Chapter 4: Attributing hypoxia responses of early life *Menidia menidia* to energetic mechanisms with Dynamic Energy Budget theory

**Abstract**

Although the Atlantic silverside (*Menidia menidia*) has proven robust to the fluctuating environmental conditions in its estuarine environment, chronic hypoxia impairs hatching, growth, and survival in the early life stages. To gain understanding of the energetic mechanisms responsible for these experimentally quantified impacts, we fitted different versions of a Dynamic Energy Budget model to data with oxygen-based correction factors applied to various DEB parameters. We sought to identify the parameters that, when adjusted with the correction factors, provided the best fit to hypoxia effects in the three state variables of total length, egg buffer mass, and survival over time. Reducing the yield coefficient for conversion of assimilates to structure (*yVA*) with hypoxia provided the best fit when combined with the parameters for pre- and post-hatching mortality rate (*μemb* and *μlar*). The maximum assimilation rate (*JaAm*) performed almost as well as *yVA* when combined with the mortality parameters, and both *yVA* and *JaAm* can independently account at least in part for the hypoxia impacts of delayed hatching, reduced size at hatching, slower growth, and lower survival to hatching. Increasing the parameter for maintenance rate with hypoxia had little impact on early life growth and egg buffer depletion and no impact on survival rates. By combining empirical data with unified principles for energetic allocation that are broadly applicable across species, we identified the uptake and conversion of assimilates into structure as a primary process by which low oxygen levels affect early life stages of *M. menidia*.

**Introduction**

Hypoxia is common in coastal and estuarine waters and is expected to intensify with global warming (Diaz and Rosenberg, 2008; Breitburg et al., 2018). Between anthropogenic influence on nearshore waters and the natural dynamics of shallow, partially enclosed water bodies, hypoxia often co-occurs with other stressors such as high temperature, carbon dioxide (CO2) acidification, and pollutants (Gruber, 2011). Along the Northeast United States coast, stratification and productivity associated with high temperatures in spring and summer cause hypoxic and eutrophic zones to form and great fluctuations in dissolved oxygen (DO) on diel to monthly time scales (O’Donnell et al., 2004; Baumann and Smith, 2018; Testa et al., 2018). While fish species that currently live in such areas tend to have mechanisms to cope with periods of hypoxia (Farrell and Brauner, 2009; Zhu et al., 2013; Baumann, 2019), these do not necessarily confer tolerance of longer durations. Fishes that spawn in the spring and summer face the additional threat of experiencing hypoxia during the particularly sensitive early life stages. Embryos and young larvae rely largely on diffusion for oxygen uptake and lack well-developed mechanisms, such as high surface area gills, to meet oxygen demands in low DO water and are not mobile enough to escape hypoxic zones. Mortality can result directly from severe hypoxia or indirectly from reduced growth increasing susceptibility to predation. Even fish that survive may incur sublethal effects with lifelong consequences for growth, development, and reproduction. Modeling the energetic mechanisms of responses to hypoxia can help connect physiology and life history to population-level changes and serve as a valuable alternative to time- and labor-intensive laboratory procedures, particularly with very small animals such as fish embryos and larvae.

Hypoxia is known to inhibit growth and survival in early life fishes, and often has interactive effects with other stressors such as temperature () and high CO2 (Miller et al., 2016). In a series of experiments, Atlantic silverside (*Menidia menidia*) offspring were reared in static or diel fluctuating combinations of oxygen and CO2 treatments to quantify their sensitivity to two co-occurring stressors prevalent in their early life estuarine habitat: hypoxia and acidification (Cross et al., 2019). Although diel fluctuations in both of these properties provided temporary relief that reduced the overall effects of hypoxia and acidification, static low DO significantly delayed hatching, reduced survival to hatching and larval survival, and reduced embryo and larval growth (Cross et al., 2019). While diel fluctuations are a realistic representation of changes in community photosynthesis and respiration between day and night, environmental change in coming years could extend hypoxic duration to reduce periods of relief. Warming reduces oxygen solubility while increasing metabolic rates of organisms that draw down oxygen when densely aggregated. At the same time, higher summer temperatures and freshwater input in some regions will intensify stratification that separates low-oxygen water from surface oxygen diffusion (Rabalais et al., 2009; Howarth et al., 2011). Currently *M. menidia* is tolerant enough that population declines are not a concern, but without knowledge of the mechanisms of early life impacts it is hard to anticipate whether this will change under increased hypoxia duration or with additional stressors (Baumann, 2019).

When targeted conservation action is desired, risks associated with stressors are important to quantify at the population level because management actions operate at this level. While many laboratory experiments have measured physiological responses at the individual-level, additional steps must be taken to translate them to demographic rates like recruitment and reproductive investment in the next generation. Care must be taken as individual-level impacts do not necessarily scale linearly to the population level (Galic et al., 2018). Models that connect physiological and energetic mechanisms of stressor effects to life history create widely applicable tools that can be used to make population-level predictions. Scaling experimental studies to population-level processes remains a challenge (but see Nisbet et al., 1989; Grear et al., 2020; Tai et al., 2021).

Dynamic Energy Budget (DEB) modeling is a bioenergetic framework designed to bridge multiple levels of biological organization in assessing stressor effects in a vast variety of species (Kooijman, 2010; AmP, 2023). This approach follows energy allocation, in the form of suborganismal metabolic fluxes, and how it leads to life history outcomes such as growth rate, reproductive output, and survival, using physical and biological concepts that are generalizable to most species (Jusup et al., 2017). It accounts for differences in the energy budget at each stage to allow modeling of life stage transition timing and stage-specific responses to stressors (Kooijman, 2010). DEB theory is often used to connect experimental observations of multiple stressor effects to both the underlying energetic mechanisms (Kooijman, 2018) and life history outcomes that feed into population dynamics (Nisbet et al., 2000; Martin et al., 2013; Smallegange et al., 2017). These capabilities make DEB theory an excellent tool for enhancing the utility of experimental hypoxia data in conservation and management (Lavaud et al., 2021).

Depending on the application and types of data available, simplified versions of the standard DEB model can be used (e.g. Kooijman and Metz, 1984; Jager, 2018; Martin et al., 2017). Although complexity can be beneficial (Evans et al., 2013), simpler models with fewer parameters are often preferable for their predictive power and ability to be applied, tested, and interpreted widely (Holling, 1966; May, 2001; Jusup et al., 2017). The DEBkiss framework (Figure 1) is a moderately simplified variation on the standard DEB model for animals that eliminates the concept of reserve, a pool of assimilates that are allocated to structure, maintenance, and reproduction in the standard DEB model (Jager et al., 2013). This framework reduces the data requirements, the role of compound parameters, and, depending on the data, the total number of parameters to be estimated (Jager et al., 2013). The simplicity of DEBkiss and its easily understandable equations make it ideal for adaptation to many species of ecological or commercial value using commonly measured variables in laboratory experiments, such as growth and survival rates.

We used a DEBkiss model to identify the bioenergetic mechanisms underlying experimental hatching, growth, and survival effects of hypoxia in early life stages of *M. menidia* observed in Cross et al., 2019. First, we fitted a base DEBkiss model to full-life data on total length, reproductive output, hatch timing, and survival and estimated or calculated parameters under fully oxygenated conditions. Second, we modified a subset of parameters with one of two oxygen-dependent correction factors and estimated a shape parameter for the correction factor to fit the model to early-life data for three low DO treatments. We evaluated which parameter or combination of parameters, when adjusted with the correction factors, was able to best account for the full set of hypoxia responses observed in experiments. We hypothesized that the following parameters would account for some or all of the hypoxia effects: maximum assimilation rate, conversion efficiency of assimilates into structure (growth), maximum somatic maintenance rate, embryo mortality rate, and post-hatch mortality rate. The maintenance rate could be elevated by the activity required for some of the behavioral responses fish exhibit under hypoxia (Thomas et al., 2019). *M. menidia* exposed to hypoxia swim to the surface to use aquatic surface respiration, taking advantage of the diffusion of oxygen from the air (Miller et al., 2016). This behavior is impossible in embryos but has been observed in larvae (Cross et al., 2019). Fishes also expend energy on faster ventilation and heartbeat to increase oxygen uptake when ambient DO is low (Kramer, 1987; Maxime et al., 2000), but these capabilities may be limited until development has progressed further. We therefore hypothesize that maintenance does not account for a substantial portion of the early life changes in growth, hatch timing, and survival.

The conversion efficiency of assimilates to structure controls growth and hatch timing because it is the fraction of assimilates that are converted into structure rather than burned on overhead costs of growth (Jager, 2018). When oxygen is low enough that anaerobic metabolism must be used, this reduces conversion efficiency so that less growth results from the same amount of yolk or food (Thomas et al., 2019). We hypothesize that this contributed to a smaller hatch size and slower growth post-hatch.

Assimilation is the transformation of food and oxygen into compounds that will go to structure, maintenance, or reproduction. Reduced food consumption is a primary mechanism by which the fish energy budget is thought to be impacted by hypoxia (Chabot and Dutil, 1999; Thomas et al., 2019). However, feeding effects cannot explain the observed hypoxia impacts on *M. menidia* hatch survival, timing, and size (Cross et al., 2019) because embryos do not yet ingest food. But because oxygen is also used in assimilation, low oxygen could reduce the assimilation rate of yolk resulting in slower depletion of the egg buffer and smaller size at hatching. Changes to assimilation efficiency under hypoxia have been recorded in other species, but the direction of that effect is species-dependent (reviewed in Thomas et al., 2019). In the base model, our fitted survival parameter for embryo mortality is greater than that of larvae. If assimilation rate or conversion efficiency of *M. menidia* decreases under hypoxia, the resulting slower egg buffer depletion would delay hatching, extending individuals’ time in the stage with greater mortality and thus accounting for reduced hatch survival under hypoxia. We therefore hypothesize that either maximum assimilation rate or conversion efficiency for growth will be the best parameter to explain the bioenergetic mechanism of early life hypoxia effects, and that modifying the embryo mortality parameter will consequently not be necessary. However, we hypothesize that this will not be the case for the post-hatch mortality parameter because none of the processes in the DEBkiss model indirectly affect mortality after hatching, so using the correction factors on either the assimilation or conversion efficiency parameter in combination with the post-hatch mortality parameter may be necessary to fully replicate the observed changes to growth, hatch timing, and survival under hypoxia.

**Methods**

*DEB Model Description*

To model the stage-specific energy budget of *M. menidia* in a way that would allow us to explain early-life hypoxia effects with bioenergetic processes, we used DEBkiss, a simplified and widely applicable DEB model (Jager et al., 2013; Jager, 2018). The full set of assumptions and equations can be found in Jager (2018). Briefly, the flux of food (*JX*) or, for embryos, the egg buffer (*WB*) is immediately converted to assimilates which are allocated to a somatic fraction (*κ*) and a reproductive fraction (1-*κ*; Figure 1); these fractions are constant throughout the life cycle. The assimilation flux (*JA*) is the product of the scaled food level (*f*), the volumetric surface area (*L2*), and the parameter maximum area-specific assimilation rate (*JaAm*):

For embryos (*WB* > 0) and under *ad libitum* feeding *f* = 1. The differential equation for change in egg buffer over time is –*JA*. Within the somatic branch, which does not change with life stage, a flux to maintenance (*JM*) is prioritized while the remainder goes to the flux for structure (*JV*) with a conversion efficiency *yVA*. The maintenance flux is the product of volume and the parameter for the volume-specific cost for maintenance (*JvM*):

The differential equation for growth is equal to *JV*. For juveniles, the non-somatic fraction of assimilates is spent on maturation, or increasing complexity through gonad development. Once the mass at puberty is reached (*WVp*), reproductive flux (*JR*) toward egg production begins in adults with a conversion efficiency *yBA*. Although *M. menidia* have a distinct larval and juvenile stage, both are treated as the juvenile stage because the relevant aspects of their energy budget for DEBkiss are identical. DEBkiss also uses an optional flux to maturity maintenance (*JJ*) that comes from the 1-*κ* fraction of assimilates (Jager, 2018), which we chose to use in our model.

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where *WV* is the structural mass, *R* is the continuous reproduction rate, and *WB0* is the initial egg mass. The equation for continuous reproduction gives the differential equation for egg production over time. Because the model equations use dry weight for body size and our growth data is total length, we calculated a shape correction coefficient (*δM*) and dry weight density (*dV*) to allow the model to convert between the two.

To address the assumption of DEBkiss that all eggs hatch when buffer is depleted, regardless of body size or developmental progress (Jager et al., 2013), we added a survival variable. In addition to allowing an alternative outcome to hatching, this allowed us to examine survival as a consequence of hypoxia effects on the energy budget. We fitted mortality parameters for embryos and post-hatch fish (*μemb* and *μlar*) to data for survival to hatching and larval/juvenile survival (Figure 1). 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. The differential equation for proportion surviving over time is:

DEBkiss uses fewer parameters than the standard DEB model, which reduces data requirements and the risk of overfitting. While the standard DEB formulation uses a state variable for maturity that triggers changes between life stages, DEBkiss instead uses a constant size at puberty to specify when reproduction is initiated (Kooijman, 2010; Jager et al., 2013). It also has no reserve compartment between food assimilation and allocation, and for embryos this means that the egg buffer is assimilated into body structure and for maintenance, with hatching occurring when the egg buffer is fully depleted, instead of following reserve dynamics of the standard DEB model (Jager et al., 2013). The lack of reserve makes DEBkiss well-suited for animals with a small ultimate body size because reserve plays a smaller role in such species under DEB theory (Nisbet et al., 2000), but DEBkiss has been successfully applied to larger animals as well (e.g. Desforges et al., 2017).

Diagram

Description automatically generated

**Figure 1.** The DEBkiss model (diagram adapted from Jager et al., 2013) with stage-specific survival parameters. The candidate parameters for hypoxia stress mechanisms are highlighted in red boxes.

*Base Model and Data*

For the base model we calculated and estimated parameters based on four types of data (state variables): total length over time, egg buffer mass over time (and through this, time to hatching), cumulative egg production over time, and proportion surviving since fertilization over time. We estimated three parameters by fitting them to data (*yVA*, *μemb*, and *μ­lar*) and fixed at suggested values parameters for which we had insufficient data to calculate or estimate. The primary parameters and their calculated or estimated values are found in Table 1. Fitting was done in Matlab with the packages BYOM v.6.4 (Jager, 2022) and DEBkiss v.2.3a (Jager, 2021). BYOM uses a Nelder-Mead simplex search to optimize the parameters for a set of ordinary differential equations (ODEs) by minimizing negative log-likelihood (NLL). The DEBkiss package works under BYOM to bring in the DEBkiss model parameters, variables, and equations so that the parameters can be estimated based on their effect on the DEBkiss equations and the ODEs derived from them. The ODEs give the predicted data for each type of observed data (length, egg production, egg buffer mass, and survival over time) the difference between which is used to calculate NLL.

BYOM allows users to turn fitting on and off for each parameter, and with fitting turned off for all parameters it runs a simulation that calculates predicted values over time for each state variable using the initial parameter values. Before estimating any parameters with the optimization described above, we ran simulations with fitting turned off using a set of recommended parameters (Jager, 2018) and parameters obtained from existing data on *M. menidia*. We visually assessed fit and checked NLL as we adjusted parameters to obtain a reasonable set of initial parameters before estimating any. This also helped us reduce the number of parameters being estimated to avoid overfitting and so that there were not multiple correlated parameters free at once, because we were able to obtain a reasonable fit using suggested default values for *yAV*, *yBA*, and *κ*. The default value for *yVA­* did not allow a realistic fit to the length data, but the length, reproduction, and egg buffer depletion data allowed it to be estimated with the BYOM optimization. Ultimate length was used to fix *JaAm* to a reasonable value before estimating *yVA* because both parameters affect growth and egg buffer depletion in the model and therefore can not be estimated simultaneously. Finally, we fixed all parameters except *μemb* and *μlar* to estimate these parameters, again using the visually best-fitting parameters from the simulations as initial values. The full-life and early-life predicted and observed data are shown in Figure 2.

**Table 1.** DEBkiss parameters, their abbreviations, and their fixed or estimated values. Units are given with the value unless the parameter is a unitless ratio.

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| **Parameter** | **Symbol** | **Fixed or estimated** | **Value** |
| Max. area-specific assimilation rate | *JaAm* | Estimated | 0.333 mg mm-2 d-1 |
| Max. volume-specific maintenance rate | *JvM* | Fixed | 0.0214 mg mm-3 d-1 |
| Initial egg weight | *WB0* | Fixed | 0.15 mg |
| Total length at puberty | *LVp* | Fixed | 102 mm |
| Yield of assimilates on volume | *yAV* | Fixed | 0.8 |
| Yield of egg buffer on assimilates | *yBA* | Fixed | 0.95 |
| Yield of structure on assimilates | *yVA* | Estimated | 0.3646 |
| Fraction of assimilates allocated to soma | *κ* | Fixed | 0.8 |
| Scaled food level | *f* | Fixed | 1 |
| Scaled food level for embryo | *fB* | Fixed | 1 |
| Half-saturation total length | *Lf* | Fixed | 0 |
| Mortality rate for embryos | *μemb* | Estimated | 0.06393 |
| Mortality rate for larvae | *μlar* | Estimated | 0.02940 |

Diagram

Description automatically generated

**Figure 2.** Predicted (lines) and observed data (dots) for the base DEBkiss model of *M. menidia*. The state variables are (A) total length (mm) over time (days), (B) cumulative reproduction (eggs) over time (days), (C) egg buffer mass (mg) over time (days), and (D) survival over time (days). Predicted data lines are calculated with the parameter values listed in Table 1.

The length and reproductive data allowed us to calculate length at puberty (*LVp*), which in this model is the length at which egg production begins. We obtained *WB0* from *M. menidia* egg dry weight data (Klahre, 1997) and calculated *δM* and *dV* from total length, egg diameter, and egg mass data (Cross et al., 2019; Klahre, 1997; Concannon et al., 2021). To calculate volume-specific maintenance costs (*JvM*), we used data on the rate of decrease in larval dry weight over a period of starvation in the congeneric species *M. beryllina* (Letcher and Bengtson, 1993). Borrowing from closely related species is a common practice in bioenergetic modeling when the species has similar habitat, life history, and physiology, as is the case here (Sibly et al., 2013; Bengtson, 1984). All *M. menidia* datasets came from experiments in which fish were fed *ad libitum* so *f* was set to 1. For experiments that exposed fish to different CO2 levels, we only used data from control groups to avoid potential stressor effects in the data.

Data for the state variable total length were sourced from three studies. Length at hatching and 15 days post-hatching (dph) came from a study that reared *M. menidia* offspring in different static oxygen levels across two experiments (Cross et al., 2019). This provided data for control oxygen levels used in the base model and three reduced oxygen treatments (Table 2). The study featured two additional experiments that exposed offspring to fluctuating oxygen and CO2 levels but the control conditions were static, so we used total length data from these treatments for the base model as well (Cross et al., 2019). We sourced additional length data for the base model from control levels of experiments that exposed *M. menidia* offspring to ambient and elevated CO2 levels (Murray and Baumann, 2018; Murray and Baumann, 2020; Concannon et al., 2021). All total length data were obtained from fish maintained in static laboratory conditions at 24°C.

The state variable cumulative egg production over time was also obtained from control groups in Concannon et al. (2021), a study in which wild-caught juveniles were held in the laboratory at 20°C in different CO2 treatments and strip-spawned once they reached reproductive maturity. Data for the state variables on egg buffer mass (i.e. time to hatching when egg buffer mass is zero) and survival to hatching and 15 dph under different oxygen levels were obtained from Cross et al. (2019). We also used survival data from the 24°C and control CO2 groups of a study on the effects of temperature and CO2 on *M. menidia* early life survival (Murray and Baumann, 2018). Four additional data points for long-term survival in laboratory conditions at 17°C were obtained from a study that exposed *M. menidia* offspring until 122 dph to two CO2 levels, of which we only used data from the control level (Murray et al., 2017).

**Table 2.** The mean survival to hatching, hatch time (at which egg buffer is zero), length at hatching, length at 15 dph, and survival to 15 dph from the different oxygen treatments in Cross et al. (2019). The control DO level means (7.7 mg l-1) also include data from Murray and Baumann (2018).

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|  | **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% |

*Hypoxia Stress*

We multiplied several DEBkiss parameters (Figure 1) by correction factors to attempt to explain observed differences in *M. menidia* length, hatching, and survival between experimental oxygen treatments (Cross et al., 2019). To summarize the experimental data on static hypoxia effects we are attempting to explain by altering these parameters, the mean values of data for each oxygen treatment are listed in Table 2. We used the parameter values from the base model, which contained full lifespan data, and altered one or more parameters at a time with oxygen-dependent correction factors, then fitted the model to data for only the first 136 days by estimating the best value of the parameter *K* that influences correction factor shape. We only used early life data to fit the hypoxia-altered parameters because we did not have late-life data for multiple oxygen treatments later in life to validate observed changes against and did not have any reproduction data for oxygen treatments. It did not make sense to include later life data in the calculations of NLL that influence the parameter estimates or to speculate about how well the predicted data match what we might expect to happen later in life if we not only lack late-life hypoxia data but also do not expect full life hypoxia to occur in nature.

A primary correction factor (*c*) that decreased exponentially with decreasing DO between (Figure 3) was calculated as:

where *K* is the shape parameter that affects the strength of the DO effect on predicted values of the state variables. DO is the treatment level of oxygen, and DOc is the critical oxygen level below which the *c* = 0. The value of *c* cannot exceed 1 with this function. A larger *K* value keeps *c* higher as oxygen decreases before a more abrupt drop, while a smaller *K* gives a more constant decline in *c* with hypoxia (Figure 3). Attempts to estimate DOc and *K* simultaneously showed that leaving DOc free did not improve the ability of the correction factor to fit the hypoxia data. Instead, DOc was fixed at a biologically relevant level of 2.044 mg L-1, which is the critical oxygen level below which embryonic routine metabolism becomes highly oxygen-dependent (Schwemmer, unpublished data). This correction factor was multiplied by *JaAm* and *yVA* because these parameters were hypothesized to decrease under hypoxia. To alter the parameters hypothesized to increase under hypoxia (*JvM*, *μemb*, and *μlar*) a secondary correction factor, *c1*, was calculated from *c*:

where *c1(max)* is the upper limit to the correction factor, or a maximum factor by which we are willing to multiply the parameters. We set *c1(max)* = 10 because the value doesn’t affect the shape of the curve below the limit and only very low *K* values would lead *c1* to reach this level at the DO treatments of the data. The correction factor *c1* was multiplied by *JvM*, *μemb*, and *μlar* to increase them with decreasing DO.

To find the best value of *K* for each DEBkiss parameter or combination of parameters, we added *K* as a model parameter and estimated it using the BYOM optimization to minimize NLL. We used initial criteria to identify the candidate parameters for inclusion in the best fitting model. The initial criteria for a given DEBkiss parameter were 1) that altering the parameter must lead to a change in at least one state variable in the same direction as the observed effect of hypoxia, and 2) that the final best model must include parameter(s) that account for the changes in all three state variables for which low oxygen data exist. For example, *yBA* does not meet the first criterion because applying *c* to it has no effect on any of the state variables. *μemb* meets the first criterion because applying *c1* to it changes the survival state variable, but it does not change total length or egg buffer mass over time so a model with a correction factor for *μemb* alone does not meet criterion 2. Once we narrowed down the list of candidate parameters that met criterion 1 (summarized in Table 3 with examples in Figure 4) we estimated *K* and calculated AIC with a correction factor applied to each individual parameter and every combination of two, three, or four parameters. We did not apply the correction factor to *JaAm* and *yVA* simultaneously because they are multiplied together to obtain *JV* and their individual contributions to the growth and egg buffer depletion can not be fully separated. Although *κ* met criterion 1, we did not include it as a candidate because we lacked the reproductive data needed to model any potential changes in relative energy allocation under hypoxia. We also did not include *f* despite it meeting criterion 1 because feeding was *ad libitum* across all experiments. We compared the AIC between each model to determine which combination of parameters best fit the data while also meeting criterion 2 and, therefore, which DEB processes best explain the hypoxia effects observed in experiments (Table 4).

Diagram

Description automatically generated

**Figure 3.** The effect of DO on correction factor *c* (A) at three different values of shape parameter *K*, and correction factor *c1* (B) as a function of *c*.

**Table 3.** Summary of impacts of altering each DEBkiss parameter on predicted data for total length, time to hatching (egg buffer mass = 0), and survival over time. We used this information to choose which parameters to which to apply hypoxia-based correction factors by identifying those that best meet our requirement of accounting for hypoxia effects on all three state variables. The last column indicates whether the effect of changing the parameter matches the overall patterns observed in the data (i.e. an increase or decrease in at least one state variable).

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|  |  | Impact on predicted values of: | | |  |
| Parameter | Hypothesized hypoxia effect on parameter | Total length (mm) | Time to hatching | Survival proportion | Initial criteria met? |
| *JaAm* | ↓ | ↓ | ↑ | ↓ | Yes |
| *JvM* | ↑ | ↓ | ↑ (weak) | ↓ (weak) | Yes |
| *WB0* | ↓ | none | none | none | No |
| *LVp* | ↓↑ | none | none | none | No |
| *yAV* | ↓ | none | none | none | No |
| *yBA* | ↓ | none | none | none | No |
| *yVA* | ↓ | ↓ | ↑ | ↓ | Yes |
| *κ* | ↓ | ↓ | ↑ | ↓ | Yes |
| *f* | ↓ | ↓ | ↑ | ↓ | Yes |
| *fB* | ↓ | ↓ (prehatch only) | ↑ | ↓ | No |
| *Lf* | ↓↑ | none | none | none | No |
| *μemb* | ↑ | none | none | ↓ | Yes |
| *μlar* | ↑ | none | none | ↓ | Yes |

Diagram

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**Figure 4.** Predicted values of total length, egg buffer mass, and survival over time for each parameter’s base model value and two levels representing hypoxia effects on the parameter. These plots use assimilation (A, B, and C), maintenance (D, E, and F), and combined embryo and post-hatch mortality rates (G, H, and I) as examples to show how we selected DEBkiss parameters that would influence at least one of the state variables that was impacted by hypoxia in experiments. State variables are total length (A, D, and G), egg buffer mass (B, E, and H), and survival (C, F, and I). Reducing *yVA* with hypoxia affects the response variables similarly to *JaAm* so it is not shown in the figure.

**Results**

*Base Model*

We obtained realistic fits to all datasets. The only exception is late-life survival, for which the mortality was too high beyond the larval stage but could not be better fit due to lack of full-life survival data. However, this did not impair our ability to model the effects of hypoxia on early life survival. Estimating *yVA* returned a lower than typical value for conversion efficiency of assimilates to growth, but this gave a realistic fit to the length data and allowed a detailed and very close fit to egg buffer mass over time (hatch timing). The observed and predicted data for full life span and early life are plotted in Figure 2.

*Hypoxia Stress*

The best model of experimental hypoxia effects on *M. menidia* early life stages had correction factors applied to *yVA*, *μemb*, and *μlar*. The correction factor *c* was used to reduce *yVA* and *c1* was used to increase both *μemb* and *μlar*. This model met the initial criteria of affecting all three state variables (total length, egg buffer mass, and survival) in the same direction as hypoxia affected them in experimental data. Although adjusting *yVA* alone met the initial criteria of affecting all three state variables, also increasing both mortality parameters improved the fit to the data. It also had a lower AIC than all but one of the other models that met the initial criteria, with an AIC of 584.75. Adding a correction factor to *JvM* in addition to these three parameters reduced AIC slightly to 584.62 (AICmin). The relative likelihood of the model with correction factors for *yVA*, *μemb*, and *μlar* has a relative likelihood (Akaike weight) of 0.937, indicating it is 0.937 times as probable as the model that applies correction factors to *JvM*, *yVA*, *μemb*, and *μlar*. It was therefore not considered to have improved the fit, and in the interest of parsimony is not beneficial enough to justify the added complexity of applying the correction factor to a fourth parameter. The estimated *K* values and AIC for each version of the model, as well as the ΔAIC for models that fit the initial criteria, are listed in Table 4. The values of *yVA*, *μemb*, and *μlar* when their respective correction factors are applied for each DO level are listed in Table 5.

Reducing *JaAm* with hypoxia using correction factor *c* also resulted in a good fit to the data across oxygen levels and fulfilled the initial criteria. Combining the adjusted *JaAm* with correction factors to increase both mortality rates improved the fit as well, but this model fit slightly less well than the version that corrected *yVA*, *μemb*, and *μlar*, with an AIC value of 586.72 in the former model compared to 584.72 in the latter. The ΔAIC for this pair of models is 2, indicating that the model with *c* multiplied by *JaAm* performs similarly to the model with *c* multiplied by *yVA*, when correction factor *c1* is also included for both mortality parameters. The ΔAIC values relative to the AICmin for the models applying correction factors to *JaAm* + *μemb* and *yVA* + *μemb* are 5.95 and 4.64, respectively (ΔAIC for all models listed in Table 4). This suggests that although these are not the best fitting models, there is a moderate level of support for them, contrary to our hypothesis that adjusting *μlar* with oxygen would be required to get a good fit.

**Table 4.** The estimated *K* value and AIC when the correction factors were applied to each parameter or combination of parameters. ΔAIC is listed only for models that satisfied the initial criteria as the ones that do not fit the criteria are not eligible to be selected as the best model, and was calculated with AICmin = 584.62 for the *yVA* + *JvM* + *μemb* + *μlar* model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter(s)** | **Correction factor(s)** | **Estimated *K* [95% CI]** | **AIC** | **ΔAIC** |
| *JaAm* | *c* | 1.698 [1.694-2.702] | 600.70 | 16.08 |
| *yVA* | *c* | 1.475 [1.197-3.205] | 602.35 | 17.73 |
| *JvM* | *c1* | 0.3646 [0.3016-0.5179] | 599.49 | - |
| *μemb* | *c1* | 0.6257 [0.4351-0.9920] | 585.73 | - |
| *μlar* | *c1* | 0.3028 [0.2009-0.4918] | 575.03 | - |
| *JaAm* + *JvM* | *c* + *c1* | 1.720 [1.716-2.686] | 600.62 | 16.00 |
| *yVA* + *JvM* | *c* + *c1* | 1.468 [1.215-3.075] | 602.20 | 17.58 |
| *JvM* + *μemb* | *c1* + *c1* | 0.5200 [0.3740-0.8511] | 582.80 | - |
| *JaAm* + *μemb* | *c* + *c1* | 1.698 [1.694-2.041] | 590.27 | 5.95 |
| *yVA* + *μemb* | *c* + *c1* | 1.308 [1.198-1.777] | 589.26 | 4.64 |
| *JvM* + *μlar* | *c1* + *c1* | 0.3541 [0.2988-0.4479] | 568.10 | - |
| *JaAm* + *μlar* | *c* + *c1* | 1.698 [1.694-2.253] | 595.42 | 10.80 |
| *yVA* + *μlar* | *c* + *c1* | 1.340 [1.195-1.981] | 594.64 | 10.02 |
| *μemb* + *μlar* | *c1* + *c1* | 0.7659 [0.5434-1.145] | 580.08 | - |
| *JaAm* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.698 [1.694-2.023] | 586.72 | 2.10 |
| *yVA* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.315 [1.196-1.756] | 584.75 | 0.13 |
| *JvM* + *μemb* + *μlar* | *c1* + *c1* + *c1* | 0.7124 [0.4823-1.087] | 578.79 | - |
| *JaAm* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.720 [1.716-2.042] | 586.83 | 2.21 |
| *yVA* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.313 [1.216-1.753] | 584.62 | 0 |

Chart, diagram

Description automatically generated

**Figure 5.** Best fit of DEBkiss model to experimental data from four DO levels, selected based on a combination of initial criteria that all three response variables’ predicted values are affected by the hypoxia correction factor and ΔAIC. (A) is total length (mm) over time (days), (B) is egg buffer mass (mg) over time (days), and (C) is survival over time (days), with means rather than all data plotted for survival for ease of viewing patterns.

**Table 5.** The value of the DEBkiss parameters that best reproduce the hypoxia effects observed experimentally, calculated (along with 95% confidence intervals in brackets) for each DO treatment level using the correction factors *c* and *c1* and the estimated value of *K* = 1.315.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **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** |
| ***yVA*** | 0.364  [0.364, 0.365] | 0.343  [0.337, 0.356] | 0.274  [0.261, 0.308] | 0.211  [0.198, 0.249] |
| ***μemb*** | 0.175  [0.175, 0.175] | 0.186  [0.179, 0.190] | 0.234  [0.207, 0.244] | 0.303  [0.256, 0.322] |
| ***μlar*** | 0.0807  [0.0806, 0.0807] | 0.0856  [0.0825, 0.0872] | 0.107  [0.0956, 0.112] | 0.139  [0.118, 0.148] |

**Discussion**

We tested the full set of DEBkiss parameters with the objective of identifying explanatory mechanisms for experimentally observed hypoxia effects on *M. menidia* early life stages. Preliminary testing ruled out seven of the parameters as having no effect on the state variables when increased or decreased based on hypothesized hypoxia effects (Table 3). We also omitted *κ* and *f* because we lacked data on reproduction and feeding rates under hypoxia. With the remaining parameters, we discovered that applying correction factors to reduce the conversion efficiency for growth (*yVA*) and increase pre- and post-hatching mortality rates (*μemb* and *μlar*) best predicted the experimental effects of hypoxia on larval length, time to hatching, and early life survival. Through this model we have found evidence that the mechanism largely responsible for the observed hypoxia impacts on growth, hatch timing, and survival is the efficiency by which assimilated food or egg yolk is converted into structure. The estimated best value of *K*, the shape parameter in the correction factor *c*, enables us to calculate that *yVA* at the lowest oxygen level is 58% of its value with no hypoxia stress. Reducing *yVA* alone produced small differences in survival at hatching because it prolongs the time spent in the embryo stage, which has a greater mortality rate than post-hatching in our model. Multiplying both the pre- and post-hatching mortality rates by the correction factor *c1* more closely predicted the reduced survival rates in the low DO treatments, resulting in a best fitting model that explained observed hypoxia effects well by altering *yVA*, *μemb*, and *μlar*. Our best fitting model, according to ΔAIC and parsimony, underestimated time to hatching and overestimated size at age, which suggests there were additional factors contributing to these differences that the model does not account for. Nonetheless, the model was able to replicate the direction of effects and even account for hypoxia effects in all three state variables simultaneously by changing only one parameter, either *yVA* or *JaAm*.

Replacing *yVA* with *JaAm* as the hypoxia-reduced parameter yielded a similar fit, likely because both parameters are used to calculate predicted growth and egg buffer depletion. However, applying correction factor *c* to *yVA* explained the data slightly better than *JaAm* based on AIC. Hypoxia could influence either the assimilation rate or the efficiency with which assimilates turn into structure could be affected by hypoxia. Under *ad libitum* feeding, differences in assimilation of hatched larvae could indicate reduced ingestion with low oxygen, a common hypoxia response in fishes (Chabot and Dutil, 1999; Thomas et al., 2019). For embryos, on the other hand, reduced assimilation rates indicate slower absorption of the yolk. Hypoxia has been shown to delay development in Atlantic salmon by reducing yolk absorption rates (Polymeropoulos et al., 2017). If assimilation rate were the only difference between hypoxia treatments, one would expect the offspring to reach the same size at hatching regardless of the timing. However, *M. menidia* larvae had significant differences in hatch lengths between DO treatments (Cross et al., 2019), indicating that *yVA* played a role in the hypoxia response as well. When oxygen is low, conversion efficiencies of assimilates can be reduced by the far less efficient production of ATP through anaerobic respiration combined with slower rates of tissue differentiation. Extending developmental time while continuing to pay maintenance costs can further increase the energy expended to produce each unit of structure (Kamler, 2008). After hatching, these mechanisms would continue to reduce *yVA* but it may also be reduced by increased ventilation required during digestion (Chabot and Claireaux, 2008). The experimental DO levels are greater than the critical oxygen levels for oxygen-independent routine metabolism (*P*crit) of 2.04 mg L-1 and 1.56 mg L-1 for embryos and 5dph larvae, respectively (Schwemmer, unpublished data). *P*crit has been assumed by some to be the oxygen level at which anaerobic metabolism is triggered, but there is abundant evidence that some level of anaerobic metabolism can occur well above *P*crit (Nonnotte et al., 1993; Maxime et al., 2000; Wood et al., 2018). Additional activity such as swimming bursts can drive up the need for anaerobiosis (Di Santo et al., 2017). Our identification of *yVA* as a primary component of the energy budget that is reduced by hypoxia suggests that anaerobic metabolism is a mechanism of hypoxia effects in *M. menidia* early life stages even at oxygen levels above *P*crit. A limitation of this study is the inability to fully separate the relative influences of *yVA* and *JaAm* from each other because flux for growth is calculated from the product of *yVA* and the somatic fraction of *JaAm*; we can adjust one or the other and get similar effects on *JV* with no way of determining which is correct.

Adding a correction factor to *JvM* in addition to this model did not substantially improve the fit according to ΔAIC, suggesting that increasing maintenance costs is not a bioenergetic mechanism underlying hypoxia response in early life stages. In this model, egg buffer depletion is insensitive to changes in volume-specific maintenance costs, requiring a quadrupling to see a noticeable delay in hatching (Figure 4). Changing *JvM* has much greater effects on length later in life while failing to explain differences in length at the time of hatching (Figure 4). One way maintenance costs could increase under hypoxia is through additional activity related to ventilation and mobility (Thomas et al., 2019), but at the embryo stage very little activity is possible so it makes sense that the correction factor for maintenance doesn’t model the hypoxia effects well. A common response to hypoxia in fish embryos is premature hatching (Kamler, 2008) which could allow swimming escape responses that increase maintenance costs, but studies on chorion removal have shown that the increased mobility can improve growth despite hypoxia exposure (Ciuhandu et al., 2005; Ninness et al., 2006). In contrast, *M. menidia* embryos’ delayed growth and hatching do not appear to be related to elevated maintenance costs, and rearing them in hypoxia did not significantly change their oxygen consumption rates as may be expected if maintenance was elevated (Cross et al., 2019; Schwemmer et al., 2020). Some studies on fish responses to hypoxia suggest maintenance may drop temporarily due to the reduced capacity for aerobic metabolism at low DO levels. Maintenance rates may then be temporarily elevated after oxygen is restored because of recovery demands such as paying oxygen debt and removing or repairing damage from anaerobic byproducts (Thomas et al., 2019). If such fluctuations were occurring in the *M. menidia* offspring from this dataset, the net effect on maintenance was not discernible by our model.

Although both *yVA* and *JaAm* can explain hypoxia effects on total length and egg buffer mass over time, reducing them only produced a small decrease in survival relative to the data. Applying correction factor *c1* to both mortality rates better captured the great reductions in survival at both hatching 15 dph with hypoxia. In the experiments, the lowest oxygen level (2.7 mg L-1) had a mean hatch survival of 30.2% while the mean survival in the other three treatments was over 70% (Cross et al., 2019). By 15 dph fish from all three low oxygen treatments had lower survival than those from the normoxic treatment (Cross et al., 2019; Table 2). Including hypoxia effects for both pre- and post-hatching mortality rates allowed the model to more closely predict these differences in hypoxia effects in both stages and improve the fit based on ΔAIC (Table 5). However, an intrinsic mortality rate isn’t as explicitly indicative of underlying energetic processes as the other DEB parameters are. The additional mortality that was not accounted for by *yVA* may have been related to tissue damage from buildup of toxic compounds during anaerobic metabolism (Richards, 2011). The mortality could also have resulted from failing to meet energetic demands with either aerobic or anaerobic metabolism (citations) and, specifically in embryos, failure to reach a viable level of complexity before the yolk is depleted (Jager et al., 2013). The latter could be an indirect effect of reduced *yVA* that the model does not account for, as mortality rates are not influenced by the other model parameters in our formulation. Measurement of anaerobic byproducts such as lactate and morphological evaluation of dead embryos and larvae could help to identify the mechanisms underlying the mortality rates in future work. Although survival does not approach 0% during the larval stage in our best fitting model (Figure 5), all experimental replicates of the 2.7 mg L-1 DO treatment had 0% survival by 15 dph, making larvae apparently more sensitive than embryos (Cross et al., 2019). The authors of the study attribute this to a possibly lower ability to suppress metabolism in larvae compared to embryos. While the increased mobility of larvae may allow escape from hypoxia in a patchy and stratified estuarine environment, activity comes with elevated maintenance costs and, regardless of escape behavior, some level of swimming is required for *M. menidia* to begin feeding almost immediately after hatching (Middaugh and Lempesis, 1976). Furthermore, swimming upward for aquatic surface respiration may inhibit feeding, thus creating a positive feedback of additional energetic costs with decreasing assimilates to meet them (Miller et al., 2016; Cross et al., 2019). Though beyond the scope of this work, a model that captures stage-specific differences in maintenance costs and links them explicitly to survival may better capture the high mortality in larvae and their reduced ability to suppress metabolism.

Understanding the mechanisms of reduced growth and survival under hypoxia through DEB theory can be useful for predicting life history effects, and although not within the scope of this study, the predictions can be used to model population growth rates, which are useful for resource management (Kooijman et al., 2020; Lavaud et al., 2021). An important assumption of our model is that several of the parameters have the same value across life stages (e.g. *JaAm*, *JvM*, *yVA*) and similarly that values of the hypoxia correction factors are the same regardless of life stage. Future work could evaluate full-life sensitivity with higher resolution data for the later life stages. We lacked reproductive data to look at hypoxia effects on the proportion of total energy allocated to reproduction (1-*κ*), which is an additional component of DEB useful in connecting organismal effects to populations, but future experimentation could provide the needed information. Nonetheless, our model fitted to early life data with a hypoxia-based correction factor predicts reductions in long-term growth and survival that would certainly be detrimental to population growth under extended periods of low oxygen. Under this model, even restoring normoxia after 15 days would result in smaller size at age and survival rates than the groups exposed to 7.7 mg L-1, although compensation of growth may be possible after exposure to hypoxia (Wei et al., 2008) and other stressors (Russell and Wootton, 1992; Nicieza and Metcalfe, 1997; Ali et al., 2003). Delayed hatching and slower growth can both lead to enhanced vulnerability to predation, which could further reduce survival rates beyond those observed in controlled laboratory conditions.

With this simple and widely applicable DEBkiss model we were able to attribute much of the hypoxia-related variability in total length, egg buffer mass, and survival over time to changes in core DEB parameters. The evidence for the mechanisms is inferred from a combination of experimentally observed responses and unified principles that apply to virtually all animal species (Jager et al., 2013). Similar approaches have applied correction factors to DEB parameters to model other species’ responses to hypoxia (Lavaud et al., 2019; Aguirre-Velarde et al., 2019) and other stressors such as seawater acidification (Jager et al., 2016; 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 levels 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). The patterns modeled in this study should not be interpreted as a direct prediction of what will happen to wild *M. menidia* populations as coastal hypoxia intensifies. Lifelong constant oxygen levels do not occur and are not expected to occur in the future, but rather fluctuating oxygen levels will provide opportunities for recovery and may confer tolerance of temporary stress (Cross et al., 2019; Baumann, 2019). Instead, this approach demonstrates the value of identifying DEB parameters responsible for whole-organism effects of hypoxia to understand underlying energetic processes that are often time, labor, and cost-intensive to measure empirically, particularly in the early life stages, when biomass available for sampling is small and developmental changes are rapid. Through doing so we were able to highlight the conversion of assimilates to structure as a primary, but not sole, mechanism by which hypoxia reduces size, delays hatching, and increases mortality in an ecologically important forage fish.

**References**

Aguirre-Velarde, A., Pecquerie, L., Frederic, J., Gerard, T., and Flye-Sainte-Marie, J. 2019. Predicting the energy budget of the scallop *Argopecten purpuratus* in an oxygen-limiting environment. *J. Sea Res.*, 143: 254-261.

Ali, M., Nicieza, A., and Wootton, R. J. 2003. Compensatory growth in fishes: a response to growth depression. *Fish and Fisheries*, 4: 147-190.

AmP. 2021. Online database of DEB parameters, implied properties and referenced underlying data. [www.bio.vu.nl/thb/deb/deblab/add\_my\_pet/](http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/) (data accessed: March 3, 2023).

Baumann, H. 2019. Experimental assessments of marine species sensitivities to ocean acidification and co-stressors: how far have we come? *Can. J. Zool.*, 97: 399-408.

Baumann, H. and Smith, E. M. 2018. Quantifying Metabolically Driven pH and Oxygen Fluctuations in US Nearshore Habitats at Diel to Interannual Time Scales. *Estuaries and Coasts*, 41: 1102-1117.

Bengtson, D. A. 1984. Resource partitioning by *Menidia menidia* and *Menidia beryllina* (Osteichthyes: Atherinidae). *Mar. Ecol. Prog. Ser.*, 18: 21-30.

Boult, V. L. and Evans, L. C. 2021. Mechanisms matter: Predicting the ecological impacts of global change. *Glob. Change Biol.*, 27(9): 1689-1691.

Breitburg, D., Levin, L. A., Oschlies, A., et al. 2018. Declining oxygen in the global ocean and coastal waters. *Science*, 359(6371): eaam7240.

Chabot, D. and Claireaux, G. 2008. Environmental hypoxia as a metabolic constraint on fish: The case of Atlantic cod, *Gadus morhua*. *Mar. Pollut. Bull.*, 57: 6-12.

Chabot, D. and Dutil, J.-D. 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. *J. Fish. Biol.*, 55: 472-491.

Ciuhandu, C. S., Stevens, E. D., and Wright, P. A. 2005. The effect of oxygen on the growth of *Oncorhynchus mykiss* embryos with and without a chorion. *J. Fish. Biol.*, 67: 1544-1551.

Cross, E. L., Murray, C. S., and Baumann, H. 2019. Diel and tidal *p*CO2 x O2 fluctuations provide physiological refuge to early life stages of a coastal forage fish. *Sci. Rep.*, 9: 18146.

Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., and Baumann, H. 2021. Temperature-dependent effects on fecundity in a serial broadcast spawning fish after whole-life high CO2 exposure. *ICES J. Mar. Sci.*, 78(10): 3724-3734.

Desforges, J.-P. W., Sonne, C., and Dietz, R. 2017. Using energy budgets to combine ecology and toxicology in a mammalian sentinel species. *Sci. Rep.*, 7: 46267. doi: 10.1038/srep46267

Di Santo, V., Kenaley, C. P., and Lauder, G. V. 2017. High postural costs and anaerobic metabolism during swimming support the hypothesis of a U-shaped metabolism–speed curve in fishes. *Proc. Nat. Acad. Sci.*, 114(49): 13048-13053.

Diaz, R. J. and Rosenberg, R. 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. *Science*, 321: 926-929.

Evans, M. R., Grimm, V., Johst, K., et al. 2013. Do simple models lead to generality in ecology? *Trends in Ecology & Evolution*, 28(10): 578-583.

Farrell, A. P. and Brauner, C. J. 2009. Fish Physiology, Vol. 27: Hypoxia. Academic Press, London.

Galic, N., Sullivan, L. L., Grimm, V., and Forbes, V. E. 2018. When things don’t add up: quantifying impacts of multiple stressors from individual metabolism to ecosystem processing. *Ecol. Lett.*, 21(4): 568-577.

Goussen, B., Rendal, C., Sheffield, D., Butler, E., Price, O. R., and Ashauer, R. 2020. Bioenergetics modelling to analyze and predict the joint effects of multiple stressors: Meta-analysis and model corroboration. *Sci. Total. Environ.*, 749: 141509.

Grear, J. S., O’Leary, C. A., Nye, J. A., Tettelbach, S. T., and Gobler, C. J. 2020. Effects of coastal acidification on North Atlantic bivalves: interpreting laboratory responses in the context of *in situ* populations. *Mar. Ecol. Prog. Ser.*, 633: 89-104.

Gruber, J. 2011. Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Phil. Trans. R. Soc. A*, 369: 1980-1996.

Holling, C. S. 1966. The strategy of building models of complex ecological systems. In: Systems Analysis in Ecology. (K. E. F. Watt, Ed.) Academic Press. Pp. 195-214.

Howarth, R., Chan, F., Conley, D. J., Garnier, J., Doney, S. C., Marino, R., and Billen, G. 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Front. Ecol. Environ.*, 9(1): 18-26. doi: 10.1890/100008

Jager, T. 2018. DEBkiss: A Simple Framework for Animal Energy Budgets. Version 2.0. Leanpub: <https://leanpub.com/debkiss_book>.

Jager, T., Martin, B. T., and Zimmer, E. I. 2013. DEBkiss or the quest for the simplest generic model of animal life history. *J. Theor. Biol.*, 328: 9-18.

Jager, T., Ravagnan, E., and Dupont, S. 2016. Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points using a DEB modelling approach. *J. Exp. Mar. Biol. Ecol.*, 474: 11-17.

Jusup, M., Sousa, T., Domingos, T., Labinac, V., Marn, N., Wang, Z., and Klanjšček, T. 2017. Physics of metabolic organization. *Physics of Life Reviews*, 20: 1-39.

Kamler, E. 2008. Resource allocation in yolk-feeding fish. *Rev. Fish. Biol. Fisheries*, 18: 143-200.

Klahre, L. E. 1997. Countergradient Variation in Egg Production Rate of the Atlantic Silverside *Menidia menidia*. [Master’s thesis]. Stony Brook University.

Kooijman, S. A. L. M. 2010. Dynamic Energy Budget Theory for Metabolic Organisation. Cambridge University Press, Cambridge.

Kooijman, S. A. L. M. 2018. Models in stress research. *Ecol. Complex.*, 34: 161-177.

Kooijman, S. A. L. M., and Metz, J. A. J. 1984. On the dynamics of chemically stressed populations: The deduction of population consequences from effects on individuals. *Ecotoxicology and Environmental Safety*, 8(3): 254-274.

Kooijman, S. A. L. M., Lika, K., Augustine, S., Marn, N., and Kooi, B. W. 2020. The energetic basis of population growth in animal kingdom. *Ecol. Model.*, 428: 109055.

Kramer, D. L. 1987. Dissolved oxygen and fish behavior. *Environmental Biology of Fishes*, 18: 81-92.

Lavaud, R., Filgueira, R., and Augustine, S. 2019. The role of Dynamic Energy Budgets in conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi: 10.1093/conphys/coab083

Lavaud, R., Filgueira, R., and Augustine, S. 2021. The role of Dynamic Energy Budgets in conservation physiology. *Conserv. Physiol.*, 9(1): coab083. doi: 10.1093/conphys/coab083.

Letcher, B. H. and Bengtson, D. A. 1993. Effects of food density and temperature on feeding and growth of young inland silversides (*Menidia beryllina*). *J. Fish Biol.*, 43: 671-686.

Martin, B. T., Jager, T., Nisbet, R. M., Preuss, T. G., and Grimm, V. 2013. Predicting Population Dynamics from the Properties of Individuals: A Cross-Level Test of Dynamic Energy Budget Theory. *The American Naturalist*, 181(4): 506-519.

Martin, B. T., Heintz, R., Danner, E. M., and Nisbet, R. M. 2017. Integrating lipid storage into general representations of fish energetics. *Journal of Animal Ecology*, 86: 812-825.

Maxime, V., Pichavant, K., Boeuf, G., and Nonnotte, G. 2000. Effects of hypoxia on respiratory physiology of turbot, *Scophthalmus maximus*. *Fish Physiology and Biochemistry*, 22: 51-59.

May, R. M. 2001. Stability and Complexity in Model Ecosystems. 2nd Edition. Princeton University Press.

Miller, S. H., Breitburg, D. L., Burrell, R. B., Keppel, A. G. 2016. Acidification increases sensitivity to hypoxia in important forage fishes. *Mar. Ecol. Prog. Ser.*, 549: 1-8.

Muller, E. B., Nisbet, R. M., and Berkley, H. A. 2010. Sublethal toxicant effects with dynamic energy budget theory: model formulation. *Ecotoxicology*, 19: 48-60.

Nicieza, A. G. and Metcalfe, N. B. 1997. Growth compensation in juvenile Atlantic salmon: Responses to depressed temperature and food availability. *Ecology*, 78(8): 2385-2400.

Ninness, M. M., Stevens, E. D., and Wright, P. A. 2006. Removal of the chorion before hatching results in increased movement and accelerated growth in rainbow trout (*Oncorhynchus mykiss*) embryos. *J. Exp. Biol.*, 209: 1874-1882.

Nisbet, R. M., Gurney, W. S. C., Murdoch, W. W., and McCauley, E. 1989. Structured population models: a tool for linking effects at individual and population level. *Biol. J. Linn. Soc.*, 37: 79-99.

Nisbet, R. M., Muller, E. B., Lika, K., and Kooijman, S. A. L. M. 2000. From molecules to ecosystems through dynamic energy budget models. *Journal of Animal Ecology*, 69: 913-926.

Nonnotte, G., Maxime, V., Truchot, J. P., Williot, P., and Peyraud, C. 1993. Respiratory responses to progressive ambient hypoxia in the sturgeon, *Acipenser baeri*. *Respir. Physiol.*, 91: 71-82.

O’Donnell, J., Dam, H. G., Bohlen, W. F., Fitzgerald, W., Gay, P. S., Houk, A. E., Cohen, D. C., and Howard-Strobel, M. M. 2008. Intermittent ventilation in the hypoxic zone of western Long Island Sound during the summer of 2004. *J. Geophys. Res.*, 113: C09025.

Polymeropoulos, E. T., Elliott, N. G., and Frappell, P. B. 2017. Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins. *Comp. Biochem. Physiol. A*, 213: 28-35.

Pousse, É., Munroe, D., Hart, D., Hennen, D., Cameron, L. P., Rheuban, J. E., Wang, Z. A., Wikfors, G. H., and Meseck, S. L. 2022. Dynamic energy budget modeling of Atlantic surfclam, *Spisula solidissima*, under future ocean acidification and warming. *Mar. Environ. Res.*, 177: 105602. https://doi.org/10.1016/j.marenvres.2022.105602

Rabalais, N. N., Turner, R. E., Díaz, R. J., and Justić, D. 2009. Global change and eutrophication of coastal waters. *ICES J. Mar. Sci.*, 66(7): 1528-1537.

Richards, J. G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *J. Exp. Biol.*, 214: 191-199.

Russell, N. R., and Wootton, R. J. 1992. Appetite and growth compensation in the European minnow, *Phoxinus phoxinus* (Cyprinidae), following short periods of food restriction. *Environ. Biol. Fishes*, 34: 277-285.

Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., and Nye, J. A. 2020. Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish *Menidia menidia*. *J. Exp. Biol.*, 223: jeb228015. doi: 10.1242/jeb.228015

Sibly, R. M., Grimm, V., Martin, B. T., Johnston, A. S. A., et al. 2013. Representing the acquisition and use of energy by individuals in agent-based models of animal populations. *Methods in Ecology and Evolution*, 4: 151-161.

Smallegange, I. M., Caswell, H., Toorians, M. E. M., and de Roos, A. M. 2017. Mechanistic description of population dynamics using dynamic energy budget theory incorporated into integral projection models. *Methods in Ecology and Evolution*, 8: 146-154.

Testa, J. M., Murphy, R. R., Brady, D. C., and Kemp, W. M. 2018. Nutrient- and Climate-Induced Shifts in the Phenology of Linked Biogeochemical Cycles in a Temperate Estuary. *Front. Mar. Sci.*, 5: 114.

Thomas, Yoann., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G. M., and Pecquerie, Laure. 2019. Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB) theory. *J. Sea Res.*, 143: 231-242.

Wei, L.-Z., Zhang, X.-M., Li, J., and Huang, G.-Q. 2008. Compensatory growth of Chinese shrimp, *Fenneropenaeus chinensis* following hypoxic exposure. *Aquacult. Int.*, 16: 455-470.

Wood, C. M. 2018. The fallacy of the *P*crit – are there more useful alternatives? *J. Exp. Biol.*, 221: jeb163717. doi: 10.1242/jeb.163717

Zhu, C.-D., Wang, Z.-H., and Yan, B. 2013. Strategies for hypoxia adaptation in fish species: a review. *J. Comp. Physiol. B*, 183: 1005-1013.