1. Direct modeling of O2 flows
   1. I am not sure if this would be useful because MO2 only changed below 2.7 mg/L (on average) so this would do nothing to explain effects on hatch timing, growth, and survival at 2.7 mg/L and above. There is also a difference in that the metabolic measurements applied acute hypoxia and the other experiments applied chronic hypoxia.
   2. The shift to O2 limited metabolism would not explain the effects because it happens at a lower oxygen level.
2. Use of a damage variable
   1. **Gene expression** to indicate which DEB fluxes could be affected
   2. **Direct biochemical information** such as lactate production. Either add an anaerobic pathway explicitly into the model or treat the anaerobic products as a measure of damage.
   3. **Metabolomics**. “Conventional wisdom among DEB workers is that data on intermediate metabolites (e.g. ATP) has little value because of their high turnover rate compared with the processes on which DEB models focus.” But Roger has been doing work with Louise Stevenson and Phil Antzsac where Phil identified important pathways through the metabolome.
3. Bringing in CO2?
   1. No, only as a discussion point because the data are too inconsistent to try and fit anything unless we just did a simulation. But also I want to be done with this and not add anything else on.

Goals

* Look for evidence for the three damage proxies.
* Understand how damage is quantitatively modeled and applied to a parameter.
* Once damage proxies or mechanisms are identified, match them up to one or more DEB parameter they would affect.

Damage Module Proxies

Hypoxia-inducible factor

* “Fish eggs are freely accessible and studies analyzing Hif expression in fish embryos during development have revealed that Hif proteins are not only controlling the hypoxic response, but are also crucial for proper development and organ differentiation.”
  + Could a limitation of this be responsible for effects? Hatching without complete development?
  + Could measures of Hif expression in fish embryos be used to parameterize a damage variable? Or would evidence of Hif expression suggest the opposite of damage is occurring? Hif helps them respond to hypoxia but are there downsides that it could be a proxy for?
  + Pelster and Egg, 2018, Hypoxia-inducible transcription factors in fish: expression, function and interconnection with the circadian clock. *J. Exp. Biol.*, 221: jeb163709
  + More info about Hif: Mandic et al., 2021. The evolutionary and physiological significance of the Hif pathway in teleost fishes. *J. Exp. Biol.*, 224(18): jeb231936
* “Hif-1α is known to control metabolism by regulating the expression of many glycolytic enzymes such as phosphofructokinase, pyruvate kinase, lactate dehydrogenase and hexokinase, and glucose transporters such as GLUT1 and GLUT3, thereby orchestrating glycolytic activity in adaptation to the availability of oxygen.”
  + LDH catalyzes the conversion of pyruvate to lactate when oxygen is absent/low, and at high concentrations of lactate it exhibits feedback inhibition, reducing the rate of conversion of pyruvate to lactate.
* Hif influences cardiovascular development and can affect gill surface area or structure to increase hypoxia tolerance.
  + But you still need some oxygen to build these things – maybe there is a critical threshold below which an increase in Hif expression or activity would not be able to help them survive. But under moderate hypoxia they are okay because of Hif.
* Hifs can also control expression of a gene that suppresses growth (igfbp-1), haematopoietic regulating hormone erythropoietin (epo, involved in blood formation), and a angiogenesis-promoting cytokine (vegf, involved in blood vessel formation).
  + Robertson et al. 2014. Hypoxia-inducible factor-1 mediates adaptive developmental plasticity of hypoxia tolerance in zebrafish, *Danio rerio*. This paper measured HIF-1 activation after acute hypoxia, and the genes related to it.
* Kajimura et al. 2005. Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. *PNAS*, 102(4): 1240-1245.
  + In zebrafish, hypoxia strongly induced the expression of insulin-like growth factor (IGF)-binding protein (IGFBP)-1, a secreted protein that binds IGFs in extracellular environments.
  + Used loss- and gain-of-function (knockdown and overexpression) approaches to show that elevated IGFBP-1 mediates hypoxia-induced embryonic growth retardation and developmental delay.
    - Then they linked this to HIF-1 in another study by looking at IGFBP-1 expression after exposure to CoCl2, which is known to induce HIF-1 (Kajimura et al., 2006).
  + Their oxygen levels were ~6.5 mg/L (normoxic) and 0.6 mg/L (hypoxic) at 28°C.
* **If we have IGFBP-1 expression for two oxygen levels, can we fit a model at a standard shape for such a function (linear or nonlinear?) and use this as a damage variable or other stress function.**
  + **Presumably, this would reduce the rate of assimilation of yolk or food into structure. Would assimilation stay the same and a greater proportion goes elsewhere (reduce conversion efficiency) or would assimilation decrease overall?**
  + **I think assimilation would decrease overall because hatching is delayed.**
  + **But I also think because they hatch at a smaller size maybe that means efficiency also decreases?**
  + **Maybe it doesn’t matter a lot because they have similar effects in this model.**
  + **But the reason they hatch at a smaller size is probably because they still have energy going towards SMR even if less is going towards growth or being taken in overall, so that means the efficiency is reduced right?**

Lactate concentration

* Barrionuevo, W. R., Fernandes, M. N., and Rocha, O. 2010. Aerobic and anaerobic metabolism for the zebrafish, *Danio rerio*, reared under normoxic and hypoxic conditions and exposed to acute hypoxia during development. *Braz. J. Biol.*, 70(2). <https://doi.org/10.1590/S1519-6984210000200027>
  + Measured lactate concentration, as well as growth, metabolism, and heart rate, of zebrafish embryos and hatchlings every 10 days up to 100dph, after rearing in two different oxygen levels.
  + Specifically, they started the embryos in normoxia or mild hypoxia, then each of these groups had a subgroup that was exposed to either normoxia or acute hypoxia.
  + **The embryos and 20 day old fish did not have any significant differences in lactate levels across treatments.**
  + However, they cite Nilsson and Östlund-Nilsson (2008) as saying **“large individuals exposed to extreme hypoxia present a clear advantage over smaller ones due to the fact that small fishes reach lethal levels of anaerobic end-products much faster because of their higher mass-specific metabolic rate.”** But that paper focuses on free swimming fish and says embryos are unable to regulate oxygen uptake because it depends on diffusion, and hypoxia tolerance decreases leading up to hatching as mass increases but surface area stays the same.
  + The results of this paper are evidence that early exposure to mild hypoxia better prepares fish for it later in life – more likely to survive, maintain metabolism at lower oxygen (lower Pcrit), maintain equilibrium (Ho and Burggren 2012).
    - But according to the Diaz and Rosenberg chapter of the Hypoxia volume of Fish Physiology, frequent acute bouts of hypoxia cause more physiological damage than chronic hypoxia.
* Wieser, W. 1995. Energetics of fish larvae, the smallest vertebrates. *Acta. Physiol. Scand.*, 154: 279-290.
  + **The capacity for anaerobic glycolysis develops gradually after termination of the yolk sac stage. Very little lactate is produced in embryos and yolk sac larvae of marine fish (Finn 1994).**
  + Exceptions:
    - Lactate peak at beginning of gastrulation in Atlantic cod, but not turbot and halibut (Finn 1994).
  + They plot energy expenditure as a function of wet weight in roach from Kaufmann (1990), assuming 0.45 Joules per µmol of O2.
  + **Need to thoroughly search the literature to see if there could be more exceptions**.
  + There are measurements of a killifish in diapause and an amphibious fish exposed to air and water, but these extreme examples don’t seem like good enough evidence that *Menidia* might also have anaerobic glycolysis during yolk sac stage.
  + Thorough searching so far has not found the evidence we would need.
  + Vodianitskyi et al (2021, Fisheries & Aquatic Life) looked at LDH in carp embryos exposed to different temperatures and oxygen levels, but not hypoxia in isolation it seems. They focused on temperature differences across ponds instead of looking at differences between ponds with dif oxygen levels.
  + Broberg and Kristoffersson looked at oxygen consumption and lactate accumulation in viviparous eelpout intra-ovarian embryos and young. Very different physiological situation.
  + The Fish Physiology chapter on early life hypoxia (Rombough 1988) indicates that embryos do use anaerobic glycolysis and produce lactate, so maybe there is enough evidence ...somewhere.
* Lactate or metabolic enzymes in larvae
  + Franklin et al 1996 – herring larvae and swimming activity
  + Gibb and Dickson 2002 – Halibut larvae and juveniles and swimming performance
  + Finn et al 1995 – respiration and lactate concentrations of embryos and larvae of Atlantic cod
  + Anderson and Podrabsky 2014 – The effects of hypoxia and temperature on killifish development, metabolic enzymes
* Any mode of action: food and oxygen gives assimilate, synthesizing unit,
  + Almost bypasses damage variable, more vague about the process
  + Synthesizing unit formulation in deb book

Metabolomics

* Is there a difference between metabolomics and measuring gene expression of metabolic enzymes?
  + Measures quantity or concentration of small moleculesinvolved in metabolism, cofactors, includes ATP and ADP, electron transport chain
  + Metabolites not enzymes
  + Concentrations relate to processes
  + Still gives a connection to pathways, but maybe tenuous connection
* Metabolomics studies exist for
  + Phoxinus lagowskii (a freshwater minnow) adults exposed to sustained and diel cycling hypoxia (Wang et al., 2023)
  + Cobia (Rachycentron canadum) adults’ intestinal tract exposed to chronic hypoxia (Yang et al., 2022)
  + Nothing on early life stages and hypoxia that I could find

Effect of hypoxia on yolk depletion? If not going to growth, where does it go? Homeostasis?

SMR – goes to maintenance of ion gradients, osmoregulation, constitutive rates of protein synthesis. Additional metabolism above SMR goes to things like physical activity, food digestion, or reproduction. SMR is generally less sensitive (lower threshold) to hypoxia than RMR. (source: Wang et al., 2009, Hypoxia: Volume 27, Chapter 8)

“The Bohr effect depends on an arteriovenous pH gradient sustained by respiratory acidosis (CO2), whereas the Root effect is activated by fixed acid (lactate) and, unless localized in specific retail tissues, may seriously compromise effective oxygen transport in hypoxic situations.” (source: Wells et al., 2009, Hypoxia: Volume 27, Chapter 6)

* I might be able to just refer to the Bohr effect in my papers on OA

Make a concise summary of notes to send Roger

He will work on synthesizing unit equations