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 and Expected Results**

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).

**Table 1.** Summary of experimental data on *M. menidia* early life effects of hypoxia. Univariate datasets (multiple observations of a response variable over time as used in model) are shortened here to specific points in time (e.g. length at hatching and survival to hatching are part of larger datasets of length and survival over time) just to give an idea of the data we can use to compare between oxygen levels.

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|  | Normoxia (~8 mg l-1) | Suboxia (~4 mg l-1) | Hypoxia (~3 mg l-1) |
| Survival to hatching | 65-90% | 55-88% | 30-85% |
| Hatch time (egg buffer=0) | 6 days | 7 days | 9 days |
| Length at hatching | 5.1-5.5 mm | 4.5-4.6 mm | 4.1-4.4 mm |
| Larval length at 16 dpf |  |  |  |
| Larval survival to 16 dpf | 32-72% | 20-45% | 0-23% |