Chapter 4 Outline

**Rationale – why do we want to do this work?**

*Rationale for this specific approach*

Hypoxia is common in the early life environment of *Menidia menidia* and is expected to intensify with global warming (Cadigan and Fell, 1985; Breitburg et al., 2018). 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 and coinciding stressors (Baumann, 2019). It is important to unify the multiple physiological responses we have documented in order to quantify population-level consequences, and a DEB model builds the foundation to do so (Lavaud et al., 2021).

A primary mechanism by which the fish energy budget is thought to be impacted by hypoxia is reduced food consumption (Chabot and Dutil, 1999; Thomas et al., 2019). However, consumption effects do not explain the observed hypoxia impacts on *M. menidia* hatch survival and size (Cross et al., 2019) because embryos do not feed. For this reason it is necessary to put a particular focus on the early life energy budget and attempt to identify alternative DEB processes in *M. menidia* that are impacted by hypoxia.

*Big picture rationale*

Developing a model that incorporates physiological and energetic mechanisms of hypoxia effects creates a widely applicable tool that can be used not only for making population-level predictions of hypoxia effects, but also be incorporated into larger models of other stressor impacts such as acidification and contaminant effects. This type of work could be continued for *M. menidia* as a model species and ecologically important fish, or it could be modified to other species for which similar data are available.

**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, in juveniles and adults the flux of food (*JX*) is converted to assimilates with an efficiency *yAX* which is then allocated to a somatic fraction (*κ*) and a reproductive fraction (1-*κ*) which are constant throughout the life cycle. 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*. For juveniles, the non-somatic fraction of assimilates is spent on maturation, or increasing complexity through gonad development. Once the size at puberty is reached, reproductive flux (*JR*) toward egg production begins in adults with a conversion efficiency *yBA*. 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.

Like the standard DEB model (Kooijman, 2010), the DEBkiss framework specifies the inputs, relative allocation, and sinks of energy and mass in a general enough manner to be applied to most animals, although it does not apply to other forms of life such as plants. However, 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.

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. 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 is means survival is indirectly affected by the assimilation rate and conversion efficiency of assimilates into structure.

*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 with the BYOM package …

*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, which in this model is the length at the age at which egg production begins. We borrowed dry weight data from the closely related species *M. beryllina* from a study in which larvae were starved over a period of seven days (Letcher and Bengtson, 1993). We used the rate of decrease in dry weight during starvation to approximate somatic maintenance (CITATION where someone does this?). The total length data allowed us to estimate maximum assimilation rate and yield of structure from assimilates 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.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Symbol** | **Fixed or estimated** | **Value** |
| Max. area-specific assimilation rate | *JaAm* | Fixed | 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 | *LMp* | Fixed | 100 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 |

*Hypoxia Stress*

We applied a stress function to several primary parameters to attempt to explain observed differences in *M. menidia* length, hatching, and survival between experimental oxygen treatments (Cross et al., 2019). 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*:

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. To increase a parameter *p* each occurrence of the parameter in the model equations was replaced with:

To decrease a parameter *p* each occurrence of the parameter in the model equations was replaced with:

We used a DEBkiss model to simulate the response of *M. menidia* to oxygen levels from experiments and identify the DEB parameter(s) that, when adjusted with a stress function, allow the model to replicate observed differences in hatch length, hatch time, and survival.

We first estimated DEBkiss parameters for *M. menidia* using data, primarily from the early life stages, to calculate some parameters and estimating others by fitting the model to the data. The univariate datasets for the model are total length, reproduction, egg buffer mass, and survival over time. We also used data on length, dry weight, length at puberty, and food level in experiments to fix some parameters, and suggested values to fix primary parameters we did not have the data to estimate.

We used a stress function to modify a parameter (yield of structure on assimilates, *yVA*, the maximum area-specific assimilation rate, *JAMa*, and/or the embryo mortality rate, *μemb*) and run the model to see how well the predicted data (length, egg buffer depletion, and survival) match observed data for the corresponding treatments. The experimental data are summarized in Table 1. The stress function was based on Jager (2018) and further developed based on measured routine metabolic rates of embryos and larvae under steadily decreasing oxygen levels, which gave thresholds for oxygen levels below which the stress function would be turned on (above the threshold oxygen-related stress would not affect the parameter).

* + Could we try using a stress function on multiple parameters (either at once or separately), and see which ones let us get the closest fit to the experimental data?
  + Do we need to fix the parameter(s) the stress function is applied to?

Adding a stress function to reduce *yVA* as oxygen decreases will result in lower length-at-age during both the pre- and post-hatching stages. We also want the stress function to reproduce delayed hatching and reduced survival to hatching that we observed in experiments. A stress function for *μemb* would directly result in lower embryonic survival to hatching but not affect hatch timing or size, and it would not get at a mechanism for this (or perhaps the mechanism is general damage). Reducing *yVA*, on the other hand, delays hatching so with a constant *μemb*, the oxygen effect on *yVA* will lead to lower survival to hatching. A plausible reason for *yVA* to be reduced under hypoxia is a reduction in aerobic metabolism and increased reliance on anaerobic metabolism, which is less efficient and would therefore reduce the yield of structure from assimilates (Thomas et al., 2019).

Reducing the assimilation rate similarly reduces growth and delays hatching, indirectly reducing survival at hatching. Assimilation affects the shape of the growth curve differently than *yVA*, however, with a lower assimilation rate limiting ultimate length more abruptly while reducing *yVA* allows growth to continue increasing for longer.

Hypoxia may change assimilation efficiency and thus the overall assimilation rate, but the direction of the effect is species-dependent (reviewed in Thomas et al., 2019). Assimilation is when food and oxygen are transformed into reserve (or in DEBkiss directly into structure) and metabolic products. So with less oxygen, less assimilation can happen and more anaerobic metabolism is used instead (also leading to the effect on conversion efficiency described above).

**References**

Jager et al 2013 – DEBkiss or the quest for simplest

Jager 2018 – DEBkiss book

Letcher and Bengtson 1993