Dissertation Introduction and Discussion

**Abstract**

**Background and Motivation**

* Describe global change and conditions in estuaries.
* Describe previously documented responses of *M. menidia* and other species a little bit (at least with examples relevant to what we measured).
* Ecological importance of *M. menidia* and its use as a model species.
* Explain the need to look at physiological mechanisms and bridge between levels of biological organization, or use models to look at whole life cycle and make more of experiments. Cite big picture type papers that talk about what is needed in this field.
* Maybe get a little into the possible physiological responses to each stressor.

**Objectives**

1. Quantify metabolic effects of three stressors in ELS with factorial combinations of stressors and microrespirometry
2. Refine understanding of metabolic effects by applying acute hypoxia and measuring critical oxygen level
3. Quantify temperature-dependent cellular mechanisms of acidification response in ELS by staining ionocytes
4. Use hypoxia as a case study in enhancing utility and understanding of experimental effects by identifying energetic mechanisms using a model that can ultimately connect physiology and life history to populations.

**Conclusions**

In Chapters 1 through 3 we gained understanding of physiological mechanisms by which *Menidia menidia* responds to elevated pCO2, which previous studies had shown to reduce growth and survival more in the early and late spawning season than in the middle (Baumann et al., 2018). Embryos at reared at 17°C had significantly higher ionocyte density in elevated pCO2 while those in other temperatures had little to no pCO2 effects, which could help explain the seasonal differences in sensitivity of growth and survival. Early in the season when temperatures are lower and slower embryos may be growing additional ionocytes as they acclimate to high pCO2, which could draw energy away from growth and, in some individuals, inhibit development of other systems needed to survive post-hatching. The elevated metabolism in the high pCO2 treatments in Chapter 1 also suggest additional energy is being used to exchange ions for acid-base balance, drawing it away from growth. Like previous findings, Chapters 1 and 3 also highlight how variable responses can be within and across experiments. Metabolic rates and ionocyte densities had high variance and metabolism was not significantly affected by pCO2 in the experiments that combined pCO2 and temperature treatments. These levels of natural variability highlight how tolerance may be facilitated by parental influences and a wide range of phenotypes (citations). [Chapter 2 results relevant in this paragraph?????]

A common element of Chapters 1 through 3 is that environmentally relevant levels of seawater acidification primarily impact *M. menidia* early life stages through interactions with hypoxia and temperature, rather than in isolation. This is consistent with findings in other species (citations). Wild *M. menidia* have historically experienced pCO2 levels in parts of their range that are similar to predictions for global ocean pH at the end of the century and beyond (citation IPCC? Baumann et al., 2014, Wallace et al., 2014; other citations). As a result they must have the physiological capability to withstand at least short term exposure, and the results in this dissertation suggest they can tolerate long term acidification as well. However, warming waters and intensifying hypoxic zones may not only pose a greater threat than high pCO2 – *M. menidia* are clearly more sensitive to these variables – but also increase vulnerability to high pCO2 through interactive effects.

* Acidification alone has variable and usually no effect on physiology
  + But combining acidification with hypoxia or temperature can elicit interactive responses.
  + In wild context, with fluctuations and different combinations, gradual global ocean acidification is unlikely to negatively impact *M. menidia*. Temperature and oxygen have much more direct effects on responses that are important for life history variables.
  + How does this compare to other species? Without getting into the weeds, just describe the range of sensitivities.
* Implications of energy budget under hypoxia for CO2 sensitivity (this is my chance to discuss this since the standalone paper doesn’t make as much sense to dwell on OA)
  + The growth efficiency or conversion efficiency coming from anaerobic metabolism being less efficient – what would that mean for acid-base balance? Are protons released from anaerobiosis that would bring down internal pH?
  + Slower differentiation could mean slower ionocyte differentiation, slower gill structure development, slower development of other homeostasis mechanisms and organ systems.
  + Energy budget doesn’t account for the concept that the most sensitive ones may die off at the earliest stages (e.g. weeded out by hatching or first feeding) and the remaining survivors are more tolerant – not a specific physiological mechanism but a result of phenotypic variability.
    - The skew in embryo but not larval skin ionocytes supports this, as Janet pointed out in comment on Ch 3.

**Future Directions**

* Future directions
  + Compare to more sensitive species
  + Take DEB model to the next step with population predictions and multistressor scenario simulations ranging from worst to best effects of OA (since it varies).
  + Apply sensitivities to ecosystem models and decision support tools (like with hotspots approach).

Considerations

-Look for similar points in the conclusions and abstract of each chapter

-How does the DEB chapter tie in with the other three?

-Go back to dissertation proposal and look at original objectives