length at hatching	5.3 mm	4.6 mm	4.4 mm	4.1 mm
Larval length at 15 dph	15.8 mm	12.2 mm	9.2 mm	-
Larval survival to 15 dph	44.0%	22.2%	20.9%	0%

490 **Table 3. Summary of experimental data for each DO level.** The mean survival to hatching,
 491 hatch time (at which egg buffer is zero), length at hatching, length at 15 dph, and survival to 15
 492 dph from the different DO treatments in Cross et al. (2019). The control DO level means (7.7 mg
 493 L⁻¹) also include data from Murray and Baumann (2018).

494
 495 We derived a correction factor for *inhibition* using the framework developed by Muller et
 496 al. (2019), in which inhibitors can act on SU dynamics in five different ways. Out of these,
 497 *noncompetitive inhibition* is well-suited to this study because of the limitations of data
 498 availability for *M. menidia*. In noncompetitive inhibition the arrival rate of substrate does not
 499 affect the binding and dissociation of inhibitors and therefore requires little information about the
 500 rate of food uptake (Muller et al., 2019). In this form of inhibition, the rate of assimilation by the
 501 SU is:

$$J_A = f J_{Am}^a L^2 \left(\frac{1}{1 + \frac{j_i}{k_i}} \right) \quad (42)$$

503 where j_i (mg d^{-1}) is the arrival flux of the inhibitor and k_i (mg d^{-1}) is the dissociation parameter.
 504 The effect of this relationship in our model is that assimilation declines as the arrival rate of
 505 hypoxia-related inhibitors increases. We set j_i to depend on DO treatment above a DO threshold,
 506 DO_c (mg L^{-1}), below which j_i is infinitely large, which would bring the rate of the process it is
 507 inhibiting to zero:

$$j_i = \begin{cases} \infty & \text{if } \text{DO} \leq \text{DO}_c \\ \frac{1}{k_i \text{BZ}(\text{DO} - \text{DO}_c)} & \text{if } \text{DO} > \text{DO}_c \end{cases} \quad (35)$$

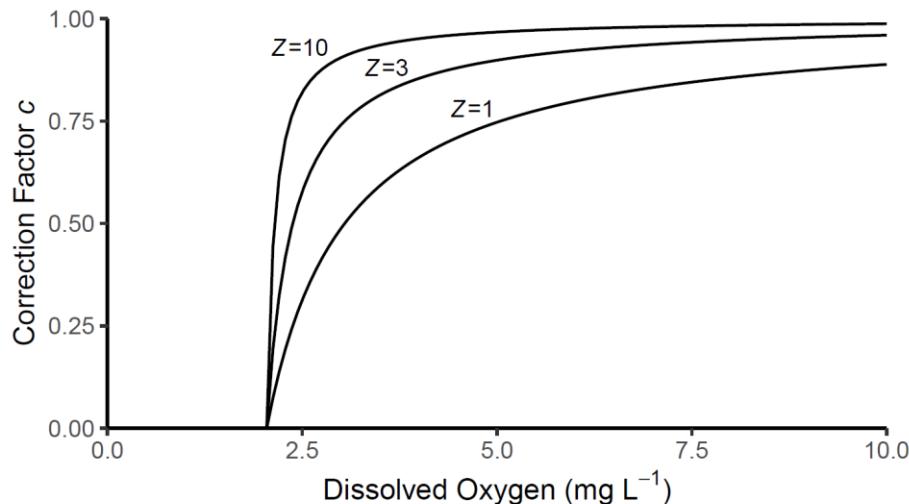
509 BZ ($\text{L} \cdot \text{d} \cdot \text{mg inhibitor}^{-1} \cdot \text{mg O}_2^{-1}$) is a parameter that influences the shape of the relationship
 510 between j_i and DO. We defined the correction factor c as the inhibition term (in parentheses in
 511 Equation 42) and replace j_i with the function from Equation 53 for $\text{DO} > \text{DO}_c$ to derive the
 512 correction factor c :

$$c = \frac{1}{1 + \frac{1}{k_i \text{BZ}(\text{DO} - \text{DO}_c)}} \quad (64)$$

513 As only the product of the parameters k_i and BZ appear in the formula and we have no need to
 514 estimate them separately, they can be combined into one parameter as Z (L mg O_2^{-1}). Simplifying
 515 Equation 64 and adding in the case for which $\text{DO} \leq \text{DO}_c$ gives us the following correction factor:
 516

$$c = \begin{cases} 0 & \text{if } \text{DO} \leq \text{DO}_c \\ \frac{Z(\text{DO} - \text{DO}_c)}{1 + Z(\text{DO} - \text{DO}_c)} & \text{if } \text{DO} > \text{DO}_c \end{cases} \quad (75)$$

517 The relationship between c and DO for three different sample values of Z , the parameter
 518 to be estimated, is shown in Figure 2. A larger Z value keeps c higher as oxygen decreases before
 519 a more abrupt drop, while a smaller Z gives a more constant decline in c with hypoxia. The value
 520 of c cannot exceed 1. DO_c was fixed at a biologically relevant level of 2.04 mg L^{-1} , which is the
 521 critical oxygen level below which embryonic routine metabolism becomes highly oxygen-
 522 dependent (Schwemmer, 2023). Attempts to estimate DO_c and Z simultaneously showed that
 523 leaving DO_c free did not improve the ability of the correction factor to fit the hypoxia data.
 525



526

527 **Figure 2. The correction factor c used to apply hypoxia effects to DEBkiss parameters.** The
 528 effect of DO on correction factor c is shown at three different values of the ~~exponential~~
 529 parameter Z . Actual estimated Z values are listed in Table 4, and the three Z values used in this
 530 figure are sample values to show how Z affects the relationship between DO and c .

531

532 Similar simplification of the reasoning by Muller et al. (2019) can be used to derive an
 533 analogous correction factor for *damage*. Assuming ~~that the~~a proportional change in the rate of
 534 damage production to the SU (e.g. via “damage inducing compounds”; Kooijman 2010a), $-j_d$ has
 535 the same form as Equation (53). If damage production is quickly balanced by repair or
 536 mitigation, then fluxes that decrease through hypoxia will again be reduced by the factor given
 537 by Equation (75). This was recognized by Muller et al. (2019) who noted that if damage
 538 production is much slower than the maximum production rate of an SU, the formalism for
 539 noncompetitive damage is equivalent to that of noncompetitive inhibition (Muller et al., 2019).
 540 Further submodels relating damage to rates that may increase in response to hypoxia (e.g.
 541 maintenance and mortality) are needed to derive functional forms for appropriate conversion
 542 factors here. Absent information to support such submodels, we hypothesize that the increase
 543 was inversely proportional to c defined by Equation (75).

544 The correction factor c was multiplied by J^a_{Am} and y_{VA} because these parameters were
545 hypothesized to decrease under hypoxia irrespective of the underlying cause (inhibition or
546 damage). Reductions in the parameter y_{VA} through hypoxia are most plausibly interpreted as
547 damage, the irreversible destruction of functionality of an SU. However, the parameters for
548 maintenance and mortality were divided by c because they were hypothesized to increase, rather
549 than decrease, with damage production and inhibition.

550 To find the best value of Z for each DEBkiss parameter or combination of parameters, we
551 added Z as a model parameter and estimated it using the BYOM optimization to minimize NLL.
552 We weighted the data equally across treatments to correct for differences in sample size across
553 treatments and prevent one treatment group from disproportionately affecting the estimation of
554 Z , so that all weights within each treatment added up to the same number. We did not apply the
555 correction factor to J^a_{Am} and y_{VA} simultaneously because they ~~are multiplied together both~~
556 ~~contribute to obtain~~ J_V and their individual contributions to the growth and egg buffer depletion
557 ~~cannot be fully separated~~ ~~are difficult to disentangle, particularly when J_M is very small as in the~~
558 ~~early life stages~~. We only compared the fit of models in which c was applied to parameter(s) that
559 resulted in all three early life datasets – total length, egg buffer mass, and survival – being
560 affected by hypoxia. As a result, either J^a_{Am} or y_{VA} is in each candidate model, because J^v_M , μ_{emb} ,
561 and μ_{lar} do not affect egg buffer depletion.

562 To identify the most likely version of the model (which parameter or combination of
563 parameters best explains the hypoxia effects on the state variables), we estimated Z for each of
564 these scenarios and calculated Akaike's Information Criterion for small sample sizes (AICc). We
565 compared the AICc between each model using the difference between AICc values (ΔAICc) and
566 the relative likelihood of each model using Akaike weights:

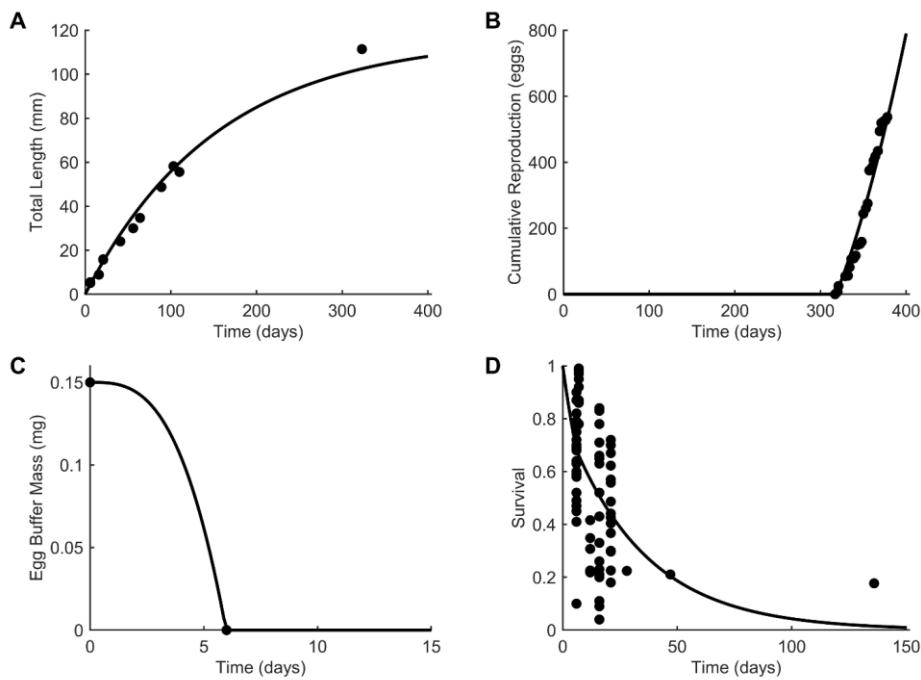
$$w_i(\text{AICc}) = e^{-0.5 \cdot \Delta_i \text{AICc}} / \sum_{k=1}^K e^{-0.5 \cdot \Delta_k \text{AICc}}, \quad (68)$$

567 where $w_i(\text{AICc})$ is the Akaike weight of each model i , $\Delta_i \text{AICc}$ is the difference between each
568 model i and the model with the lowest AICc (AICc_{\min}), and the denominator calculates the sum
569 of relative likelihoods for every model starting at the first model k (Wagenmakers and Farrell,
570 2004). We used ΔAICc and ratios of Akaike weights to determine which combination of
571 parameters best fit the data when inhibition or damage was applied and, therefore, which DEB
572 processes best explain the hypoxia effects observed in experiments (Table 4).

574

575 **3. Results**576 *3.1. DEBkiss mModel*

577 We obtained realistic fits to the full life cycle data (Figure 3). The only exception is late-
 578 life survival, for which the mortality was too high beyond the larval stage but could not be better
 579 fit due to lack of full-life survival data (Figure 3D). Silversides are an annual species so survival
 580 should be greater than 0% after 150 days. However, this did not impair our ability to model the
 581 effects of hypoxia on early life survival, which is most important given that the present study
 582 focuses on hypoxia in the early life stages. Estimating y_{VA} returned a value much lower than 0.8,
 583 which is the value suggested by Jager (2018) and has been applied in DEBkiss models of other
 584 species (e.g. Jager et al., 2018; Hamda et al., 2019). However, our value of $y_{VA} = 0.365$ is close to
 585 the maximum growth efficiency of 0.375 measured in the closely related *M. beryllina* (Letcher
 586 and Bengtson, 1993). This gave a realistic fit to the length data and allowed a detailed and very
 587 close fit to egg buffer mass over time (hatch timing). The observed and predicted data for full life
 588 span are plotted in Figure 3.



589

590
 591 **Figure 3. Full life model fits to data for four state variables.** Predicted (lines) and observed
 592 data (dots) for the DEBkiss model of *M. menidia* are shown. The state variables are (A) total
 593 length (mm) over time (days), (B) cumulative reproduction (eggs) over time (days), (C) egg
 594 buffer mass (mg) over time (days), and (D) survival over time (days). Predicted data lines were
 595 calculated with the parameter values listed in Table 1.
 596
 597

598 *3.2. Hypoxia eEffects*
 599

599 Applying the oxygen-dependent correction factor to the parameter combinations listed in
 600 Table 4 reproduced the direction of experimentally observed hypoxia effects, e.g. ~~de~~increasing
 601 J^a_{Am} reduced total length, increased time until egg buffer mass reaches 0, and reduced survival.
 602 The best model of experimental hypoxia effects on *M. menidia* early life stages simultaneously
 603 had y_{VA} multiplied by c , and μ_{emb} and μ_{lar} divided by c (Figure 4, Table 4, Figure S1). Although
 604 applying damage to y_{VA} alone affected all three state variables, concurrently increasing both
 605 mortality parameters improved the fit to the data (Table 4). The model in which the correction
 606 factor was applied to y_{VA} , μ_{emb} , and μ_{lar} also had the lowest AICc of all candidate models, with an
 607 AICc of 794.03 (AICc_{min}). Adding a correction factor to J^v_M in simultaneously with these three
 608 parameters yielded a slightly higher AICc of 795.97 (Table 4). The ratio of Akaike weights
 609 shows that the model with c applied to y_{VA} , μ_{emb} , and μ_{lar} , is 2.67 times as likely as the one with c
 610 concurrently applied to J^v_M (Table 4). Applying a damage effect to maintenance was therefore
 611 not considered to have improved the fit. After estimating Z_e we calculated the values of y_{VA} , μ_{emb} ,
 612 and μ_{lar} when their respective correction factors are applied for each DO level (Table 5).

613
 614

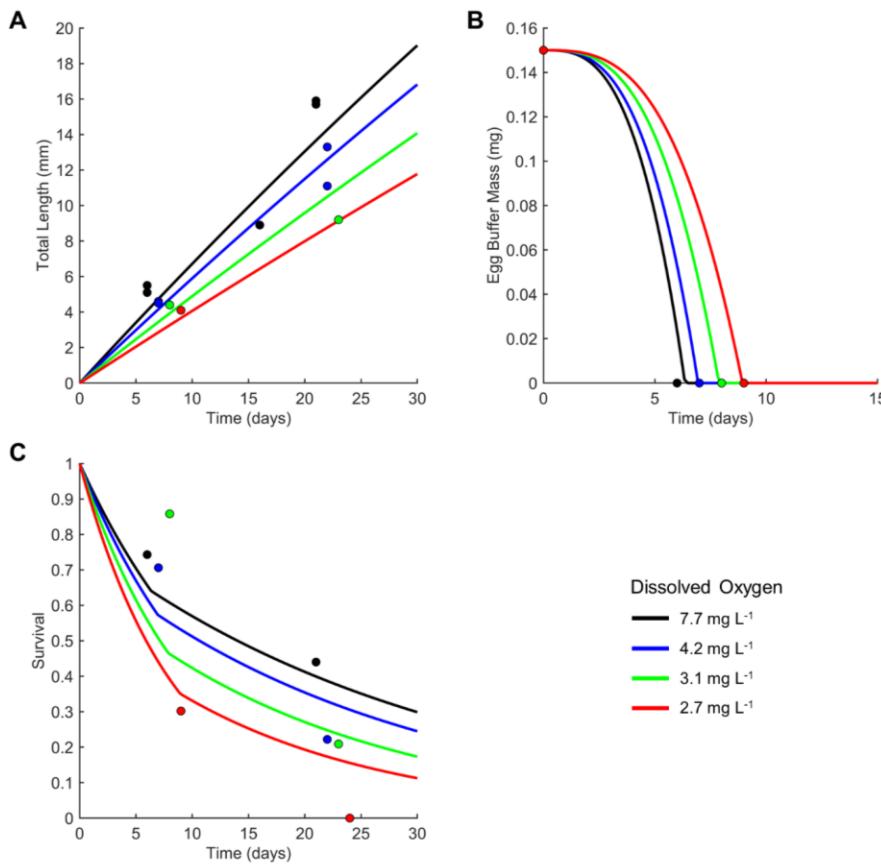
Parameter(s) affected by hypoxia correction factor	Estimated Z [95% CI]	AICc	ΔAICc	Akaike weight
J^a_{Am}	3.019 [2.512-3.612]	856.06	62.03	2.5e-14
y_{VA}	1.818 [1.601-2.342]	848.65	54.62	1.0e-12
$J^a_{Am} + J^v_M$	3.105 [2.651-3.726]	855.00	60.97	4.2e-14
$y_{VA} + J^v_M$	1.985 [1.688-2.774]	850.64	56.61	3.7e-13
$J^a_{Am} + \mu_{emb}$	2.804 [1.605-3.287]	823.24	29.21	3.3e-7
$y_{VA} + \mu_{emb}$	1.801 [1.570-2.167]	808.12	14.09	6.3e-4

$J^a_{Am} + \mu_{lar}$	2.930 [2.165-3.428]	838.17	44.14	1.9e-10
$y_{VA} + \mu_{lar}$	1.767 [1.536-2.111]	821.30	27.27	8.7e-7
$J^a_{Am} + \mu_{emb} + \mu_{lar}$	2.819 [1.920-3.286]	810.21	16.18	2.2e-4
$y_{VA} + \mu_{emb} + \mu_{lar}$	1.827 [1.620-2.269]	794.03	0	0.72
$J^a_{Am} + J^r_M + \mu_{emb} + \mu_{lar}$	2.913 [2.288, 3.387]	809.96	15.93	2.5e-4
$y_{VA} + J^r_M + \mu_{emb} + \mu_{lar}$	1.981 [1.700, 2.456]	795.97	1.94	0.27

615 **Table 4. Parameter Z estimates and model selection results.** The estimated Z value, AICc,
 616 ΔAICc, and Akaike weights when the correction factors were applied to each parameter or
 617 combination of parameters. ΔAICc and Akaike weights were calculated with $AICc_{min} = 794.03$
 618 for the $y_{VA} + \mu_{emb} + \mu_{lar}$ model (bold).

619

620



621
622
623 **Figure 4. Best fit of DEBkiss model to experimental data from four DO levels.** The best fit of
624 the predicted data (lines) to the observed data (dots) for four DO levels is shown, for early life
625 data only. The best fitting model was selected based on lowest AICc. (A) is total length (mm)
626 over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time
627 (days), with means rather than all data plotted for survival for ease of viewing. Full datasets used
628 to estimate the correction factor parameter Z are plotted in Figure S1.

629

630 Interestingly, although J^a_{Am} affects the variables similarly to y_{VA} , the ratio of Akaike
631 weights showed that the best fitting model is about 3000 times as likely as the version applying

632 inhibition to J^a_{Am} , μ_{emb} , and μ_{lar} (Table 4). Reducing J^a_{Am} with hypoxia using the correction factor
633 resulted in a visually good fit to the data across oxygen levels and variables. Simultaneously
634 applying c to J^a_{Am} and both mortality parameters improved the fit compared to only applying it to
635 J^a_{Am} , but this model fit less well than the version that applied c to y_{VA} , μ_{emb} , and μ_{lar} , with an AIC
636 value of 810.21 in the former model compared to 794.03 in the latter.

637 The estimated best value of Z , the exponential coefficient in the correction factor c ,
638 enables us to calculate that y_{VA} at the lowest oxygen level is 55% of its value with no hypoxia
639 stress. Reducing conversion efficiency alone produced small differences in survival at hatching
640 because it prolongs the time spent in the embryo stage, which has a greater mortality rate than
641 post-hatching in our model. Dividing both the pre- and post-hatching mortality rates by c more
642 closely predicted the reduced survival rates in the low DO treatments, resulting in a best fitting
643 model that explained observed hypoxia effects well by altering conversion efficiency, embryo
644 mortality, and post-hatch mortality.

645

	Product of correction factor and initial parameter value			
	7.7 mg L ⁻¹	4.2 mg L ⁻¹	3.1 mg L ⁻¹	2.7 mg L ⁻¹
y_{VA}	0.333 [0.329, 0.339]	0.291 [0.284, 0.303]	0.240 [0.230, 0.257]	0.199 [0.188, 0.218]
μ_{emb}	0.0701 [0.0689, 0.0709]	0.0801 [0.0770, 0.0822]	0.0970 [0.0906, 0.101]	0.117 [0.107, 0.124]
μ_{lar}	0.0322 [0.0317, 0.0326]	0.0369 [0.0354, 0.0378]	0.0446 [0.0417, 0.0466]	0.0539 [0.0492, 0.0571]

646 **Table 5. Effects of correction factor c on parameters.** The value of the DEBkiss parameters
647 that best reproduce the hypoxia effects observed experimentally, calculated (with 95%
648 confidence intervals in brackets) for each DO treatment level using the correction factor c and
649 the estimated value of $Z = 1.827$.

650

651

652 4. Discussion

653 By combining experimental data with unified principles for energetic allocation that are
654 broadly applicable across species, we identified the conversion efficiency of assimilates into
655 structure as the most likely process by which low oxygen levels affect early life stages of *M.*
656 *menidia*. In comparing combinations of DEBkiss parameters that influence the ecological
657 endpoints (total length, hatch timing, and survival), we discovered that applying correction

658 factors based on damage production to the growth SU to reduce conversion efficiency (y_{VA}) and
659 increase pre- and post-hatching mortality rates (μ_{emb} and μ_{lar}) best predicted the experimental
660 effects of hypoxia on larval length, time to hatching, and early life survival. Through this model
661 we have found evidence that the mechanism largely responsible for the observed hypoxia
662 impacts on growth, hatch timing, and survival is the efficiency with which assimilated food or
663 egg yolk is converted into structure. The limitations of this inference are discussed later.

664 Changes to assimilation in response to hypoxia have been recorded in other species, but
665 the direction of that effect is species-dependent (reviewed in Thomas et al., 2019). In *M.*
666 *menidia*, however, reducing assimilation with hypoxia rather than conversion efficiency yielded
667 a worse fit despite the two parameters' similar contributions to the DEBkiss model in that both
668 parameters are used to calculate predicted growth and egg buffer depletion. ~~In our
669 implementation of hypoxia effects on SUs, these two parameters are assumed to be impacted by
670 different molecular mechanisms but the same noncompetitive inhibition based correction factor,
671 due to the assumption that the maximum rate at which the SUs form product is much greater than
672 the damage production rate (Muller et al., 2019).~~ Reducing either assimilation or conversion
673 efficiency would extend developmental time, which is consistent with previous work showing
674 yolk absorption slows under hypoxia (Polymeropoulos et al., 2017). As maintenance costs must
675 continue to be paid, this would increase the energy expended to produce each unit of structure
676 (Kamler, 2008). ~~Unlike assimilation, However,~~ the mechanism for reduced conversion efficiency
677 is most plausibly interpreted as damage to the synthesizing unit, perhaps from buildup of
678 anaerobic byproducts, ~~along with far less efficient ATP production through anaerobic respiration
679 and slower rates of tissue differentiation and repairing the SU incurs additional maintenance
680 costs compared to inhibition which requires no repair~~ (Bouma et al., 1994; Kooijman, 2010a;
681 Muller et al., 2019). ~~Conversion efficieney can also be reduced by the far less efficient
682 production of ATP through anaerobic respiration combined with slower rates of tissue
683 differentiation.~~ The experimental DO levels are greater than the critical oxygen levels for
684 oxygen-independent routine metabolism (P_{crit}) of 2.04 mg L^{-1} and 1.56 mg L^{-1} for embryos and
685 5dph larvae, respectively (Schwemmer, 2023). P_{crit} has been assumed by some to be the oxygen
686 level at which anaerobic metabolism is triggered, but there is abundant evidence that some level
687 of anaerobic metabolism can occur well above P_{crit} (Nonnotte et al., 1993; Maxime et al., 2000;
688 Wood et al., 2018). Additional activity such as swimming bursts can drive up the need for

689 anaerobiosis (Di Santo et al., 2017). Our evidence that conversion efficiency is reduced by
690 hypoxia-induced damage suggests that anaerobic metabolism may be a mechanism of hypoxia
691 effects in *M. menidia* early life stages even at oxygen levels above P_{crit} .

692 While y_{VA} is the best parameter to explain the hypoxia effects according to our model and
693 AICc, it is nonetheless possible that J^{aAm} is responsible for an unknown portion of the hypoxia
694 effects. Because of near collinearity between J^{aAm} and y_{VA} , our model does not allow us to test for
695 the possibility that both parameters are simultaneously contributing to the observed hypoxia
696 effects. It is not possible to simultaneously estimate both parameters, particularly when J_M is
697 negligible as in the early life stages and as their product is used J^{aAm} and y_{VA} are directly
698 multipled to calculate growth in the model; we can adjust one or the other with the correction
699 factor and get similar effects on the flux for growth with no way of determining which is correct.
700 We therefore cannot test for partial contribution of the two parameters to hypoxia effects or
701 quantify their relative contributions. If conversion efficiency were the only parameter varying
702 across hypoxia treatments, one might expect all the offspring to fully deplete the egg buffer and
703 hatch at the same time, but with hatch size increasing with DO level. However, adjusting
704 conversion efficiency with hypoxia does account for the observed significant differences in hatch
705 timing between DO treatments in *M. menidia* larvae (Cross et al., 2019) because y_{VA} reduces the
706 body size at a given time, indirectly reducing the assimilation flux due to smaller body volume.
707 Future work examining the effects of hypoxia on ingestion, defecation, respiration, and growth
708 could help tease apart the relative contributions of y_{VA} and J^{aAm} by allowing direct calculation of
709 y_{VA} . Data on fecundity at different DO levels would provide information on the contribution of
710 J^{aAm} , although constant hypoxia through adulthood is unrealistic and this would assume the
711 energy budget is impacted similarly across life stages.

712 Although both conversion efficiency and assimilation can explain hypoxia effects on total
713 length and egg buffer mass over time, reducing them only produced a small decrease in survival
714 relative to the data. Simultaneously applying c to both mortality rates better captured predicted
715 the great reductions in survival at both hatching and 15 dph with hypoxia and improved the fit
716 based on ΔAICc (Table 4). In the experiments, the lowest oxygen level (2.7 mg L^{-1}) had a mean
717 hatch survival of 30.2% while the mean survival in the other three treatments was over 70%
718 (Cross et al., 2019). By 15 dph fish from all three low oxygen treatments had lower survival than
719 those from the normoxic treatment (Cross et al., 2019; Table 3). Including hypoxia effects for

720 both pre- and post-hatching mortality rates allowed the model to predict these stage-specific
721 differences in hypoxia effects more closely and improve the fit based on AICc (Table 4). The
722 additional mortality that was not accounted for by y_{VA} may have been related to unrepaired
723 damage from buildup of toxic compounds during anaerobic metabolism (Richards, 2011). The
724 mortality could also have resulted from failing to meet energetic demands with either aerobic or
725 anaerobic metabolism (Richards, 2009) and, specifically in embryos, failure to reach a viable
726 level of complexity before the yolk is depleted (Jager et al., 2013). Measurement of anaerobic
727 byproducts such as lactate and morphometric assessment of dead embryos and larvae could help
728 to identify the mechanisms underlying the mortality rates in future work. Although survival does
729 not approach 0% during the larval stage in our best fitting model (Figure 4), all experimental
730 replicates of the 2.7 mg L⁻¹ DO treatment had 0% survival by 15 dph, making larvae apparently
731 more sensitive than embryos (Cross et al., 2019). The authors of the study attribute this to a
732 possibly lower ability to suppress metabolism in larvae compared to embryos. While the
733 increased mobility of larvae may allow aquatic surface respiration (Miller et al., 2016; Cross et
734 al., 2019) and escape from hypoxia in a patchy and stratified estuarine environment, activity
735 comes with elevated maintenance costs in addition to those required to begin feeding almost
736 immediately after hatching (Middaugh and Lempesis, 1976). This may also be a crucial time to
737 repair damage to the SU (Muller et al., 2019), and the combination of these additional
738 maintenance demands may be too great to meet without restoration of normoxia. Though beyond
739 the scope of this work, a model that captures stage-specific differences in maintenance costs and
740 links them explicitly to survival may better capture the mechanism of high mortality in larvae.

741 Adding a correction factor to the volume-specific maintenance rate in addition to y_{VA} ,
742 μ_{emb} , and μ_{lar} did not substantially improve the fit according to AICc, suggesting that increasing
743 maintenance costs is not an important bioenergetic mechanism underlying hypoxia response in
744 early life stages. This is consistent with laboratory measurements showing no effect of these
745 hypoxia levels on embryonic or larval metabolic rates (Schwemmer et al., 2020), but as noted
746 earlier interpretation of respiration data is challenging and there was high individual variability
747 in the data. In our model, egg buffer depletion is insensitive to changes in volume-specific
748 maintenance costs, requiring a quadrupling to see a noticeable delay in hatching. Changing
749 maintenance has much greater effects on length later in life while failing to explain differences in
750 length at the time of hatching. Because maintenance is dependent on volume, it is a relatively

751 small portion of the energy budget in the very small early life stages but increases substantially
752 relative to the surface area-specific assimilation when larger sizes are reached, increasing its
753 relative role in determining growth rate and, indirectly, all size-specific fluxes. Repairing
754 damage and increasing ventilation and swimming activity could both increase maintenance costs
755 (Thomas et al., 2019), but at the embryo stage very little activity is possible. A common response
756 to hypoxia in some fish embryos is premature hatching (Kamler, 2008) which could allow
757 swimming escape responses that increase maintenance costs and in theory reduce growth, but
758 studies on chorion removal have shown that the increased mobility can improve growth despite
759 hypoxia exposure (Ciuhandu et al., 2005; Ninness et al., 2006). Some studies on fish responses to
760 hypoxia suggest maintenance may drop temporarily due to the reduced capacity for aerobic
761 metabolism at low DO levels, then subsequently be temporarily elevated after oxygen is restored
762 because of recovery demands such as paying oxygen debt and removing or repairing damage
763 from anaerobic byproducts (Heath and Pritchard, 1965; Claireaux and Chabot, 2016; Thomas et
764 al., 2019). Such fluctuations in maintenance were not discernible in the time scale of our model,
765 but future work should attempt to model the *M. menidia* early life energy budget during recovery
766 from hypoxia.

767 Understanding the mechanisms of reduced growth and survival under hypoxia through
768 DEB theory is useful for predicting life history effects, and although modeling population growth
769 rates was not within the scope of this study, our results have implications for processes that
770 influence fish population dynamics. The predicted data resulting from fitting best fitting model to
771 early life data with a hypoxia-based correction factor predicts hypoxia-related reductions in long-
772 term growth and survival that would certainly be detrimental to population growth under
773 extended periods of low oxygen. Under this model, even restoring normoxia after 15 days would
774 result in smaller size at age and survival rates than the control group, and damage to the SU is
775 not reversed upon return to normoxia, but rather requires energy to repair (Muller et al., 2019).

776 Delayed hatching and slower growth can lead to enhanced vulnerability to predation (Chambers
777 and Leggett, 1987; Takasuka et al., 2007), further reducing fish survival rates beyond those
778 observed in controlled laboratory conditions, although this is not always the case (Lankford et
779 al., 2001; Robert et al., 2023). However, compensation of growth may be possible in aquatic
780 ectotherms after exposure to hypoxia (Wei et al., 2008), and other stressors (Russell and
781 Wootten, 1992; Niebla and Metcalfe, 1997; Ali et al., 2003). Delayed hatching and slowe

782 growth can lead to enhanced vulnerability to predation (Chambers and Leggett, 1987; Takesuka
783 et al., 2007), further reducing fish survival rates beyond those observed in controlled laboratory
784 conditions, although this is not always the case (Lankford et al., 2001; Robert et al., 2023). An
785 important assumption of our model is that several of the parameters have the same value across
786 life stages (e.g. J^a_{Am} , J^r_M , y_{VA}) and similarly that values of the hypoxia correction factors are the
787 same regardless of life stage. We lacked data on the effects of hypoxia on the proportion of total
788 energy allocated to reproduction ($1-\kappa$), which is an additional component of DEB useful in
789 connecting organismal effects to populations. Future experimentation could provide the adult-
790 stage information that is needed to extend this DEB model to predict population growth, which
791 would be useful for resource management applications (Kooijman et al., 2020; Lavaud et al.,
792 2021), given the ecological importance of forage fishes and the value of model species like *M.*
793 *menidia*.

794 The oxygen levels in the estuaries inhabited by *M. menidia* undergo great diel and
795 seasonal fluctuations (Baumann et al., 2015). The effects of fluctuating DO cannot be resolved in
796 the time scales used by our DEBkiss model, so we assumed constant DO levels. As a result, the
797 model is more useful in identifying mechanisms than in quantitatively predicting how *M.*
798 *menidia* will respond to realistic hypoxia scenarios, as lifelong constant hypoxia is unrealistic
799 and this assumption may lead to overestimation of hypoxia effects. Studies comparing fish
800 responses to static and fluctuating hypoxia treatments have suggested that fluctuations provided
801 temporary relief and reduced sensitivity (Cross et al., 2019; Williams et al., 2019; Wang et al.,
802 2021), although conflicting results also exist (Morrell and Gobler, 2020). It is also unrealistic for
803 only a single environmental factor, in this case hypoxia, to influence the energy budget. Other
804 studies have applied correction factors to DEB parameters to model other species' responses to
805 hypoxia (Lavaud et al., 2019; Aguirre-Velarde et al., 2019), seawater acidification (Jager et al.,
806 2016; Moreira et al., 2022; Pousse et al., 2022) and pollutants (Muller et al., 2010; Desforges et
807 al., 2017). The success of this approach with a wide variety of stressors makes it an ideal
808 supplement to multistressor experiments, which are limited by logistical constraints. Modeling
809 stressor effects with DEBkiss parameters can yield additional information about energetic
810 mechanisms of responses and, with careful attention to the assumptions being made, may be
811 useful in extrapolating stressor effects to additional magnitudes or combinations of stressors that
812 would have been impractical to test experimentally, or to species with certain shared physiology

813 or life history traits (Goussen et al., 2020; Boult and Evans, 2021). In the case of *M. menidia*,
814 previous work showed that high CO₂ increases oxygen dependence of metabolism under both
815 chronic (Schwemmer et al., 2020) and acute hypoxia (Schwemmer, 2023). Adding oxygen as a
816 second substrate in the SU would allow a DEB model to incorporate the oxygen limitation that is
817 evidently induced by acidification.

818 Our best fitting model overestimated time to hatching at 7.7 mg L⁻¹ DO and
819 overestimated survival at age for the 2.7 and 4.2 mg L⁻¹ treatments, which suggests there either
820 may be a different nonlinear correction factor function that better fits the relationship between
821 DO and the DEBkiss parameters or that there were additional factors contributing to these
822 differences that the model does not account for. For example, hypoxia can reduce gonadosomatic
823 index and gonad development in fish (Wu et al., 2002; Thomas et al., 2006; Landry et al., 2007),
824 but we do not have data on gonad development or reproductive output ~~later in life~~ after rearing
825 *M. menidia* in hypoxia, which would allow us to investigate if κ is an affected parameter.

826 ~~Hypoxia can reduce gonadosomatic index and gonad development in fish, suggesting that the~~
827 ~~reproductive branch of the energy budget might require additional energy to be redirected from~~
828 ~~the somatic branch (Wu et al., 2002; Thomas et al., 2006; Landry et al., 2007)~~. Despite the
829 potential for improvements with more data, the model was able to replicate the direction of
830 effects and even account for some hypoxia effects in all three state variables simultaneously by
831 changing only one parameter, either conversion efficiency or assimilation. Further, it provided
832 these reasonable fits using an SU model based in well-studied and widely applicable Michaelis-
833 Menten-Briggs-Haldane enzyme kinetics (Muller et al., 2019) rather than a more specialized or
834 complex correction factor. While the generalized framework allows this model to be applied to
835 other species, one species-specific assumption is that birth occurs at hatching. This is a fitting
836 assumption for *M. menidia*, which are known to hatch with no detectable yolk (Bayliff, 1950)
837 and begin feeding the day of hatching (Middaugh and Lempesis, 1976). However, investigators
838 would need to alter the model or use different types of data before applying this approach to
839 species that have an extended yolk-sac larval stage before feeding begins.

840 We end with a comment on the limitation of the “DEBtox” approach (Kooijman et al.,
841 2009), a toxicology application of DEB from which DEBkiss stems, to identifying physiological
842 modes of action in response to environmental stress. In Section 1 we cite the paper by Romoli et
843 al. (2024) that highlighted the difficult modeling choices that are required. Here we chose to use

844 DEBkiss coupled with several hypothesized responses to hypoxia. We selected the combination
845 of DEB model and response hypothesis that best described given data (in an information
846 theoretic sense using AICc), *conditional on the “correctness” of the model and of assumed*
847 *values for some parameters*. Yet, for a case study in ecotoxicology, Romoli et al. showed that a
848 different dominant physiological model of action was selected when using two different
849 underlying DEB models that both give visually good fits to control data. Muller et al. (2010)
850 demonstrated a related issue for a study of early life stage growth by identifying best fit
851 submodel for larval growth of two closely related bivalve species exposed to mercury.
852 Implausibly, the selected submodels were different to the extent that the best fit for one species
853 was the worst for the other.

854 In the preceding discussion, we have offered a few suggestions for empirical work on
855 whole organisms that would significantly help narrow down the DEB processes responsible for
856 responses to hypoxia. However, it is likely that an additional, very promising way forward is to
857 determine *suborganismal* processes co-occurring with the observed whole-organism responses.
858 Transcriptomic data represent a particularly promising candidate (Murphy et al 2018). We
859 recognized this qualitatively in Section 2.4 when invoking genes controlling cell division and
860 protein synthesis that are regulated by hypoxia-inducible factors. Stevenson et al. (2023)
861 demonstrated the power of transcriptomic data in a study of killifish embryos exposed to a
862 toxicant. The molecular data helped to identify damage mechanisms that in turn led to changes in
863 DEB parameters. There are many further exciting possibilities for integrating suborganismal
864 (molecular) data with DEBtox modeling.
865

866 **5. Conclusions**

867 With this simple and widely applicable DEBkiss model we were able to attribute
868 hypoxia-related variability in *M. menidia* growth, hatch timing, and survival to damage-induced
869 reductions in conversion efficiency of assimilates into structure. Applying hypoxia corrections
870 simultaneously to conversion efficiency and the mortality parameters for embryos and larvae
871 provided the best fit, suggesting that hypoxia leads to both wasted energy and damage that
872 cannot be sufficiently repaired in the early life stages. As lifelong, constant oxygen conditions
873 are unrealistic in nature, the patterns modeled in this study should not be interpreted as a
874 standalone prediction of what will happen to wild *M. menidia* populations as coastal hypoxia

875 intensifies. Instead, this approach demonstrates the value of identifying energetic processes
876 responsible for whole-organism effects of hypoxia to understand underlying energetic processes
877 that are often time, labor, and cost-intensive to measure empirically, particularly in the early life
878 stages, when biomass available for sampling is small and developmental changes are rapid.
879 Through doing so we were able to support the utility of modeling inhibition and damage to
880 synthesizing units and highlight conversion efficiency of food into growth as a primary
881 mechanism by which hypoxia impacts an ecologically important forage fish and model species.
882 Measuring suborganismal processes to identify physiological modes of action can refine this
883 model so that it can better model this species' response to realistic hypoxia scenarios and,
884 ultimately, how reductions in conversion efficiency could affect energy flow through food webs.
885

886 **Declaration of Competing Interest**

887 The authors do not declare any competing interests.
888

889 **Acknowledgements**

890 The authors would like to acknowledge the researchers who collected the data used in this
891 model, and without whose hard work this study could not exist: Hannes Baumann, Christopher
892 S. Murray, Emma L. Cross, Callie Concannon, Lucas F. Jones, Catherine M. Matassa, and
893 Richard S. McBride. We would also like to thank Robert Cerrato, Michael Frisk, Amy Maas and
894 Louise Stevenson for valuable feedback on this research and manuscript. Finally, we would like
895 to express our gratitude to the guest editor Dina Lika and an anonymous reviewer for their
896 constructive and insightful feedback on this manuscript.
897

898 **Author Contributions**

899 Conceptualization – T.G.S., R.M.N., J.A.N.; Data curation – T.G.S.; Methodology – R.M.N.,
900 T.G.S.; Formal analysis – T.G.S.; Funding acquisition – T.G.S., J.A.N.; Visualization – T.G.S.,
901 Writing, original draft – T.G.S., R.M.N.; Writing, reviewing and editing – T.G.S., R.M.N.,
902 J.A.N.
903

904 **Funding Sources**

905 This research and the preparation of this article were supported by NOAA Sea Grant; NOAA
906 Ocean Acidification Program [NA19OAR170349]; the New York State Department of
907 Environmental Conservation [AM10560].

908

909 **Data and Code**

910 The datasets used for modeling can be found on BCO-DMO: early life total length, survival, and
911 hatching: doi: 10.1575/1912/bco-dmo.742200; early life total length with oxygen treatments: doi:
912 10.1575/1912/bco-dmo.777130.1; hatching and survival with oxygen treatments: doi:
913 10.1575/1912/bco-dmo.777117.1; total length of adults: doi: 10.26008/1912/bco-dmo.845906.1;
914 total length of larvae and juveniles: doi: 10.1575/1912/bco-dmo.652124; egg production: doi:
915 10.26008/1912/bco-dmo.845633.1. The BYOM and DEBkiss packages can be found at
916 <https://www.debtox.info/byom.html>. The code for inputting data, parameter estimation, and
917 plotting, for both the normoxic model and the model with hypoxia effects, can be found at
918 github.com/tschwemmer/MenidiaDEB.

919

920 **References**

- 921
922 Aguirre-Velarde, A., Pecquerie, L., Frederic, J., Gerard, T., and Flye-Sainte-Marie, J. 2019.
923 Predicting the energy budget of the scallop *Argopecten purpuratus* in an oxygen-limiting
924 environment. *J. Sea Res.*, 143: 254-261. <https://doi.org/10.1016/j.seares.2018.09.011>
925
926 ~~Ali, M., Niebla, A., and Woottton, R. J. 2003. Compensatory growth in fishes: a response to
927 growth depression. *Fish and Fisheries*, 4: 147-190. [https://doi.org/10.1046/j.1467-2979.2003.00120.x](https://doi.org/10.1046/j.1467-
928 2979.2003.00120.x)~~
929
930 AmP. 2021. Online database of DEB parameters, implied properties and referenced underlying
931 data. www.bio.vu.nl/thb/deb/deblab/add_my_pet/ (data accessed: March 3, 2023).
932
933 AmPtool, 2022. Software package, <https://github.com/add-my-pet/AmPtool/>
934
935 Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean
936 acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97: 399-408.
937 dx.doi.org/10.1139/cjz-2018-0198
938
939 Baumann, H. and Smith, E. M. 2018. Quantifying Metabolically Driven pH and Oxygen
940 Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuaries and
941 Coasts*, 41: 1102-1117. <https://doi.org/10.1007/s12237-017-0321-3>
942
943 [dataset] Baumann, H., Nye, J. (2016) Laboratory study of long-term growth in juvenile *Menidia
944 menidia* (Atlantic silverside) at contrasting CO₂ levels for 16 to 122 days in 2015.
945 Biological and Chemical Oceanography Data Management Office (BCO-DMO).
946 (Version final) Version Date 2016-07-07. doi:10.1575/1912/bco-dmo.652124 [accessed
947 2022-03-30]
948
949 [dataset] Baumann, H., Cross, E. (2019) Growth data from static and fluctuating pCO₂ x
950 dissolved oxygen (DO) experiments on *Menidia menidia*. Biological and Chemical
951 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2019-
952 09-20. doi:10.1575/1912/bco-dmo.777130.1 [accessed 2022-03-30]
953
954 [dataset] Baumann, H., Cross, E. (2019) Survival data from static and fluctuating pCO₂ x
955 dissolved oxygen (DO) experiments on *Menidia menidia*. Biological and Chemical
956 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2019-
957 09-20. doi:10.1575/1912/bco-dmo.777117.1 [accessed 2022-03-30]
958
959 [dataset] Baumann, H., Nye, J. (2021) Data from the spawning trial in a study of CO₂ and
960 temperature-specific reproductive traits in *Menidia menidia*. Biological and Chemical
961 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-
962 04-23. doi:10.26008/1912/bco-dmo.845633.1 [accessed 2022-03-30]
963
964 [dataset] Baumann, H., Nye, J. (2021) Data from the fecundity trial in a study of CO₂ and
965 temperature-specific reproductive traits in *Menidia menidia*. Biological and Chemical

- 966 Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-
967 03-18. doi:10.26008/1912/bco-dmo.845906.1 [accessed 2022-03-30]
- 968
- 969 Baumann, H., Wallace, R. B., Tagliaferri, T., and Gobler, C. J. 2015. Large Natural pH, CO₂ and
970 O₂ Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time
971 Scales. *Estuaries Coasts*, 38: 220-231. doi: 10.1007/s12237-014-9800-y
- 972
- 973 Bayliff, W. H. (1950). *The life history of the silverside Menidia menidia (Linnaeus)*. Chesapeake
974 Bay Laboratory, Solomons Island, Maryland: State of Maryland Board of Natural
975 Resources, Department of Research and Education.
- 976
- 977 Bengtson, D. A. 1984. Resource partitioning by *Menidia menidia* and *Menidia beryllina*
978 (Osteichthyes: Atherinidae). *Mar. Ecol. Prog. Ser.*, 18: 21-30.
- 979
- 980 Bianchini, K. and Wright, P. A. 2013. Hypoxia delays hematopoiesis: retention of embryonic
981 hemoglobin and erythrocytes in larval rainbow trout, *Oncorhynchus mykiss*, during
982 chronic hypoxia exposure. *J. Exp. Biol.*, 216(23): 4415-4425.
983 https://doi.org/10.1242/jeb.083337
- 984
- 985 Bigelow, H. B. and Schroeder, W. C. (1953). *Fishes of the Gulf of Maine*. U.S. Fish and Wildlife
986 Service, Fish. Bull., 53(74). 577 pp.
- 987
- 988 Boult, V. L. and Evans, L. C. 2021. Mechanisms matter: Predicting the ecological impacts of
989 global change. *Glob. Change Biol.*, 27(9): 1689-1691. https://doi.org/10.1111/gcb.15527
- 990
- 991 Bouma, T. J., De Visser, R., Janssen, J. H. J. A., De Kock, M. J., Van Leeuwen, P. H., and
992 Lambers, H. 1994. Respiratory energy requirements and rate of protein turnover in vivo
993 determined by the use of an inhibitor of protein synthesis and a probe to assess its effect.
994 *Physiol. Plant.*, 92: 585-594. https://doi.org/10.1111/j.1399-3054.1994.tb03027.x
- 995
- 996 Brandt, S. B., Gerken, M., Hartman, K. J., and Demers, E. 2009. Effects of hypoxia on food
997 consumption and growth of juvenile striped bass (*Morone saxatilis*). *J. Exp. Mar. Biol.*
998 Ecol., 381: S143-S149. doi: 10.1016/j.jembe.2009.07.028
- 999
- 1000 Breitburg, D., Levin, L. A., Oschlies, A., et al. 2018. Declining oxygen in the global ocean and
1001 coastal waters. *Science*, 359(6371): eaam7240. doi: 10.1126/science.aam7240
- 1002
- 1003 Chabot, D. and Dutil, J. D. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic
1004 conditions. *J. Fish. Biol.*, 55: 472-491. https://doi.org/10.1111/j.1095-
- 1005 8649.1999.tb00693.x
- 1006
- 1007 Chambers, R. C. and Leggett, W. C. 1987. Size and age at metamorphosis in marine fishes – an
1008 analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a
1009 review of variation in other species. *Can. J. Fish. Aquat. Sci.*, 44(11): 1936-1947.
1010 https://doi.org/10.1139/f87-238
- 1011

Formatted: Font: Italic

- 1012 Chapman, L. J. and McKenzie, D. J. 2009. Behavioral responses and ecological consequences.
1013 In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. Jeffrey G. Richards, Anthony P. Farrell, and
1014 Colin J. Brauner), pp. 25-77. San Diego: Academic Press.
- 1015
- 1016 ~~Ciuhandu, C. S., Stevens, E. D., and Wright, P. A. 2005. The effect of oxygen on the growth of
1017 *Oncorhynchus mykiss* embryos with and without a chorion. *J. Fish. Biol.*, 67: 1544-1551.
1018 <https://doi.org/10.1111/j.1095-8649.2005.00856.x>~~
- 1019
- 1020 Claireaux, G. and Chabot, D. 2016. Responses by fishes to environmental hypoxia: integration
1021 through Fry's concept of aerobic metabolic scope. *J. Fish Biol.*, 88: 232-251.
1022 <https://doi.org/10.1111/jfb.12833>
- 1023
- 1024 Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., and
1025 Baumann, H. 2021. Temperature-dependent effects on fecundity in a serial broadcast
1026 spawning fish after whole-life high CO₂ exposure. *ICES J. Mar. Sci.*, 78(10): 3724-3734.
1027 <https://doi.org/10.1093/icesjms/fsab217>
- 1028
- 1029 Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal pCO₂ x O₂ fluctuations
1030 provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9:
1031 18146. <https://doi.org/10.1038/s41598-019-53930-8>
- 1032
- 1033 Del Rio, A. M., Davis, B. E., Fangue, N. A., and Todgham, A. E. 2019. Combined effects of
1034 warming and hypoxia on early life stage Chinook salmon physiology and development.
1035 *Conserv. Physiol.*, 7(1): coy078. doi: 10.1093/conphys/coy078
- 1036
- 1037 DePasquale, E., Baumann, H., and Gobler, C. J. 2015. Vulnerability of early life stage Northwest
1038 Atlantic forage fish to ocean acidification and low oxygen. *Mar. Ecol. Prog. Ser.*, 523:
1039 145-156. doi: 10.3354/meps11142
- 1040
- 1041 Desforges, J.-P. W., Sonne, C., and Dietz, R. 2017. Using energy budgets to combine ecology
1042 and toxicology in a mammalian sentinel species. *Sci. Rep.*, 7: 46267. doi:
1043 10.1038/srep46267
- 1044
- 1045 Di Santo, V., Kenaley, C. P., and Lauder, G. V. 2017. High postural costs and anaerobic
1046 metabolism during swimming support the hypothesis of a U-shaped metabolism-speed
1047 curve in fishes. *Proc. Nat. Acad. Sci.*, 114(49): 13048-13053.
1048 <https://doi.org/10.1073/pnas.1715141114>
- 1049
- 1050 Diaz, R. J. and Rosenberg, R. 2008. Spreading Dead Zones and Consequences for Marine
1051 Ecosystems. *Science*, 321: 926-929. doi: 10.1126/science.1156401
- 1052
- 1053 ~~Earhart, M. L., Blanchard, T. S., Harman, A. A., and Schulte, P. M. 2022. Hypoxia and High
1054 Temperature as Interacting Stressors: Will Plasticity Promote Resilience of Fishes in a
1055 Changing World? *Biol. Bull.*, 243: 149-170. <https://doi.org/10.1086/722115>~~
- 1056

- 1057 Evans, M. R., Grimm, V., Johst, K., et al. 2013. Do simple models lead to generality in ecology?
1058 *Trends in Ecology & Evolution*, 28(10): 578-583.
1059 <https://doi.org/10.1016/j.tree.2013.05.022>
- 1060
1061 Farrell, A. P. and Brauner, C. J. 2009. Fish Physiology, Vol. 27: Hypoxia. Academic Press,
1062 London.
- 1063
1064 **Finn, R. N., Fyhn, H. J., and Evjen, M. S. 1995. Physiological energetics of developing embryos**
1065 **and yolk sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen**
1066 **metabolism.** *Mar. Biol.*, 124: 355-369. <https://doi.org/10.1007/BF00363909>
- 1067
1068 Goussen, B., Rendal, C., Sheffield, D., Butler, E., Price, O. R., and Ashauer, R. 2020.
1069 Bioenergetics modelling to analyze and predict the joint effects of multiple stressors:
1070 Meta-analysis and model corroboration. *Sci. Total. Environ.*, 749: 141509.
1071 <https://doi.org/10.1016/j.scitotenv.2020.141509>
- 1072
1073 Grear, J. S., O'Leary, C. A., Nye, J. A., Tettelbach, S. T., and Gobler, C. J. 2020. Effects of
1074 coastal acidification on North Atlantic bivalves: interpreting laboratory responses in the
1075 context of *in situ* populations. *Mar. Ecol. Prog. Ser.*, 633: 89-104.
1076 <https://doi.org/10.3354/meps13140>
- 1077
1078 Gruber, J. 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global
1079 change. *Phil. Trans. R. Soc. A*, 369: 1980-1996. <https://doi.org/10.1098/rsta.2011.0003>
- 1080
1081 Hamda, N. T., Martin, B., Poletto, J. B., Cocherell, D. E., Fangue, N. A., Van Eenennaam, J.,
1082 Mora, E. A., and Danner, E. 2019. Applying a simplified energy-budget model to explore
1083 the effects of temperature and food availability on the life history of green sturgeon
1084 (*Acipenser medirostris*). *Ecol. Model.*, 395: 1-10.
1085 <https://doi.org/10.1016/j.ecolmodel.2019.01.005>
- 1086
1087 **Haneoek, J. R. and Place, S. P. 2016. Impact of ocean acidification on the hypoxia tolerance of**
1088 **the woolly sculpin, *Clinocottus analis*.** *Conserv. Physiol.* 4, e0040.
1089 doi:10.1093/conphys/eow040
- 1090
1091 Heath, A. G. and Pritchard, A. W. 1965. Effects of severe hypoxia on carbohydrate energy stores
1092 and metabolism in two species of fresh-water fish. *Physiol. Zool.*, 38(4): 325-334.
1093 <https://doi.org/10.1086/physzool.38.4.30152409>
- 1094
1095 Holling, C. S. 1966. The strategy of building models of complex ecological systems. In: Systems
1096 Analysis in Ecology. (K. E. F. Watt, Ed.) Academic Press. Pp. 195-214.
- 1097
1098 **Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., and Billen, G. 2011.**
1099 **Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and**
1100 **coastal marine ecosystems.** *Front. Ecol. Environ.*, 9(1): 18-26. doi: 10.1890/100008
- 1101

- 1102 Jager, T. 2018. DEBkiss: A Simple Framework for Animal Energy Budgets. Version 2.0.
1103 Leanpub: https://leanpub.com/debkiss_book.
- 1104
- 1105 Jager, T., Martin, B. T., and Zimmer, E. I. 2013. DEBkiss or the quest for the simplest generic
1106 model of animal life history. *J. Theor. Biol.*, 328: 9-18.
1107 <https://doi.org/10.1016/j.jtbi.2013.03.011>
- 1108
- 1109 Jager, T., Ravagnan, E., and Dupont, S. 2016. Near-future ocean acidification impacts
1110 maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points
1111 using a DEB modelling approach. *J. Exp. Mar. Biol. Ecol.*, 474: 11-17.
1112 <https://doi.org/10.1016/j.jembe.2015.09.016>
- 1113
- 1114 Jager, T., Nepstad, R., Hansen, B. H., and Farkas, J. 2018. Simple energy-budget model for yolk-
1115 feeding stages of Atlantic cod (*Gadus morhua*). *Ecol. Model.*, 385: 213-219.
1116 <https://doi.org/10.1016/j.ecolmodel.2018.08.003>
- 1117
- 1118 Jager, T., Malzahn, A. M., Hagemann, A., and Hansen, B. H. 2022. Testing a simple energy-
1119 budget model for yolk-feeding stages of cleaner fish. *Ecol. Model.*, 469: 110005.
1120 <https://doi.org/10.1016/j.ecolmodel.2022.110005>
- 1121
- 1122 Jusup, M., Sousa, T., Domingos, T., Labinac, V., Marn, N., Wang, Z., and Klanjšek, T. 2017.
1123 Physics of metabolic organization. *Physics of Life Reviews*, 20: 1-39.
1124 <https://doi.org/10.1016/j.plrev.2016.09.001>
- 1125
- 1126 ~~Kajimura, S., Aida, K., and Duan, C. 2005. Insulin-like growth factor binding protein 1 (IGFBP-4) mediates hypoxia induced embryonic growth and developmental retardation. *Proc. Natl. Acad. Sci.*, 102(4): 1240-1245. <https://doi.org/10.1073/pnas.0407443102>~~
- 1127
- 1128
- 1129
- 1130 ~~Kajimura, S., Aida, K., and Duan, C. 2006. Understanding Hypoxia Induced Gene Expression in Early Development: In Vitro and In Vivo Analysis of Hypoxia Inducible Factor 1-Regulated Zebra Fish Insulin Like Growth Factor Binding Protein 1 Gene Expression. *Mol. Cell. Biol.*, 26(3): 1142-1155. <https://doi.org/10.1128/MCB.26.3.1142-1155.2006>~~
- 1131
- 1132
- 1133
- 1134
- 1135 Kamler, E. 2008. Resource allocation in yolk-feeding fish. *Rev. Fish. Biol. Fisheries*, 18: 143-
1136 200. <https://doi.org/10.1007/s11160-007-9070-x>
- 1137
- 1138 Klahre, L. E. 1997. Countergradient Variation in Egg Production Rate of the Atlantic Silverside
1139 *Menidia menidia*. [Master's thesis]. Stony Brook University.
- 1140
- 1141 ~~Kooijman, S. A. L. M. 1998. The Synthesizing Unit as model for the stoichiometric fusion and branching of metabolic fluxes. *Biophys. Chem.*, 73: 179-188. [https://doi.org/10.1016/S0301-4622\(98\)00162-8](https://doi.org/10.1016/S0301-4622(98)00162-8)~~
- 1142
- 1143
- 1144
- 1145 Kooijman, S. A. L. M. 2010a. Dynamic Energy Budget Theory for Metabolic Organisation.
1146 Cambridge University Press, Cambridge.
- 1147

- 1148 Kooijman, S. A. L. M. 2010b. Comments on Dynamic Energy Budget Theory for Metabolic
1149 Organisation. Cambridge University Press, Cambridge.
1150
- 1151 Kooijman, S. A. L. M. 2018. Models in stress research. *Ecol. Complex.*, 34: 161-177.
1152 <https://doi.org/10.1016/j.ecocom.2017.07.006>
1153
- 1154 Kooijman, S. A. L. M., and Metz, J. A. J. 1984. On the dynamics of chemically stressed
1155 populations: The deduction of population consequences from effects on individuals.
1156 *Ecotoxicology and Environmental Safety*, 8(3): 254-274. [https://doi.org/10.1016/0147-6513\(84\)90029-0](https://doi.org/10.1016/0147-6513(84)90029-0)
1157
- 1158 Kooijman, S. A. L. M., Baas, J., Bontje, D., Broerse, M., van Gestel, C. A. M., and Jager, T.
1159 2009. Ecotoxicological Applications of Dynamic Energy Budget Theory. In: *Emerging
1160 Topics in Ecotoxicology, Vol. 2: Ecotoxicology Modeling* (Ed. Devillers, J.), pp. 237-259.
1161 Boston, MA: Springer. https://doi.org/10.1007/978-1-4419-0197-2_9
1162
- 1163 Kooijman, S. A. L. M., Lika, K., Augustine, S., Marn, N., and Kooi, B. W. 2020. The energetic
1164 basis of population growth in animal kingdom. *Ecol. Model.*, 428: 109055.
1165 <https://doi.org/10.1016/j.ecolmodel.2020.109055>
- 1166
- 1167 ~~Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18:
1168 81-92. <https://doi.org/10.1007/BF00002597>~~
- 1169
- 1170 Landry, C. A., Steele, S. L., Manning, S., and Cheek, A. O. 2007. Long term hypoxia suppresses
1171 reproductive capacity in the estuarine fish, *Fundulus grandis*. *Comp. Biochem. Physiol.
1172 Part A: Mol. Integr. Physiol.*, 148(2): 317-323.
1173 <https://doi.org/10.1016/j.cbpa.2007.04.023>
- 1174
- 1175 Lankford, T. E., Billerbeck, J. M., and Conover, D. O. 2001. Evolution of intrinsic growth and
1176 energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia
1177 menidia*. *Evolution*, 55(9): 1873-1881. <https://doi.org/10.1111/j.0014-3820.2001.tb00836.x>
1178
- 1179
- 1180 Lavaud, R., Filgueira, R., and Augustine, S. 2019. The role of Dynamic Energy Budgets in
1181 conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi:
1182 [10.1093/conphys/coab083](https://doi.org/10.1093/conphys/coab083)
- 1183
- 1184 Lavaud, R., Filgueira, R., and Augustine, S. 2021. The role of Dynamic Energy Budgets in
1185 conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi:
1186 [10.1093/conphys/coab083](https://doi.org/10.1093/conphys/coab083).
- 1187
- 1188 Letcher, B. H. and Bengtson, D. A. 1993. Effects of food density and temperature on feeding and
1189 growth of young inland silversides (*Menidia beryllina*). *J. Fish Biol.*, 43: 671-686.
1190 <https://doi.org/10.1111/j.1095-8649.1993.tb01145.x>
- 1191
- 1192

- 1193 Lika, K., Kearney, M. R., Freitas, V., van der Veer, H. W., van der Meer, J., Mijisman, J. W. M.,
1194 Pecqueries, L., and Kooijman, S. A. L. M. 2011. The “covariation method” for estimating
1195 the parameters of the standard Dynamic Energy Budget model I: Philosophy and
1196 approach. *J. Sea Res.*, 66(4): 270-277. <https://doi.org/10.1016/j.seares.2011.07.010>
1197
- 1198 Marques, G. M., Augustine, S., Lika, K., Pecquerie, L., Domingos, T., and Kooijman, S. A. L.
1199 M. 2018. The AmP project: Comparing species on the basis of dynamic energy budget
1200 parameters. *PLoS Comput. Biol.*, 14(5): e1006100.
1201 <https://doi.org/10.1371/journal.pcbi.1006100>
- 1202
- 1203 Martin, B. T., Jager, T., Nisbet, R. M., Preuss, T. G., and Grimm, V. 2013. Predicting Population
1204 Dynamics from the Properties of Individuals: A Cross-Level Test of Dynamic Energy
1205 Budget Theory. *The American Naturalist*, 181(4): 506-519.
1206 <https://doi.org/10.1086/669904>
- 1207
- 1208 Martin, B. T., Heintz, R., Danner, E. M., and Nisbet, R. M. 2017. Integrating lipid storage into
1209 general representations of fish energetics. *Journal of Animal Ecology*, 86: 812-825.
1210 <https://doi.org/10.1111/1365-2656.12667>
- 1211
- 1212 Maury, O., Poggiale, J. C., and Aumont, O. 2019. Damage related protein turnover explains
1213 inter-specific patterns of maintenance rate and suggest modifications of the DEB theory. *J. Sea
1214 Res.*, 143: 35-47. <https://doi.org/10.1016/j.seares.2018.09.021>
- 1215
- 1216 Maxime, V., Pichavant, K., Boeuf, G., and Nonnotte, G. 2000. Effects of hypoxia on respiratory
1217 physiology of turbot, *Scophthalmus maximus*. *Fish Physiology and Biochemistry*, 22: 51-
1218 59. <https://doi.org/10.1023/A:1007829214826>
- 1219
- 1220 May, R. M. 2001. *Stability and Complexity in Model Ecosystems*. 2nd Edition. Princeton
1221 University Press.
- 1222
- 1223 McBryan, T. L., Anttila, K., Healy, T. M., and Schulte, P. M. 2013. Responses to temperature
1224 and hypoxia as interacting stressors in fish: implications for adaptation to environmental change.
1225 *Integr. Comp. Biol.*, 53: 648-659. doi: 10.1093/icb/ict066
- 1226
- 1227 Middaugh, D. P. 1981. Reproductive Ecology and Spawning Periodicity of the Atlantic
1228 Silverside, *Menidia menidia* (Pisces: Atherinidae). *Copeia*, 1981(4): 766-776.
1229 <https://doi.org/10.2307/1444176>
- 1230
- 1231 Middaugh, D. P. and Lempesis, P. W. 1976. Laboratory spawning and rearing of a marine fish,
1232 the silverside *Menidia menidia menidia*. *Mar. Biol.*, 35: 295-300.
1233 <https://doi.org/10.1007/BF00386640>
- 1234
- 1235 Middaugh, D. P. and Hemmer, M. J. 1992. Reproductive Ecology of the Inland Silverside,
1236 *Menidia beryllina*, (Pisces: Atherinidae) from Blackwater Bay, Florida. *Copeia*, 1992(1):
1237 53-61. <https://doi.org/10.2307/1446535>
- 1238

Formatted: Indent: Left: 0", First line: 0"

Formatted: Indent: Left: 0", First line: 0"

- 1239 Miller, S. H., Breitburg, D. L., Burrell, R. B., Keppel, A. G. 2016. Acidification increases
1240 sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.*, 549: 1-8.
1241 <https://doi.org/10.3354/meps11695>
- 1242
- 1243 Moreira, J. M., Candeias Mendes, A., Maulvault, A. L., Marques, A., Rosa, R., Pousão-Ferreira,
1244 P., Sousa, T., Anacleto, P., and Marques, G. M. 2022. Impacts of ocean warming and
1245 acidification on the energy budget of three commercially important fish species. *Conserv.*
1246 *Physiol.*, 10(1): coac048. <https://doi.org/10.1093/conphys/coac048>
- 1247
- 1248 Morrell, B. K. and Gobler, C. J. 2020. Negative Effects of Diurnal Changes in Acidification and
1249 Hypoxia on Early-Life Stage Estuarine Fishes. *Diversity*, 12: 25. doi: 10.3390/d12010025
- 1250
- 1251 Muller, E. B., Nisbet, R. M., and Berkley, H. A. 2010. Sublethal toxicant effects with dynamic
1252 energy budget theory: model formulation. *Ecotoxicology*, 19: 48-60.
1253 <https://doi.org/10.1007/s10646-009-0385-3>
- 1254
- 1255 Muller, E. B., Klanjšček, T., and Nisbet, R. M. 2019. Inhibition and damage schemes within the
1256 synthesizing unit concept of dynamic energy budget theory. *J. Sea Res.*, 143: 165-172.
1257 <https://doi.org/10.1016/j.seares.2018.05.006>
- 1258
- 1259 Murphy, C. A., Nisbet, R. M., Antczak, P., Garcia-Reyero, N., Gergs, A., Lika, K., Mathews, T.,
1260 Muller, E. B., Nacci, D., Peace, A., Remien, C. H., Schultz, I. R., Stevenson, L. M., and
1261 Watanabe, K. H. 2018. Incorporating Suborganismal Processes into Dynamic Energy
1262 Budget Models for Ecological Risk Assessment. *Integr. Environ. Assess. Manag.*, 14(5):
1263 615-624. doi: 10.1002/ieam.4063
- 1264
- 1265 Murray, C. S. and Baumann, H. 2018. You Better Repeat It: Complex CO₂ × Temperature
1266 Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*,
1267 10: 69. doi: 10.3390/d10030069
- 1268
- 1269 [dataset] Murray, C., Baumann, H. (2018) CO₂ × temperature specific early life survival and
1270 growth of *Menidia menidia* assessed by 5 factorial experiments. Biological and Chemical
1271 Oceanography Data Management Office (BCO-DMO). (Version 05 April 2018) Version
1272 Date 2018-04-05. doi:10.1575/1912/bco-dmo.742200 [accessed 2022-03-30]
- 1273
- 1274 Murray, C. S. and Baumann, H. 2020. Are long-term growth responses to elevated pCO₂ sex-
1275 specific in fish? *PLoS ONE*, 15(7): e0235817.
1276 <https://doi.org/10.1371/journal.pone.0235817>
- 1277
- 1278 Murray, C. S., Fuiman, L. A., and Baumann, H. 2017. Consequences of elevated CO₂ exposure
1279 across multiple life stages in a coastal forage fish. *ICES J. Mar. Sci.*, 74(4): 1051-1061.
1280 doi: 10.1093/icesjms/fsw179
- 1281
- 1282 Niebla, A. G. and Metcalfe, N. B. 1997. Growth compensation in juvenile Atlantic salmon:
1283 Responses to depressed temperature and food availability. *Ecology*, 78(8): 2385-2400.
1284 [https://doi.org/10.1890/0012-9658\(1997\)078\[2385:GCJAS\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[2385:GCJAS]2.0.CO;2)

- 1|285
1286 Niklitschek, E. J. and Secor, D. H. 2005. Modeling spatial and temporal variation of suitable
1287 nursery habitats for Atlantic sturgeon in the Chesapeake Bay. *Estuar. Coast. Shelf Sci.*,
1288 64: 135-148. <https://doi.org/10.1016/j.ecss.2005.02.012>
- 1|289
1290 Nilsson, G. E. and Östlund Nilsson, S. 2008. Does size matter for hypoxia tolerance in fish?
1291 *Biol. Rev.*, 83: 173-189. <https://doi.org/10.1111/j.1469-185X.2008.00038.x>
- 1|292
1293 Ninness, M. M., Stevens, E. D., and Wright, P. A. 2006. Removal of the chorion before hatching
1294 results in increased movement and accelerated growth in rainbow trout (*Oncorhynchus*
1295 *mykiss*) embryos. *J. Exp. Biol.*, 209: 1874-1882. <https://doi.org/10.1242/jeb.02200>
- 1|296
1297 Nisbet, R. M., Gurney, W. S. C., Murdoch, W. W., and McCauley, E. 1989. Structured
1298 population models: a tool for linking effects at individual and population level. *Biol. J.
1299 Linn. Soc.*, 37: 79-99. <https://doi.org/10.1111/j.1095-8312.1989.tb02006.x>
- 1300
1301 Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to
1302 ecosystems through dynamic energy budget models. *Journal of Animal Ecology*, 69: 913-
1303 926.
- 1304
1305 Nonnotte, G., Maxime, V., Truchot, J. P., Williot, P., and Peyraud, C. 1993. Respiratory
1306 responses to progressive ambient hypoxia in the sturgeon, *Acipenser baeri*. *Respir.
1307 Physiol.*, 91: 71-82. [https://doi.org/10.1016/0034-5687\(93\)90090-W](https://doi.org/10.1016/0034-5687(93)90090-W)
- 1308
1309 O'Donnell, J., Dam, H. G., Bohlen, W. F., Fitzgerald, W., Gay, P. S., Houk, A. E., Cohen, D. C.,
1310 and Howard-Strobel, M. M. 2008. Intermittent ventilation in the hypoxic zone of western
1311 Long Island Sound during the summer of 2004. *J. Geophys. Res.*, 113: C09025.
1312 <https://doi.org/10.1029/2007JC004716>
- 1313
1314 Perry, S. F., Jonz, M. G., and Gilmour, K. M. 2009. Oxygen Sensing and the Hypoxic
1315 Ventilatory Response. In: *Fish Physiology, Vol. 27: Hypoxia*. (Ed. J. G. Richards, A. P.
1316 Farrell and C. J. Brauner), pp. 193-253. San Diego: Academic Press.
- 1317
1318 Polymeropoulos, E. T., Elliott, N. G., and Frappell, P. B. 2017. Hypoxic acclimation leads to
1319 metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comp.
1320 Biochem. Physiol. A*, 213: 28-35. <https://doi.org/10.1016/j.cbpa.2017.08.011>
- 1321
1322 Pousse, É., Munroe, D., Hart, D., Hennen, D., Cameron, L. P., Rheuban, J. E., Wang, Z. A.,
1323 Wikfors, G. H., and Meseck, S. L. 2022. Dynamic energy budget modeling of Atlantic
1324 surfclam, *Spisula solidissima*, under future ocean acidification and warming. *Mar.
1325 Environ. Res.*, 177: 105602. <https://doi.org/10.1016/j.marenvres.2022.105602>
- 1326
1327 Rabalaïs, N. N., Turner, R. E., Díaz, R. J., and Justié, D. 2009. Global change and eutrophication
1328 of coastal waters. *ICES J. Mar. Sci.*, 66(7): 1528-1537.
1329 <https://doi.org/10.1093/icesjms/fsp047>
- 1330

Formatted: Indent: Left: 0", First line: 0"

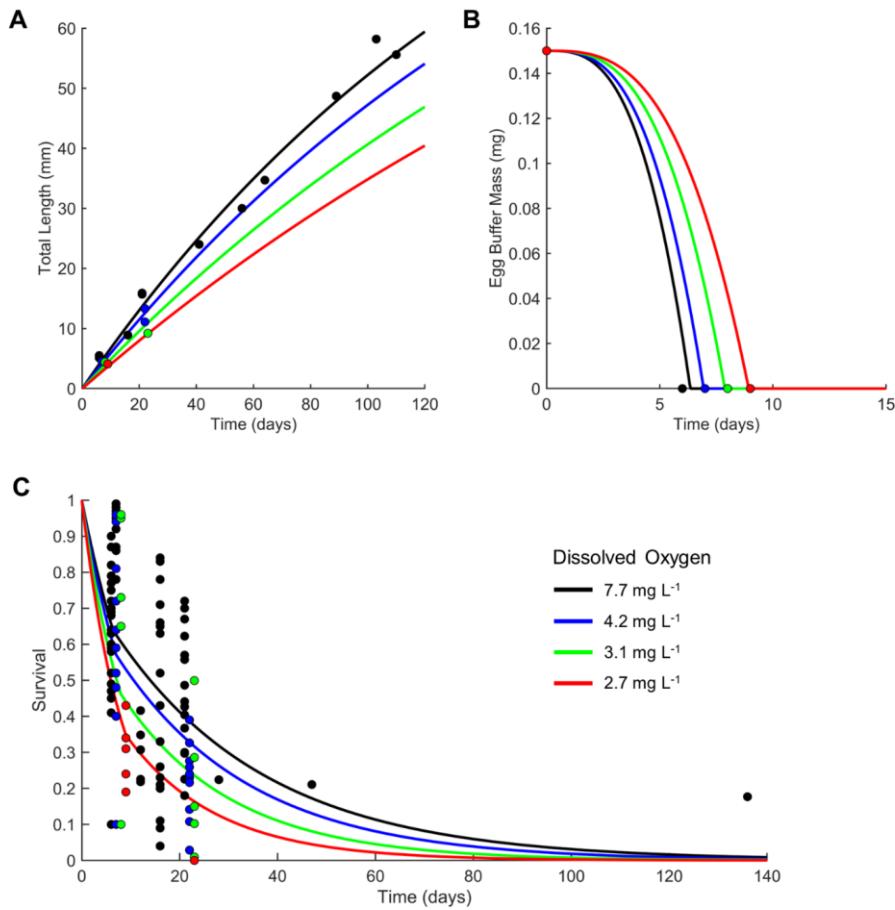
- 1331 Richards, J. G. 2009. Metabolic and Molecular Responses of Fish to Hypoxia. In: *Fish Physiology*, Vol. 27: *Hypoxia*. (Ed. J. G. Richards, A. P. Farrell and C. J. Brauner), pp.
1332 443-485. San Diego: Academic Press.
- 1333
- 1334 Richards, J. G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes
1335 to hypoxia. *J. Exp. Biol.*, 214: 191-199. <https://doi.org/10.1242/jeb.047951>
- 1336
- 1337 Robert, D., Shoji, J., Sirois, P., Takasuka, A., Catalán, I. A., et al. 2023. Life in the fast lane:
1338 Revisiting the fast growth—High survival paradigm during the early life stages of fish.
1339 *Fish and Fisheries*, 24: 863-888. <https://doi.org/10.1111/faf.12774>
- 1340
- 1341 Rombough, P. J. 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia
1342 during early life. In: *Fish Physiology, Vol. 11: The Physiology of Developing Fish, Part*
1343 *A: Eggs and Larvae*. (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego:
1344 Academic Press.
- 1345
- 1346 Romoli, C., Jager, T., Trijau, M., Goussen, B., and Gergs, A. 2024. Environmental Risk
1347 Assessment with Energy Budget Models: A Comparison Between Two Models of
1348 Different Complexity. *Environ. Toxicol. Chem.*, 43(2): 440-449. doi: 10.1002/etc.5795
- 1349
- 1350
- 1351 Russell, N. R., and Wootten, R. J. 1992. ~~Appetite and growth compensation in the European
1352 minnow, Phoxinus phoxinus (Cyprinidae), following short periods of food restriction.~~
1353 *Environ. Biol. Fishes*, 34: 277-285. <https://doi.org/10.1007/BF00004774>
- 1354
- 1355 Schwemmer, T. G. 2023. Early Life Physiological and Energetic Responses of Atlantic
1356 Silversides (*Menidia menidia*) to Ocean Acidification, Warming, and Hypoxia. Doctoral
1357 dissertation. ProQuest Dissertations Publishing. State University of New York at Stony
1358 Brook, Stony Brook, NY.
- 1359
- 1360 Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., and Nye, J. A. 2020.
1361 Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of
1362 the coastal forage fish *Menidia menidia*. *J. Exp. Biol.*, 223: jeb228015. doi:
1363 10.1242/jeb.228015
- 1364
- 1365 Sibly, R. M., Grimm, V., Martin, B. T., Johnston, A. S. A., et al. 2013. Representing the
1366 acquisition and use of energy by individuals in agent-based models of animal
1367 populations. *Methods in Ecology and Evolution*, 4: 151-161.
1368 <https://doi.org/10.1111/2041-210X.12002>
- 1369
- 1370 Smallegange, I. M., Caswell, H., Toorians, M. E. M., and de Roos, A. M. 2017. Mechanistic
1371 description of population dynamics using dynamic energy budget theory incorporated
1372 into integral projection models. *Methods in Ecology and Evolution*, 8: 146-154.
1373 <https://doi.org/10.1111/2041-210X.12675>
- 1374
- 1375 Stevenson, L. M., Muller, E. B., Nacci, D., Clark, B. W., Whitehead, A., and Nisbet, R. M. 2023.
1376 Connecting Suborganismal Data to Bioenergetic Processes: Killifish Embryos Exposed to

- 1377 a Dioxin-Like Compound. *Environ. Toxicol. Chem.*, 42(9): 2040-2053. doi:
1378 10.1002/etc.5680
- 1379
1380 Stierhoff, K. L., Targett, T. E., and Miller, K. 2006. Ecophysiological responses of juvenile
1381 summer and winter flounder to hypoxia: experimental and modeling analyses of effects
1382 on estuarine nursery quality. *Mar. Ecol. Prog. Ser.*, 325: 255-266.
1383 doi:10.3354/meps325255
- 1384
1385 Stierhoff, K. L., Targett, T. E., and Power, J. H. 2009. Hypoxia-induced growth limitation of
1386 juvenile fishes in an estuarine nursery: assessment of small-scale temporal dynamics
1387 using RNA:DNA. *Can. J. Fish. Aquat. Sci.*, 66(7): 1033-1047.
1388 https://doi.org/10.1139/F09-066
- 1389
1390 Sun, C. F., Tao, Y., Jiang, X. Y., and Zou, S. M. 2011. IGF binding protein 1 is correlated with ▶ Formatted: Indent: Left: 0", First line: 0"
1391 hypoxia induced growth reduce and developmental defects in grass carp (*Ctenopharyngodon*
1392 *idellus*) embryos. *Gen. Comp. Endocrinol.*, 172(3): 409-415.
1393 https://doi.org/10.1016/j.ygeen.2011.04.005
- 1394
1395 Tai, T. C., Sumaila, U. R., and Cheung, W. W. L. 2021. Ocean Acidification Amplifies Multi-
1396 Stressor Impacts on Global Marine Invertebrate Fisheries. *Front. Mar. Sci.*, 8: 596644.
1397 doi: 10.3389/fmars.2021.596644
- 1398
1399 Takasuka, A., Aoki, I., and Oozeki, Y. 2007. Predator-specific growth-selective predation on
1400 larval Japanese anchovy *Engraulis japonicus*. *Mar. Ecol. Prog. Ser.*, 350: 99-107.
1401 https://doi.org/10.3354/meps07158
- 1402
1403 Taylor, J. C. and Miller, J. M. 2001. Physiological performance of juvenile southern flounder,
1404 *Paralichthys lethostigma* (Jordan and Gilbert, 1884), in chronic and episodic hypoxia. *J.*
1405 *Exp. Mar. Biol. Ecol.*, 258: 195-214. https://doi.org/10.1016/S0022-0981(01)00215-5
- 1406
1407 Testa, J. M., Murphy, R. R., Brady, D. C., and Kemp, W. M. 2018. Nutrient- and Climate-
1408 Induced Shifts in the Phenology of Linked Biogeochemical Cycles in a Temperate
1409 Estuary. *Front. Mar. Sci.*, 5: 114. https://doi.org/10.3389/fmars.2018.00114
- 1410
1411 Thomas, P., Rahman, M. S., Kummer, J. A., and Lawson, S. 2006. Reproductive endocrine
1412 dysfunction in Atlantic croaker exposed to hypoxia. *Mar. Environ. Res.*, 62: S249-S252.
1413 https://doi.org/10.1016/j.marenvres.2006.04.031
- 1414
1415 Thomas, Y., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G. M., and
1416 Pecquerie, Laure. 2019. Effects of hypoxia on metabolic functions in marine organisms:
1417 Observed patterns and modelling assumptions within the context of Dynamic Energy
1418 Budget (DEB) theory. *J. Sea Res.*, 143: 231-242.
1419 https://doi.org/10.1016/j.seares.2018.05.001
- 1420
1421 Tian, Y. M., Chen, J., Tao, Y., Jiang, X. Y., and Zou, S. M. 2014. Molecular cloning and
1422 function analysis of insulin-like growth factor binding protein 1a in blunt snout bream

- 1423 (*Megalobrama amblycephala*). *Dongwuxue Yanjiu*, 35(4): 300-306.
1424 [10.13918/j.issn.2095-8137.2014.4.300](https://doi.org/10.13918/j.issn.2095-8137.2014.4.300)
- 1425
1426 Ton, C., Stamatou, D., and Liew, C. C. 2003. Gene expression profile of zebrafish exposed to
1427 hypoxia during development. *Physiol. Genomics*, 13(2): 97-106.
1428 <https://doi.org/10.1152/physiolgenomics.00128.2002>
- 1429
1430 Vanderplancke, G., Claireaux, G., Quazuguel, P., Madec, L., Ferrarese, S., Sévère, A.,
1431 Zambonino-Infante, J.-L., and Mazurais, D. 2015. Hypoxic episode during the larval
1432 period has long-term effects on European sea bass juveniles (*Dicentrarchus labrax*). *Mar.*
1433 *Biol.*, 162: 367-376. <https://doi.org/10.1007/s00227-014-2601-9>
- 1434
1435 Wagenmakers, E.-J. and Farrell, S. 2004. AIC model selection using Akaike weights. *Psychon.*
1436 *Bull. Rev.*, 11(1): 192-196. <https://doi.org/10.3758/BF03206482>
- 1437
1438 Wang, J., Yang, Y., Wang, Z., Xu, K., Xiao, X., and Mu, W. 2021. Comparison of effects in
1439 sustained and diel-cycling hypoxia on hypoxia tolerance, histology, physiology, and
1440 expression of clock genes in high latitude fish *Phoxinus lagowskii*. *Comp. Biochem.*
1441 *Physiol. A Mol. Integr. Physiol.*, 260: 111020.
1442 <https://doi.org/10.1016/j.cbpa.2021.111020>
- 1443
1444 Wei, L.-Z., Zhang, X.-M., Li, J., and Huang, G.-Q. 2008. Compensatory growth of Chinese
1445 shrimp, *Fenneropenaeus chinensis* following hypoxic exposure. *Aquacult. Int.*, 16: 455-
1446 470. <https://doi.org/10.1007/s10499-007-9158-2>
- 1447
1448 Wieser, W. 1995. Energetics of fish larvae, the smallest vertebrates. *Acta Physiol. Scand.*, 154:
1449 279-290. <https://doi.org/10.1111/j.1748-1716.1995.tb09912.x>
- 1450
1451 Williams, K. J., Cassidy, A. A., Verhille, C. E., Lamarre, S. G., and MacCormack, T. J. 2019.
1452 Diel cycling hypoxia enhances hypoxia tolerance in rainbow trout (*Oncorhynchus*
1453 *mykiss*): evidence of physiological and metabolic plasticity. *J. Exp. Biol.*, 222(14):
1454 jeb206045. <https://doi.org/10.1242/jeb.206045>
- 1455
1456 Wood, C. M. 2018. The fallacy of the P_{crit} – are there more useful alternatives? *J. Exp. Biol.*,
1457 221: jeb163717. doi: 10.1242/jeb.163717
- 1458
1459 Wu, R. S. S., Zhou, B. S., Randall, D. J., Woo, N. Y. S., and Lam, P. K. S. 2003. Aquatic
1460 Hypoxia Is an Endocrine Disruptor and Impairs Fish Reproduction. *Environ. Sci.*
1461 *Technol.*, 37(6): 1137-1141. <https://doi.org/10.1021/es0258327>
- 1462
1463 Zambonino-Infante, J. L., Mazurais, D., Dubuc, A., Quéau, P., Vanderplancke, G., Servili, A.,
1464 Cahu, C., Le Bayon, N., Huelvan, C., and Claireaux, G. 2017. An early life hypoxia event
1465 has a long-term impact on protein digestion and growth in juvenile European sea bass. *J.*
1466 *Exp. Biol.*, 220(10): 1846-1851. <https://doi.org/10.1242/jeb.154922>
- 1467

Formatted: Indent: Left: 0", First line: 0"

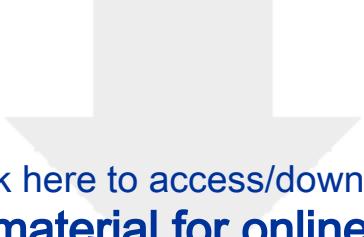
1468 Zhu, C.-D., Wang, Z.-H., and Yan, B. 2013. Strategies for hypoxia adaptation in fish species: a
1469 review. *J. Comp. Physiol. B*, 183: 1005-1013. <https://doi.org/10.1007/s00360-013-0762-3>
1470

1471 **Supplementary Figure**

1472

1473 **Figure S1. Best fit of DEBkiss model to all experimental data from four DO levels.** The
 1474 model was fitted to early life data (embryos, larvae, and juveniles) and the best fitting model was
 1475 selected based on lowest AICc. (A) is total length (mm) over time (days), (B) is egg buffer mass
 1476 (mg) over time (days), and (C) is survival over time (days).

1477



Click here to access/download

Supplementary material for online publication only
Schwemmer et al Supplementary Materials - clean.docx



[Click here to access/download](#)

Supplementary material for online publication only
Schwemmer et al Supplementary Materials - track
changes.docx

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: