Notes for meeting with Roger

June 1, 2023

Notes from emails and defense

* Want to bring in the idea that below a certain level of a stressor there can be a breaking point or reversal of how something works, e.g. an enzyme’s activity crashes
* Toxic metabolic products from anaerobic metabolism causing damage
* Use oxygen consumption, changes in growth from metabolic measurements
* Is there a mass balance calculation that could get at the magnitude of the shift to anaerobic metabolism?
* Simpler: hypothesize chemical reactions for assimilation and growth to get explicit formulae for changes in yield (yVA) with onset of oxygen limitation

Notes on new ideas

* How to include a mechanism for mortality? A certain amount of damage causes a certain mortality rate? Fit it so that damage at lowest oxygen level can cause 100% mortality somehow.
* Does our model allow for metabolic acceleration for embryo or larval stage? Could this explain why the best fitting yVA is so low?
* Simple mass balance equation:
  + Consumption = metabolism + waste + growth
  + Consumption = (resp + active metabolism + SDA) + (egestion + excretion) + (somatic growth + gonad production)
  + For embryos, consumption = resp + active metabolism + waste + somatic growth
  + For larvae, consumption = resp + active metabolism + SDA + waste + somatic growth
  + If we can estimate the metabolism, growth, and assimilation (feeding?) we can get yield (what percent did not go to waste)?
* DEBkiss – where does waste fit in? Is it the remainder of what doesn’t go to yield? What is the difference between feeding flux and assimilation?
* Enzyme activity
  + In *Fundulus grandis* enzyme activity of glycolysis and glycogen metabolism were strongly suppressed by hypoxia exposure in skeletal muscle, but in liver several enzymes increased their activity.
  + In *Cyprinodon variegatus* larvae hypoxia depresses CYP1A induction and enhances DNA damage caused by exposure to crude oil.

Notes for meeting

* I won’t have time to get new results for my talk but I can present something on what we have so far and what we are working on next.
  + We got good fits to growth and egg buffer mass but for survival there is no mechanism, just changing the mortality rate to fit the data.
* How would damage be different than correction factor? What would we apply the damage to?
  + Would it be helpful to see how gene expression in other species was affected by hypoxia?
    - *Core metabolism is pretty similar across species, pathways at least. If can see any upregulation of genes associated with enzymes that would clear lactate or equivalent substances (how is lactate cleared?)*
    - *Some evidence of a direction to push rather than just speculating would be good in talk. But can still talk to people who may know more about hypoxia than said in papers.*
    - *We have demonstrated with changes in model params can capture some of what’s going on, now need suggestions of how to nail things down.*
    - *Will I be talking about co2? Can set the scene with what I have already got from defense.*
    - *Cyp1a is implicated in removing toxins, protective proteins, has complicated effects can worsen toxicant effects as well.*
    - *With mollusks its mostly about calcium carbonate.*
* I started thinking on and reading about the idea of trying to calculate yield from the info we have but didn’t get far, one near future step is figuring out the exact data needs.
  + Is yield/conversion efficiency the same thing as assimilation efficiency?
    - I think no, but DEBkiss seems to disregard the assimilation efficiency by letting you directly estimate or calculate assimilation rate rather than ingestion rate. Assimilation is proportional to ingestion, so if assimilation goes down it could be either due to lower ingestion or lower assimilation efficiency.
  + Assimilation efficiency is the percent that is not going to egestion (defecation)
  + What does assimilation mean in embryos? What does conversion efficiency mean? If the yolk is already reserves, but assimilation is defined as the conversion of food into reserve, then the assimilation efficiency is 100% but conversion into growth is not. What happens to the waste in the yield coefficient?
    - *Daphnia work – moved to using carbon as currency,*
    - *Don’t worry about distinction between assimilation efficiency and growth efficiency (conversion)*
    - *With aerobic processes the waste is CO2 primarily, converting reserves into biomass requires ATP, with aerobic you get ~36 per unit of food but for anaerobic you get 2. So with anaerobic it may not be expressed in terms of CO2 but instead lactate, etc. Switch from food limited to oxygen limited.*
    - *Does Lavaud’s emphasis on ingestion come from experimental observations of feeding or assimilation efficiency?*
    - *What would I need to know to make this calculation easy. Ask “why is this hard?” we don’t have consumption for each hypoxia level.*
    - According to Reviewer #1 of Giacoletti and Sarà (Predicting the multiple effects of acidification and hypoxia on *Mytilus galloprovincialis* life history traits), “In the DEB framework, respiration is emphatically not the same as maintenance, but also include energetic overheads, such as that of growth. Respiration is a function of the commitment rate in DEB, of which maintenance could be a minor part, depending on size and nutritional status of the animal.”
  + The typical way to calculate that would be to measure food consumed and egestion, so how can we get at it without having either?
    - Metabolism = Consumption – rest of energy budget

Data we have for getting at conversion efficiency and/or anaerobic metabolism

* Metabolic rates of individuals across a range of DO levels (acute hypoxia)
* Metabolic rates of fish reared in one of four DO levels (chronic hypoxia)
* Total length of larvae from all of these experiments – converted to DW using an equation
* Total length at 2 and 5 dph (not for same individuals though)
* Differences in growth between chronic DO treatments (Cross et al., 2019)

Get in touch later next week about what to include/not include, other sticking points, if needed.

Papers to read

Vanderplancke et al. (2015), Exposure to chronic moderate hypoxia impacts physiological and developmental traits of European sea bass (*Dicentrarchus labrax*) larvae.

* Chronic moderate hypoxia (40% air sat) between 30 and 38 dph
  + Before metamorphosis they are offshore so wouldn’t experience hypoxia.
* Wanted to investigate survival, growth rates, energy metabolism (citrate synthase and cytochrome-c oxidase activities), and maturation of digestive function (pancreatic and intestinal enzyme activities: trypsin and amylase, and alkaline phosphatase and amino-peptidase-N)
  + “[Hypoxia effects] occur due to disturbances in a series of programmed, highly intricate, and energy-consuming processes.” – what does programmed mean?
* Results:
  + No significant increase in mortality
  + Growth rate was significantly reduced
  + CS/COX activity ratio was significantly reduced, i.e. there was less CS activity relative to COX or more COX relative to CS.
    - CS and COX are mitochondrial enzymes and their fluctuations provide information on the properties and numbers of mitochondria present.
    - CS/COX ratio estimates changes in mitochondrial size and shape in response to energy-demand conditions.
  + Intestinal enzyme activities were lower under hypoxia
  + The digestive enzyme activities are indicators of developmental status.
* Conclusions:
  + The larvae cope with hypoxia by reducing processes that are costly in energy (digestion) and regulate mitochondria functions in order to respond to energy-demand conditions.
  + Result is delayed maturation of digestive function.
* Other info
  + In intro they say that saltwater fish larvae are more sensitive to hypoxia than freshwater larvae, because they hatch at earlier developmental stages.
  + Particular sensitivity during metamorphosis because metabolic rates increase just before and after the flexion stage, which is a time of molecular, physiological, and behavioral change.
    - Their experiments are several days after flexion, but ours are before flexion.
  + After returning to normoxic conditions they showed compensatory growth.

Dasgupta et al. (2016). Hypoxia depresses CYP1A induction and enhances DNA damage, but has minimal effects on antioxidant responses in sheepshead minnow (*Cyprinodon variegatus*) larvae exposed to dispersed crude oil.

Lavaud et al. (2019). Modeling the impact of hypoxia on the energy budget of Atlantic cod in two populations of the Gulf of Saint-Lawrence, Canada.

Fish Physiology, Vol. 27, Ch. 10.

Fish Bioenergetics Section 1 Chapter 2 – Core Processes in Bioenergetics

Wisconsin Fish Bioenergetics Model documentation

Thomas et al. (2019). Effects of hypoxia on metabolic functions in marine organisms: Observed patterns and modeling assumptions within the context of Dynamic Energy Budget (DEB) theory.

Nilsson and Östlund-Nilsson (2008). Does size matter for hypoxia tolerance in fish?

Next steps forward:

* How would a damage module be different than the correction factors we used?
* What process(es) would the damage variable for hypoxia affect?
  + Gene expression
    - HIF-1?
  + Enzyme activity
    - CS/COX activity ratio in European sea bass later larvae, from higher COX activity rather than lower CS activity, suggests lower citrate synthesis over respiratory chain capacities of mitochondria and modified size and shape of mitochondria. COX is the terminal electron acceptor of the electron transport chain. Reduced CS/COX ratio has been associated with reduced growth rate in saithe (Mathers et al. 1992).
    - In European seabass, trypsin-specific activity, was reduced in larvae, which suggests ingestion was impaired because trypsin activity is related to dietary protein intake after pancreatic maturation.
    - Higher amylase-specific activity in European seabass larvae, or the decline was delayed. Lower AP- and N-LAP-specific activities as well, all of which suggest impaired maturation of enterocytes (cells of intestinal lining).
  + Other molecular pathways/evidence
  + Other physiological pathways/evidence
    - Vertebral column deformities in yellowfin tuna (Wexler et al. 2011) and Atlantic salmon (Sanchez et al. 2011). This could affect survival.
    - Reduced food ingestion (Lakani et al. 2013; Pichavant et al. 2000, 2001; Thetmeyer et al. 1999). This could affect growth and survival.
    - Lactate clearance
* What parameter(s) of the DEBkiss model would be affected?
* Trying to quantify consumption or defecation under hypoxia, or get around it.
* How to get from gene expression or enzyme activity to damage?
  + At X oxygen level, fish have X change in gene expression or enzyme activity, and that causes X amount of damage? Or affects X process by X %?
  + How did they do this in other damage work?
  + Can blood lactate concentration be used the same as internal concentration of some other toxicant?
  + Remember that changing maintenance didn’t account for the changes – does this imply that energy is not going into lactate removal?
    - How is lactate (and other byproducts like protons) removed following or during hypoxia?
* What else do I want to include in my presentation that wasn’t included in defense?
  + Table of parameters and AICs (or at least explain how we used AIC)
  + Plot of correction factors
  + Include Jager’s stress functions as an extra slide in case there is a question about that
  + Another potential improvement could be not tying together the different parameters – have a different correction factor for each parameter instead of the same one, so that hypoxia can affect each parameter by a different magnitude. But this would change implication of AIC I think it would be truly adding an additional parameter for each parameter altered, so need to be aware of that.
  + Make sure I can explain why we used DEBkiss