Dissertation Defense Cheat Sheet

**Chapter 1**

Hypotheses

* Metabolism increases with temperature and with CO2.
* Metabolism decreases under low oxygen.
* Multiple combined stressors may have non-additive interacting effects.
  + Looking at interactions can help us understand the underlying physiological mechanisms because the different stressors may affect different processes.

Methods, assumptions, and possible improvements

* Used linear regression with continuous (rather than categorical) independent variables because not every treatment group was replicated at least once over time. This means no pairwise comparisons, but better predictive value.
  + Assumes linear relationships though.
* Future directions
  + Let oxygen go to zero so can assess acute hypoxia effects, Pcrit.

Results

* Temperature x CO2: temperature is significant, CO2 is not.
* DO x CO2: interaction is significant.
  + Mean RMR in high CO2 is about 30% higher than in control.
    - Suggests additional energy being used on acid-base balance.
  + As CO2 increases, RMR becomes more oxygen-dependent.
    - Suggests increased hypoxia sensitivity.
    - Pcrit is one way to measure hypoxia sensitivity, but this experiment we used static long term DO treatments instead of acute hypoxia for each individual.
* Overall conclusions
  + Embryos are more sensitive.
  + High CO2 by itself has little effect on metabolism, it is only through its interaction with DO that it does.
  + Now that we know Pcrit wasn’t affected by CO2 in embryos, an alternative explanation is that low DO inhibits the mechanisms for pH regulation so that they aren’t possible (as much), and oxygen demand is lower. Implication: silverside embryos can’t cope with high CO2 as well in low DO (as opposed to vice versa).
    - Low DO could eventually give them more Hb which can buffer pH.

Why it’s important

* Evidence for additional energy being spent on coping with high CO2, but only in embryos and not in every experiment.
* Evidence that hypoxia can counteract this and not allow additional energy to go to pH regulation. Also evidence that we need to further investigate the interaction between acidification and hypoxia.

Notes for committee discussion

* How might body size have affected results?

**Chapter 2**

Hypotheses

* Pcrit increases at high CO2.
* RMR increases with CO2.

Methods and assumptions

* This had treatment replicates in the form of completely independent tanks in Exp. 1 (Dana Hall, 24C) which only shared CO2 tank and air pump, and same water flowing into each separate rearing container in Exp. 2 (Flax, 22C).
* Can’t compare temperature effects because of different methods between the two experiments.
* Because of methodological differences, the
* Future directions
  + Quantify survival in the same experiment (I didn’t have time to do this or space for more individual embryos) so we can figure out if there may have been a survivor bias effect influencing their sensitivity.

Results

* Embryo RMR increases with CO2.
  + I think it was because it has higher CO2 at highest level, and in theory maybe the other experiment would have had this effect if the highest CO2 level was higher, but Baoshan says that doesn’t make sense.
  + May be a temperature interaction
    - In Chapter 1 the temperature experiment had no CO2 effect but the DO one, which was done at 20°C, did have one. Maybe lower temperatures have more of a response? This
      * This would correspond well to ionocyte results.
* 5dph RMR decreases at high CO2.
  + Maybe a survivor effect
  + Maybe after hatching they are more tolerant, can use buffers or something else less energetically demanding to regulate pH. Reduced metabolism could be a stress response though.
* No significant effect of CO2 on embryo Pcrit
  + So this does not agree with our hypothesis and does not help explain the interaction in Chapter 1.
    - What does it mean then?
* After hatching, Pcrit decreases with increasing CO2.
  + Possible survivor effect.
  + What else?
* Transient low-DO increase in MO2
  + No significant effects of CO2
  + More frequent in larvae, and decreased slightly with increasing CO2 but not significant.
* Oxyconformity in embryos
  + Experiment 2 had significantly more embryonic oxyconformity in high CO2 treatments. This could have been a higher Pcrit which would mean we do confirm the hypothesis, but unfortunately I didn’t start respiration at a higher DO level.
* Overall conclusions
  + Embryos still more sensitive than larvae.
  + Temperature seems to influence CO2 effects.
  + Silversides may have an advantage when dealing with combined CO2 and hypoxia, or at least acute hypoxia, and somehow save energy.

Why it’s important

* High CO2 evidently does not increase hypoxia sensitivity, which is important for Chapter 4 and for the outlook for this species under global change.

Notes for committee discussion

* I didn’t calculate alpha because of low-DO transient increase in MO2. The Pcrit had to be identified with more attention because the segmented regression was finding multiple breakpoints and false Pcrits. So I would need to go back and use Pcrit and the DO at which MO2 = 0 to get alpha I think. Or is it just assumed that (0,0) is the other point to get the slope?
* Effect of oxyconformity on results?

**Chapter 3**

Results

* Shift in distribution at hatching: before hatching, mostly low ionocyte density with strong positive skew, after hatching more normally distributed with a higher mean.
  + Means before hatching only a few have a greater capacity for pH regulation with ionocytes but this small amount is closer to after hatching.
  + Then at hatching either the ionocytes proliferate quickly or the lowest ionocyte density ones die off.
* Embryo results: interaction between temperature and CO2
  + Ionocyte density decreased with increasing temperature
  + Ionocyte density increased with CO2 but mainly at low temperature
  + CO2 effect stronger on yolk sac, suggests this is an important site for pH regulation before gills develop and activity begins.
  + Temperature interaction suggests that at low temperature, more energy available for creating ionocytes or the longer incubation time gives more time to differentiate ionocytes in response to env conditions.

Why it’s important

* The embryo results suggest that they are better able to adjust their ionocyte density to deal with high CO2 at lower temperatures, which could suggest that at higher temperatures they are less likely to have more ionocytes to help them survive high CO2 – for example, may explain the reduced survival late in the spawning season.

**Chapter 4**

Methods and Assumptions

* Assume same correction factor with same Z parameter (exponential coefficient) for multiple parameters at once (e.g. when do both mortality rates with c1).
* Might need to try putting in raw length data instead of means – survival could be having a disproportionate effect on the estimated Z. OR don’t assume same Z applies to both parameters, and have a different one for c1?
  + Should I have the same number of data points for each treatment? Remove the normoxic ones that aren’t repeated for hypoxia?
* Used AICc even though the sample sizes are greater than 40 because AICc converges with AIC at high sample sizes, so it made sense to use AICc. It also doesn’t really affect the results because there is only one free parameter in each scenario, so the small sample size correction is always the same.
* Assumption that correction factor c = 0 at 2.44, the embryonic Pcrit, which affects the shape of the relationship.

Results

* Best model according to AIC has the correction factor applied to yVA, mu\_emb, and mu\_lar.
* The one with sJM, yVA, mu\_emb, and mu\_lar was actually a little better but does not improve it enough to make it worth the added complexity.
* Next best was sJAm, mu\_emb, and mu\_lar, which has a very similar effect on the state variables.
  + This is because sJAm gives JA, which influences growth and depletion of egg yolk, while yVA also influences growth.
  + Reduced sJAm means less assimilation flux overall, which slows down egg buffer depletion, but the same amount ultimately goes to growth, it just takes longer to deplete the egg buffer and reach that same size at hatching.
  + Meanwhile, reducing yVA means less overall growth can come from the egg buffer, so size at hatching would be lower but in theory it should take the same amount of time to deplete the egg buffer. In the model though, changing yVA also changes hatch timing. WHY?
    - Because JA is calculated with not only f and sJAm, but also L2. L comes from WV, which comes from dWV, which comes from JV, which is influenced by yVA.
    - So the reduced body size at any given time from reduced yVA indirectly results in a lower assimilation flux.
    - More briefly put, the assimilation rate is size-specific and yVA affects size at time.
    - So this means that reducing yVA can also delay hatching by slowing the egg buffer depletion rate (assimilation).
* These results indicate that the hypoxia treatments reduced yVA by up to 58% and increased mortality rate by up to 73%.
* What about for the version with sJAm?
* The substantial improvement from adding correction factors to the mortality parameters indicates that the energetic allocation is not accounting for all of the hypoxia effects, at least for mortality. What could be causing this?

**Mistakes I already found**

* Chapter 2: Used wrong pCO2 values when plotting the data means (but the tables are correct and the analysis used ANOVA so actual pCO2 values don’t matter).
* Chapter 3: Figures show significance asterisks based on emmeans (estimated marginal means pairwise comparisons test), which is not valid for this dataset because not every treatment group is replicated. The figure caption also mentions this test.