Chapter 4 Draft

**Abstract**

**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. Understanding the mechanistic responses to hypoxia can help predict how tolerant fishes will be to intensifying hypoxic zones and how their sensitivity to predation and additional environmental stressors could change.

When targeted conservation action is desired, risks associated with stressors are important to quantify at the population-level because management actions act at this. While many laboratory experiments have measured physiological responses at the individual-level, additional steps must be taken to translate demographic rates like recruitment and reproductive investment in the next generation. 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 Grear et al. 2020 and maybe references within).

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 mortality (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 (Martin et al., 2013; Smallegange et al., 2017). These capabilities make DEB theory an excellent tool for enhancing the utility of experimental stressor 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). While in the standard DEB model reserve controls embryonic growth and hatch timing, DEBkiss deals with this stage using a state variable for egg buffer mass. Body size increases as egg buffer mass (yolk) is converted into structure and used for somatic maintenance, and hatching occurs when the egg buffer mass reaches zero (Jager et al., 2013). A potential downside to not using reserve is low resolution for modeling fluctuations in food level on small time scales, but this should not be a concern when working with constant feeding over time or when small changes in feeding are not vital to the research question, the model has clear assumptions for sustained starvation (Jager, 2018). The lack of reserve also means that DEBkiss is best 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). DEBkiss also differs from standard DEB theory by using body size thresholds to trigger life stage transitions, while DEB theory does this by having a state variable for ‘maturity’ (Kooijman, 2010; Jager et al., 2013).

We used a DEBkiss model to identify the bioenergetic mechanisms underlying observed growth and survival effects of hypoxia in early life stages of the Atlantic silverside, *Menidia menidia*. In a series of experiments, *M. 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 acidfication (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. At the same time, higher summer temperatures and precipitation in some regions will intensify stratification that separates low-oxygen water from surface oxygen diffusion. Using DEB theory to model the metabolic mechanisms behind the early-life responses to chronic hypoxia can help build understanding of full-life consequences for individuals and the life history traits that feed into population dynamics (Nisbet et al., 2000; Lavaud et al., 2021). Currently the species is tolerant enough that population declines are not a concern, but without knowledge of the mechanisms of early life impacts it is hard to predict whether this will change under increased hypoxia duration or with additional stressors (Baumann, 2019). Hypoxia is a widespread condition that often co-occurs with other stressors, but logistical constraints generally prevent the experimental testing of more than a handful of levels of two or three different stressors at once. A DEB model of hypoxia effects could be incorporated into future models with other stressors, such as acidification or contaminants, because knowing the underlying mechanisms can help researchers predict how multiple stressors interact without having to conduct enormous multistressor experiments and sacrifice large numbers of animals. Furthermore, the DEBkiss framework is simple enough to be adapted to other species and the types of data we used – growth and survival – are some of the most commonly measured variables in laboratory experiments, so this method could easily be applied to other species of ecological or commercial importance.

We aimed to explain with DEB processes the observed hypoxia effects on early life *M. menidia* growth, survival, and hatching. 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 a hypoxia-based stress function parameterized to replicate the early-life data for three low DO treatments. We evaluated the extent to which each parameter or combination of parameters 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). However, this behavior is impossible in embryos and the tendency of larvae to attempt this (successfully or not) has not been documented. 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 as well 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 for growth 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. This would lead to a smaller hatch size and slower growth post-hatch.

Maximum assimilation may best explain the observed hypoxia effects. 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 can not explain the observed hypoxia impacts on *M. menidia* hatch survival, timing, and size (Cross et al., 2019) because embryos do not yet feed. 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). Our fitted survival parameter for embryo mortality is greater than that of larvae. If assimilation rate 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 maximum assimilation rate 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 affect mortality after hatching, so using the stress function on assimilation and post-hatch mortality parameters may be necessary to fully replicate the observed hypoxia data.

**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*); these fractions are constant throughout the life cycle (Figure 1). 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*):

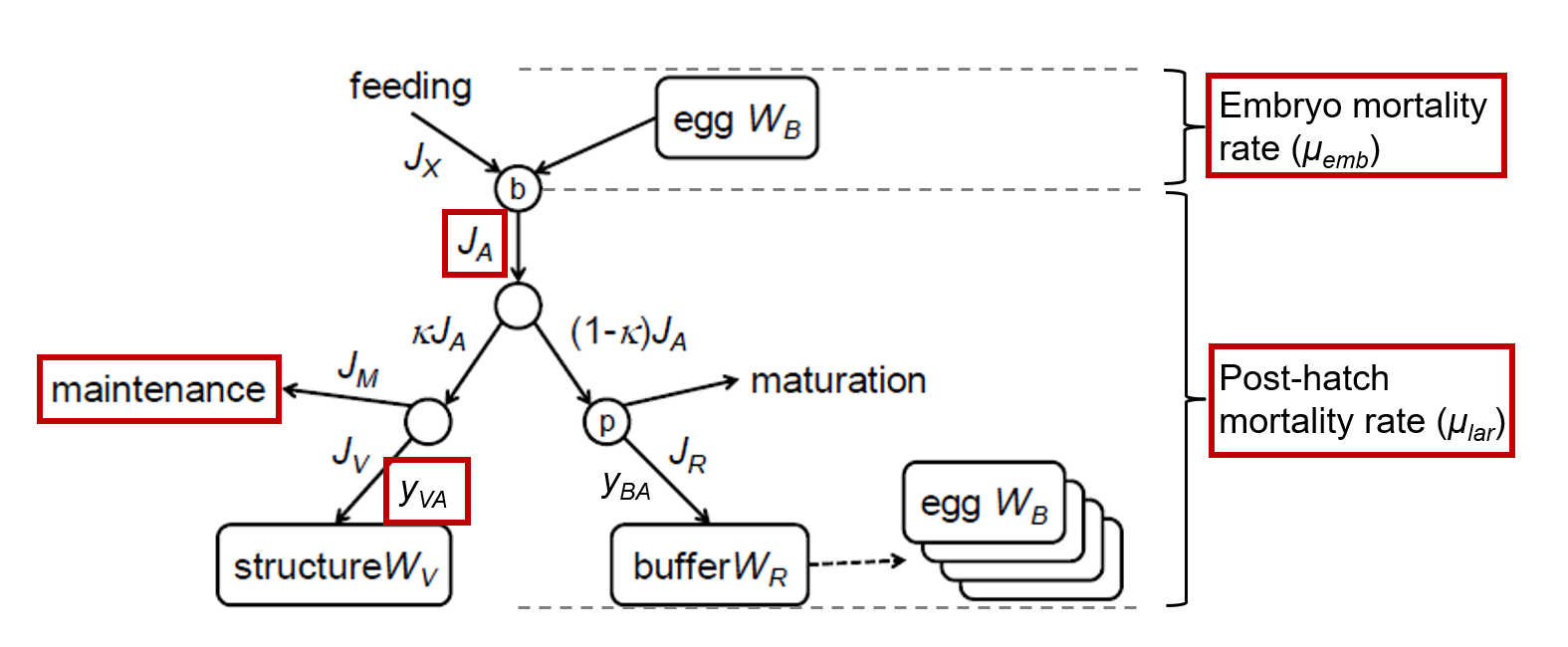
The differential equation for growth is equal to *JV*. For juveniles, the non-somatic fraction of assimilates is spent on maturation, or increasing complexity through gonad development. Once the mass at puberty is reached (*WVp*), reproductive flux (*JR*) toward egg production begins in adults with a conversion efficiency *yBA*. Although *M. menidia* have a distinct larval and juvenile stage, here the energy budget of each stage is assumed to be identical and both are referred to as the juvenile stage. 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.

,

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 data was 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. We fitted mortality parameters for embryos and post-hatch fish (*μemb* and *μlar*) to data for survival to hatching and larval/juveniles survival (Figure 1). In addition to allowing an alternative outcome to hatching when the egg buffer is depleted, this allowed us to examine survival as a consequence of hypoxia effects on the energy budget. 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. It lacks a state variable for maturity that triggers changes between life stages, instead using a constant size at puberty to specify when reproduction is initiated. 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 fully depleted immediately before hatching instead of following reserve dynamics of the standard DEB model.



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

*Data*

For the base model we calculated and fitted parameters based on total length over time, initial egg buffer mass, time from fertilization to hatching (when egg buffer mass equals zero), cumulative egg production over time, and proportion surviving since fertilization over time. This allowed us to estimate length at puberty (*LVp*), which in this model is the length at the age 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). We borrowed data on change in larval dry weight over a period of starvation from the closely related species *M. beryllina* (Letcher and Bengtson, 1993). We used the rate of decrease in dry weight during starvation to approximate maintenance costs (*JvM*). The total length data allowed us to estimate *JaAm* and *yVA* by adjusting these parameters to simulate a growth curve similar to the data, fix *JaAm* to a reasonable value based on ultimate length, then estimate *yVA* using the BYOM solver. All datasets came from experiments in which fish were fed *ad libitum* so *f* was set to 1.

Total length data came from three studies. Length at hatching and 15 days post-hatching (dph) came from a study that reared *M. menidia* offspring in three 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. The study featured two additional experiments that exposed offspring to fluctuating oxygen and carbon dioxide (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.

Cumulative egg production over time was also obtained from 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. We only used data from control fish. Data for time to hatching (i.e., time at which 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 levels of a study on the effects of different temperatures and CO2 levels on *M. menidia* early life survival from several experiments (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 1.** DEBkiss parameters, their abbreviations, and their fixed or estimated values. Units are given with the value unless the parameter is a unitless ratio.

|  |  |  |  |
| --- | --- | --- | --- |
| **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 |

*Base Model Calibration*

We used experimental data on *M. menidia* and the closely related inland silverside *M. beryllina* to calculate core DEBkiss parameters, estimated three parameters by fitting them to data, and fixed parameters for which we had insufficient data to calculate or estimate at suggested values (Jager, 2018). 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. 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 negative log likelihood.

Before estimating any parameters with the optimization described above, we ran simulations of the predicted data with a set of default parameters and parameters sourced 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 solver. We estimated *yVA* then fixed its value as the estimated value to estimate *JaAm*. Both of these parameters affect growth and egg buffer depletion so they could not be estimated simultaneously, but we did not have sufficient data to calculate them as we did *JvM*. 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.

*Hypoxia Stress*

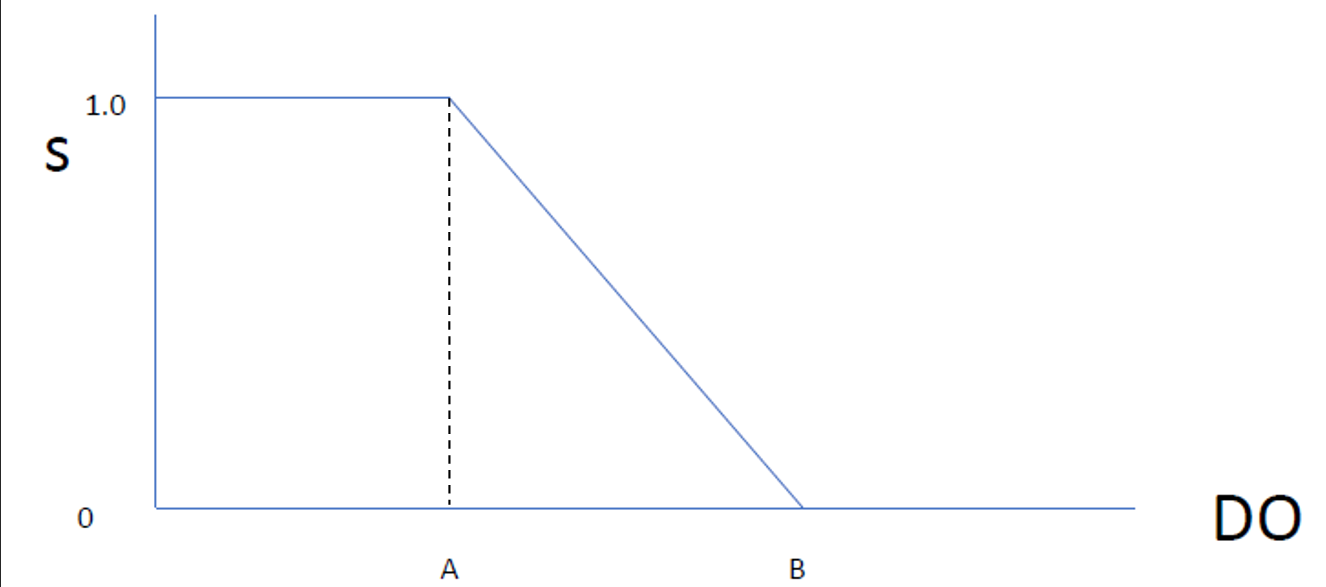
We applied a stress function to several primary parameters (Figure 1) to attempt to explain observed differences in *M. menidia* length, hatching, and survival between experimental oxygen treatments (Cross et al., 2019). To summarize the experimental data on chronic hypoxia effects, the mean values of each data type for the different oxygen treatments are listed in Table 2. We used the parameter values from the base model that contained full life data and altered one or more parameters at a time with an oxygen-dependent stress variable, then fitted the model to data for only the first 136 days. 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 and AIC 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.

The stress function calculated a stress variable (*s*) that increased linearly with DO between an upper and lower oxygen threshold, *A* and *B* (Figure 2):

The stress variable was applied to the parameter(s) of interest using functions that either increased or decreased the parameter with increasing stress, depending on the hypothesis for each parameter. We increased the parameters *JvM*, *μemb*, and *μlar* with the stress function by replacing the parameter (p) with:

The parameters we decreased with the stress function were *JaAm* and *yVA* by replacing them with:

Because the thresholds *A* and *B* affect the slope of the stress function and the location of the DO treatments within the window, changing *A* and *B* affected the fit to the data for each DO level as evidenced by the NLL in simulations (without estimation turned off). To find the best values of *A* and *B* for each parameter or combination of parameters according to NLL and AIC, we set *A* and *B* as primary parameters and estimated them. Once we found the best *A* and *B* for each parameter and pair of parameters, we compared the AIC between each stress function scenario to determine which one best fits the data and, therefore, which DEB processes best explain the hypoxia effects observed in experiments.



**Figure 2.** The increase in stress (*s*) from 0 to 1 with decreasing DO from upper threshold *B* to lower threshold *A*. This stress variable is implemented through functions to increase or decrease one or more parameters based on level of stress from DO.

**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).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **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% |

**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 data types. 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 data type).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Impact on predicted values of: | | |  |
| Parameter | Hypothesized hypoxia effect on parameter | Total length (mm) | Time to hatching | Survival proportion | Matches data? |
| *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

Description automatically generated

**Figure 3.** 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 (*JaAm*), maintenance (*JvM*), and combined embryo and post-hatch mortality rates (*μemb* and *μlar*) as examples to show how we selected DEBkiss parameters that would influence at least one of the data types that was impacted by hypoxia in experiments. 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 depletion (time to hatch). The observed and predicted data for full life span and early life are plotted in Figure 4.

Diagram

Description automatically generated

**Figure 4.** Predicted and observed data for the base DEBkiss model of *M. menidia*.

*Hypoxia Stress*

The best model of experimental hypoxia effects on *M. menidia* early life stages had correction factors applied to *yVA*, *μemb*, and *μlar*. This model met the initial criteria of affecting all three response variables (total length, egg buffer mass, and survival) in the same direction as hypoxia affected them in experimental data. It also had a lower AIC than other models that met the initial criteria, with an AIC of 584.48. Adding a correction factor to *JvM* in addition to these three parameters reduced AIC negligibly to 584.38 so it was not considered to have improved the fit, and thus not beneficial enough to justify the added complexity. The values of *yVA*, *μemb*, and *μlar* when their respective correction factors are applied for each DO level are listed in Table 5.

**Table 4.** The estimated *K* value and AIC when the correction factors were applied to each parameter or combination of parameters.

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter(s)** | **Correction factor(s)** | **Estimated *K* [95% CI]** | **AIC** |
| *JaAm* | *c* | 1.698 [1.694-2.880] | 600.21 |
| *yVA* | *c* | 1.504 [1.196-3.972] | 601.96 |
| *JvM* | *c1* | 0.3634 [0.3040-0.4995] | 597.77 |
| *μemb* | *c1* | 0.6257 [0.4351-0.9920] | 584.59 |
| *μlar* | *c1* | 0.3028 [0.2009-0.4918] | 573.90 |
| *JaAm* + *JvM* | *c* + *c1* | 1.720 [1.716-2.867] | 600.14 |
| *yVA* + *JvM* | *c* + *c1* | 1.497 [1.214-3.795] | 601.84 |
| *JvM* + *μemb* | *c1* + *c1* | 0.5066 [0.3686-0.8370] | 581.55 |
| *JaAm* + *μemb* | *c* + *c1* | 1.698 [1.694-2.058] | 589.78 |
| *yVA* + *μemb* | *c* + *c1* | 1.318 [1.198-1.805] | 588.99 |
| *JvM* + *μlar* | *c1* + *c1* | 0.3543 [0.3012-0.4429] | 566.37 |
| *JaAm* + *μlar* | *c* + *c1* | 1.698 [1.694-2.294] | 594.93 |
| *yVA* + *μlar* | *c* + *c1* | 1.356 [1.195-2.040] | 594.34 |
| *μemb* + *μlar* | *c1* + *c1* | 0.7660 [0.5434-1.145] | 578.94 |
| *JaAm* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.698 [1.694-2.037] | 586.23 |
| *yVA* + *μemb* + *μlar* | *c* + *c1* + *c1* | 1.326 [1.195-1.779] | 584.48 |
| *JvM* + *μemb* + *μlar* | *c1* + *c1* + *c1* | 0.7091 [0.4752-1.086] | 577.74 |
| *JaAm* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.720 [1.716-2.056] | 586.35 |
| *yVA* + *JvM* + *μemb* + *μlar* | *c* + *c1* + *c1* + *c1* | 1.323 [1.215-1.777] | 584.38 |

Chart

Description automatically generated

**Figure 5.** Best fit of DEBkiss model to experimental data from four DO levels, selected based on a combination of a requirement that all three response variables’ predicted values are affected by the hypoxia correction factor and lowest AIC.

**Table 5.** The value of the DEBkiss parameters that best reproduce the hypoxia effects observed experimentally, calculated for each DO treatment level using the correction factors *c* and *c1* and the estimated value of *K* = 1.326.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Correction factor** | **7.7 mg L-1** | **4.2 mg L-1** | **3.1 mg L-1** | **2.7 mg L-1** |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
|  |  |  |  |  |  |

This

Assimilation

**References**

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.

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

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.

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.

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.

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.

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.

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.

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

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.

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.

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.

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.

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.