Dissertation Introduction and Discussion

**Background and Motivation**

Ocean warming, acidification, and deoxygenation are concurrently affecting the coastal waters globally and especially in the Northeast United States (Wallace et al., 2014; Gledhill et al., 2015; Saba et al., 2016; Breitburg et al., 2018). The Earth’s atmosphere is warming as greenhouse gases from anthropogenic emissions accumulate and increasingly trap heat from solar radiation near the Earth’s surface (IPCC, 2022). The oceans take up most of the heat from the atmosphere, resulting in gradually rising temperatures in addition to increasing frequency and intensity of marine heatwaves (Scannell et al., 2016). Similarly, the carbon dioxide (CO2) from the atmosphere dissolves into seawater and changes ocean chemistry including the gradual decline in ocean pH, a process called ocean acidification (Feely et al., 2004; Doney et al., 2009). While anthropogenic CO2 emissions are driving global trends, community respiration in shallow coastal waters and estuaries leads to coastal acidification, more extreme high CO2 levels that fluctuate on diel to seasonal time scales (Cai et al., 2011). This results from a combination of natural stratification and upwelling patterns, freshwater input, and densely aggregated marine life in shallower waters relative to the open ocean, but is also caused by eutrophication from high nutrient inputs by humans (Cai et al., 2021).

Reduced levels of dissolved oxygen (DO) occur in combination with both warming and acidification. Warmer water can hold less DO and hence global warming is thought to be the primary cause of ocean deoxygenation worldwide (Diaz and Rosenberg, 2008; Breitburg et al., 2018). Climate change is also causing increased precipitation in many regions, and the freshwater input along with nutrient pollution causes eutrophication. During the day, algal blooms caused by the excess nutrients can take up CO2 and release oxygen but at night or in shaded subsurface waters, respiration overtakes photosynthesis and depletes oxygen – particularly when stratification, which is also intensified by climate change, traps layers of water from oxygen at the surface (Breitburg et al., 2018).

Assessing species’ biological sensitivity is necessary to determine vulnerability to these concurrent stressors and to conserve marine resources, ecosystems, and biodiversity. A challenge to quantifying biological sensitivity is the potential for non-additive interactions between multiple stressors that can be experienced simultaneously in nature, which include warming, acidification, hypoxia, contaminants, food availability, fishing pressure, and noise (Crain et al., 2008). Multistressor studies of biological responses provide information that can help predict not only a species’ sensitivity but also its ability to acclimate or adapt (Foo and Byrne, 2016; Orr et al., 2020) and even, when steps are taken to bridge individuals to the population and ecosystem levels, the secondary impacts on humans and other species.

Warming and hypoxia have well-studied, straightforward effects on fishes (Fry, 1947; Claireaux and Lagardère, 1999; Pörtner and Knust, 2007; Chabot and Claireaux, 2008; Richards et al., 2009; Claireaux et al., 2016). Rates of physiological processes depend directly on temperature and fishes have ranges of temperatures between which they can function at optimal capacity as well as upper and lower thermal limits beyond which they cannot survive for long (Pörtner, 2010). Oxygen is required for conversion of stored energy for activity, homeostasis, and growth, and hypoxia occurs when the supply of oxygen in the water is exceeded by organismal or community demand (Diaz and Breitburg, 2009). Hypoxia elicits temporary metabolic suppression to reduce oxygen demand, increased ventilation and heart rate to maximize oxygen uptake, and swimming to search for more oxygenated water (Kramer, 1987; Taylor and Miller, 2001; Chapman and McKenzie, 2009; Gamperl and Driedzic, 2009; Stierhoff et al., 2009). Larvae are generally the most sensitive stage, with oxygen demand very low in embryos and coping mechanisms better developed in juveniles and adults (Rombough, 1988).

Ocean acidification, however, has only recently been studied in fishes at relevant levels for global change (Fabry et al., 2008; Ishimatsu et al., 2008), with the number of studies rapidly increasing in the past 20 years and no widely applicable pattern of negatively or positively affecting fish (Kroeker et al., 2010; Kroeker et al., 2013; Heuer and Grosell, 2014; Clements et al., 2022). High CO2 reduces blood pH, and fish can remove CO2 from their blood to regulate internal pH by increasing ventilation as long as external partial pressure of CO2 (pCO2) does not exceed internal. Fish can rapidly correct small changes to internal pH with buffers in the blood and use active and passive transport in the gill and kidney cells to remove hydrogen ions and increase pH (Deigweiher et al., 2008; Brauner et al., 2019). What is less well-understood in fishes is whether there are widely applicable patterns in how the energetic and biochemical costs of acid-base regulation impact traits that relate to fitness and population status, such as growth, survival, behavior, and reproduction. Experimental exposure to near-future levels of acidification has shown effects such as detrimental sensory behavior (Munday et al., 2014; Porteus et al., 2021), reduced growth and survival (Baumann et al., 2012; Stiasny et al., 2016), and reproductive impacts (Welch and Munday, 2016; Concannon et al., 2021), but effects vary within and among species (Kroeker et al., 2010; Baumann et al., 2018; Cattano et al., 2018; Esbaugh, 2018). Measuring multiple physiological mechanisms underlying whole-organism responses is important for understanding the capacity for acclimation (Melzner et al., 2009), even when effects are not detected, because compensation by one physiological mechanism could allow other variables to remain unchanged (Sunday et al., 2014). In this way a lack of effects in experiments do not necessarily mean there are no energetic costs or tradeoffs that may affect fitness and population growth. Comprehensive assessment of responses related to fitness and adaptation is a research priority for anticipating acidification impacts on fished populations and ecosystem health.

The Atlantic silverside, *Menidia menidia*, is an ecologically important forage fish that has frequently been used as a model species to study impacts of environmental stressors (Bengtson et al., 1987; Schultz et al., 1998; Dixon et al., 2017; Baumann et al., 2018). Its annual life cycle, small size, and nearshore abundance make it an ideal species for laboratory experiments (Middaugh et al., 1987). *M. menidia* lives in the coastal and estuarine waters of eastern North America, including in Long Island Sound, NY, USA, and the smaller bays and estuaries attached to it. Long Island Sound is located within one of the most rapidly warming regions of the global oceans and near densely populated urban areas, but the coastal waters have improved in water quality over the last few decades, with nutrient input and hypoxic extent steadily decreasing (Gledhill et al., 2015; Whitney and Vlahos, 2021). The smaller more enclosed bays where *M. menidia* spawn have more extreme conditions, and the spawning season from April to early July results in larvae experiencing some of the most severe fluctuations in temperature, DO, and pCO2 (Murray et al., 2014; Baumann et al., 2015).

Natural variability in sensitivity to three co-occurring stressors makes predicting broader consequences of *M. menidia* responses challenging, but revealing the underlying physiological and energetic mechanisms can improve utility of results. Experiments on *M. menidia* offspring reared in different levels of pCO2 have found reduced early life growth and survival (Murray et al., 2014; Murray et al., 2017; Baumann et al., 2018), but *M. menidia* has remarkably high natural variability in sensitivity to acidification and co-occurring stressors (Murray and Baumann, 2018; Baumann et al., 2018; Cross et al., 2019; Morrell and Gobler, 2020). This species is often tolerant of high pCO2 alone but displays interactive effects when temperature or hypoxia treatments are simultaneously applied (Murray and Baumann, 2018; Cross et al., 2019; Morrell and Gobler, 2020; Concannon et al., 2021). Even small changes in growth and survival can have strong effects on recruitment (Houde, 1989). Quantifying the mechanisms underlying individual-level responses can improve understanding of ways in which stressors interact (Boyd et al., 2018; Baumann, 2019). Establishing mechanisms of response throughout the life cycle can also help connect individual responses to population-level consequences (Nisbet et al., 2000; National Research Council, 2005; Watson et al., 2020). Characterizing the full distribution of responses to global change stressors through multiple replicated studies can reveal whether a great enough proportion of a given species is substantially impacted enough to affect population size and, subsequently, the species that consume or are preyed upon by that species (Wittman and Pörtner, 2013; Baumann, 2019). This dissertation addresses these needs by quantifying responses of *M. menidia* to acidification, temperature, and hypoxia and determining the mechanisms responsible for whole-organism hypoxia effects.

**Objectives**

1. Chapter 1 quantifies the routine metabolic rates of *M. menidia* embryos and larvae reared in factorial combinations of CO2 and temperature, and CO2 and oxygen. This work was driven by previously observed declines in growth and survival under high CO2, and the desire to understand the physiological mechanisms responsible as well as how these may vary across temperature and DO levels periodically experienced in the early life environment of this species.
2. Chapter 2 characterizes the relationship between ambient oxygen and routine metabolism in *M. menidia* embryos and larvae, and how that relationship is impacted by high CO2. The objective was to refine understanding of metabolic interactions between DO and CO2 observed in Chapter 1 by recording responses to acute hypoxia.
3. In Chapter 3, I aimed to quantify the relationship between exposure to temperature and CO2 on the structures that largely control acid-base balance in fishes, the as a function of age in *M. menidia*. This chapter sought to elucidate a cellular mechanism underlying other responses to acidification and the role developmental stage, which is influenced by temperature, plays in ionoregulatory capacity.
4. Chapter 4 used hypoxia as a case study in enhancing utility and understanding of experimental effects by identifying the energetic mechanisms responsible for impacts in *M. menidia* early life stages. Using Dynamic Energy Budget (DEB) theory, which can ultimately connect physiology and life history to populations, I changed bioenergetic parameters based on hypothesized hypoxia effects and evaluated which parameters best accounted for differences in three response variables: total length over time, time to hatching, and survival rates.

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**Conclusions**

In Chapters 1 through 3 I quantified physiological mechanisms that help explain why early and late-spawned larvae exhibited reduced growth and survival when exposed to high CO2, but not those spawned within the peak season (Baumann et al., 2018). Embryos reared at 17°C, the temperature experienced by early spawners, had significantly greater ionocyte density in elevated pCO2. Early in the season when temperatures are lower and hatching takes longer, embryos may produce 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 embryonic metabolism in the high pCO2 treatments in Chapters 1 and 2 also suggest additional energy is being used for acid-base balance, drawing it away from growth. Like previous results on *M. menidia*, Chapters 1 through 3 highlight the incredible variability in metabolic rates and ionocyte densities overall and some experiments and age groups showing no pCO2 effects on metabolism. These levels of natural variability highlight how tolerance may be facilitated by genetic influences and a wide range of phenotypes (Sunday et al., 2014; Foo and Byrne, 2016).

Throughout Chapters 1 through 3 I showed 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 a wide variety of species (Harvey et al., 2013) such as Atlantic cod (Stiasny et al., 2019), mummichog (Targett et al., 2019), inland silverside, and sheepshead minnow (Gobler et al., 2018). 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 (Baumann et al., 2015; Wallace et al., 2014; Cooley et al., 2022). 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 to fishes than high pCO2 (Sampaio et al., 2021) – *M. menidia* are clearly more sensitive to these variables – but also increase fish vulnerability to high pCO2 through interactive effects (Depasquale et al., 2015). Warming increases metabolic rates in *M. menidia* as well as most species and is expected to reduce average fish sizes worldwide (Cheung et al., 2013). Even moderate but static reductions in oxygen can be detrimental to early life stages (Depasquale et al., 2015; Cross et al., 2019) and alter behavior in ways that increase energy use and predation vulnerability (Miller et al., 2016). In Chapter 2, unexpectedly, I did find an exception to the idea that combining stressors increases sensitivity. At 2 days post-hatching rearing *M. menidia* in high pCO2 decreased the critical oxygen level of routine metabolism, contrary to what I hypothesized. This response has also been documented in European sea bass (Montgomery et al., 2019) and may indicate that using additional energy on maintaining homeostasis could lead to modifications that enhance oxygen uptake capacity in some way. Compared to species that naturally occur in habitats with relatively static conditions, the results of this dissertation suggest that *M. menidia* has mechanisms to tolerate anticipated multistressor challenges (Baumann, 2019).

In Chapter 4, I demonstrated that conversion of assimilates into somatic growth and maximum assimilation rate are two primary energetic mechanisms mostly likely underlying hypoxia effects on hatching, growth, and survival in *M. menidia* early life stages. This model provided an important step towards better understanding acidification sensitivity by creating a foundation to investigate multiple stressors and by identifying processes impacted by hypoxia that may tie into internal pH regulation. Reduced conversion efficiency could be indicative of slower rates of differentiation in development, which could impair formation of ionocytes, gills, and organ systems that aid tolerance of acidified seawater. Hypoxia-related reductions in both the conversion efficiency and assimilation rate could also result in less energy for ionic homeostasis. Maintenance is often considered to be the process most likely affected by acidification in DEB modeling (Jager et al., 2016; Moreira et al., 2022; Pousse et al., 2022), and I did not find elevated maintenance rates to be a primary source of hypoxia effects. This evidence that both acidification and hypoxia would not be simultaneously demanding energy for maintenance bodes well for *M. menidia* as global change intensifies both in coastal waters.

This dissertation quantifies the physiological and energetic mechanisms underlying observed tolerance and sensitivity to various combinations of pCO2, oxygen, and temperature for the model species *M. menidia* to gain a fuller understanding of species sensitivity and capacity for acclimation. While doing the same for all fishes is not possible, the mechanisms revealed in this study are broadly applicable to other species. However, because *M. menidia* has evolved in ecosystems with naturally varying hypoxia and acidification the adaptability demonstrated here might be seen as the upper limits of adaptability. Even when traits such as hatch survival and hatch length are unaffected by stressors (Murray and Baumann, 2018), I show here by looking at fish from the same experiments that the lack of effects on important traits for fitness is sometimes supported by physiological tradeoffs such as increased embryonic ionocyte abundance (Chapter 3). Identifying these mechanisms can help researchers understand the limits of acclimation and what tradeoffs fish may incur to maintain performance under multiple stressors (Sunday et al., 2014). The wide variability in sensitivity of the responses I measured over the course of eight different experiments also highlights the high level of phenotypic variability in multiple processes, which increases the chances that at least some individuals will survive. For example, great positive skew in embryo ionocyte densities may have yielded a subset that went on to be measured as insensitive to high pCO2 after hatching. These survivors possibly pass on modes of transgenerational acclimation to offspring and their tolerance buys time for genetic adaptation to occur (Chevin et al., 2010).

**Future Directions**

There are two pressing demands in global change research that this dissertation sets a foundation to investigate in the future. One is the need to bridge cellular, physiological, whole-organismal, and life history impacts of multiple stressors to the population level . The second, which builds upon the first, is the need to integrate biological responses of individual species with biological, physical, and chemical oceanography monitoring and social science to support ecosystem-based management (Leslie and McLeod, 2007; Saba et al., 2019).

Energy budget models are a well-established approach to connect individual effects to the population level (Nisbet et al., 2000) and incorporate organismal impacts into conservation efforts (Watson et al., 2020). Future work should build on this DEB model by exploring the incorporation of additional stressors and using it to model population growth rates under different scenarios of global change conditions. Modeling acidification effects in this DEB model was not within the scope of this dissertation because of the inconsistency of acidification effects on *M. menidia* throughout multiple experiments and to allow sufficient attention to hypoxia effects on the energy budget. Now that the parameters have been estimated and correction factors established for one stressor, this work should be built on to obtain population-level predictions for multiple stressors.

I have documented the range of sensitivity levels *M. menidia* early life stages have to pCO2, oxygen, and temperature. Coastal and estuarine monitoring of environmental conditions and projections for future conditions could be combined with these sensitivity levels to identify regions with most and least suitable habitat, keeping in mind that sensitivities – especially for acidification – tend to be low and seasonally dependent. The high possibility for tolerance could be considered as a competitive advantage that may allow *M. menidia* to live in areas other species would avoid. As an abundant forage fish that provides an important food source for fish, birds, and mammals, predicting population growth and preferred habitat could contribute an important link in ecosystem-level studies.

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